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Handbook of Enzymes

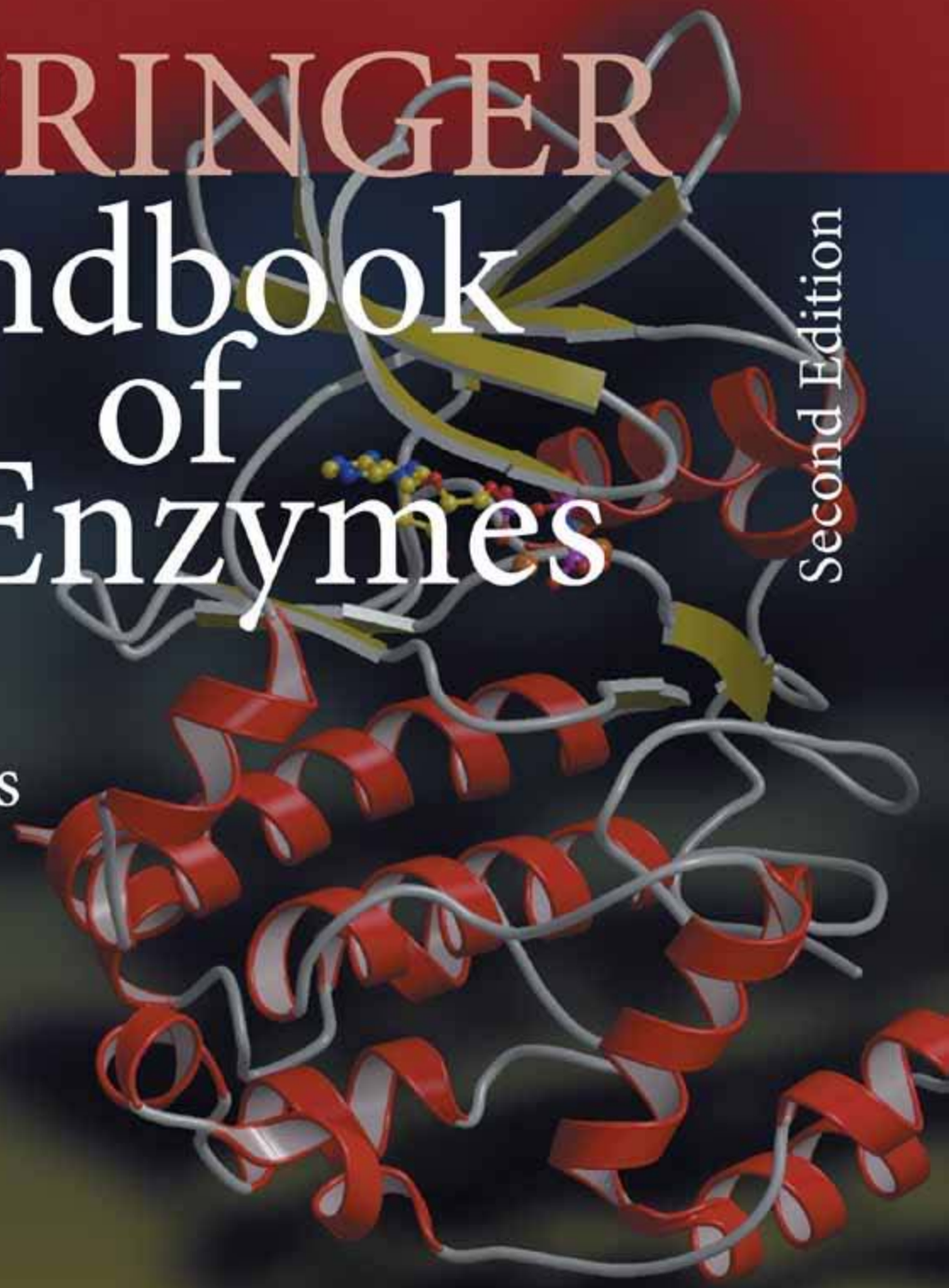
Second Edition

SUPPLEMENT VOLUME S5

CLASS 3

Hydrolases

EC 3.1–3.4.21



Springer

Springer Handbook of Enzymes
Supplement Volume S5

Dietmar Schomburg and
Ida Schomburg (Eds.)

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Hydrolases

EC 3.1–3.4.21

coedited by Antje Chang

Second Edition

Springer

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Preface

Today, as the full information about the genome is becoming available for a rapidly increasing number of organisms and transcriptome and proteome analyses are beginning to provide us with a much wider image of protein regulation and function, it is obvious that there are limitations to our ability to access functional data for the gene products – the proteins and, in particular, for enzymes. Those data are inherently very difficult to collect, interpret and standardize as they are widely distributed among journals from different fields and are often subject to experimental conditions. Nevertheless a systematic collection is essential for our interpretation of genome information and more so for applications of this knowledge in the fields of medicine, agriculture, etc. Progress on enzyme immobilisation, enzyme production, enzyme inhibition, coenzyme regeneration and enzyme engineering has opened up fascinating new fields for the potential application of enzymes in a wide range of different areas.

The development of the enzyme data information system BRENDA was started in 1987 at the German National Research Centre for Biotechnology in Braunschweig (GBF), continued at the University of Cologne from 1996 to 2007, and then returned to Braunschweig, to the Technical University, Institute of Bioinformatics & Systems Biology. The present book "Springer Handbook of Enzymes" represents the printed version of this data bank. The information system has been developed into a full metabolic database.

The enzymes in this Handbook are arranged according to the Enzyme Commission list of enzymes. Some 5,000 "different" enzymes are covered. Frequently enzymes with very different properties are included under the same EC-number. Although we intend to give a representative overview on the characteristics and variability of each enzyme, the Handbook is not a compendium. The reader will have to go to the primary literature for more detailed information. Naturally it is not possible to cover all the numerous literature references for each enzyme (for some enzymes up to 40,000) if the data representation is to be concise as is intended.

It should be mentioned here that the data have been extracted from the literature and critically evaluated by qualified scientists. On the other hand, the original authors' nomenclature for enzyme forms and subunits is retained. In order to keep the tables concise, redundant information is avoided as far as possible (e.g. if K_m values are measured in the presence of an obvious cosubstrate, only the name of the cosubstrate is given in parentheses as a commentary without reference to its specific role).

The authors are grateful to the following biologists and chemists for invaluable help in the compilation of data: Cornelia Munaretto and Dr. Antje Chang.

Braunschweig
Spring 2009

Dietmar Schomburg, Ida Schomburg

List of Abbreviations

A	adenine
Ac	acetyl
ADP	adenosine 5'-diphosphate
Ala	alanine
All	allose
Alt	altrose
AMP	adenosine 5'-monophosphate
Ara	arabinose
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine 5'-triphosphate
Bicine	N,N'-bis(2-hydroxyethyl)glycine
C	cytosine
cal	calorie
CDP	cytidine 5'-diphosphate
CDTA	trans-1,2-diaminocyclohexane-N,N,N,N-tetraacetic acid
CMP	cytidine 5'-monophosphate
CoA	coenzyme A
CTP	cytidine 5'-triphosphate
Cys	cysteine
d	deoxy-
D-	(and L-) prefixes indicating configuration
DFP	diisopropyl fluorophosphate
DNA	deoxyribonucleic acid
DPN	diphosphopyridinium nucleotide (now NAD^+)
DTNB	5,5'-dithiobis(2-nitrobenzoate)
DTT	dithiothreitol (i.e. Cleland's reagent)
EC	number of enzyme in Enzyme Commission's system
E. coli	Escherichia coli
EDTA	ethylene diaminetetraacetate
EGTA	ethylene glycol bis(-aminoethyl ether) tetraacetate
ER	endoplasmic reticulum
Et	ethyl
EXAFS	extended X-ray absorption fine structure
FAD	flavin-adenine dinucleotide
FMN	flavin mononucleotide (riboflavin 5'-monophosphate)
Fru	fructose
Fuc	fucose
G	guanine
Gal	galactose

GDP	guanosine 5'-diphosphate
Glc	glucose
GlcN	glucosamine
GlcNAc	N-acetylglucosamine
Gln	glutamine
Glu	glutamic acid
Gly	glycine
GMP	guanosine 5'-monophosphate
GSH	glutathione
GSSG	oxidized glutathione
GTP	guanosine 5'-triphosphate
Gul	gulose
h	hour
H4	tetrahydro
HEPES	4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid
His	histidine
HPLC	high performance liquid chromatography
Hyl	hydroxylysine
Hyp	hydroxyproline
IAA	iodoacetamide
IC 50	50% inhibitory concentration
Ig	immunoglobulin
Ile	isoleucine
Ido	idose
IDP	inosine 5'-diphosphate
IMP	inosine 5'-monophosphate
ITP	inosine 5'-triphosphate
K_m	Michaelis constant
L-	(and D-) prefixes indicating configuration
Leu	leucine
Lys	lysine
Lyx	lyxose
M	mol/l
mM	millimol/l
m-	*meta*-
Man	mannose
MES	2-(N-morpholino)ethane sulfonate
Met	methionine
min	minute
MOPS	3-(N-morpholino)propane sulfonate
Mur	muramic acid
MW	molecular weight
NAD^+	nicotinamide-adenine dinucleotide
NADH	reduced NAD
$NADP^+$	NAD phosphate
NADPH	reduced NADP
NAD(P)H	indicates either NADH or NADPH

NBS	N-bromosuccinimide
NDP	nucleoside 5'-diphosphate
NEM	N-ethylmaleimide
Neu	neuraminic acid
NMN	nicotinamide mononucleotide
NMP	nucleoside 5'-monophosphate
NTP	nucleoside 5'-triphosphate
o-	*ortho*-
Orn	ornithine
p-	*para*-
PBS	phosphate-buffered saline
PCMB	*p*-chloromercuribenzoate
PEP	phosphoenolpyruvate
pH	-log10[H^+]
Ph	phenyl
Phe	phenylalanine
PHMB	*p*-hydroxymercuribenzoate
PIXE	proton-induced X-ray emission
PMSF	phenylmethane-sulfonylfluoride
p-NPP	*p*-nitrophenyl phosphate
Pro	proline
Q_{10}	factor for the change in reaction rate for a 10°C temperature increase
Rha	rhamnose
Rib	ribose
RNA	ribonucleic acid
mRNA	messenger RNA
rRNA	ribosomal RNA
tRNA	transfer RNA
Sar	N-methylglycine (sarcosine)
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Ser	serine
T	thymine
t_H	time for half-completion of reaction
Tal	talose
TDP	thymidine 5'-diphosphate
TEA	triethanolamine
Thr	threonine
TLCK	N^{α}-*p*-tosyl-L-lysine chloromethyl ketone
T_m	melting temperature
TMP	thymidine 5'-monophosphate
Tos-	tosyl- (*p*-toluenesulfonyl-)
TPN	triphosphopyridinium nucleotide (now $NADP^+$)
Tris	tris(hydroxymethyl)-aminomethane
Trp	tryptophan
TTP	thymidine 5'-triphosphate
Tyr	tyrosine
U	uridine

U/mg	μmol/(mg*min)
UDP	uridine 5'-diphosphate
UMP	uridine 5'-monophosphate
UTP	uridine 5'-triphosphate
Val	valine
Xaa	symbol for an amino acid of unknown constitution in peptide formula
XAS	X-ray absorption spectroscopy
Xyl	xylose

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Description of Data Fields

All information except the nomenclature of the enzymes (which is based on the recommendations of the Nomenclature Committee of IUBMB (**I**nternational **U**nion of **B**iochemistry and **M**olecular **B**iology) and IUPAC (**I**nternational **U**nion of **P**ure and **A**pplied **C**hemistry) is extracted from original literature (or reviews for very well characterized enzymes). The quality and reliability of the data depends on the method of determination, and for older literature on the techniques available at that time. This is especially true for the fields *Molecular Weight* and *Subunits*.

The general structure of the fields is: **Information – Organism – Commentary – Literature**

The information can be found in the form of numerical values (temperature, pH, K_m etc.) or as text (cofactors, inhibitors etc.).

Sometimes data are classified as *Additional Information*. Here you may find data that cannot be recalculated to the units required for a field or also general information being valid for all values. For example, for *Inhibitors*, *Additional Information* may contain a list of compounds that are not inhibitory.

The detailed structure and contents of each field is described below. If one of these fields is missing for a particular enzyme, this means that for this field, no data are available.

1 Nomenclature

EC number
The number is as given by the IUBMB, classes of enzymes and subclasses defined according to the reaction catalyzed.

Systematic name
This is the name as given by the IUBMB/IUPAC Nomenclature Committee

Recommended name
This is the name as given by the IUBMB/IUPAC Nomenclature Committee

Synonyms
Synonyms which are found in other databases or in the literature, abbreviations, names of commercially available products. If identical names are frequently used for different enzymes, these will be mentioned here, cross references are given. If another EC number has been included in this entry, it is mentioned here.

CAS registry number

The majority of enzymes have a single chemical abstract (CAS) number. Some have no number at all, some have two or more numbers. Sometimes two enzymes share a common number. When this occurs, it is mentioned in the commentary.

2 Source Organism

For listing organisms their systematic name is preferred. If these are not mentioned in the literature, the names from the respective literature are used. For example if an enzyme from yeast is described without being specified further, yeast will be the entry. This field defines the code numbers for the organisms in which the enzyme with the respective EC number is found. These code numbers (form <_>) are displayed together with each entry in all fields of BRENDA where organism-specific information is given.

3 Reaction and Specificity

Catalyzed reaction

The reaction as defined by the IUBMB. The commentary gives information on the mechanism, the stereochemistry, or on thermodynamic data of the reaction.

Reaction type

According to the enzyme class a type can be attributed. These can be oxidation, reduction, elimination, addition, or a name (e.g. Knorr reaction)

Natural substrates and products

These are substrates and products which are metabolized in vivo. A natural substrate is only given if it is mentioned in the literature. The commentary gives information on the pathways for which this enzyme is important. If the enzyme is induced by a specific compound or growth conditions, this will be included in the commentary. In *Additional information* you will find comments on the metabolic role, sometimes only assumptions can be found in the references or the natural substrates are unknown.

In the listings, each natural substrate (indicated by a bold **S**) is followed by its respective product (indicated by a bold **P**). Products are given with organisms and references included only if the respective authors were able to demonstrate the formation of the specific product. If only the disappearance of the substrate was observed, the product is included without organisms of references. In cases with unclear product formation only a ? as a dummy is given.

Substrates and products

All natural or synthetic substrates are listed (not in stoichiometric quantities). The commentary gives information on the reversibility of the reaction,

on isomers accepted as substrates and it compares the efficiency of substrates. If a specific substrate is accepted by only one of several isozymes, this will be stated here.

The field *Additional Information* summarizes compounds that are not accepted as substrates or general comments which are valid for all substrates.

In the listings, each substrate (indicated by a bold **S**) is followed by its respective product (indicated by a bold **P**). Products are given with organisms and references included if the respective authors demonstrated the formation of the specific product. If only the disappearance of the substrate was observed, the product will be included without organisms or references. In cases with unclear product formation only a **?** as a dummy is given.

Inhibitors

Compounds found to be inhibitory are listed. The commentary may explain experimental conditions, the concentration yielding a specific degree of inhibition or the inhibition constant. If a substance is activating at a specific concentration but inhibiting at a higher or lower value, the commentary will explain this.

Cofactors, prosthetic groups

This field contains cofactors which participate in the reaction but are not bound to the enzyme, and prosthetic groups being tightly bound. The commentary explains the function or, if known, the stereochemistry, or whether the cofactor can be replaced by a similar compound with higher or lower efficiency.

Activating Compounds

This field lists compounds with a positive effect on the activity. The enzyme may be inactive in the absence of certain compounds or may require activating molecules like sulfhydryl compounds, chelating agents, or lipids. If a substance is activating at a specific concentration but inhibiting at a higher or lower value, the commentary will explain this.

Metals, ions

This field lists all metals or ions that have activating effects. The commentary explains the role each of the cited metal has, being either bound e.g. as Fe-S centers or being required in solution. If an ion plays a dual role, activating at a certain concentration but inhibiting at a higher or lower concentration, this will be given in the commentary.

Turnover number (min^{-1})

The k_{cat} is given in the unit min^{-1}. The commentary lists the names of the substrates, sometimes with information on the reaction conditions or the type of reaction if the enzyme is capable of catalyzing different reactions with a single substrate. For cases where it is impossible to give the turnover number in the defined unit (e.g., substrates without a defined molecular weight, or an undefined amount of protein) this is summarized in *Additional Information*.

Specific activity (U/mg)

The unit is micromol/minute/milligram of protein. The commentary may contain information on specific assay conditions or if another than the natural substrate was used in the assay. Entries in *Additional Information* are included if the units of the activity are missing in the literature or are not calculable to the obligatory unit. Information on literature with a detailed description of the assay method may also be found.

K_m-Value (mM)

The unit is mM. Each value is connected to a substrate name. The commentary gives, if available, information on specific reaction condition, isozymes or presence of activators. The references for values which cannot be expressed in mM (e.g. for macromolecular, not precisely defined substrates) are given in *Additional Information*. In this field we also cite literature with detailed kinetic analyses.

K_i-Value (mM)

The unit of the inhibition constant is mM. Each value is connected to an inhibitor name. The commentary gives, if available, the type of inhibition (e.g. competitive, non-competitive) and the reaction conditions (pH-value and the temperature). Values which cannot be expressed in the requested unit and references for detailed inhibition studies are summerized under *Additional information*.

pH-Optimum

The value is given to one decimal place. The commentary may contain information on specific assay conditions, such as temperature, presence of activators or if this optimum is valid for only one of several isozymes. If the enzyme has a second optimum, this will be mentioned here.

pH-Range

Mostly given as a range e.g. 4.0–7.0 with an added commentary explaining the activity in this range. Sometimes, not a range but a single value indicating the upper or lower limit of enzyme activity is given. In this case, the commentary is obligatory.

Temperature optimum (°C)

Sometimes, if no temperature optimum is found in the literature, the temperature of the assay is given instead. This is always mentioned in the commentary.

Temperature range (°C)

This is the range over which the enzyme is active. The commentary may give the percentage of activity at the outer limits. Also commentaries on specific assay conditions, additives etc.

4 Enzyme Structure

Molecular weight
This field gives the molecular weight of the holoenzyme. For monomeric enzymes it is identical to the value given for subunits. As the accuracy depends on the method of determination this is given in the commentary if provided in the literature. Some enzymes are only active as multienzyme complexes for which the names and/or EC numbers of all participating enzymes are given in the commentary.

Subunits
The tertiary structure of the active species is described. The enzyme can be active as a monomer a dimer, trimer and so on. The stoichiometry of subunit composition is given. Some enzymes can be active in more than one state of complexation with differing effectivities. The analytical method is included.

Posttranslational modifications
The main entries in this field may be proteolytic modification, or side-chain modification, or no modification. The commentary will give details of the modifications e.g.:
- proteolytic modification <1> (<1>, propeptide Name) [1];
- side-chain modification <2> (<2>, N-glycosylated, 12% mannose) [2];
- no modification [3]

5 Isolation / Preparation / Mutation / Application

Source / tissue
For multicellular organisms, the tissue used for isolation of the enzyme or the tissue in which the enzyme is present is given. Cell-lines may also be a source of enzymes.

Localization
The subcellular localization is described. Typical entries are: cytoplasm, nucleus, extracellular, membrane.

Purification
The field consists of an organism and a reference. Only references with a detailed description of the purification procedure are cited.

Renaturation
Commentary on denaturant or renaturation procedure.

Crystallization
The literature is cited which describes the procedure of crystallization, or the X-ray structure.

Cloning
Lists of organisms and references, sometimes a commentary about expression or gene structure.

Engineering
The properties of modified proteins are described.

Application
Actual or possible applications in the fields of pharmacology, medicine, synthesis, analysis, agriculture, nutrition are described.

6 Stability

pH-Stability
This field can either give a range in which the enzyme is stable or a single value. In the latter case the commentary is obligatory and explains the conditions and stability at this value.

Temperature stability
This field can either give a range in which the enzyme is stable or a single value. In the latter case the commentary is obligatory and explains the conditions and stability at this value.

Oxidation stability
Stability in the presence of oxidizing agents, e.g. O_2, H_2O_2, especially important for enzymes which are only active under anaerobic conditions.

Organic solvent stability
The stability in the presence of organic solvents is described.

General stability information
This field summarizes general information on stability, e.g., increased stability of immobilized enzymes, stabilization by SH-reagents, detergents, glycerol or albumins etc.

Storage stability
Storage conditions and reported stability or loss of activity during storage.

References
Authors, Title, Journal, Volume, Pages, Year.

Polyneuridine-aldehyde esterase 3.1.1.78

1 Nomenclature

EC number
3.1.1.78

Systematic name
polyneuridine aldehyde hydrolase (decarboxylating)

Recommended name
polyneuridine-aldehyde esterase

Synonyms
polyneuridine aldehyde esterase

CAS registry number
87041-55-2

2 Source Organism

<1> *Rauvolfia serpentina Benth. ex Kurz* (no sequence specified) [1, 2, 3, 4]

3 Reaction and Specificity

Catalyzed reaction
polyneuridine aldehyde + H_2O = 16-epivellosimine + CO_2 + methanol

Reaction type
decarboxylation
ester hydrolysis

Natural substrates and products
- **S** polyneuridine aldehyde + H_2O <1> (<1> central enzyme of indole alkaloid biosynthesis [1]; <1> key enzyme in the biosynthesis of sarpagine/ajmaline type alkaloids [3,4]) (Reversibility: ?) [1, 2, 3, 4]
- **P** 16-epivellosimine + CO_2 + methanol

Substrates and products
- **S** akuammidine aldehyde + H_2O <1> (Reversibility: ?) [3]
- **P** vellosiminol + CO_2 + methanol
- **S** polyneuridine aldehyde + H_2O <1> (<1> intermediate is polyneuridine aldehyde acid [2]; <1> central enzyme of indole alkaloid biosynthesis

[1]; <1> key enzyme in the biosynthesis of sarpagine/ajmaline type alkaloids [3,4]) (Reversibility: ?) [1, 2, 3, 4]

P 16-epivellosimine + CO_2 + methanol <1> (<1> 16-epivellosimine reacts to vellosimine [2,4]) [1, 2, 3, 4]

Inhibitors

2,2'-dithiodipyridine <1> [3]
5,5'-dithio-bis-(2-nitrobenzoate) <1> [3]
diethyldicarbonate <1> (<1> 100% inhibition at 0.8 mM [1]) [1]
$HgCl_2$ <1> (<1> 100% inhibition at 0.2 mM [1]) [1]
iodoacetamide <1> (<1> 100% inhibition at 5 mM [3]) [3]
L-chloro-3-[4-tosyl-amido]-7-amido-2-heptanone <1> (<1> 12% inhibition at 0.12 mM [1]) [1]
$MgCl_2$ <1> (<1> 50% inhibition at 0.25 mM [3]) [3]
$MnCl_2$ <1> (<1> 50% inhibition at 0.25 mM [3]) [3]
phenylmethanesulfonyl fluoride <1> (<1> 20% inhibition at 1 mM [1]) [1]
$ZnCl_2$ <1> (<1> 50% inhibition at 0.25 mM [3]) [3]

K_m-Value (mM)

0.83 <1> (polyneuridine aldehyde) [2]
1.25 <1> (polyneuridine aldehyde) [3]

pH-Optimum

6.5 <1> [3]
8 <1> (<1> assay conditions [1]) [1]

pH-Range

4-8.5 <1> [3]

Temperature optimum (°C)

30 <1> (<1> assay conditions [1]) [1]

4 Enzyme Structure

Molecular weight

60000 <1> (<1> gel filtration [1]) [1]

Subunits

homodimer <1> (<1> 2 * 30000, SDS-PAGE [1,2]) [1, 2]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [1, 2, 3]

Cloning

<1> (expressed in Escherichia coli M15) [1]
<1> (fused with a C-terminal His-tag) [2]

Engineering

C132A <1> (<1> 755% lower activity than wild-type [1]) [1]
C170A <1> (<1> 80% lower activity than wild-type [1]) [1]
C213S/G152Q <1> (<1> 35% lower activity than wild-type [1]) [1]
C257A <1> (<1> 35% lower activity than wild-type [1]) [1]
H17A <1> (<1> 97% lower activity than wild-type [1]) [1]

6 Stability

Storage stability

<1>, 0°C, 5 months, 0.2 M 2-mercaptoethanol, 100% activity [3]

References

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[4] Pfitzner, A.; Stoeckigt, J.: Polyneuridine aldehyde esterase: an unusually specific enzyme involved in the biosynthesis of sarpagine-type alkaloids. J. Chem. Soc. Chem. Commun., **1983**, 459-460 (1983)

Hormone-sensitive lipase 3.1.1.79

1 Nomenclature

EC number
3.1.1.79

Systematic name
diacylglycerol acylhydrolase

Recommended name
hormone-sensitive lipase

Synonyms
HSL <1, 2, 3, 4, 5> [1, 4, 5, 8, 11, 12, 13, 14, 15, 16, 20, 21, 22, 23, 24, 27, 29]

CAS registry number
9001-62-1

2 Source Organism

<1> *Mus musculus* (no sequence specified) [4, 7, 21, 22, 39]
<2> *Homo sapiens* (no sequence specified) [4, 9, 10, 16, 17, 22, 27, 31, 32, 33, 36, 37, 38, 41]
<3> *Rattus norvegicus* (no sequence specified) [4, 6, 8, 12, 13, 14, 15, 17, 18, 22, 23, 25, 27, 28, 29, 30, 35, 40]
<4> *Sus scrofa* (no sequence specified) [20, 24]
<5> *Bos taurus* (no sequence specified) [1,2,3,5,11,19]
<6> *Trematomus newnesi* (no sequence specified) [26]
<7> *Rattus norvegicus* (UNIPROT accession number: Q6T861) [34]

3 Reaction and Specificity

Catalyzed reaction
diacylglycerol + H_2O = monoacylglycerol + a carboxylate
monoacylglycerol + H_2O = glycerol + a carboxylate
triacylglycerol + H_2O = diacylglycerol + a carboxylate

Natural substrates and products
S 1,2-dioleoylglycerol + H_2O <3> (Reversibility: ?) [25]
P 2-monooleoylglycerol (<3> mayor end product of trioleoylglycerol and 1,2-dioleoylglycerol hydrolysis [25])

S 1-monooleoylglycerol + H_2O <3> (<3> 3times higher activity as with 2-oleoylglycerol [25]) (Reversibility: ?) [25]
P glycerol + oleate
S 2-monooleoylglycerol + H_2O <3> (Reversibility: ?) [25]
P glycerol + oleate
S cholesteryl ester + H_2O <1> (Reversibility: ?) [22]
P cholesterol + a carboxylate
S cholesteryl oleate + H_2O <3> (Reversibility: ?) [15]
P cholesterol + oleate
S diacylglycerol + H_2O <3, 5> (Reversibility: ?) [5, 22]
P monoacylglycerol + a carboxylate
S dioleoylglycerol + H_2O <4> (Reversibility: ?) [20]
P oleoylglycerol + oleate
S monacylglycerol + H_2O <3> (Reversibility: ?) [22]
P glycerol + a carboxylate
S monoacylglycerol + H_2O <5> (Reversibility: ?) [5]
P glycerol + a carboxylate
S oleoylglycerol + H_2O <4> (Reversibility: ?) [20]
P glycerol + oleate
S triacylglycerol + H_2O <3, 5> (Reversibility: ?) [5, 22]
P diacylglycerol + a carboxylate
S trioleoylglycerol + H_2O <4> (Reversibility: ?) [20]
P dioleoylglycerol + oleate
S trioleoylglycerol + H_2O <3> (Reversibility: ?) [25]
P 1,2-dioleoylglycerol + oleate
S Additional information <1, 2, 3, 7> (<3> expressed at a higher level in oxidative fibres than in glycolytic fibres. The two physiological stimuli adrenaline and contraction increase enzyme activity in muscle, and the effects are partially additive. Adrenaline acts via β-adrenergic activation of cAMP-dependent protein kinase, while the effect of contractions is mediated by protein kinase C, at least partly via the extracellular signal-regulated kinase pathway. Endurance training diminishes the sensitivity of muscle hormone-sensitive lipase to adrenaline but increases the contraction-mediated activation [40]; <1> hormone-sensitive lipase is required for high-density lipoprotein cholesteryl ester-supported adrenal steroidogenesis [39]; <2> hormone-sensitive lipase may be the rate-limiting enzyme controlling the cellular catabolism of diacylglycerol in adipose tissue and that of cholesteryl esters in macrophage foam cells and steroidogenic tissues releasing the cholesterol required for adrenal steroidogenesis [36]; <2> in the skeletal muscle at rest there is a high constitutive level of hormone-sensitive lipase activity, which is not a function of biopsy freezing. The combination of low adrenaline and Ca^{2+} levels and resting levels of insulin appears to dictate the levels of activity of hormone-sensitive lipase at rest. During the onset of low and moderate aerobic exercise enzyme activity is activated by contractions, in the apparent absence of increases in circulating adrenaline. However, adrenaline may contribute to the early activation of the enzyme during intense aerobic exercise. The

contraction-induced activation appears to be related to the increase in protein kinase C and extracellular signal regulated kinase activity associated with Ca^{2+} and/or other unknown activators. As low- and moderate-intensity exercise continues beyond a few min, activation by adrenaline through the cAMP cascade and protein kinase A also appears to occur. With prolonged moderate-intensity exercise beyond 1-2 h and sustained high-intensity exercise, HSL activity decreases despite continuing increases in adrenaline, possibly as a result of increasing accumulation of free AMP, activation of AMP-activated kinase and phosphorylation of inhibitory sites on the hormone-sensitive lipase. Intramuscular factors dominate the control of hormone-sensitive lipase activity, with hormonal factors playing a smaller role [41]; <7> key enzyme in fatty acid mobilization [34]; <3> rate-limiting enzyme in lipolysis [30]; <2> the enzyme catalyses the hydrolysis of myocellular triacylglycerol, which is a potential energy source diuring exercise [38]; <2> the enzyme is involved in the hormone regulated release of fatty acids and glycerol from adipocyte lipid stores [37]) (Reversibility: ?) [30, 34, 36, 37, 38, 39, 40, 41]

P ?

Substrates and products

S 1,2-dioleoyl-sn-glycerol + H_2O <3> (Reversibility: ?) [35]
P ?
S 1,2-dioleoylglycerol + H_2O <3> (Reversibility: ?) [25]
P 2-monooleoylglycerol (<3> mayor end product of trioleoylglycerol and 1,2-dioleoylglycerol hydrolysis [25])
S 1-monooleoylglycerol + H_2O <3> (<3> 3times higher activity as with 2-oleoylglycerol [25]) (Reversibility: ?) [25]
P glycerol + oleate
S 1-oleoyl-2-O-oleylglycerol + H_2O <2, 3, 5> (Reversibility: ?) [9, 18, 19]
P 2-O-oleylglycerol + oleate
S 1-oleoyl-2-oleylglycerol + H_2O <2, 3, 5> (Reversibility: ?) [2, 6, 8, 11, 12, 17, 22, 23]
P 2-oleylglycerol + oleate
S 2-monooleoylglycerol + H_2O <3> (Reversibility: ?) [25]
P glycerol + oleate
S cholesterol oleate + H_2O <3> (Reversibility: ?) [18]
P cholesterol + oleate
S cholesteryl ester + H_2O <1> (Reversibility: ?) [22]
P cholesterol + a carboxylate
S cholesteryl oleate + H_2O <3> (Reversibility: ?) [30]
P cholesterol + oleic acid
S cholesteryl oleate + H_2O <2, 3, 5> (<5> 18.5% of activity with 1-oleoyl-2-oleylglycerol [2]; <3> approx. 55% of activity with dioleoylglcerol [14]) (Reversibility: ?) [1, 2, 3, 9, 11, 14, 15, 19, 22, 36]
P cholesterol + oleate
S dehydroepiandrosterone oleate + H_2O <5> (<5> 12.9% of activity with 1-oleoyl-2-oleylglycerol [2]) (Reversibility: ?) [2]

P dehydroepiandrosterone + oleate
S diacylglycerol + H_2O <1, 2, 3, 5> (Reversibility: ?) [3, 5, 10, 21, 22]
P monoacylglycerol + a carboxylate
S dioleoyl glycerol + H_2O <2> (<2> 21% of activity with vinylbutyrate [16]) (Reversibility: ?) [16]
P monooleoylglycerol + oleate
S dioleoylglycerol + H_2O <3> (Reversibility: ?) [14]
P monooleoylglycerol + oleate
S dioleoylglycerol + H_2O <4> (Reversibility: ?) [20]
P oleoylglycerol + oleate
S estradiol-17-β-oleate + H_2O <5> (<5> 17.9% of activity with 1-oleoyl-2-oleylglycerol [2]) (Reversibility: ?) [2]
P 17-β-estradiol + oleate
S estradiol-17-β-palmitate + H_2O <5> (<5> 18.3% of activity with 1-oleoyl-2-oleylglycerol [2]) (Reversibility: ?) [2]
P 17-β-estradiol + palmitate
S lipid emulsion + H_2O <2, 3> (<2,3> recombinant HSL, the release of fatty acids is only slightly affected by their degree of unsaturation [27]) (Reversibility: ?) [27]
P fatty acids (<2,3> fatty acids ranging in chain length from 12 to 24 carbon atoms and in unsaturation from 0 to 6 double bonds [27])
S monacylglycerol + H_2O <3> (Reversibility: ?) [22]
P glycerol + a carboxylate
S monoacylglycerol + H_2O <1, 5> (Reversibility: ?) [3, 5, 21]
P glycerol + a carboxylate
S oleoylglycerol + H_2O <4> (Reversibility: ?) [20]
P glycerol + oleate
S olive oil + H_2O <2> (<2> 3% of activity with vinylbutyrate [16]) (Reversibility: ?) [16]
P ?
S *p*-nitrophenyl acetate + H_2O <5> (Reversibility: ?) [3]
P *p*-nitrophenol + acetate
S *p*-nitrophenyl butyrate + H_2O <2, 3, 5> (Reversibility: ?) [3, 5, 22, 30, 31]
P *p*-nitrophenol + butanoate
S *p*-nitrophenyl laurate + H_2O <5> (Reversibility: ?) [3]
P *p*-nitrophenol + laurate
S *p*-nitrophenyl palmitate + H_2O <5> (Reversibility: ?) [3]
P *p*-nitrophenol + palmitate
S *p*-nitrophenylbutyrate + H_2O <5> (Reversibility: ?) [1]
P *p*-nitrophenol + butanoate
S testosterone oleate + H_2O <5> (<5> 1.4% of activity with 1-oleoyl-2-oleylglycerol [2]) (Reversibility: ?) [2]
P testosterone + oleate
S triacylglycerol + H_2O <1, 2, 3, 5> (Reversibility: ?) [3, 4, 5, 13, 21, 22, 29]
P diacylglycerol + a carboxylate
S triacylglycerol + H_2O <1> (Reversibility: ?) [4]
P diacylglycerol + a carboxlate

S tributanoyl glycerol + H_2O <2> (<2> 8% of activity with vinylbutyrate [16]) (Reversibility: ?) [16]
P dibutanoylglycerol + butanoate
S tributanoylglycerol + H_2O <2> (Reversibility: ?) [22]
P dibutanoylglycerol + butanoate
S tributyrin + H_2O <2> (Reversibility: ?) [31]
P dibutanoylglycerol + butanoate
S trioctanoin + H_2O <2> (Reversibility: ?) [31]
P diactanoylglycerol + octanoate
S trioctanoyl glycerol + H_2O <2> (<2> 3% of activity with vinylbutyrate [16]) (Reversibility: ?) [16]
P dioctanoylglycerol + octanoate
S triolein + H_2O <1, 3, 5> (Reversibility: ?) [1, 3, 7, 12]
P dioleoylglycerol + oleate
S triolein + H_2O <6> (Reversibility: ?) [26]
P 1,2-dioleoylglycerol + oleate
S trioleoylglycerol + H_2O <3> (Reversibility: ?) [25]
P 1,2-dioleoylglycerol + oleate
S trioleoylglycerol + H_2O <3, 4> (<3> approx. 16% of activity with dioleoylglcerol [14]; <3> the type of dietary fat has no effect on kinetic properties of hormone-sensitive lipase [28]) (Reversibility: ?) [6, 14, 20, 28]
P dioleoylglycerol + oleate
S trioleoylglycerol + H_2O <2, 4> (<4> suspended in glycerol/phospholipid, 30-50% of a gum-based substrate suspension [24]) (Reversibility: ?) [24, 32]
P dioleoylglcerol + oleate
S tripropionin + H_2O <2> (Reversibility: ?) [31]
P dipropanoylglycerol + propanoate
S tripropionyl glycerol + H_2O <2> (<2> 4% of activity with vinylbutyrate [16]) (Reversibility: ?) [16]
P dipropionylglycerol + propionate
S vinyl acetate + H_2O <2> (Reversibility: ?) [31]
P acetate + ethylenol
S vinyl acetate + H_2O <2> (<2> 67% of activity with vinylbutyrate [16]) (Reversibility: ?) [16]
P vinyl alcohol + acetate
S vinyl butyrate + H_2O <2> (Reversibility: ?) [16]
P vinyl alcohol + butanoate
S vinyl butyrate + H_2O <2> (Reversibility: ?) [31]
P butanoate + ethylenol
S vinyl laurate + H_2O <2> (Reversibility: ?) [31]
P laurate + ethylenol
S vinyl laurate + H_2O <2> (<2> 15% of activity with vinylbutyrate [16]) (Reversibility: ?) [16]
P vinyl alcohol + laurate
S vinyl propionate + H_2O <2> (Reversibility: ?) [31]
P propanoate + ethylenol

S vinyl propionate + H_2O <2> (<2> 50% of activity with vinylbutyrate [16]) (Reversibility: ?) [16]
P vinyl alcohol + propionate
S Additional information <1, 2, 3, 7> (<3> expressed at a higher level in oxidative fibres than in glycolytic fibres. The two physiological stimuli adrenaline and contraction increase enzyme activity in muscle, and the effects are partially additive. Adrenaline acts via β-adrenergic activation of cAMP-dependent protein kinase, while the effect of contractions is mediated by protein kinase C, at least partly via the extracellular signal-regulated kinase pathway. Endurance training diminishes the sensitivity of muscle hormone-sensitive lipase to adrenaline but increases the contraction-mediated activation [40]; <1> hormone-sensitive lipase is required for high-density lipoprotein cholesteryl ester-supported adrenal steroidogenesis [39]; <2> hormone-sensitive lipase may be the rate-limiting enzyme controlling the cellular catabolism of diacylglycerol in adipose tissue and that of cholesteryl esters in macrophage foam cells and steroidogenic tissues releasing the cholesterol required for adrenal steroidogenesis [36]; <2> in the skeletal muscle at rest there is a high constitutive level of hormone-sensitive lipase activity, which is not a function of biopsy freezing. The combination of low adrenaline and Ca^{2+} levels and resting levels of insulin appears to dictate the levels of activity of hormone-sensitive lipase at rest. During the onset of low and moderate aerobic exercise enzyme activity is activated by contractions, in the apparent absence of increases in circulating adrenaline. However, adrenaline may contribute to the early activation of the enzyme during intense aerobic exercise. The contraction-induced activation appears to be related to the increase in protein kinase C and extracellular signal regulated kinase activity associated with Ca^{2+} and/or other unknown activators. As low- and moderate-intensity exercise continues beyond a few min, activation by adrenaline through the cAMP cascade and protein kinase A also appears to occur. With prolonged moderate-intensity exercise beyond 1-2 h and sustained high-intensity exercise, HSL activity decreases despite continuing increases in adrenaline, possibly as a result of increasing accumulation of free AMP, activation of AMP-activated kinase and phosphorylation of inhibitory sites on the hormone-sensitive lipase. Intramuscular factors dominate the control of hormone-sensitive lipase activity, with hormonal factors playing a smaller role [41]; <7> key enzyme in fatty acid mobilization [34]; <3> rate-limiting enzyme in lipolysis [30]; <2> the enzyme catalyses the hydrolysis of myocellular triacylglycerol, which is a potential energy source diuring exercise [38]; <2> the enzyme is involved in the hormone regulated release of fatty acids and glycerol from adipocyte lipid stores [37]) (Reversibility: ?) [30, 34, 36, 37, 38, 39, 40, 41]
P ?

Inhibitors

(3,4-dihydro-1H-isoquinolin-2-yl)-carbamic acid 4-tert-butoxycarbonylamino-phenyl ester <2> (<2> IC50: 3 nM [32]) [32]

2-methoxyphenyl 3,4-dihydroisoquinolin-2(1H)-ylcarbamate <2> (<2> IC50: 6400 nM [32]) [32]
4-(acetylamino)phenyl morpholin-4-ylcarbamate <2> [32]
4-(butyrylamino)phenyl morpholin-4-ylcarbamate <2> (<2> IC50: 3200 nM [32]) [32]
4-[(2,2-dimethylpropanoyl)amino]phenyl morpholin-4-ylcarbamate <2> (<2> IC50: 47 nM [32]) [32]
4-[(cyclohexylcarbonyl)amino]phenyl morpholin-4-ylcarbamate <2> (<2> IC50: 10 nM [32]) [32]
4-benzoylphenyl 3,4-dihydroisoquinolin-2(1H)-ylcarbamate <2> [32]
4-benzylphenyl 3,4-dihydroisoquinolin-2(1H)-ylcarbamate <2> (<2> IC50: 97 nM [32]) [32]
4-chlorophenyl (1-methyl-3,4-dihydroisoquinolin-2(1H)-yl)carbamate <2> (<2> IC50: 32 nM [32]) [32]
4-chlorophenyl (3-methyl-3,4-dihydroisoquinolin-2(1H)-yl)carbamate <2> (<2> IC50: 35 nM [32]) [32]
4-chlorophenyl 2,2-bis(2-ethoxyethyl)hydrazinecarboxylate <2> (<2> IC50: 184 nM [32]) [32]
4-chlorophenyl 2,2-dipentylhydrazinecarboxylate <2> (<2> IC50: 50 nM [32]) [32]
4-chlorophenyl 3,4-dihydroisoquinolin-2(1H)-ylcarbamate <2> (<2> IC50: 19 nM [32]) [32]
4-methoxyphenyl 3,4-dihydroisoquinolin-2(1H)-ylcarbamate <2> (<2> IC50: 360 nM [32]) [32]
diethyl *p*-nitrophenyl phosphate <2> (<2> 100fold molar excess, complete inactivation after 15 min [16]) [16]
diethyl-*p*-nitrophenyl phosphate <2> (<2> 0.1 mM, 94% inhibition [9]) [9]
diisopropyl fluorophosphate <2, 3, 5> (<5> complete inhibition [19]; <3> 0.1 mM, 98% inhibition [17]; <2> 0.1 mM, 92% inhibition [17]; <3> serine hydrolase with the active-site serine at position 423 [4]; <2> serine hydrolase, active-site at position 424 [4]) [4, 17, 19]
diisopropylfluorophosphate <3, 4, 5> (<4> 0.0075 mM, 50% inhibition [20]; <3> 0.1 mM, 98% inhibition [23]; <5> 0.012 mM, 50% inhibition after 30 min incubation [3]) [3, 11, 20, 23]
Emulgen 120 <5> (<5> 0.1%, 955 of cholesteryl oleate hydrolysis [3]) [3]
Hg^{2+} <2, 3, 5> (<3> 0.1 mM, complete inhibition [17]; <2> 0.1 mM, 91% inhibition [17]; <3> 0.1 mM, 96% inhibition [23]; <5> 0.005 mM, approx. 50% inhibition, 0.02 mM, approx. 70% inhibition [2]) [2, 17, 23]
$HgCl_2$ <2, 4> (<4> 0.01 mM, 50% inhibition [20]; <2> 0.1 mM, 93% inhibition [9]) [9, 20]
N',N'-bis-(2-butylamino-ethyl)-hydrazinecarboxylic acid 4-chloro-phenyl ester <2> (<2> IC50: 24 nM [32]) [32]
N',N'-bis-[2-(ethyl-methyl-amino)-ethyl]-hydrazinecarboxylic acid 4-chlorophenyl ester <2> (<2> IC50: 515 nM [32]) [32]
NaF <2, 3, 4, 5> (<2> 100 mM, 67% inhibition [9]; <4> 4 mM, 50% inhibition [20]; <3> 0.1 mM, 90% inhibition [17]; <2> 0.1 mM, 91% inhibition [17];

<5> 20 mM, 50% inhibition of *p*-nitrophenyl butyrate hydrolysis, 70% of triolein hydrolysis, 60% of cholesteryl hydrolysis [3]) [3, 9, 17, 20]
phenylmethanesulfonyl fluoride <5> (<5> 0.01 mM, approx. 75% inhibition, 0.01 mM, approx. 90% inhibition [2]) [2]
benzyl (4-[[(morpholin-4-ylamino)carbonyl]oxy]phenyl)acetate <2> (<2> IC50: 5 nM [32]) [32]
ethyl (4-[[(morpholin-4-ylamino)carbonyl]oxy]phenyl)acetate <2> (<2> IC50: 138 nM [32]) [32]
methyl 4-[[(3,4-dihydroisoquinolin-2(1H)-ylamino)carbonyl]oxy]benzoate <2> (<2> IC50: 9952 nM [32]) [32]
morpholin-4-yl-carbamic acid 4-[(4-tert-butyl-cyclohexanecarbonyl)-amino]-phenyl ester <2> (<2> IC50: 1 nM [32]) [32]
phenyl 3,4-dihydroisoquinolin-2(1H)-ylcarbamate <2> (<2> IC50: 205 nM [32]) [32]
phenylmethylsulfonyl fluoride <2> (<2> 100fold molar excess, complete inactivation after 15 min [16]) [16]
Additional information <6> (<6> polyunsaturated species of triacylglycerols containing 18:2 or 20:4 double bonds depress rates of oleate release by 70-75% below control values [26]) [26]

Activating compounds

HSL inhibitor <5> (<5> reduced activity in macrophage foam cells due to increased activity of an inhibitor protein [5]) [5]
phosphatidylcholine <5> (<5> 0.075 mM, slight activation upon incorporation into cholestryl oleate liquid crystals [1]) [1]
phosphatidylethanolamine <5> (<5> 0.075 mM, approx. 6fold activation upon incorporation into cholestryl oleate liquid crystals [1]) [1]
phospholipid <5> [5]
cyclic AMP-dependent protein kinase <5> (<5> phosphorylation, 40% activation of triolein hydrolyzing activity [3]) [3]
trimyrestoyl glycerol <6> (<6> 3.7fold increase of triolein hydrolysis rate [26]) [26]
tripalmitoyl glycerol <6> (<6> 2.1fold increase of triolein hydrolysis rate [26]) [26]
tristearoyl glycerol <6> (<6> 1.9fold increase of triolein hydrolysis rate [26]) [26]
Additional information <3> (<3> activity in incubated soleus muscle increases in response to adrenaline [12]) [12]

Turnover number (min^{-1})

6 <2> (trioctanoin) [31]
7 <2> (tripropionin) [31]
18 <2> (tributyrin) [31]
170 <2> (vinyl acetate) [31]
190 <2> (*p*-nitrophenyl butyrate) [31]
375 <2> (vinyl laurate) [31]
625 <2> (vinyl butyrate) [31]
800 <2> (vinyl propionate) [31]

Specific activity (U/mg)

0.068 <5> (<5> cholesteryl oleate hydroylsis [11]) [11]
0.3 <5> (<5> hydrolysis of estradiol-17-β-oleate [2]) [2]
0.8 <5> (<5> hydrolysis of *p*-nitrophenyl laurate [3]) [3]
0.9 <5> (<5> hydrolysis of *p*-nitrophenyl palmitate [3]) [3]
1.45 <5> (<5> hydrolysis of cholesteryl oleate [19]) [19]
2.6 <5> (<5> hydrolysis of 1-oleoyl-2-oleylglycerol [2]) [2]
2.8 <5> (<5> 1-oleoyl-2-oleylglycerol [11]) [11]
3.8 <5> (<5> hydrolysis of cholesteryl oleate [3]) [3]
4.7 <5> (<5> hydrolysis of *p*-nitrophenyl acetate [3]) [3]
18 <2> [36]
21 <4> [20]
22 <5> (<5> hydrolysis of *p*-nitrophenyl butyrate [3]) [3]
30 <5> (<5> hydrolysis of 1-oleoyl-2-O-oleylglycerol [19]) [19]
100 <2> [22]
143 <2> (<2> hydrolysis of vinyl butyrate [16]) [16]

K_m-Value (mM)

0.0022 <3> (cholesteryl oleate) [15]
0.0034 <3> (trioleoylglycerol, <3> 37°C, pH 7.0 [14]) [14]
0.0047 <3> (cholesteryl oleate, <3> 37°C, pH 7.0 [14]) [14]
0.011 <5> (cholesteyl oleate, <5> cholesteryl oleate vesicles, 37°C, pH 6.8 [1]) [1]
0.012 <5> (cholesteyl oleate, <5> cholesteryl oleate emulsion, 37°C, pH 6.8 [1]) [1]
0.015 <3> (cholesteryl oleate, <3> wild-type enzyme [30]) [30]
0.021 <3> (cholesteryl oleate, <3> $\Delta^6$66-683 [30]) [30]
0.024 <3> (cholesteryl oleate, <3> $\Delta^6$44-658 [30]) [30]
0.025 <3> (cholesteryl oleate, <3> $\Delta^6$57-664 [30]) [30]
0.03 <3> (dioleoylglycerol, <3> 37°C, pH 7.0 [14]) [14]
0.033 <3> (1,2-dioleoylglycerol, <3> 37°C [25]) [25]
0.099 <5> (cholesteyl oleate, <5> liquid crystals of cholesteryl oleate, 37°C, pH 6.8 [1]) [1]
0.164 <5> (*p*-nitrophenyl butyrate, <5> 37°C, pH 6.8 [3]) [3]
0.268 <3> (*p*-nitrophenyl oleate, <3> $\Delta^6$66-683 [30]) [30]
0.389 <3> (*p*-nitrophenyl oleate, <3> $\Delta^6$44-658 [30]) [30]
0.4 <2> (tributanoyl glycerol, <2> 37°C, pH 8.0 [16]) [16]
0.4 <2> (tributyrin) [31]
0.427 <3> (*p*-nitrophenyl oleate, <3> $\Delta^6$57-664 [30]) [30]
0.462 <3> (*p*-nitrophenyl oleate, <3> wild-type enzyme [30]) [30]
4 <2> (vinyl laurate) [31]
10 <2> (vinyl butyrate, <2> 37°C, pH 8.0 [16]) [16]
15 <2> (tripropionin) [31]
15 <2> (tripropionyl glycerol, <2> 37°C, pH 8.0 [16]) [16]
25 <2> (vinyl butyrate) [31]
70 <2> (vinyl propionate, <2> 37°C, pH 8.0 [16]) [16]
75 <2> (vinyl propionate) [31]

170 <2> (vinyl acetate, <2> 37°C, pH 8.0 [16]) [16]
315 <2> (vinyl acetate) [31]

pH-Optimum
6.8 <5> [1]
7 <3> [12]

pH-Range
5-9 <5> (<5> approx. 50% of maximal activity at pH 5.5 and pH 8.5, respectively [1]) [1]

4 Enzyme Structure

Molecular weight
89000 <7> [34]
160000 <3> (<3> sucrose density gradient centrifugation [15]) [15]

Subunits
? <2, 3, 4, 5> (<3,4,5> x * 84000, SDS-PAGE [2,4,18,19,20]; <2> x * 88000, SDS-PAGE [4]; <2> x * 116000, SDS-PAGE, testis HSL [22]; <3> x * 82820, deduced from nucleotide sequence [17]; <3> x * 84000, immunoblot [6,8,12]; <5> x * 84000, immunoprecipitation [11]; <2> x * 84032, deduced from nucleotide sequence [4,22]; <2> x * 88200, SDS-PAGE, immunoblot [17]) [2, 4, 6, 8, 11, 12, 17, 18, 19, 20, 22]
dimer <2, 3> (<2> 2 * 84000, SDS-PAGE, recombinant HSL [16]; <3> 2 * 85000, immunoblotting, 40fold higher hydrolysis rate for cholesteryl ester than monomer [15]; <3> x * 84073, deduced from nucleotide sequence, HSL might be a homodimer in solution [22]) [15, 16, 22]

Posttranslational modification
phosphoprotein <2, 3, 4, 5> (<3> basal phosphorylation site at S565, regulatory phosphorylation site at S563, S659 and S660 may also be phosphorylated, HSL is probably also phosphorylated by extracellular-signal-regulated kinase [4]; <4> phosphorylation by cyclic AMP-dependent protein kinase [20]; <3> phosphorylation of HSL appears to be important in enabling dimerization to occur [15]; <2> phosphorylation of HSL leads to its translocation from the cytoplasm to the lipid droplet [16]; <3> phosphorylation sites at S563 and S565 [22]; <3> recombinant HSL can be phosphorylated and activated in vitro by cAMP-dependent protein kinase [29]; <2> 5AMP-activated protein kinase phosphorylates hormone-sensitive lipase on Ser565 in human skeletal muscle during exercise with reduced muscle glycogen. Ser565 phosphorylation by 5AMP-activated protein kinase during exercise has no effect on activity of hormone-sensitive lipase [38]) [2, 3, 4, 5, 6, 14, 15, 16, 17, 18, 19, 20, 22, 23, 25, 29, 38, 40, 41]

5 Isolation/Preparation/Mutation/Application

Source/tissue

HT-29 cell <2> [9]
adipocyte <3, 7> [34, 35]
adipose tissue <1, 2, 3, 4, 5, 6> (<5> perirenal [19]) [1, 4, 5, 8, 15, 16, 17, 19, 20, 21, 22, 25, 26, 28]
adrenal <3> [8]
adrenal gland <1, 7> [21, 34]
brown adipose tissue <1> [7, 22]
cardiac muscle <5> [11]
heart <3> [8]
macrophage <1, 5> [4, 5, 21]
mammary epithel <3> [18]
mammary gland <3> (<3> in virgin rats, immunoreactive hormone-sensitive lipase is observed in mammary adipocytes, diffuse staining is detected in epithelial cells. Positive staining for hormone-sensitive lipase is detected in the two types of cells in pregnant and lactating animals. As pregnancy advances, the staining intensity of immunoreactive hormone-sensitive lipase increases in the epithelial cells parallel to their proliferation, attaining the maximum during lactation. Both hormone-sensitive lipase protein and activity are lower in mammary glands from 20 and 21 day pregnant rats than from those of virgin rats. They return to virgin values on days 1 and 4 of lactation [35]) [35]
milk <3> [35]
ovary <3, 7> [8, 34]
pancreatic β cell <7> [34]
placenta <5> (<5> cotyledon [2]) [2]
skeletal muscle <2, 3> (<2> enzyme activity increases early in exercise and remains elevated for the duration of the exercise [33]; <3> expressed at a higher level in oxidative fibres than in glycolytic fibres. The two physiological stimuli adrenaline and contraction increase enzyme activity in muscle, and the effects are partially additive. Adrenaline acts via β-adrenergic activation of cAMP-dependent protein kinase, while the effect of contractions is mediated by protein kinase C, at least partly via the extracellular signal-regulated kinase pathway. Endurance training diminishes the sensitivity of muscle hormone-sensitive lipase to adrenaline but increases the contraction-mediated activation [40]; <2> in the skeletal muscle at rest there is a high constitutive level of hormone-sensitive lipase activity, which is not a function of biopsy freezing. The combination of low adrenaline and Ca^{2+} levels and resting levels of insulin appears to dictate the levels of activity of hormone-sensitive lipase at rest. During the onset of low and moderate aerobic exercise enzyme activity is activated by contractions, in the apparent absence of increases in circulating adrenaline. However, adrenaline may contribute to the early activation of the enzyme during intense aerobic exercise. The contraction-induced activation appears to be related to the increase in protein kinase

C and extracellular signal regulated kinase activity associated with Ca^{2+} and/or other unknown activators. As low- and moderate-intensity exercise continues beyond a few min, activation by adrenaline through the cAMP cascade and protein kinase A also appears to occur. With prolonged moderate-intensity exercise beyond 1-2 h and sustained high-intensity exercise, HSL activity decreases despite continuing increases in adrenaline, possibly as a result of increasing accumulation of free AMP, activation of AMP-activated kinase and phosphorylation of inhibitory sites on the hormone-sensitive lipase. Intramuscular factors dominate the control of hormone-sensitive lipase activity, with hormonal factors playing a smaller role [41]) [6, 8, 12, 33, 38, 40, 41]
small intestine <1> (<1> HSL accounts for the entire neutral cholesteryl ester hydrolase activity of enterocytes [21]) [21]
testis <1, 2> [10, 21, 22]
white adipose tissue <3> [35]

Localization

cytoplasm <2> [10]
cytosol <2, 3> (<3> HSL can translocate from the cytosol to the lipid droplet surface upom protein kinase phosphorylation [22]) [14, 15, 16, 22]
lipid particle <2> [16]

Purification

<2> (partially purified, Q-Sepharose FF) [9]
<2> (recombinant HSL) [16]
<3> [8, 25]
<3> (recombinant HSL, detergent-solubilization, Q-Sepharose, Phenyl-Sepharose) [29]
<4> [20]
<4> (partially purified) [24]
<5> [5]
<5> (pH 5.0, DE-52, Phenyl-Sepharose, Mono Q, Mono S) [3]
<5> (pH 5.0, DE-52, hydroxyapatite) [2]
<5> (pH 5.0, DE-52, hydroxyapatite, Phenyl-Sepharose, Heparin-Sepharose) [11]
<5> (pH 5.0, DE-52, hydroxylapatite, Phenyl-Sepharose, Heparin-Sepharose) [19]

Cloning

<2> [27]
<2> (cloning of cDNA) [10]
<2> (expression Sf9 insect cells) [16]
<3> [27]
<3> (expression Sf9 insect cells) [29]
<3> (expression in CHO cells) [14]
<3> (expression in COS and insect cells) [22]
<3> (expression in Sf9 insect cells and COS cells) [15]
<3> (expression of HSL cDNA in HepG2 cells) [13]
<3> (expression of cDNA in COS cells) [23]

Engineering

Δ644-658 <3> (<3> reduced basal hydrolytic activity, no activation by cyclic AMP-dependent protein kinase [30]) [30]
Δ657-664 <3> (<3> reduced basal hydrolytic activity, no activation by cyclic AMP-dependent protein kinase [30]) [30]
Δ666-683 <3> (<3> very low basal and stimulated hydrolytic activity. V_{max} with cholesteryl ester is 29fold lower than wild-type value. V_{max} with *p*-nitrophenylbutyrate is 8fold lower than wild-type value [30]) [30]
F650C <3> (<3> reduced activity against lipid-soluble but not water-soluble substrates [30]) [30]
F654D <3> (<3> reduced activity against lipid-soluble but not water-soluble substrates [30]) [30]
P651A <3> (<3> reduced activity against lipid-soluble but not water-soluble substrates [30]) [30]
S423A <3> (<3> 99% loss of cholesteryl oleate hydrolysis, more than 80% loss of trioleoylglycerol hydrolysis and more than 90% loss of dioleoylglycerol hydrolysis [14]) [14]
S563A <3> (<3> 98% loss of cholesteryl oleate hydrolysis, more than 80% loss of trioleoylglycerol hydrolysis and more than 90% loss of dioleoylglycerol hydrolysis [14]) [14]
S563A/S565A <3> (<3> 70% loss of cholesteryl oleate hydrolysis, 10% loss of trioleoylglycerol hydrolysis and 80% loss of dioleoylglycerol hydrolysis [14]) [14]
S563D <3> (<3> approx. 20% increase in cholesteryl oleate hydrolysis compared to wild-type [14]) [14]
S565A <3> (<3> 70% loss of cholesteryl oleate hydrolysis, 35% loss of trioleoylglycerol hydrolysis and 30% loss of dioleoylglycerol hydrolysis [14]) [14]
Additional information <3> (<3> substitution of S423 by several different amino acids completey abolishes lipase and esterase activity [29]) [29]

Application

medicine <2> (<2> inhibition of hormone-sensitive lipase may improve insulin sensitivity and blood glucose handling in type 2 diabetes. The utility of enzyme inhibition in diabetes treatment will depend on the presence or absence of adverse effects of inhibition in tissues beyond the adipocyte [37]) [37]

6 Stability

Storage stability

<5>, -20°C, 500 mM phosphate buffer, pH 7.0, 50% glycerol, 100 mM benzamidine, 1 mM dithiothreitol, 0.2% C13E12, 5 mg/ml leupeptin and 1 mg/ml pepstatin, at least 2 months, no loss of activity [19]

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Acetylajmaline esterase 3.1.1.80

1 Nomenclature

EC number
3.1.1.80

Systematic name
17-O-acetylajmaline O-acetylhydrolase

Recommended name
acetylajmaline esterase

CAS registry number
110183-46-5

2 Source Organism

<1> *Rauvolfia serpentina* (no sequence specified) [1]
<2> *Rauvolfia serpentina* (no sequence specified) [2]
<3> *Rauvolfia verticillata* (no sequence specified) [1]

3 Reaction and Specificity

Catalyzed reaction
17-O-acetylajmaline + H_2O = ajmaline + acetate
17-O-acetylnorajmaline + H_2O = norajmaline + acetate

Substrates and products
S 17-O-acetylajmaline + H_2O <2, 3> (<2> 84.2% of the activity with 17-O-acetylnorajmaline [2]) (Reversibility: ?) [1, 2]
P ajmaline + acetate
S 17-O-acetylnorajmaline + H_2O <2, 3> (<3> 70.2% of the activity with 17-O-acetylajmaline [1]) (Reversibility: ?) [1, 2]
P norajmaline + acetate
S 17-O-acetyltetraphyllicine + H_2O <2> (<2> 86% of the activity with 17-O-acetylnorajmaline [2]) (Reversibility: ?) [2]
P ?

Inhibitors
2,2'-dithiodipyridine <2> (<2> 0.5 mM, 30-40% inhibition [2]) [2]
5,5'-dithiobis(2-nitrobenzoic acid) <2> (<2> 0.5 mM, 30-40% inhibition [2]) [2]
iodoacetamide <2> (<2> 0.5 mM, 30-40% inhibition [2]) [2]

Specific activity (U/mg)
0.0364 <2> [2]
2.484 <3> [1]

K_m-Value (mM)
0.12 <3> (17-O-acetylajmaline) [1]
0.7 <3> (17-O-acetylnorajmaline) [1]

pH-Optimum
7.5 <2> [2]
8 <3> [1]

pH-Range
6.3-8.5 <2> (<2> pH 6.3: about 50% of maximal activity, pH 8.5: about 50% of maximal activity [2]) [2]
6.5-9.8 <3> (<3> pH 6.5 and pH 9.8: about 50% of maximal activity [1]) [1]

Temperature optimum (°C)
37 <2> [2]
53 <3> [1]

4 Enzyme Structure

Molecular weight
33000 <2> (<2> gel filtration [2]) [2]
40000 <3> (<3> gel filtration [1]) [1]

Subunits
monomer <3> (<3> 1 * 40000, SDS-PAGE [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue
cell suspension culture <2, 3> [1, 2]
leaf <2> [2]

Purification
<2> (partial) [2]
<3> [1]

Cloning
<3> (the expression in Escherichia coli failed although a wide range of conditions were tested. With a virus-based plant expression system, it was possible to express the enzyme functionally in leaves of Nicotiana benthamiana Domin. An extraordinarily high enzyme activity is detected in the Nicotiana tissue, which exceeds that in Rauvolfia cell suspension cultures about 20fold) [1]

6 Stability

General stability information

<2>, 4°C, at the stage of a 150fold enrichment in 0.1 M phosphate buffer, pH 7.0, 1 mM dithiothreitol, half-life: 21 days [2]

References

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Quorum-quenching N-acyl-homoserine lactonase 3.1.1.81

1 Nomenclature

EC number
3.1.1.81

Systematic name
N-acyl-L-homoserine-lactone lactonohydrolase

Recommended name
quorum-quenching N-acyl-homoserine lactonase

Synonyms
AHL lactonase <6, 12, 13, 15, 16, 17, 18, 19, 20, 43> [1, 7, 12, 14]
AHL-degrading enzyme <6, 14, 38, 39, 42> [4, 8, 11]
AHL-inactivating enzyme <6, 12, 16, 17, 18, 19, 20, 43> [1]
AHL-lactonase <1, 2, 3, 4, 7, 9, 10, 13, 16, 17, 18, 19, 20, 35, 40, 42, 43> [1, 8, 9, 10, 12, 13, 15]
AHLase <38, 39> [11]
AhlD <38> [11]
AhlK <39> [11]
AiiA <1, 2, 3, 4, 9, 10, 35, 40> [9, 15, 16]
AiiA lactonase <6, 7, 14> [4, 6]
AiiA-like protein <15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> [2]
AiiB <11> [3]
AiiC <11> [3]
AttM <11> [3]
N-acyl homoserine lactonase <11, 35> [3, 13]
N-acyl homoserine lactone hydrolase <11> [3]
N-acyl-homoserine lactonase <13> [12]
N-acyl-homoserine lactone lactonase <13> [12]
N-acylhomoserine lactonase <38, 39> [11]
PvdQ <5> [5]
QuiP <5> [5]
acyl homoserine degrading enzyme <38> [11]
acyl-homoserine lactone acylase <5> [5]
delactonase <35> [13]
lactonase-like enzyme <1, 2, 3, 4, 9, 10> [9]
quorum-quenching N-acyl homoserine lactonase <6, 12, 16, 17, 18, 19, 20, 43> [1]
quorum-quenching N-acyl homoserine lactone hydrolase <15> [14]
quorum-quenching N-acyl homoserine lactone lactonase <7, 40> [10, 15]

quorum-quenching enzyme <7, 13, 35> [10, 12, 13]
quorum-quenching lactonase <15> [7]
quorum-sensing enzyme
Additional information <13> (<13> the enzyme belongs to the quorum-quenching enzymes [12]) [12]

CAS registry number
389867-43-0

2 Source Organism

<1> *Mus musculus* (no sequence specified) [9]
<2> *Homo sapiens* (no sequence specified) [9]
<3> *Bos taurus* (no sequence specified) [9]
<4> *Oryctolagus cuniculus* (no sequence specified) [9]
<5> *Pseudomonas aeruginosa* (no sequence specified) [5]
<6> *Bacillus cereus* (no sequence specified) [1,4]
<7> *Bacillus sp.* (no sequence specified) [6,10]
<8> no activity in *Bacillus sphaericus* [1]
<9> *Capra hircus* (no sequence specified) [9]
<10> *Equus caballus* (no sequence specified) [9]
<11> *Agrobacterium tumefaciens* (no sequence specified) [3]
<12> *Bacillus mycoides* (no sequence specified) [1]
<13> *Ralstonia sp.* (no sequence specified) [12]
<14> *Bacillus anthracis* (no sequence specified) [4]
<15> *Bacillus thuringiensis* (UNIPROT accession number: Q7B8B9) [2, 7, 14]
<16> *Bacillus sp.* (UNIPROT accession number: Q8RPW9) [1]
<17> *Bacillus thuringiensis* (UNIPROT accession number: Q8RPW7) [1]
<18> *Bacillus thuringiensis* (UNIPROT accession number: Q8RPW6) [1]
<19> *Bacillus thuringiensis* (UNIPROT accession number: Q8RPW5) [1]
<20> *Bacillus thuringiensis* (UNIPROT accession number: Q8RJA0) [1]
<21> *Bacillus thuringiensis* (UNIPROT accession number: Q7B8C1) [2]
<22> *Bacillus thuringiensis* (UNIPROT accession number: Q7B8C2) [2]
<23> *Bacillus thuringiensis* (UNIPROT accession number: Q7B8C0) [2]
<24> *Bacillus thuringiensis* (UNIPROT accession number: Q7B8C4) [2]
<25> *Bacillus thuringiensis* (UNIPROT accession number: Q7KI79) [2]
<26> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTX1) [2]
<27> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTW4) [2]
<28> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTW7) [2]
<29> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTW6) [2]
<30> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTW8) [2]
<31> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTX0) [2]
<32> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTC3) [2]
<33> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTC5) [2]
<34> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTW9) [2]
<35> *Bacillus sp.* (UNIPROT accession number: Q9L8R8) [13, 16]

<36> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTW5) [2]
<37> *Bacillus sp.* (UNIPROT accession number: Q8KTW3) [2]
<38> *Arthrobacter sp.* (UNIPROT accession number: Q7X3T2) [11]
<39> *Klebsiella pneumoniae* (UNIPROT accession number: Q7X477) [11]
<40> *Bacillus thuringiensis subsp. kurstaki* (no sequence specified) [15]
<41> no activity in *Gallus gallus serum* [9]
<42> *Bacillus thuringiensis subsp. kurstaki* (UNIPROT accession number: Q7B8B9) [8]
<43> *Bacillus thuringiensis* (UNIPROT accession number: Q8RPW8) [1]
<44> no activity in *Bacillus fusiformis* (no sequence specified) [1]

3 Reaction and Specificity

Catalyzed reaction

an N-acyl-L-homoserine lactone + H_2O = an N-acyl-L-homoserine (<15> active site structure and substrate binding, catalytic mechanism, overview [14]; <38,39> hydrolysis of the lactone ring [11]; <7> residues H106, D108, and H109, as well as H169 are important for catalytic activity [10]; <40> substrate binding and catalytic mecanism, overview [15])

Natural substrates and products

S N-3-oxododecanoyl-L-homoserine lactone + H_2O <1, 2, 3, 4, 5, 9, 10> (<5> QuiP is required for utilization and growth on long-chain acylhomoserine lactones of Pseudomonas aeruginosa, while PvdQis not [5]) (Reversibility: ?) [5, 9]
P N-3-oxododecanoyl-L-homoserine
S N-3-oxooctanoyl-L-homoserine lactone + H_2O <11> (Reversibility: ?) [3]
P N-3-oxooctanoyl-L-homoserine
S N-acyl-(S)-homoserine lactone + H_2O <6, 7, 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 43> (<7> N-acylhomoserine lactones, AHL, regulate protease production, swarming motility, biofilm formation, and Caenorhabditis elegans killing efficiency in most strains of the Burkholderia complex organisms, which is inhibited by Bacillus AHL lactonase acting as a quorum-quenching enzyme [6]; <6,12,16,17,18,19,20,43> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence bacteria on plants [1]; <15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora strain IBN98 on Solanum tuberosum, acylhomoserine lactones are autoinducers of quorum-sensing signaling [2]; <38> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora strain N98 [11]; <7, 35> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora, acylhomoserine lactones are autoinducers of quorum-sensing signaling, mechanism [10, 16];

<35> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora, acylhomoserine lactones are autoinducers of quorum-sensing signaling, the inhibition of which is a feasible approach for prevention of bacterial infection [13]; <39> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of pathogenic bacteria [11]; <11> the isozymes AttM, AiiB, and AiiC inactivate the acylhomoserine lactone quorum-sensing signaling and attenuate the virulence of Erwinia carotovora subsp. atroseptica strain 6276 on Solanum tuberosum, function and regulation of quorum-sensing signaling, overview [3]) (Reversibility: ?) [1, 2, 3, 6, 10, 11, 13, 16]

P N-acyl-(S)-homoserine

S N-decanoyl-L-homoserine lactone + H_2O <5> (Reversibility: ?) [5]

P N-decanoyl-L-homoserine

S N-hexanoyl-L-homoserine lactone + H_2O <11> (Reversibility: ?) [3]

P N-hexanoyl-L-homoserine

S Additional information <1, 2, 3, 4, 5, 7, 9, 10, 13, 15, 38, 39, 42> (<4> acylhomoserine lactone quorum-sensing signals play a key role in synchronizing virulence gene expression during bloodstream infections of mammals, the enzyme inactivates the AHL signaling by hydrolysis of the lacton ring thus acting as quorum-quenching enzyme [9]; <1, 2, 3, 9, 10> acyl-homoserine lactone quorum-sensing signals play a key role in synchronizing virulence gene expression during bloodstream infections of mammals, the enzyme inactivates the AHL signaling by hydrolysis of the lactone ring thus acting as quorum-quenching enzyme [9]; <7,15> N-acyl homoserine lactone quorum-sensing signals are the vital elements of bacterial quorum-sensing systems, which regulate diverse biological functions, including virulence [7, 10]; <5> N-acyl homoserine lactone quorum-sensing signals are the vital elements of bacterial quorum-sensing systems, which regulate diverse biological functions, including virulence, regulation quorum-sensing signaling and quorum-quenching in Pseudomonas aeruginosa [5]; <42> N-acyl homoserine lactone, AHL, quorum-sensing signals are the vital elements of bacterial quorum-sensing systems, which regulate diverse biological functions, including virulence and biofilm formation of Gram-negative bacteria, the enzyme deactivates the signaling by degradation of AHLs via lactone ring hydrolysis [8]; <38, 39> quorum-sensing is a signaling mechanism, that controls diverse biological functions, including virulence, via N-acylhomoserine lactone signal molecules in Gram-negative bacteria, overview [11]; <13> the enzyme is involved in inactivation of N-acylhomoserine lactone signaling in regulation of infection and virulence functions [12]) (Reversibility: ?) [5, 7, 8, 9, 10, 11, 12]

P ?

Substrates and products

S N-3-hydroxybutanoyl-L-homoserine lactone + H_2O <7> (Reversibility: ?) [10]

P N-3-hydroxybutanoyl-L-homoserine

S N-3-oxobutanoyl-L-homoserine lactone + H_2O <7> (Reversibility: ?) [10]
P N-3-oxobutanoyl-L-homoserine
S N-3-oxodecanoyl-L-homoserine lactone + H_2O <4, 7, 35> (<4,7> high activity [9,10]) (Reversibility: ?) [9, 10, 16]
P N-3-oxodecanoyl-L-homoserine
S N-3-oxododecanoyl-L-homoserine lactone + H_2O <1, 2, 3, 4, 5, 7, 9, 10, 35, 38> (<4> best substrate [9]; <5> QuiP is required for utilization and growth on long-chain acylhomoserine lactones of Pseudomonas aeruginosa, while PvdQis not [5]; <35> the substrate regulates production of virulence determinants of the human pathogen Pseudomonas aeruginosa [13]) (Reversibility: ?) [5, 9, 10, 11, 13]
P N-3-oxododecanoyl-L-homoserine (<35> product identification by mass spectrometry [13])
S N-3-oxohexanoyl-L-homoserine lactone + H_2O <4, 7, 15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39> (Reversibility: ?) [2, 9, 10, 11, 13, 16]
P N-3-oxohexanoyl-L-homoserine (<35> product identification by mass spectrometry [13])
S N-3-oxooctanoyl-L-homoserine lactone + H_2O <4, 7, 11, 35> (<7> high activity [10]; <35> the substrate controls Ti plasmid conjugal transfer in Agrobacterium tumefaciens [13]) (Reversibility: ?) [3, 9, 10, 13, 16]
P N-3-oxooctanoyl-L-homoserine (<35> product identification by mass spectrometry [13])
S N-acyl-(S)-homoserine lactone + H_2O <6, 7, 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 43> (<7> N-acylhomoserine lactones, AHL, regulate protease production, swarming motility, biofilm formation, and Caenorhabditis elegans killing efficiency in most strains of the Burkholderia complex organisms, which is inhibited by Bacillus AHL lactonase acting as a quorum-quenching enzyme [6]; <6, 12, 16, 17, 18, 19, 20, 43> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence bacteria on plants [1]; <15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora strain IBN98 on Solanum tuberosum, acylhomoserine lactones are autoinducers of quorum-sensing signaling [2]; <38> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora strain N98 [11]; <7, 35> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora, acylhomoserine lactones are autoinducers of quorum-sensing signaling, mechanism [10, 16]; <35> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora, acylhomoserine lactones are autoinducers of quorum-sensing signaling, the inhibition of which is a feasible approach for prevention of bacterial infection [13]; <39> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of pathogenic

bacteria [11]; <11> the isozymes AttM, AiiB, and AiiC inactivate the acyl-homoserine lactone quorum-sensing signaling and attenuate the virulence of Erwinia carotovora subsp. atroseptica strain 6276 on Solanum tuberosum, function and regulation of quorum-sensing signaling, overview [3]; <6, 12, 16, 17, 18, 19, 20, 43> the acylhomoserine lactones are inducers of quorum-sensing signaling [1]) (Reversibility: ?) [1, 2, 3, 6, 10, 11, 13, 16]

P N-acyl-(S)-homoserine

S N-butanoyl-L-homoserine lactone + H_2O <7, 35, 38> (<7> high activity [10]; <35> the substrate regulates production of virulence determinants of the human pathogen Pseudomonas aeruginosa [13]) (Reversibility: ?) [10, 11, 13]

P N-butanoyl-L-homoserine (<35> product identification by mass spectrometry [13])

S N-decanoyl-L-homoserine lactone + H_2O <4, 5, 7, 38> (<4,7> high activity [9,10]) (Reversibility: ?) [5, 9, 10, 11]

P N-decanoyl-L-homoserine

S N-hexanoyl-(S)-homoserine lactone <15> (Reversibility: ?) [7]

P N-hexanoyl-(S)-homoserine

S N-hexanoyl-(S)-homoserine lactone + H_2O <15> (<15> no activity with N-hexanoyl-(R)-homoserine lactone [7]) (Reversibility: ?) [7]

P N-hexanoyl-(S)-homoserine

S N-hexanoyl-L-homoserine lactone + H_2O <4, 7, 11, 38, 39, 40> (<7> best substrate [10]) (Reversibility: ?) [3, 9, 10, 11, 15]

P N-hexanoyl-L-homoserine (<38> product analysis of recombinant enzyme expressed in Escherichia coli strain JM109 [11])

S N-octanoyl-L-homoserine lactone + H_2O <4, 7, 38> (<7> high activity [10]) (Reversibility: ?) [9, 10, 11]

P N-octanoyl-L-homoserine

S Additional information <1, 2, 3, 4, 5, 7, 9, 10, 13, 15, 35, 38, 39, 42> (<4> acyl-homoserine lactone quorum-sensing signals play a key role in synchronizing virulence gene expression during bloodstream infections of mammals, the enzyme inactivates the AHL signaling by hydrolysis of the lacton ring thus acting as quorum-quenching enzyme [9]; <1,2,3,9,10> acyl-homoserine lactone quorum-sensing signals play a key role in synchronizing virulence gene expression during bloodstream infections of mammals, the enzyme inactivates the AHL signaling by hydrolysis of the lactone ring thus acting as quorum-quenching enzyme [9]; <7,15> N-acyl homoserine lactone quorum-sensing signals are the vital elements of bacterial quorum-sensing systems, which regulate diverse biological functions, including virulence [7,10]; <5> N-acyl homoserine lactone quorum-sensing signals are the vital elements of bacterial quorum-sensing systems, which regulate diverse biological functions, including virulence, regulation quorum-sensing signaling and quorum-quenching in Pseudomonas aeruginosa [5]; <42> N-acyl homoserine lactone, AHL, quorum-sensing signals are the vital elements of bacterial quorum-sensing systems, which regulate diverse biological functions, including virulence and biofilm formation of Gram-negative bacteria, the enzyme deactivates

the signaling by degradation of AHLs via lactone ring hydrolysis [8]; <38,39> quorum-sensing is a signaling mechanism, that controls diverse biological functions, including virulence, via N-acylhomoserine lactone signal molecules in Gram-negative bacteria, overview [11]; <13> the enzyme is involved in inactivation of N-acylhomoserine lactone signaling in regulation of infection and virulence functions [12]; <4> substrate specificity, N-butanoyl-L-homoserine lactone is a poor substrate [9]; <7> substrate specificity, poor activity with non-acyl lactones and no activity with non-cyclic esters, but strong activity with all N-acyl-(S)-homoserine lactones of different chain length and nature, the amide group and the ketone at C_1 position of the substrates acyl chain might be important for substrate-enzyme interaction, overview [10]; <35> the substrate N-acyl-L-homoserine lactones show negligible enzyme-independent delactonization [13]) (Reversibility: ?) [5, 7, 8, 9, 10, 11, 12, 13]

P ?

Inhibitors

Ag^+ <7> (<7> complete inhibition at 0.2 mM [10]) [10]
Cr^{3+} <7> (<7> 72% inhibition at 2 mM [10]) [10]
Cu^{2+} <7> (<7> complete inhibition at 0.2 mM [10]) [10]
EDTA <4> (<4> in vitro and in serum [9]) [9]
Fe^{2+} <7> (<7> 48% inhibition at 2 mM [10]) [10]
Pb^{2+} <7> (<7> 67% inhibition at 2 mM [10]) [10]
TPEN <40> (<40> i.e. N,N,N,N-tetrakis-(2-pyridylmethyl)-ethylene-diamine [15]) [15]
Additional information <7> (<7> enzyme is not affected by EDTA, 2,2-bipyridine, and *o*-phenanthroline at 2 mM [10]) [10]

Activating compounds

Additional information <7> (<7> enzyme is not affected by EDTA, 2,2-bipyridine, and *o*-phenanthroline at 2 mM [10]) [10]

Metals, ions

Ca^{2+} <1, 2, 3, 4, 9, 10> (<1,2,3,4,9,10> activates, required [9]) [9]
Zn^{2+} <6, 12, 15, 16, 17, 18, 19, 20, 35, 40, 43> (<15> dinuclear form, 2 Zn^{2+} ions per enzyme molecule, preparation of the apoenzyme, which is inactive, and reconstitution with Zn^{2+} at pH 7.1 restoring about 60% of native enzyme activity, metal content determination, overview [7]; <35> metallohydrolase containing the conserved HXHXDH zinc-binding motif required for full enzyme activity [16]; <6, 12, 17, 18, 19, 20, 43> metallohydrolase containing the conserved zinc-binding motif [1]; <16> metallohydrolase containing the conserved zinc-binding motif, residues H106, H109, D108, and H169 of the strain 240B1 enzyme are required for Zn^{2+} binding and full enzyme activity, overview [1]; <40> the enzyme is a metallohydrolase and contains 2 Zn^{2+} ions per enzyme molecule involved in catalysis in a dinuclear zinc-binding center involving residues H104, H106, H169 for coordination of the first Zn^{2+}, and H109, H235, and D108 for coordination of the second Zn^{2+}, determination of metal content of wild-type and mutant enzymes, AiiA has a very high affi-

nity for Zn^{2+}, overview [15]; <15> the enzyme is a metalloprotein [7]) [1, 7, 15, 16]
Additional information <4, 7> (<4> no activation by Mg^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, and Pb^{2+} [9]; <7> no effect by Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}, the enzyme contains the putative zinc-binding motif 104HXHXDH109, but is no metallohydrolase since it does not contain and require Zn^{2+} or other metal ions, only trace amounts of Zn^{2+}, the 104HXHXDH109 sequence is a catalytic motif of the AHL-lactonase [10]) [9, 10]

Turnover number (min^{-1})

20.2 <7> (N-3-oxodecanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
22.2 <7> (N-3-oxooctanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
22.7 <7> (N-3-oxohexanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
27.5 <7> (N-octanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
28.6 <7> (N-3-oxobutanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
29.3 <7> (N-3-hydroxybutanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
35.7 <7> (N-hexanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
117 <15> (N-hexanoyl-(S)-homoserine lactone, <15> pH 7.4, 28°C, purified detagged recombinant enzyme [7]) [7]

Specific activity (U/mg)

0.012 <2> (<2> serum [9]) [9]
0.013 <3, 9> (<3,9> serum [9]) [9]
0.014 <10> (<10> serum [9]) [9]
0.016 <1> (<1> serum [9]) [9]
0.017 <4> (<4> serum, substrate N-3-oxododecanoyl-L-homoserine lactone [9]) [9]
Additional information <7, 15, 38> [7, 10, 11]

K_m-Value (mM)

0.0014 <7> (N-3-oxodecanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
0.0023 <7> (N-3-oxooctanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
0.0026 <7> (N-octanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
0.003 <7> (N-3-oxohexanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
0.0038 <7> (N-hexanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]

0.0047 <7> (N-3-oxobutanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
0.0051 <7> (N-butanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
0.0075 <7> (N-3-hydroxybutanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
6.7 <15> (N-hexanoyl-(S)-homoserine lactone, <15> pH 7.4, 28°C, purified detagged recombinant enzyme [7]) [7]
Additional information <7, 11, 15> (<11> kinetics [3]; <7> kinetics and thermodynamics [10]; <15> steady-state kinetics, different enzyme constitutions [7]) [3, 7, 10]

pH-Optimum

6.5 <11, 15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> (<11, 15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> in vivo assay at [2,3]) [2, 3]
7.4 <1, 2, 3, 4, 9, 10, 15> (<1,2,3,4,9,10,15> assay at [7,9]) [7, 9]
8 <7> (<7> broad optimum of pH 7.0 to pH 9.0 [10]) [10]

pH-Range

6-9 <7> (<7> complete loss of activity at pH 5.5 and above pH 9.0, pH-profile [10]) [10]

Temperature optimum (°C)

22 <7> (<7> assay at [10]) [10]
25 <11> (<11> in vivo assay at [3]) [3]
28 <6, 12, 15, 16, 17, 18, 19, 20, 43> (<15> assay at [7]; <6, 12, 16, 17, 18, 19, 20, 43> in vivo assay at [1]) [1, 7]
37 <1, 2, 3, 4, 9, 10, 15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> (<1, 2, 3, 4, 9, 10> assay at [9]; <15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> in vivo assay at [2]) [2, 9]

Temperature range (°C)

6-37 <7> [10]

4 Enzyme Structure

Subunits

? <15, 38> (<15> x * 29000, SDS-PAGE [7]; <15> x * 29000, recombinant detagged enzyme, SDS-PAGE [7]; <38> x * 30000, native enzyme, SDS-PAGE, x * 36000, recombinant enzyme, SDS-PAGE [11]) [7, 11]
Additional information <7, 15, 38> (<38> AhlD contains the conserved HXDH-H-D motif required for full enzyme activity [11]; <7> analysis of secondary wild-type and mutant enzyme structures, comparison, overview [10]; <15> enzyme secondary structure analysis and oligomeric state, overview [14]) [10, 11, 14]

5 Isolation/Preparation/Mutation/Application

Source/tissue

culture condition:N-3-oxododecanoyl-L-homoserine lactone/ammonium chloride-grown cell <38, 39> [11]
culture condition:N-decanoyl-L-homoserine lactone-grown cell <5> [5]
serum <1, 2, 3, 4, 9, 10> [9]

Purification

<4> [9]
<7> (recombinant GST-tagged wild-type and mutant enzymes from Escherichia coli by glutathione affinity chromatography) [10]
<15> [7]
<15> (recombinant maltose binding protein-fusion enzyme from Escherichia coli strain DH5α to over 95% purity, the fusion tag is cleaved off by TEV protease, the detagged enzyme is purified by DEAE ion exchange chromatography, and amylose affinity chromatography to remove traces of MBP, further purification by hydrophobic interaction chromatography in presence of 1.6-2.0 M ammonium sulfate, and dialysis) [7]
<35> (recombinant GST-tagged AiiA from Escherichia coli strain DH5α) [16]
<35> (recombinant GST-tagged AiiA from Escherichia coli strain DH5α by glutathione affinity chromatography, the GST-atg is cleaved off by thrombin) [13]
<38> (partially from strain IBN110 by ammonium sulfate fractionation and two steps of ion exchange chromatography, full purification failed due to enzyme instability) [11]
<42> (recombinant His6-tagged maltose binding protein-fusion enzyme from Escherichia coli strain XL 1-Blue by amylose and nickel affinity chromatography and gel filtration, the fusion tag is cleaved off by TEV protease) [8]

Crystallization

<15> (purified recombinant detagged enzyme, hanging drop vapour diffusion method, 10 mg/ml protein in 20% glycerol, 80 mM Tris-HCl, pH 8.5, 24% PEG 4000, and 160 mM $MgCl_2$, in a ratio of 4:1 mixed with well solution, room temperature, 2 weeks, X-ray diffraction structure determination and analysis at 1.6 A resolution using single-wavelength anomalous dispersion phasing, modeling) [14]
<40> (native and selenomethionine-labeled enzyme, free or in complex with L-homoserine lactone, X-ray diffraction structure determination and analysis at 1.7-2.0 A resolution, structure modeling) [15]
<42> (purified detagged recombinant native and selenomethionine-labeled enzyme, sitting drop vapour diffusison method, 7 mg/ml protein in 20 mM Tris-HCl, pH 7.5, mixed with and equilibrated against the reservoir solution containing 0.1 M Tris-HCl, pH 8.0, 30% PEG 4000, and 0.2 M $MgCl_2$, 21°C, 5 days, X-ray diffraction structure determination and analysis at 2.0 A resolution) [8]

Cloning

<6> (functional expression of AiiA lactonase in Burkholderia thailandensis) [4]
<6> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in Escherichia coli strain DH5α) [1]
<7> (gene aiiA, expression in Burkholderia cepacia, an organism of the Burkholderia complex, from a broad-host range plasmid, the expression abolishes or greatly reduces the accumulation of N-acyl-homoserine lactone molecules in the Burkholderia cepacia complex strains, phenotype, overview) [6]
<7> (gene aiiA, expression of wild-type and mutant enzymes in Escherichia coli as GST-tagged proteins) [10]
<11> (genes attM, aiiB, and aiiC, DNA and amino acid sequence determination and analysis, expression in Escherichia coli) [3]
<12> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in Escherichia coli strain DH5α) [1]
<14> (functional expression of AiiA lactonase in Burkholderia thailandensis) [4]
<15> (expression in Escherichia coli strain DH5α) [7]
<15> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<15> (gene aiiA, expression in Escherichia coli strain DH5α fused to the maltose binding protein with the TEV protease cleavage site ENLYFQ*G) [7]
<16> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in Escherichia coli strain DH5α) [1]
<17> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in Escherichia coli strain DH5α) [1]
<18> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in Escherichia coli strain DH5α) [1]
<19> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in Escherichia coli strain DH5α) [1]
<20> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in Escherichia coli strain DH5α) [1]
<21> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<22> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<23> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<24> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]

<25> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<26> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<27> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<28> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<29> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<30> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<31> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<32> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<33> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<34> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<35> (expression of GST-tagged AiiA containing a thrombin cleavage site in Escherichia coli strain DH5α, construction of transgenic Solanum tuberosum and Nicotiana tabacum plants expressing gene aiiA) [13]
<35> (gene aiiA, DNA and amino acid sequence determination and analysis, expression of GST-tagged AiiA in Escherichia coli strain DH5α, expression in Erwinia carotovora strain SCG1 leading to reduction of autoinducer release thereby cedreasing extracellular pectolytic enzyme activities, and attenuating pathogenicity on potato, eggplant, Chinese cabbage, carrot, celery, cauliflower, and tobacco) [16]
<36> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]
<37> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in Escherichia coli strain DH5α and BL21(DE3)) [2]

<38> (gene ahlD, DNA and amino acid sequence determination and analysis, expression in Escherichia coli strains DH5α, BL21(DE3) as His-tagged enzyme, and JM109) [11]
<39> (gene ahlK, DNA and amino acid sequence determination and analysis, expression in Escherichia coli strain JM109) [11]
<40> (expression of wild-type and mutant enzymes as His-tagged maltose binding protein-fusion proteins) [15]
<42> (gene aiiA, expression of His6-tagged maltose binding protein-fusion enzyme with the TEV protease cleavage site in Escherichia coli strain XL 1-Blue) [8]
<43> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in Escherichia coli strain DH5α) [1]

Engineering

D108A <40> (<40> site-directed mutagenesis, the mutant enzyme shows a reduced Zn^{2+} content compared to the wild-type enzyme [15]) [15]
D108E <35> (<35> site-directed mutagenesis, mutation of a zinc-binding motif residue, the mutant shows 90.8% of wild-type enzyme activity [16]) [16]
D108S <7, 35> (<35> site-directed mutagenesis, mutation of a zinc-binding motif residue, inactive mutant [16]; <7> site-directed mutagenesis, the mutant shows no activity, and altered secondary structure [10]) [10, 16]
D108S/H109S <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, inactive mutant [16]) [16]
D191A <40> (<40> site-directed mutagenesis, the mutant enzyme shows a reduced Zn^{2+} content compared to the wild-type enzyme [15]) [15]
D191S <16> (<16> site-directed mutagenesis, mutation of a zinc-binding motif residue, inactive mutant [1]) [1]
D236S <16> (<16> site-directed mutagenesis, the mutation does not affect the enzyme activity [1]) [1]
H104L/H106L/D108L <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, the mutant shows 61.4% of wild-type enzyme activity [16]) [16]
H104L/H106L/D108L/H109L <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, the mutant shows 37.9% of wild-type enzyme activity [16]) [16]
H104S <35> (<35> site-directed mutagenesis, mutation of a zinc-binding motif residue, the mutant is as active as the wild-type enzyme [16]) [16]
H104S/H106S <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, the mutant shows 51.1% of wild-type enzyme activity [16]) [16]
H104S/H106S/D108S/H109Sc <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, inactive mutant [16]) [16]
H104S/H106S/H109S <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, inactive mutant [16]) [16]
H106S <7, 35> (<35> site-directed mutagenesis, mutation of a zinc-binding motif residue, the mutant shows 61.4% of wild-type enzyme activity [16];

<7> site-directed mutagenesis, the mutant shows 53.5% of wild-type enzyme activity, and altered secondary structure [10]) [10, 16]
H109A <40> (<40> site-directed mutagenesis, the mutant enzyme shows a reduced Zn^{2+} content compared to the wild-type enzyme [15]) [15]
H109S <7, 35> (<35> site-directed mutagenesis, mutation of a zinc-binding motif residue, inactive mutant [16]; <7> site-directed mutagenesis, the mutant shows no activity, and altered secondary structure [10]) [10, 16]
H169S <7> (<7> site-directed mutagenesis, the mutant shows 53.1% of wild-type enzyme activity, and altered secondary structure [10]) [10]
H235A <40> (<40> site-directed mutagenesis, the mutant enzyme shows a reduced Zn^{2+} content compared to the wild-type enzyme [15]) [15]
H235S <16> (<16> site-directed mutagenesis, the mutation does not affect the enzyme activity [1]) [1]
Additional information <5, 6, 14, 35> (<35> construction of transgenic Solanum tuberosum and Nicotiana tabacum plants via Agrobacterium tumefaciens transfection with Bacillus sp. gene aiiA inserted into their genome, the transgenic plants express the recombinant aiiA gene and show increased AHL-lactonase activity compared to the wild-type plants, as well as enhanced resistance to plant pathogen Erwinia carotovora, the recombinant enzyme might be trapped in microsomes [13]; <14> expression of AiiA lactonase in Burkholderia thailandensis completely abolishes the accumulation of N-decanoyl-L-homoserine lactone and N-octanoyl-L-homoserine lactone, reduces N-hexanoyl-L-homoserine lactone levels, alters both swarming and twitching motility, causes a significant increase in generation time, and affects carbon metabolism, but the AHL lactonase activity does not enhance β-hemolytic activity in the transgenic bacteria, overview [4]; <6> expression of AiiA lactonase in Burkholderia thailandensis reduces the concentrations of N-decanoyl-L-homoserine lactone, N-octanoyl-L-homoserine lactone, and N-hexanoyl-L-homoserine lactone, alters both swarming and twitching motility, and caused fluctuations in carbon utilization, but the AHL lactonase activity does not enhance β-hemolytic activity in the transgenic bacteria, overview [4]; <5> transposon mutants of quiP are defective in growth when Pseudomonas aeruginosa is cultured on decanoyl-(S)-homoserine lactone as sole carbon and energy source, the growth-defect mutant strain can be rescued by complementation with a functional copy of quiP, constitutive expression of quiP leads to decreased accumulation of the quorum signal molecule N-3-oxododecanoyl-L-homoserine lactone [5]) [4, 5, 13]

6 Stability

Temperature stability

37 <7> (<7> purified recombinant wild-type enzyme, completely stable below [10]) [10]
45 <7> (<7> purified recombinant wild-type enzyme, inactivation, 2 h [10]) [10]
95 <4> (<4> inactivation within 3 min [9]) [9]

General stability information

<38>, the enzyme is instable during purification [11]

Storage stability

<7>, 4°C or 21°C, purified recombinant enzyme, 10 days, 99% remaining activity [10]

References

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Pheophorbidase 3.1.1.82

1 Nomenclature

EC number
3.1.1.82

Systematic name
pheophorbide-a hydrolase

Recommended name
pheophorbidase

Synonyms
PPD type 1 <4> [6]
PPD type 2 <4> [6]
Phedase type 1 <4> [2]
Phedase type 2 <4> [2]
Ppd <4> [1]
phedase <2, 3, 4, 5, 6, 7, 8, 9, 12, 22> [1, 2]

CAS registry number
186207-01-2

2 Source Organism

<1> *Chlamydomonas reinhardtii* (no sequence specified) [5]
<2> *Arabidopsis thaliana* (no sequence specified) (<2> DXR [2]) [2]
<3> *Brassica napus* (no sequence specified) [2]
<4> *Raphanus sativus* (no sequence specified) [1, 2, 6]
<5> *Brassica oleracea* (no sequence specified) [2]
<6> *Brassica campestris* (no sequence specified) [2]
<7> *Brassica pekinensis* (no sequence specified) (<7> DXR [2]) [2]
<8> *Nasturtium officinale* (no sequence specified) [2]
<9> *Chenopodium album* (no sequence specified) [2,3,4]
<10> no activity in *Hordeum vulgare* [1, 2]
<11> no activity in *Equisetum arvense* [2]
<12> *Brassica chinensis* (no sequence specified) [2]
<13> no activity in *Pisum sativum* [2]
<14> no activity in *Chlamydomonas reinhardtii* [1, 2]
<15> no activity in *Spinacia oleracea* [2]
<16> no activity in *Rumex acetosa* [2]
<17> no activity in *Camellia sinensis* [2]

<18> no activity in *Corchorus olitorius* [2]
<19> no activity in *Perilla frutescens* [2]
<20> no activity in *Chrysanthemum coronarium* [2]
<21> no activity in *Petroselinum sativum* [2]
<22> *Hypolepis punctata* (no sequence specified) [2]
<23> no activity in *Lepisorus thunbergianus* [2]
<24> no activity in *Ginkgo biloba* [2]

3 Reaction and Specificity

Catalyzed reaction

pheophorbide a + H_2O = C-132-carboxypyropheophorbide a + methanol
C-132-carboxypyropheophorbide a = pyropheophorbide a + CO_2 (spontaneous)
pheophorbide a + H_2O = pyropheophorbide a + methanol + CO_2 (overall reaction)

Natural substrates and products

S pheophorbide a + H_2O <4, 9> (<9> constitutively expressed [3]; <4> Phedase type 1 is senescence-induced [2]; <4> Phedase type 2 is constitutively produced [2]) (Reversibility: ?) [2, 3]
P pyropheophorbide a + methanol + CO_2 (<9> overall reaction [3])

Substrates and products

S bacteriopheophorbide a + H_2O <4> (<4> PPD type 1 [6]; <4> PPD type 2 [6]) (Reversibility: ?) [6]
P ?
S pheophorbide a + H_2O <1, 4, 9> (<9> constitutively expressed [3]; <4> Phedase type 1 is senescence-induced [2]; <4> Phedase type 2 is constitutively produced [2]; <4> PPD type 1 [6]; <4> PPD type 2 [6]; <9> the enzyme catalyzes the conversion of pheophorbide a to a precursor of pyropheophorbide a (C-132-carboxyl-pyropheophorbide a). The precursor is then decarboxylated non-enzymatically to yield pyropheophorbide [3]; <4> the enzyme catalyzes the conversion of pheophorbide a to a precursor of pyropheophorbide a, C-132-carboxylpyropheophorbide a, by demethylation, and then the precursor is decarboxylated non-enzymatically to yield pyropheophorbide a, Phedase type 1 [2]; <4> the enzyme catalyzes the conversion of pheophorbide a to a precursor of pyropheophorbide a, C-132-carboxylpyropheophorbide a, by demethylation, and then the precursor is decarboxylated non-enzymatically to yield pyropheophorbide a, Phedase type 2 [2]) (Reversibility: ?) [2, 3, 5, 6]
P pyropheophorbide a + methanol + CO_2 (<9> overall reaction [3])
S pheophorbide b + H_2O <4> (<4> PPD type 1 [6]; <4> PPD type 2 [6]) (Reversibility: ?) [6]
P ?

S Additional information <4> (<4> the enzyme is extremely specific for pheophorbides of the dihydroporphyrin and tetrahydroporphyrin type [6]) (Reversibility: ?) [6]
P ?

Inhibitors
cycloheximide <1> [5]
methanol <4, 9> (<9> uncompetitive [3]; <4> 1 M, 20% inhibition, Phedase type 1 [2]; <4> 1 M, 20% inhibition, Phedase type 2 [2]) [2, 3]
N-ethylmaleimide <4> (<4> 1 mM, 11% inhibition, Phedase type 1 [2]) [2]
phenylmethylsulfonic fluoride <4> (<4> 1 mM, 26.4% inhibition, Phedase type 2 [2]; <4> 1 mM, 61% inhibition, Phedase type 1 [2]) [2]

Activating compounds
EDTA <4> (<4> 1 mM, 22% activation, Phedase type 1 [2]; <4> 1 mM, 9% activation, Phedase type 2 [2]) [2]

Specific activity (U/mg)
0.0137 <4> [6]
0.021 <4> [6]
5.75 <9> [4]

K_m-Value (mM)
0.0125 <9> (pheophorbide a, <9> pH 7.0 [3]) [3]
0.0141 <4> (pheophorbide a, <4> 30°C, pH 7.0, Phedase type 1 [2]; <4> PPD type 1 [6]) [2, 6]
0.0151 <4> (pheophorbide a, <4> 30°C, pH 7.0, Phedase type 2 [2]; <4> PPD type 2 [6]) [2, 6]
0.037 <4> (bacteriopheophorbide a, <4> PPD type 2 [6]) [6]
0.039 <4> (bacteriopheophorbide a, <4> PPD type 1 [6]) [6]
0.232 <4> (pheophorbide b, <4> PPD type 2 [6]) [6]
0.243 <4> (pheophorbide b, <4> PPD type 1 [6]) [6]
0.283 <1> (pheophorbide a) [5]

K_i-Value (mM)
71.6 <9> (Methanol) [3]

pH-Optimum
6.5 <4> (<4> Phedase type 2 [2]) [2]
7-7.5 <4> (<4> Phedase type 1 [2]) [2]
7.5 <9> [3]

4 Enzyme Structure

Molecular weight
105000 <9> (<9> gel filtration [4]) [4]
113000 <4> (<4> Phedase type 1, gel filtration [2]; <4> Phedase type 2, gel filtration [2]; <4> PPD type 1, gel filtration [6]; <4> PPD type 2, gel filtration [6]) [2, 6]
170000 <1> (<1> gel filtration [5]) [5]

Subunits
dimer <1, 4> (<1> 1 * 87000 + 1 * 83000, SDS-PAGE [5]; <4> 2 * 57000, Phedase type 1, SDS-PAGE [2]) [2, 5]
tetramer <9> (<9> x * 28000 + x + 29000, SDS-PAGE [4]) [4]

5 Isolation/Preparation/Mutation/Application

Source/tissue
cotyledon <4> (<4> PPD type 1 [6]; <4> PPD type 2 [6]) [2, 6]
leaf <9> [3, 4]
Additional information <1> (<1> enzyme activity increases dependent on the age of the cell [5]) [5]

Localization
Additional information <9> (<9> extraplastidic part of the cell [4]) [4]

Purification
<1> (partial) [5]
<4> [1]
<4> (PPD type 1) [6]
<4> (PPD type 2) [6]
<4> (Phedase type 1) [2]
<4> (Phedase type 2) [2]
<9> [4]

Cloning
<4> [1]
<4> (PPD type 1) [6]
<4> (PPD type 2) [6]

6 Stability

Temperature stability
68 <4> (<4> 50% loss of activity at 67.5°C, Phedase type 1 [2]; <4> 50% loss of activity at 67.5°C, Phedase type 2 [2]) [2]

Storage stability
<9>, -20°C, stable for over a year [4]
<9>, 4°C, stable for a few months [4]

References

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Phenylacetyl-CoA hydrolase 3.1.2.25

1 Nomenclature

EC number
3.1.2.25

Systematic name
phenylglyoxylyl-CoA hydrolase

Recommended name
phenylacetyl-CoA hydrolase

Synonyms
phenylacetyl coenzyme A hydrolase <1> [3]
Additional information <2> (<2> an enzyme preparation of phenylacetyl-CoA:acceptor oxidoreductase, EC 1.17.5.1, catalyzes the second step in the conversion of phenylacetyl-CoA to phenylglyoxylate, the first step being carried out by EC 1.17.5.1 [2]; <2> an enzyme preparation of phenylacetyl-CoA:acceptor oxidoreductase, EC 1.17.5.1, catalyzes the second step in the conversion of phenylacetyl-CoA to phenylglyoxylate, the first step being carried out by EC 1.17.5.1, may be an additional membrane-bound enzyme or more likely one of the subunits of phenylacetyl-CoA:acceptor oxidoreductase is responsible for the hydrolysis of phenylglyoxylyl-CoA [1]) [1, 2]

CAS registry number
210756-43-7 (cf. EC 1.17.5.1)
57219-72-4

2 Source Organism

<1> *Penicillium chrysogenum* (no sequence specified) [3]
<2> *Thauera aromatica* (no sequence specified) [1, 2]

3 Reaction and Specificity

Catalyzed reaction
phenylglyoxylyl-CoA + H_2O = phenylglyoxylate + CoA (<2> This is the second step in the conversion of phenylacetyl-CoA to phenylglyoxylate, the first step being carried out by EC 1.17.5.1, phenylacetyl-CoA dehydrogenase [2])

Reaction type
hydrolysis

Natural substrates and products

- **S** phenylglyoxylyl-CoA + H_2O <2> (<2> involved in anaerobic metabolism of phenylalanine, catalyzes the second step in the conversion of phenylacetyl-CoA to phenylglyoxylate, the first step being carried out by EC 1.17.5.1 [1]) (Reversibility: ?) [1]
- **P** phenylglyoxylate + CoA <2> [1]
- **S** Additional information <2> (<2> catalyzes the second step in the conversion of phenylacetyl-CoA to phenylglyoxylate, the first step being carried out by EC 1.17.5.1 [2]) (Reversibility: ?) [2]
- **P** ? <2> [2]

Substrates and products

- **S** *p*-nitrophenyl acetate + H_2O <1> (<1> hydrolyzes *p*-nitrophenyl acetate at the same rate as phenylacetyl-CoA or phenoxyacetylglycine [3]) (Reversibility: ?) [3]
- **P** *p*-nitrophenol + acetate <1> [3]
- **S** phenoxyacetyl-CoA + H_2O <1> (<1> approximately 5fold higher activity as with phenylacetyl-CoA [3]) (Reversibility: ?) [3]
- **P** phenoxyacetate + CoA <1> [3]
- **S** phenoxyacetylglycine + H_2O <1> (<1> hydrolyzes phenoxyacetylglycine at the same rate as phenylacetyl-CoA or *p*-nitrophenyl acetate [3]) (Reversibility: ?) [3]
- **P** ? <1> [3]
- **S** phenylacetyl-CoA + H_2O <1> (<1> hydrolyzes phenylacetyl-CoA at the same rate as *p*-nitrophenyl acetate or phenoxyacetylglycine, approximately 5fold lower activity as with phenoxyacetyl-CoA [3]) (Reversibility: ?) [3]
- **P** phenylacetate + CoA <1> [3]
- **S** phenylglyoxylyl-CoA + H_2O <2> (<2> hydrolytic release of coenzyme A from phenylglyoxylyl-CoA [1]; <2> involved in anaerobic metabolism of phenylalanine, catalyzes the second step in the conversion of phenylacetyl-CoA to phenylglyoxylate, the first step being carried out by EC 1.17.5.1 [1]) (Reversibility: ?) [1, 2]
- **P** phenylglyoxylate + CoA <2> [1, 2]
- **S** Additional information <2> (<2> not: phenylacetyl-CoA [1]; <2> catalyzes the second step in the conversion of phenylacetyl-CoA to phenylglyoxylate, the first step being carried out by EC 1.17.5.1 [2]) (Reversibility: ?) [1, 2]
- **P** ? <2> [1, 2]

Inhibitors

Additional information <1> (<1> sensitive to SH group inhibitors [3]) [3]

K_m-Value (mM)

3.95 <1> (phenylacetyl-CoA) [3]

pH-Optimum

7.5 <2> (<2> assay at [1,2]) [1, 2]
7.6-7.8 <1> [3]

Temperature optimum (°C)
30 <2> (<2> assay at [1,2]) [1, 2]

4 Enzyme Structure

Molecular weight
25000 <1> (<1> gel filtration [3]) [3]

5 Isolation/Preparation/Mutation/Application

Localization
membrane <2> (<2> bound [1]) [1]

Purification
<1> (130fold) [3]

6 Stability

Oxidation stability
<1>, thiols are necessary during purification to prevent oxidation [3]

References

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Bile-acid-CoA hydrolase 3.1.2.26

1 Nomenclature

EC number
3.1.2.26

Systematic name
deoxycholoyl-CoA hydrolase

Recommended name
bile-acid-CoA hydrolase

Synonyms
baiF <1> (<1> gene name [1]) [1]

CAS registry number
220983-07-3

2 Source Organism

<1> *Eubacterium sp.* (no sequence specified) (<1> α-subunit OST1 [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
deoxycholoyl-CoA + H_2O = CoA + deoxycholate

Natural substrates and products
- **S** 7α,12α-dihydroxy-3-oxo-4-cholenoyl-CoA + H_2O <1> (<1> probably the physiological substrate [1]) (Reversibility: ?) [1]
- **P** CoA + 7α,12α-dihydroxy-3-oxo-4-cholenoate

Substrates and products
- **S** 7α,12α-dihydroxy-3-oxo-4-cholenoyl-CoA + H_2O <1> (<1> probably the physiological substrate [1]) (Reversibility: ?) [1]
- **P** CoA + 7α,12α-dihydroxy-3-oxo-4-cholenoate
- **S** chenodeoxycholoyl-CoA + H_2O <1> (Reversibility: ?) [1]
- **P** CoA + chenodeoxychlate
- **S** choloyl-CoA + H_2O <1> (Reversibility: ?) [1]
- **P** cholate + CoA
- **S** deoxycholoyl-CoA + H_2O <1> (Reversibility: ?) [1]
- **P** CoA + deoxycholate

Specific activity (U/mg)
61 <1> [1]

K_m-Value (mM)
0.18 <1> (choloyl-CoA, <1> 37°C, pH 7.0 [1]) [1]

pH-Optimum
6-7 <1> [1]

pH-Range
4-9 <1> (<1> no activity below pH 4.0 and above pH 9.0 [1]) [1]

4 Enzyme Structure

Molecular weight
72000 <1> (<1> gel filtration [1]) [1]

Subunits
dimer <1> (<1> 2 * 47500, SDS-PAGE [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> (recombinant baiF, DEAE, Mono-Q chromatography) [1]

Cloning
<1> (expression in Escherichia coli) [1]

6 Stability

Storage stability
<1>, -20°C, 50% glycerol, several months, no loss of activity [1]

References

[1] Ye, H.Q.; Mallonee, D.H.; Wells, J.E.; Bjorkhem, I.; Hylemon, P.B.: The bile acid-inducible baiF gene from Eubacterium sp. strain VPI 12708 encodes a bile acid-coenzyme A hydrolase. J. Lipid Res., **40**, 17-23 (1999)

Choloyl-CoA hydrolase 3.1.2.27

1 Nomenclature

EC number
3.1.2.27

Systematic name
choloyl-CoA hydrolase

Recommended name
choloyl-CoA hydrolase

Synonyms
PTE-2 <4> [1]
acyl-CoA thioesterase <4> [1]
bile acid-CoA thioesterase <2> [4]
chenodeoxycholic acid-CoA thioesterase <2> [5]
chenodeoxycholoyl-coenzyme A thioesterase <2> [5]
choloyl-coenzyme A thioesterase <2> [5]
coenzyme A thioesterase 2 <1, 2> [2]
peroxisomal acyl-coA thioesterase <4> [1]

CAS registry number
37270-64-7
9025-87-0

2 Source Organism

<1> *Mus musculus* (no sequence specified) [2]
<2> *Homo sapiens* (no sequence specified) [2, 4, 5]
<3> *Eubacterium sp.* (no sequence specified) (<3> α-subunit OST1 [3]) [3]
<4> *Mus musculus* (UNIPROT accession number: P58137) [1]

3 Reaction and Specificity

Catalyzed reaction
choloyl-CoA + H_2O = cholate + CoA (<2,4> the catalytic triad is formed by Asp233, Ser255, and Gln305 [1,5])

Natural substrates and products
S 2-trans-decenoyl-CoA + H_2O <4> (<4> substrate is a β-oxidation intermediate, low activity [1]) (Reversibility: ?) [1]
P 2-trans-decenoate + CoA

S 3-hydroxypalmitoyl-CoA + H_2O <4> (<4> substrate is a β-oxidation intermediate, low activity [1]) (Reversibility: ?) [1]
P 3-hydroxypalmitate + CoA
S chenodeoxycholoyl-CoA + H_2O <2, 4> (Reversibility: ?) [1, 4, 5]
P chenodeoxycholate + CoA (<2> free chenodeoxycholate activates the farnesoid X receptor [5])
S choloyl-CoA + H_2O <1, 2, 4> (<1,2> enzyme is involved in the regulation of free to conjugated bile acid ratio, overview [2]) (Reversibility: ?) [1, 2, 4, 5]
P cholate + CoA
S hydroxymethylglutaryl-CoA + H_2O <4> (Reversibility: ?) [1]
P hydroxymethylglutarate + CoA
S trihydroxycoprostanoyl-CoA + H_2O <4> (Reversibility: ?) [1]
P trihydroxycoprostanate + CoA
S Additional information <2, 4> (<2> enzyme is involved in the regulation of free to conjugated bile acid ratio, overview [4]; <4> the enzyme functions as a major regulator of peroxisomal lipid metabolism, the enzyme competes with the bile acid-CoA:amino acid N-acyltranferase for bile acid-CoAs as substrates [1]; <2> the enzyme has a regulatory function in bile acid metabolism [5]) (Reversibility: ?) [1, 4, 5]
P ?

Substrates and products

S 2-trans-decenoyl-CoA + H_2O <4> (<4> substrate is a β-oxidation intermediate, low activity [1]) (Reversibility: ?) [1]
P 2-trans-decenoate + CoA
S 3-dehydrocholoyl-CoA + H_2O <3> (Reversibility: ?) [3]
P 3-dehydrocholate + CoA
S 3-hydroxypalmitoyl-CoA + H_2O <4> (<4> substrate is a β-oxidation intermediate, low activity [1]) (Reversibility: ?) [1]
P 3-hydroxypalmitate + CoA
S chenodeoxycholoyl-CoA + H_2O <2, 3, 4> (<4> best substrate [1]; <2> preferred substrate [5]) (Reversibility: ?) [1, 3, 4, 5]
P chenodeoxycholate + CoA (<2> free chenodeoxycholate activates the farnesoid X receptor [5])
S choloyl-CoA + H_2O <1, 2, 3, 4> (<4> best substrate [1]; <1,2> enzyme is involved in the regulation of free to conjugated bile acid ratio, overview [2]) (Reversibility: ?) [1, 2, 3, 4, 5]
P cholate + CoA
S hydroxymethylglutaryl-CoA + H_2O <4> (Reversibility: ?) [1]
P hydroxymethylglutarate + CoA
S trihydroxycoprostanoyl-CoA + H_2O <4> (Reversibility: ?) [1]
P trihydroxycoprostanate + CoA
S Additional information <2, 3, 4> (<2> enzyme is involved in the regulation of free to conjugated bile acid ratio, overview [4]; <4> the enzyme functions as a major regulator of peroxisomal lipid metabolism, the enzyme competes with the bile acid-CoA:amino acid N-acyltranferase for

bile acid-CoAs as substrates [1]; <2> the enzyme has a regulatory function in bile acid metabolism [5]; <4> the enzyme shows broad specificity for substrates and (branched) acyl-CoA chain lengths [1]; <3> no activity with acetyl-CoA, isovaleryl-CoA, palmitoyl-CoA or phenylacetyl-CoA are used as substrates [3]) (Reversibility: ?) [1, 3, 4, 5]

P ?

Inhibitors

4-chloromercuribenzoate <4> (<4> 50% inhibition at 0.001 mM [1]) [1]
acyl-CoA <4> (<4> enzyme is substrate-inhibited at 0.005-0.01 mM of acyl-CoAs with chain length longer than C_{10} [1]) [1]
CoA <2, 4> (<4> product inhibition with regulatory function, 50% inhibition at 0.01-0.015 mM [1]; <2> slight inhibition of activity with choloyl-CoA [5]) [1, 5]
DTNB <2, 4> (<4> 50% inhibition at 0.15 mM [1]; <2> 75% inhibition at 0.3 mM, complete inhibition at 1.3 mM [5]) [1, 5]
phospholipids <2> (<2> at high concentrations [5]) [5]
Additional information <2> (<2> substrate competition between choloyl-CoA and chenodeoxycholoyl-CoA, overview [5]) [5]

Activating compounds

CoA <2> (<2> slight activation of activity with chenodeoxycholoyl-CoA [5]) [5]
DTT <2> (<2> highly stimulating on the activity with both choloyl-CoA and chenodeoxycholoyl-CoA [5]) [5]
PPARII <1, 2, 4> (<1,2> peroxisomal proliferator activated receptor-II activates the enzyme, fibrates are antagonizing the receptor activity [2]; <4> peroxisomal proliferator activated receptor-II activates the enzyme, regulatory function, fasting-mediated induction in vivo, dependent on the peroxisomal proliferator WY-14,643 [1]) [1, 2]
Additional information <4> (<4> fasting induces the enzyme [1]) [1]

Specific activity (U/mg)

0.00118 <2> (<2> nuclear fraction of liver homogenate, substrate choloyl-CoA [5]) [5]
0.00127 <2> (<2> cytosolic fraction of liver homogenate, substrate choloyl-CoA [5]) [5]
0.0014 <2> (<2> subcellular fractions, overview [4]) [4]
0.00186 <2> (<2> nuclear fraction of liver homogenate, substrate chenodeoxycholoyl-CoA [5]) [5]
0.00203 <2> (<2> microsomal fraction of liver homogenate, substrate chenodeoxycholoyl-CoA [5]; <2> mitochondrial fraction of liver homogenate, substrate choloyl-CoA [5]) [5]
0.00227 <2> (<2> cytosolic fraction of liver homogenate, substrate chenodeoxycholoyl-CoA [5]) [5]
0.00247 <2> (<2> light mitochondrial fraction of liver homogenate, substrate choloyl-CoA [5]) [5]

0.00248 <2> (<2> microsomal fraction of liver homogenate, substrate choloyl-CoA [5]) [5]
0.00263 <2> (<2> mitochondrial fraction of liver homogenate, substrate chenodeoxycholoyl-CoA [5]) [5]
0.00276 <2> (<2> postnuclear fraction of liver homogenate, substrate choloyl-CoA [5]) [5]
0.00297 <2> (<2> postnuclear fraction of liver homogenate, substrate chenodeoxycholoyl-CoA [5]) [5]
0.00334 <2> (<2> light mitochondrial fraction of liver homogenate, substrate chenodeoxycholoyl-CoA [5]) [5]
0.0133 <2> (<2> peroxisomal fraction of a metastatic liver homogenate, substrate chenodeoxycholoyl-CoA [5]) [5]
0.01602 <2> (<2> peroxisomal fraction of a metastatic liver homogenate, substrate choloyl-CoA [5]) [5]
61 <3> [3]
Additional information <2> [5]

K_m-Value (mM)

0.011 <2> (DTT, <2> pH 8.0, 37°C, with chenodeoxycholoyl-CoA [5]) [5]
0.018 <2> (DTT, <2> pH 8.0, 37°C, with choloyl-CoA [5]) [5]
0.071 <2> (chenodeoxycholoyl-CoA, <2> pH 8.0, 37°C, postnuclear fraction of liver homogenate [5]) [5]
0.077 <2> (choloyl-CoA, <2> pH 8.0, 37°C, postnuclear fraction of liver homogenate [5]) [5]
0.175 <3> (choloyl-CoA, <3> pH 7.0, 37°C [3]) [3]
Additional information <2, 4> (<2> kinetics [4]) [1, 4, 5]

pH-Optimum

6-7 <3> [3]
7-9 <2> (<2> broad optimum with substrate choloyl-CoA [5]) [5]
7.4 <4> (<4> assay at [1]) [1]
7.5-9 <2> (<2> broad optimum with substrate chenodeoxycholoyl-CoA [5]) [5]
8 <2> (<2> assay at [4]) [4]

pH-Range

4-9 <3> (<3> no activity detected below pH 4.0 or above pH 9.0 [3]) [3]

Temperature optimum (°C)

37 <2> (<2> assay at [4,5]) [4, 5]

4 Enzyme Structure

Molecular weight

70000 <4> (<4> recombinant enzyme, gel filtration [1]) [1]

Subunits

dimer <4> (<4> 2 * 35886, amino acid sequence calculation, 2 * 36000, recombinant enzyme, SDS-PAGE [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue

brain <1, 2> [2]
heart <4> (<4> lower enzyme content [1]) [1]
kidney <4> (<4> high enzyme content [1]) [1]
liver <1, 2, 4> (<2> healthy and metastatic [5]) [1, 2, 4, 5]
muscle <4> (<4> lower enzyme content [1]) [1]
testis <4> [1]

Localization

cytosol <2> (<2> high activity [4]) [4, 5]
microsome <2> [4, 5]
mitochondrion <2> (<2> especially in the light mitochondrial fraction [4]) [4, 5]
nucleus <2> [4, 5]
peroxisome <1, 2, 4> (<2> highest enzyme activity [4]) [1, 2, 4, 5]
Additional information <2> (<2> subcellular distribution, overview [4]; <2> multiorganelle distribution, overview [5]) [4, 5]

Purification

<2> (partially, subcellular fractionation) [4, 5]
<3> (recombinant) [3]

Cloning

<3> (expression in Escherichia coli) [3]
<4> (gene PTE-2, DNA and amino acid sequence determination and analysis, location on chromosome 20q12-q13, expression in Escherichia coli, expression as GFP-fusion protein in human skin fibroblasts showing targeting to the peroxisomes) [1]

6 Stability

Storage stability

<3>, -20°C, enzyme is stable for several months in 50% glycerol [3]

References

[1] Hunt, M.C.; Solaas, K.; Kase, B.F.; Alexson, S.E.: Characterization of an acyl-coA thioesterase that functions as a major regulator of peroxisomal lipid metabolism. J. Biol. Chem., **277**, 1128-1138 (2002)

[2] Russell, D.W.: The enzymes, regulation, and genetics of bile acid synthesis. Annu. Rev. Biochem., **72**, 137-174 (2003)
[3] Ye, H.Q.; Mallonee, D.H.; Wells, J.E.; Bjorkhem, I.; Hylemon, P.B.: The bile acid-inducible baiF gene from Eubacterium sp. strain VPI 12708 encodes a bile acid-coenzyme A hydrolase. J. Lipid Res., **40**, 17-23 (1999)
[4] Solaas, K.; Ulvestad, A.; Soreide, O.; Kase, B.F.: Subcellular organization of bile acid amidation in human liver: a key issue in regulating the biosynthesis of bile salts. J. Lipid Res., **41**, 1154-1162 (2000)
[5] Solaas, K.; Sletta, R.J.; Soreide, O.; Kase, B.F.: Presence of choloyl- and chenodeoxycholoyl-coenzyme A thioesterase activity in human liver. Scand. J. Clin. Lab. Invest., **60**, 91-102 (2000)

Mannosyl-3-phosphoglycerate phosphatase 3.1.3.70

1 Nomenclature

EC number
3.1.3.70

Systematic name
α-D-mannosyl-3-phosphoglycerate phosphohydrolase

Recommended name
mannosyl-3-phosphoglycerate phosphatase

Synonyms
MPG phosphatase
MPGP
mannosyl-3-phosphoglycerate phosphatase
phosphatase, mannosyl-3-phosphoglycerate

CAS registry number
393512-74-8

2 Source Organism

<1> *Pyrococcus horikoshii* (no sequence specified) [1]

3 Reaction and Specificity

Catalyzed reaction
2(α-D-mannosyl)-3-phosphoglycerate + H_2O = 2(α-D-mannosyl)-D-glycerate + phosphate

Reaction type
hydrolysis of phosphate bond

Natural substrates and products
S 2(α-D-mannosyl)-3-phosphoglycerate + H_2O <1> (<1> enzyme is involved in biosynthesis of α-mannosylglycerate [1]) (Reversibility: ?) [1]
P 2(α-D-mannosyl)-D-glycerate + phosphate <1> [1]

Substrates and products
S 2(α-D-mannosyl)-3-phosphoglycerate + H_2O <1> (<1> enzyme is highly specific for D-mannosyl-3-phosphoglycerate [1]; <1> enzyme is involved in biosynthesis of α-mannosylglycerate [1]) (Reversibility: ?) [1]
P 2(α-D-mannosyl)-D-glycerate + phosphate <1> [1]

Metals, ions
Mg^{2+} <1> (<1> activity without Mg^{2+} is 58% of the activity with 15 mM [1]) [1]

K_m-Value (mM)
0.134 <1> (2(α-D-mannosyl)-3-phosphoglycerate) [1]

pH-Optimum
5.2-6.4 <1> [1]

pH-Range
4.2-7.5 <1> (<1> pH 4.2: 60% of maximal actuvity, pH 7.5: about 45% of maximal activity [1]) [1]

Temperature optimum (°C)
95-100 <1> [1]

Temperature range (°C)
70-108 <1> (<1> 70°C: about 45% of maximal activity, 108°C: about 20% of maximal activity [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> [1]

Cloning
<1> [1]

6 Stability

Temperature stability
98 <1> (<1> half-life: 15.6 min [1]) [1]

References

[1] Empadinhas, N.; Marugg, J.D.; Borges, N.; Santos, H.; Da Costa, M.S.: Pathway for the synthesis of mannosylglycerate in the hyperthermophilic archaeon Pyrococcus horikoshii. Biochemical and genetic characterization of key enzymes. J. Biol. Chem., **276**, 43580-43588 (2001)

2-Phosphosulfolactate phosphatase 3.1.3.71

1 Nomenclature

EC number
3.1.3.71

Systematic name
(R)-2-phospho-3-sulfolactate phosphohydrolase

Recommended name
2-phosphosulfolactate phosphatase

Synonyms
(2R)-phosphosulfolactate phosphohydrolase
ComB phosphatase
phosphosulfolactate phosphohydrolase

CAS registry number
409095-18-7

2 Source Organism

<1> *Methanococcus jannaschii* (no sequence specified) [1]
<2> *Methanosarcina thermophila* (no sequence specified) [1]

3 Reaction and Specificity

Catalyzed reaction
(2R)-2-phospho-3-sulfolactate + H_2O = (2R)-3-sulfolactate + phosphate

Reaction type
phosphoric ester hydrolysis

Natural substrates and products
- **S** 2-phospho-3-sulfolactate + H_2O <1> (<1> the enzyme catalyzes the second step in biosynthesis of coenzyme M [1]) (Reversibility: ?) [1]
- **P** 3-sulfolactate + phosphate

Substrates and products
- **S** (R)-2-phospholactate + H_2O <1> (Reversibility: ?) [1]
- **P** lactate + phosphate
- **S** (S)-2-phospholactate + H_2O <1> (<1> no activity with (R)-phospholactate [1]) (Reversibility: ?) [1]

P lactate + phosphate
S (S)-2-phosphomalate + H_2O <1> (Reversibility: ?) [1]
P malate + phosphate
S 2-phospho-3-sulfolactate + H_2O <1> (<1> the enzyme catalyzes the second step in biosynthesis of coenzyme M [1]) (Reversibility: ?) [1]
P 3-sulfolactate + phosphate
S phosphoglycolate + H_2O <1> (Reversibility: ?) [1]
P glycolate + phosphate
S phosphoglycolate + H_2O <1> (Reversibility: ?) [1]
P lactate + phosphate
S rac-2-phosphosulfolactate + H_2O <1, 2> (Reversibility: ?) [1]
P sulfolactate + phosphate <1, 2> [1]
S Additional information <1> (<1> in presence of (S)-lactate the histidine-tagged enzyme can dephosphorylate phosphoglycolate and produce 2-phospholactate [1]) (Reversibility: ?) [1]
P ?

Inhibitors

2-carboxyethylphosphonate <1> (<1> weak, polyhistidine-tagged enzyme [1]) [1]
Ca^{2+} <1> (<1> in presence of equimolar Mg^{2+}, polyhistidine-tagged enzyme [1]) [1]
Cd^{2+} <1> (<1> in presence of equimolar Mg^{2+}, polyhistidine-tagged enzyme [1]) [1]
Cu^{+} <1> (<1> in presence of equimolar Mg^{2+}, polyhistidine-tagged enzyme [1]) [1]
Fe^{2+} <1> (<1> in presence of equimolar Mg^{2+}, polyhistidine-tagged enzyme [1]) [1]
Fe^{3+} <1> (<1> in presence of equimolar Mg^{2+}, polyhistidine-tagged enzyme [1]) [1]
Mg^{2+} <1> (<1> in presence of equimolar Mg^{2+}, polyhistidine-tagged enzyme [1]) [1]
vanadate <1> [1]

Metals, ions

Co^{2+} <1> (<1> can fully substitute for Mg^{2+} [1]) [1]
Cu^{2+} <1> (<1> weak activation [1]) [1]
Mg^{2+} <1> (<1> dependent on, at a concentration approximately equimolar with substrate [1]) [1]
Mn^{2+} <1> (<1> can fully substitute for Mg^{2+} [1]) [1]
Ni^{2+} <1> (<1> can fully substitute for Mg^{2+} [1]) [1]
Zn^{2+} <1> (<1> can fully substitute for Mg^{2+} [1]) [1]

Specific activity (U/mg)

48 <1> (<1> rac-phosphosulfolactate [1]) [1]

K_m-Value (mM)

0.009 <1> ((S)-phosphomalate) [1]
0.011 <1> ((R)-phosphomalate) [1]

0.034 <1> (phosphoglycolate) [1]
0.063 <1> (rac-phosphosulfolactate) [1]
0.148 <1> ((S)-phospholactate) [1]

pH-Optimum
5.5 <1> (<1> polyhistidine-tagged enzyme, hydrolysis of rac-phosphosulfolactate [1]) [1]

pH-Range
4.5-7 <1> (<1> pH 4.5: about 80% of maximal activity, pH 7.0: about 55%of maximal activity, polyhistidine-tagged enzyme, hydrolysis of rac-phosphosulfolactate [1]) [1]

Temperature optimum (°C)
75 <1> (<1> polyhistidine-tagged enzyme, hydrolysis of rac-phosphosulfolactate [1]) [1]

Temperature range (°C)
65-85 <1> (<1> 65°C: about 50% of maximal activity, 85°C: about 70% of maximal activity, polyhistidine-tagged enzyme, hydrolysis of rac-phosphosulfolactate [1]) [1]

4 Enzyme Structure

Subunits
? <1> (<1> x * 30000, SDS-PAGE [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> [1]

Cloning
<1> (expression in Escherichia coli) [1]

6 Stability

Storage stability
<1>, -30°C, in presence of 50% v/v glycerol, 25% loss of activity after 3 weeks [1]
<1>, 4°C, 7% loss of activity after 3 weeks [1]

References

[1] Graham, D.E.; Graupner, M.; Xu, H.; White, R.H.: Identification of coenzyme M biosynthetic 2-phosphosulfolactate phosphatase. A member of a new class of Mg^{2+}-dependent acid phosphatases. Eur. J. Biochem., **268**, 5176-5188 (2001)

5-Phytase 3.1.3.72

1 Nomenclature

EC number
3.1.3.72

Systematic name
myo-inositol-hexakisphosphate 5-phosphohydrolase

Recommended name
5-phytase

Synonyms
alkaline phytase <1, 2, 3, 4> [3, 4, 5]
pH 8 phytase <4> [2]
phosphatase, phytate
phytase
phytase novo L
phytaseed
phytate phosphatase

CAS registry number
37341-58-5

2 Source Organism

<1> *Phaseolus vulgaris* (no sequence specified) [4]
<2> *Pisum sativum* (no sequence specified) [4]
<3> *Medicago sativa* (no sequence specified) [4]
<4> *Lilium longiflorum* (no sequence specified) [1, 2, 3, 5]
<5> *Pinus ponderosa* (no sequence specified) [2]
<6> *Typha latifolia* (no sequence specified) [2]

3 Reaction and Specificity

Catalyzed reaction
myo-inositol hexakisphosphate + H_2O = 1L-myo-inositol 1,2,3,4,6-pentakisphosphate + phosphate

Reaction type
phosphoric ester hydrolysis

Natural substrates and products

- **S** myo-inositol-1,2,3,4,5,6-hexakisphosphate + H_2O <4> (<4> enzyme activity may be regulated by the intracellular Ca^{2+} concentration [1]; <4> important component of phytic acid metabolism, constitutive enzyme [2]; <4> phytic acid is a major component of seeds and pollen grains, localized in membrane-bound phytic-rich granules [3]) (Reversibility: ?) [1, 2, 3]
- **P** myo-inositol-1,2,3,4,6-pentakisphosphate <4> [3]

Substrates and products

- **S** myo-inositol-1,2,3,4,5,6-hexakisphosphate + H_2O <1, 2, 3, 4> (<4> hydrolysis of the phosphate ester at the D-5 position [3]; <4> high substrate specificity for phytic acid [2,3]; <1,2,3,4> myo-inositol-1,2,3,4,5,6-hexakisphosphate is identical with phytic acid [3,4]; <4> enzyme activity may be regulated by the intracellular Ca^{2+} concentration [1]; <4> important component of phytic acid metabolism, constitutive enzyme [2]; <4> phytic acid is a major component of seeds and pollen grains, localized in membrane-bound phytic-rich granules [3]) (Reversibility: ?) [1, 2, 3, 4, 5]
- **P** myo-inositol-1,2,3,4,6-pentakisphosphate <4> (<4> symmetrical product, only isomer of IP5 as product [3]) [3]
- **S** *p*-nitrophenyl phosphate + H_2O <4> (Reversibility: ?) [5]
- **P** *p*-nitrophenol + phosphate
- **S** Additional information <4> (<4> myo-inositol pentakisphosphates are no good substrates, terminal hydrolysis to myo-inositol trisphosphate [2]; <4> two subsequent dephosphorylations occur adjacent to the D-5 hydroxyl group to yield myo-inositol-1,2,3-trikisphosphate as the final product, rates of removal of the second and third phosphates are significantly lower, possibly due to reduced specificity for IP5 and IP4 and inhibition by the phosphate released, IP4: only one isomer, either I-1,2,3,4-P4 or its enantiomer I-1,2,3,6-P4, no further hydrolysis of IP3 [3]) (Reversibility: ?) [2, 3]
- **P** ?

Inhibitors

Ca^{2+} <1, 2, 3, 4> (<4> at concentrations equimolar to the substrate: 313% activation, but inhibitory at higher concentrations [1]; <1,2,3> activates at a concentration equal to the substrate, but higher concentrations result in the precipitation of the substrate as calcium phytate and strongly inhibit enzyme activity [4]) [1, 4]
EDTA <4> (<4> 5 mM, complete inhibition due to a dependence on Ca^{2+} [2]; <4> 0.5 mM: 35% inhibition, 5 mM: 94% inhibition [1]) [1, 2, 5]
Mg^{2+} <4> [1]
MgCl <4> (<4> 0.166 M [5]) [5]
$MgSO_4$ <4> (<4> 0.5 M [5]) [5]
Mn^{2+} <4> [1]
phosphate <4> (<4> inhibition by the phosphate released from enzyme [3]) [3]

vanadate <4> (<4> 0.25-3 mM, 78-83% inhibition [5]) [5]
Additional information <1, 2, 3, 4> (<4> not inhibited by 10 mM flouride [3]; <1,2,3,4> not inhibited by flouride [2,4]) [2, 3, 4]

Metals, ions

$(NH_4)_2SO_4$ <4> (<4> 0.166 M, enhances activity [5]) [5]
Ca^{2+} <1, 2, 3, 4> (<4> activates [2,3]; <4> at concentrations equimolar to the substrate: 313% activation, but inhibitory at higher concentrations [1]; <1,2,3> activates at a concentration equal to the substrate, but higher concentrations result in the precipitation of the substrate as calcium phytate and strongly inhibit enzyme activity, 10 mM EGTA prevents the calcium effect [4]; <4> dependence on Ca^{2+} [2]; <4> activates, enzyme retains low level of activity even in Ca^{2+}-free medium [5]) [1, 2, 3, 4, 5]
K_2SO_4 <4> (<4> 0.166 M, enhances activity [5]) [5]
KCl <4> (<4> 0.5 M, activates [5]) [5]
NH_4Cl <4> (<4> 0.5 M, enhances activity [5]) [5]
Na_2SO_4 <4> (<4> 0.166 M, enhances activity [5]) [5]
NaCl <4> (<4> 0.5 M, activates [5]) [5]
Sr^{2+} <4> (<4> activates [1]) [1]
Additional information <4> (<4> not activated by Mg^{2+}, Mn^{2+}, Cu^{2+} and Co^{2+} [1]) [1]

Specific activity (U/mg)

0.006 <4> (<4> in crude extracts [3]) [3]
0.019 <4> (<4> in DEAE-purified preparations [3]) [3]
0.066 <4> [1]
Additional information <1, 2, 3> [4]

K_m-Value (mM)

0.0072 <4> (myo-inositol-1,2,3,4,5,6-hexakisphosphate, <4> in presence of Ca^{2+} [1]) [1]
0.081 <4> (myo-inositol-1,2,3,4,5,6-hexakisphosphate, <4> 37°C, pH 8.0 [5]) [5]
0.372 <4> (*p*-nitrophenyl phosphate, <4> 37°C, pH 7.0 [5]) [5]

pH-Optimum

7 <4> (<4> activity with *p*-nitrophenyl phosphate [5]) [5]
8 <1, 2, 3, 4> (<4> most active in Tris-maleate, rather than in Bicine buffer [1]; <4> activity with myo-inositol-1,2,3,4,5,6-hexakisphosphate [5]) [1, 2, 3, 4, 5]

Temperature optimum (°C)

37 <1, 2, 3, 4> (<1,2,3,4> assay at [2,3,4]) [2, 3, 4]
55 <4> [5]
55-60 <4> [1]

4 Enzyme Structure

Molecular weight

88000 <4> (<4> gel filtration [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue

pollen <4, 5, 6> (<4> localized in mature pollen, germination results in a rapid increase of enzyme that persists at the higher level for at least 6 h [2]; <4> at the inner surfaces of storage bodies, the phytin-containing organelles in pollen, integral component of the membrane [2]) [1, 2, 3, 5]
seed <1, 2, 3> (<1,2,3> dormant seeds [4]) [4]

Localization

membrane <4> (<4> integral component of the storage body membrane [2]) [2]

Purification

<1> (in absence of Triton X-100, very little enzyme activity is extracted from seeds) [4]
<2> (in absence of Triton X-100, very little enzyme activity is extracted from seeds) [4]
<3> (in absence of Triton X-100, very little enzyme activity is extracted from seeds) [4]
<4> [3, 5]
<4> (partial purification, extraction with 0.1% Triton X-100 increases recovery by nearly 4fold) [1]
<4> (recovery is most complete when detergent or phospholipase C is included in extracting medium) [2]

6 Stability

Temperature stability

50 <4> (<4> 10 min, stable [5]) [5]
55 <4> (<4> 40 min: no loss of activity, 80 min: 50% loss of activity [2]; <4> 10 min, 60% loss of activity in assay containing $CaCl_2$, compared to 40% in absence of $CaCl_2$ [5]) [2, 5]
Additional information <4> (<4> heat stable [2]) [2]

General stability information

<4>, unstable, large loss of activity during the purification procedures [3]

References

[1] Scott, J.J.; Loewus, F.A.: A calcium-activated phytase from pollen of Lilium longiflorum. Plant Physiol., **82**, 333-335 (1986)
[2] Baldi, B.G.; Scott, J.J.; Everard, J.D.; Loewus, F.A.: Localization of constitutive phytases in lily pollen and properties of the pH 8 form. Plant Sci., **56**, 137-147 (1988)
[3] Barrientos, L.; Scott, J.J.; Murthy, P.P.N.: Specificity of hydrolysis of phytic acid by alkaline phytase from lily pollen. Plant Physiol., **106**, 1489-1495 (1994)
[4] Scott, J.J.: Alkaline phytase activity in nonionic detergent extracts of legume seeds. Plant Physiol., **95**, 1298-1301 (1991)
[5] Jog, S.P.; Garchow, B.G.; Mehta, B.D.; Murthy, P.P.N.: Alkaline phytase from lily pollen: Investigation of biochemical properties. Arch. Biochem. Biophys., **440**, 133-140 (2005)

α-Ribazole phosphatase 3.1.3.73

1 Nomenclature

EC number
3.1.3.73

Systematic name
α-ribazole-5'-phosphate phosphohydrolase

Recommended name
α-ribazole phosphatase

Synonyms
CobC <1, 3> [1, 2]
Mm2058 protein <2> [3]
α-ribazole-5'-P phosphatase <3> [2]
α-ribazole-5'-phosphate phosphatase <2> [3]
cobC product <3> [2]

CAS registry number
251991-06-7

2 Source Organism

<1> *Salmonella typhimurium* (no sequence specified) [1, 2]
<2> *Methanosarcina mazei* (no sequence specified) [3]
<3> *Salmonella typhimurium* (UNIPROT accession number: P39701) [2]

3 Reaction and Specificity

Catalyzed reaction
α-ribazole 5'-phosphate + H_2O = α-ribazole + phosphate

Natural substrates and products
S α-ribazole 5'-phosphate + H_2O <1, 3> (<1> de novo synthesis of adenosylcobalamin under anaerobic conditions, nucleotide loop assembly pathway, last steps in adenosylcobalamin biosynthesis [1]; <3> involved in the assembly of the nucleotide loop of cobalamin [2]) (Reversibility: ?) [1, 2]
P α-ribazole + phosphate <1, 3> [1, 2]

Substrates and products

- **S** N^1-(5-phospho-α-D-ribosyl)-5,6-dimethylbenzimidazole + H_2O <3> (Reversibility: ?) [2]
- **P** N^1-α-D-ribosyl-5,6-dimethylbenzimidazole + phosphate <3> [2]
- **S** adenosylcobalamin 5'-phosphate + H_2O <1> (Reversibility: ?) [1]
- **P** adenosylcobalamin + phosphate <1> [1]
- **S** α-ribazole 5'-phosphate + H_2O <1, 2, 3> (<1> de novo synthesis of adenosylcobalamin under anaerobic conditions, nucleotide loop assembly pathway, last steps in adenosylcobalamin biosynthesis [1]; <3> involved in the assembly of the nucleotide loop of cobalamin [2]) (Reversibility: ?) [1, 2, 3]
- **P** α-ribazole + phosphate <1, 3> [1, 2]

4 Enzyme Structure

Molecular weight

26000 <3> (<3> SDS-PAGE [2]) [2]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [2]
<2> (recombinant) [3]

Cloning

<1> (cobC gene mapped, cloned, sequenced and overexpressed) [2]
<2> (Mm2058 protein is expressed with a decahistidine tag at its N-terminus) [3]

References

[1] Maggio-Hall, L.A.; Escalante-Semerena, J.C.: In vitro synthesis of the nucleotide loop of cobalamin by Salmonella typhimurium enzymes. Proc. Natl. Acad. Sci. USA, **96**, 11798-11803 (1999)
[2] O'Toole, G.A.; Trzebiatowski, J.R.; Escalante-Semerena, J.C.: The cobC gene of Salmonella typhimurium codes for a novel phosphatase involved in the assembly of the nucleotide loop of cobalamin. J. Biol. Chem., **269**, 26503-26511 (1994)
[3] Zayas, C.L.; Woodson, J.D.; Escalante-Semerena, J.C.: The cobZ gene of encodes the nonorthologous replacement of the α-ribazole-5'-phosphate phosphatase (CobC) enzyme of Salmonella enterica. J. Bacteriol., **188**, 2740-2743 (2006)

Pyridoxal phosphatase 3.1.3.74

1 Nomenclature

EC number
3.1.3.74

Systematic name
pyridoxal-5'-phosphate phosphohydrolase

Recommended name
pyridoxal phosphatase

Synonyms
PDXP <4> [13]
PLP phosphatase <1, 2, 3, 4, 5, 6, 7, 8, 9, 13> [3, 14]
PLPase <14> [11]
PNP phosphatase <3, 10> [3, 12]
YZGD <14> [11]
pyridoxal phosphatase <4> [13]
pyridoxal-5'-phosphate phosphatase <1, 2, 4, 5, 6, 7, 8, 9, 10, 13, 14> [11, 12, 14]
pyridoxal-specific phosphatase <4> [7]
pyridoxine phosphate phosphatase <5> [10]
vitamin B_6-phosphate phosphatase <3> [3]
vitamine B_6 (pyridoxine) phosphatase <3> [3]

CAS registry number
9076-92-0

2 Source Organism

<1> *Gallus gallus* (no sequence specified) [14]
<2> *Mus musculus* (no sequence specified) [14]
<3> *Escherichia coli* (no sequence specified) [3]
<4> *Homo sapiens* (no sequence specified) [1, 2, 4, 5, 6, 7, 9, 13, 14]
<5> *Rattus norvegicus* (no sequence specified) [10,14]
<6> *Sus scrofa* (no sequence specified) [14]
<7> *Bos taurus* (no sequence specified) [14]
<8> *Canis familiaris* (no sequence specified) [14]
<9> *Felis catus* (no sequence specified) [14]
<10> *Sinorhizobium meliloti* (no sequence specified) [12]
<11> *Mus musculus* (UNIPROT accession number: P60487) [8]

<12> *Homo sapiens* (UNIPROT accession number: Q96GD0) [8]
<13> *Brachylagus idahoensins* (no sequence specified) [14]
<14> *Paenibacillus thiaminolyticus* (no sequence specified) [11]

3 Reaction and Specificity

Catalyzed reaction

pyridoxal 5'-phosphate + H_2O = pyridoxal + phosphate (<4> mechanism [1]; <4> kinetic mechanism [2]; <3> requires Mg^{2+}, this enzyme is specific for phosphorylated vitamin B_6 compounds: it acts not only on pyridoxal phosphate (PLP), but also on pyridoxine phosphate (PNP), pyridoxamine phosphate (PMP), 4-pyridoxic acid phosphate and 4-deoxypyridoxine phosphate, this reaction can also be carried out by EC 3.1.3.1 (alkaline phosphatase) and EC 3.1.3.2 (acid phosphatase), but these enzymes have very broad substrate specificities [3])

Reaction type

hydrolysis of phosphoric ester

Natural substrates and products

- **S** pyridoxal 5'-phosphate + H_2O <4> (<4> enzyme probably plays an important role in the hydrolysis of pyridoxal 5'-phosphate to pyridoxal in erythrocytes, may be important in the regulation of pyridoxal 5'-phosphate concentration [1]) (Reversibility: ?) [1]
- **P** pyridoxal + phosphate
- **S** pyridoxal-5'-phosphate + H_2O <1, 2, 4, 5, 6, 7, 8, 9, 13> (Reversibility: ?) [13, 14]
- **P** pyridoxal + phosphate
- **S** Additional information <4, 12> (<4> probably plays an important role in the regulation of vitamin B_6 metabolism [2]; <4> vitamin B_6 metabolism [5]; <12> vitamin B_6 metabolism, catabolism of pyridoxal 5'-phosphate [8]) (Reversibility: ?) [2, 5, 8]
- **P** ?

Substrates and products

- **S** 4-deoxypyridoxine 5'-phosphate + H_2O <4> (Reversibility: ?) [5]
- **P** 4-deoxypyridoxine + phosphate
- **S** 4-pyridoxic acid 5'-phosphate + H_2O <4> (<4> highest catalytic efficiency with 4-pyridoxic acid 5'-phosphate and pyridoxal 5'-phosphate [6]; <4> the catalytic efficiency decreases in the following order: pyridoxal 5'-phosphate, 4-pyridoxic acid 5'-phosphate, pyridoxine 5'-phosphate and pyridoxamine 5'-phosphate [5]) (Reversibility: ?) [5, 6]
- **P** 4-pyridoxic acid + phosphate
- **S** N-(5'-phospho-4'-pyridoxyl)benzylamine + H_2O <4> (Reversibility: ?) [6]
- **P** 4'-pyridoxylbenzylamine + phosphate
- **S** N-(5'-phospho-4'-pyridoxyl)ethanolamine + H_2O <4> (Reversibility: ?) [6]

P 4'-pyridoxylethanolamine + phosphate
S N-(5'-phospho-4'-pyridoxyl)glycine + H_2O <4> (Reversibility: ?) [6]
P 4'-pyridoxylglycine + phosphate
S N-(5'-phospho-4'-pyridoxyl)phenylalanine + H_2O <4> (<4> much higher catalytic efficiency than with pyridoxine 5'-phosphate [6]) (Reversibility: ?) [6]
P 4'-pyridoxylphenylalanine + phosphate
S *p*-nitrophenyl phosphate + H_2O <4> (<4> slow hydrolysis [5]) (Reversibility: ?) [5]
P *p*-nitrophenol + phosphate
S *p*-nitrophenyl-phosphate + H_2O (?) <14> (Reversibility: ?) [11]
P *p*-nitrophenol + phosphate
S pyridoxal 5'-phosphate + H_2O <3, 4, 5, 11, 12> (<4> best substrate [9]; <4> enzyme probably plays an important role in the hydrolysis of pyridoxal 5'-phosphate to pyridoxal in erythrocytes, may be important in the regulation of pyridoxal 5'-phosphate concentration [1]; <4> best substrate, a cysteinyl residue at or near the active site is essential for activity, there may be only one free Cys per subunit [7]; <4> highest catalytic efficiency with pyridoxal 5'-phosphate and 4-pyridoxic acid 5'-phosphate [6]; <12> highest specificity constant followed by pyridoxine 5'-phosphate [8]; <4> kinetic mechanism, random binding of pyridoxal phosphate and Mg^{2+}, formation of a dead-end complex of phosphate with the enzyme-Mg complex [2]; <4> mechanism, a covalent phosphoenzyme intermediate is formed during catalysis, may be an acylphosphate intermediate [1]; <4> the catalytic efficiency decreases in the following order: pyridoxal 5'-phosphate, 4-pyridoxic acid 5'-phosphate, pyridoxine 5'-phosphate and pyridoxamine 5'-phosphate [5]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10]
P pyridoxal + phosphate
S pyridoxal-5'-phosphate + H_2O <1, 2, 4, 5, 6, 7, 8, 9, 10, 13, 14> (Reversibility: ?) [11, 12, 13, 14]
P pyridoxal + phosphate
S pyridoxamine 5'-phosphate + H_2O <4, 12> (<4> low hydrolysis rate [6]; <4> the catalytic efficiency decreases in the following order: pyridoxal 5'-phosphate, 4-pyridoxic acid 5'-phosphate, pyridoxine 5'-phosphate and pyridoxamine 5'-phosphate [5]) (Reversibility: ?) [5, 6, 8]
P pyridoxamine + phosphate
S pyridoxine 5'-phosphate + H_2O <11> (Reversibility: ?) [8]
P pyridoxal + phosphate
S pyridoxine 5'-phosphate + H_2O <4, 12> (<12> second highest specificity constant after pyridoxal 5'-phosphate [8]; <4> the catalytic efficiency decreases in the following order: pyridoxal 5'-phosphate, 4-pyridoxic acid 5'-phosphate, pyridoxine 5'-phosphate and pyridoxamine 5'-phosphate [5]) (Reversibility: ?) [1, 2, 5, 6, 7, 8]
P pyridoxine + phosphate
S pyridoxine phosphate + H_2O <5> (Reversibility: ?) [10]
P pyridoxine + phosphate

S pyridoxine-5'-phosphate + H_2O <10> (Reversibility: ?) [12]
P pyridoxine + phosphate
S Additional information <4, 12> (<4> probably plays an important role in the regulation of vitamin B_6 metabolism [2]; <4> vitamin B_6 metabolism [5]; <12> vitamin B_6 metabolism, catabolism of pyridoxal 5'-phosphate [8]; <4> enzyme has phosphotransferase activity and transfers 20-25% of the phosphoryl group from either substrate to ethanol [1]; <4> hydrolyzes ten organic phosphates, but has maximum activity against pyridoxal phosphate [9]; <4> specifically dephosphorylates vitamin B_6-phosphates, not: phenylphosphate, nucleotide phosphates, such as ATP, ADP, AMP, cAMP, FMN, phosphoamino acids, such as phosphoserine, phosphothreonine, phosphotyrosine, phosphoglycolate [5]; <4> specificity and active site properties, enzyme also catalyzes the dephosphorylation of 4'-secondary amine derivatives of vitamin B_6 phosphate, enzyme has the greatest catalytic efficiency with substrates that contain a negatively charged group on the 4'-position of the pyridine ring, one or two positively charged groups at the active site of enzyme interacts with the substrate's phosphate ester and 4'-substituent, Arg and His residues are at or near the active site and may play roles in substrate binding and/or catalysis, very low activity with *p*-nitrophenylphosphate [6]; <12> very low activity with with *p*-nitrophenyl phosphate [8]) (Reversibility: ?) [1, 2, 5, 6, 8, 9]
P ?

Inhibitors

2,2'-dithiodipyridine <4> (<4> 0.005 mM, 50% inhibition [7]) [7]
2-ethyl-5-phenylisoxazolium-3'-sulfonate <4> (<4> 0.25 mM, 5 min at 22°C, 60% loss of activity, inactivates in a concentration- and time-dependent manner, which follows pseudo-first-order kinetics, pyridoxal 5'-phosphate, pyridoxine 5'-phosphate or phosphate protects [1]; <4> inhibition is potentiated by $MgCl_2$ [2]) [1, 2]
4,4'-dithiodipyridine <4> (<4> 0.005 mM, 50% inhibition [7]) [7]
4-pyridoxic acid 5'-phosphate <4> (<4> very effective inhibitor, 0.02 mM, 50% inhibition of pyridoxine 5'-phosphate hydrolysis [5]) [5]
5,5'-dithiobis(2-nitrobenzoate) <4> (<4> inhibition is potentiated by $MgCl_2$ [2]) [2, 6]
5,5'-dithiobis(2-nitrobenzoic acid) <4> (<4> 50 nM, 50% inhibition, incorporation of 1 mol per mol of subunit leads to complete inactivation, phosphate or dithiothreitol protects [7]) [7]
CN^- <4> [9]
Ca^{2+} <4> (<4> competitive inhibition versus Mg^{2+}, noncompetitive versus substrate [2]) [2]
Cd^{2+} <4> [2]
Cu^{2+} <4> [2]
cysteine <4> [9]
diethyldicarbonate <4> (<4> inactivates by reacting with a group with a p$K\alpha$ of 6.7, kinetics, pyridoxine 5'-phosphate protects, 100 mM neutral hydroxyla-

mine partially reactivates [6]; <4> inhibition is not potentiated by $MgCl_2$ [2]) [2, 6]
EDTA <4> (<4> 0.2 mM, complete inhibition in the absence of Mg^{2+}, 50% inhibition in the presence of 1 mM Mg^{2+} [5]) [5]
F^- <4> [9]
Fe^{2+} <4> [2]
fluoride <4> (<4> 2 mM, 50% inhibition [5]) [5]
iodoacetamide <4> (<4> weak [2]) [2]
iodoacetate <4> (<4> 0.075 mM, 50% inhibition, incorporation of 0.6 mol per mol of subunit leads to complete inactivation, phosphate protects, inhibition kinetics [7]; <4> enzyme is very sensitive to [5]; <4> inhibition is potentiated by $MgCl_2$ [2]) [2, 5, 7]
KH_2PO_4 <10> (<10> 56% inhibition at 5 mM [12]) [12]
levamisole <10> (<10> 26% inhibtion at 5 mM [12]) [12]
Mn^{2+} <4> (<4> inhibits above 0.05 mM, activates below 0.05 mM [2]) [2]
molybdate <4> (<4> competitive inhibition versus substrate, noncompetitive versus Mg^{2+} [2]; <4> very effective inhibitor, 0.0029 mM, 50% inhibition [5]) [2, 5]
N-(5'-phospho-4'-pyridoxyl)ethanolamine <4> (<4> 0.05 mM, 12% inhibition [6]) [6]
N-(5'-phospho-4'-pyridoxyl)glycine <4> (<4> 0.05 mM, 32% inhibition [6]) [6]
N-(5'-phospho-4'-pyridoxyl)phenylalanine <4> (<4> 0.05 mM, 51% inhibition [6]) [6]
N-ethylmaleimide <4, 10> (<4> 0.1 mM, 50% inhibition, incorporation of 0.6 mol per mol of subunit leads to complete inactivation, inhibition kinetics, phosphate protects [7]; <4> enzyme is very sensitive to [5]; <4> inactivated by low concentrations, low concentrations of a substrate, pyridoxine phosphate, or phosphate protect from inactivation, inhibition is not potentiated by $MgCl_2$ [2]; <10> 46% inhibiton at 5 mM [12]) [2, 5, 7, 12]
NaF <10> (<10> 24% inhibition at 5 mM [12]) [12]
Pb^{2+} <4> [2]
phenyl phosphate <4> (<4> very poor inhibitor of pyridoxine 5'-phosphate hydrolysis [5]) [5]
phenylglyoxal <4> (<4> inhibition is not potentiated by $MgCl_2$ [2]; <4> the incorporation of 1 mol per subunit inactivates, pyridoxal 5'-phosphate protects, kinetics [6]) [2, 6]
phosphate <4> (<4> competitive inhibitor [1,7]; <4> competitive inhibition versus substrate, noncompetitive versus Mg^{2+} [2]; <4> competitive inhibitor with respect to pyridoxine 5'-phosphate, product inhibition [5]) [1, 2, 5, 7]
pyridoxal <4> (<4> weak, 11 mM, 50% inhibition of hydrolysis of pyridoxal 5'-phosphate or pyridoxine 5'-phosphate [5]) [5]
sodium molybdate <10> (<10> 79% inhibition at 5 mM [12]) [12]
tetranitromethane <4> (<4> inactivates in a time-dependent manner, 10 mM, 70% inhibition in the absence of pyridoxal 5'-phosphate and 30% in the presence of 0.15 mM pyridoxal 5'-phosphate [6]) [6]

Zn^{2+} <4> (<4> inhibits at higher concentrations, activates somewhat at low concentrations [2]; <4> very potent inhibitor, 50% inhibition in the presence of $MgCl_2$ by 0.01 mM $ZnCl_2$ [5]) [2, 5]
disulfide reagent <4> (<4> reactivation by excess dithiothreitol, inactivation is due to formation of a mixed disulfide between the reagent and a free cysteinyl residue at or near the active site of enzyme [7]) [7]
iodacetate <10> (<10> 52% inhibition at 5 mM [12]) [12]
p-chloromercuribenzoate <4> (<4> 250 nM, 50% inhibition [7]; <4> enzyme is very sensitive to [5]) [5, 7]
p-nitrophenyl phosphate <4> (<4> poor, 4 mM, 50% inhibition of pyridoxine 5'-phosphate hydrolysis [5]) [5]
pyridoxal 5'-phosphate <4> (<4> very effective inhibitor, 0.03 mM, 50% inhibition of pyridoxine 5'-phosphate hydrolysis [5]) [5]
pyridoxamine 5'-phosphate <4> (<4> 0.5 mM, 50% inhibition of pyridoxine 5'-phosphate hydrolysis, less effective than pyridoxal 5'-phosphate or 4-pyridoxic acid 5'-phosphate [5]) [5]
pyridoxine 5'-phosphate <4> (<4> 0.05 mM, 45% inhibition [6]) [6]
thiol-specific reagent <4> (<4> a variety of thiol-specific reagents inactivate in a time- and concentration-dependent manner, pyridoxal phosphate or phosphate protects [7]) [7]
Additional information <4> (<4> not inhibited by iodoacetamide or cystamine [7]; <4> not inhibited by nucleotide phosphates, phosphoamino acids, levamisole, L-phenylalanine, L(+)-tartrate, 5 mM ATP, 5 mM phosphoglycolate, 0.5 mM α- or β-glycerophosphate, 0.5 mM 3-phosphoglycerate, 0.5 mM 2,3-bisphosphoglycerate, 5 mM pyridoxine, 5 mM pyridoxamine, 5 mM 4-pyridoxic acid, 5 mM 4-pyridine-carboxaldehyde, 5 mM isonicotinate, 5 mM 3-hydroxypyridine, 5 mM salicylaldehyde, 5 mM benzaldehyde [5]) [5, 7]

Activating compounds

Additional information <3> (<3> the presence of glucose in the growth medium increases the amount of pyridoxal phosphate phosphatase compared with pyridoxamine oxidase activity [3]) [3]

Metals, ions

$CaCl_2$ <4> (<4> enzyme has 17% as much activity with 1 mM $CaCl_2$ than with $MgCl_2$ [5]) [5]
Co^{2+} <4, 14> (<4> similar activation as by Mg^{2+} and Ni^{2+}, kinetics [2]; <14> highest activity at 5 mM, either Mg^{2+} or Co^{2+} required [11]) [2, 11]
Cu^{2+} <4> (<4> enzyme has 4% as much activity with 1 mM Cu^{2+} than with $MgCl_2$ [5]) [5]
Mg^{2+} <4, 10, 12, 14> (<4> requirement [1,6,7]; <4> required for maximum activity [9]; <4> $MgCl_2$, required for activity, best activator [5]; <12> requirement for a divalent cation, Mg^{2+} is most effective in catalyzing the dephosphorylation of pyridoxal 5'-phosphate [8]; <4> similar activation as by Co^{2+} and Ni^{2+}, kinetics, probably the physiological activator [2]; <10> either Mg^{2+} or Mn^{2+} required, highest activity at 2 mM [12]; <14> highest activity at 15 mM, either Mg^{2+} or Co^{2+} required [11]) [1, 2, 5, 6, 7, 8, 9, 11, 12]

Mn^{2+} <4, 10> (<4> enzyme has 2% as much activity with 1 mM Mn^{2+} than with $MgCl_2$ [5]; <4> less activatory than Mg^{2+}, Co^{2+} or Ni^{2+}, inhibits above 0.05 mM, kinetics [2]; <10> either Mg^{2+} or Mn^{2+} required, highest activity at 2 mM [12]) [2, 5, 12]
Ni^{2+} <4> (<4> similar activation as by Mg^{2+} and Co^{2+}, kinetics [2]) [2]
Zn^{2+} <4> (<4> activates somewhat at low concentrations, inhibits at higher concentrations [2]) [2]
Additional information <4> (<4> essential requirement for divalent cations [2]; <4> not activated by 100 mM NaCl, KCl, NaBr, NaI, $NaNO_3$ or sodium acetate [5]) [2, 5]

Turnover number (min^{-1})

0.45 <12> (pyridoxamine 5'-phosphate, <12> pH 7.4, 37°C, recombinant enzyme [8]) [8]
1.25 <12> (pyridoxine 5'-phosphate, <12> pH 7.4, 37°C, recombinant enzyme [8]) [8]
1.52 <12> (pyridoxal 5'-phosphate, <12> pH 7.4, 37°C, recombinant enzyme [8]) [8]
19 <14> (Pyridoxal-5'-phosphate, <14> 5 mM $CoCl_2$, 1 mM dithiothreitol, 50 mM Tris/maleate pH 5.0, 37°C [11]) [11]

Specific activity (U/mg)

0.575 <4> (<4> pH 7.4, 37°C [5]) [5]
1.4 <4> (<4> pH 7.4, 37°C [6]; <4> pH 7.4, 37°C, hydrolysis of pyridoxal 5'-phosphate, recombinant enzyme [8]) [6, 8]
5.3 <4> (<4> pH 7.4, 37°C [2]) [2]
7 <14> (<14> 5 mM $CoCl_2$, 1 mM dithiothreitol, 50 mM Tris/maleate pH 5.0, 37°C [11]) [11]
20 <14> (<14> 5 mM $CoCl_2$, 1 mM dithiothreitol, 50 mM Tris/maleate pH 5.0, 37°C [11]) [11]
42 <10> (<10> 1 mM $MnCl_2$, 50 mM Tris-HCl pH 7.0 [12]) [12]
105 <10> (<10> 1 mM $MnCl_2$, 50 mM Tris-HCl pH 7.0 [12]) [12]
Additional information <3, 4> [3, 4, 5]

K_m-Value (mM)

0.00112 <4> (4-pyridoxic acid 5'-phosphate, <4> pH 7.4, 37°C [6]) [6]
0.00129 <4> (pyridoxal 5'-phosphate, <4> pH 7.4, 37°C [6]) [6]
0.00147 <4> (pyridoxal 5'-phosphate, <4> pH 7.4, 37°C [5]) [5]
0.00154 <4> (pyridoxal 5'-phosphate, <4> pH 7.4, 37°C, 0.188 mM Mg^{2+} [2]) [2]
0.00166 <4> (pyridoxal 5'-phosphate, <4> pH 7.4, 37°C, 0.06 mM Co^{2+} [2]) [2]
0.0025 <4> (pyridoxal 5'-phosphate, <4> pH 7.4, 37°C, recombinant enzyme [8]) [8]
0.00255 <4> (4-pyridoxic acid 5'-phosphate, <4> pH 7.4, 37°C [5]) [5]
0.0043 <4> (pyridoxine 5'-phosphate, <4> pH 7.4, 37°C, 0.39 mM Mg^{2+} [2]) [2]
0.00519 <4> (pyridoxine 5'-phosphate, <4> pH 7.4, 37°C [5]) [5]

0.00812 <4> (pyridoxine 5'-phosphate, <4> pH 7.4, 37°C, 0.13 mM Co^{2+} [2]) [2]
0.00824 <4> (N-(5'-phospho-4'-pyridoxyl)phenylalanine, <4> pH 7.4, 37°C [6]) [6]
0.0106 <4> (pyridoxine 5'-phosphate, <4> pH 7.4, 37°C [6]) [6]
0.0143 <4> (N-(5'-phospho-4'-pyridoxyl)glycine, <4> pH 7.4, 37°C [6]) [6]
0.034 <4> (pyridoxamine 5'-phosphate, <4> pH 7.4, 37°C [5]) [5]
0.0434 <4> (pyridoxine 5'-phosphate, <4> pH 7.4, 37°C, recombinant enzyme [8]) [8]
0.0547 <4> (pyridoxamine 5'-phosphate, <4> pH 7.4, 37°C [6]) [6]
0.0635 <4> (N-(5'-phospho-4'-pyridoxyl)benzylamine, <4> pH 7.4, 37°C [6]) [6]
0.0786 <4> (N-(5'-phospho-4'-pyridoxyl)ethanolamine, <4> pH 7.4, 37°C [6]) [6]
0.0806 <4> (pyridoxamine 5'-phosphate, <4> pH 7.4, 37°C, recombinant enzyme [8]) [8]
0.33 <14> (pyridoxal-5'-phosphate, <14> 5 mM $CoCl_2$, 1 mM dithiothreitol, 50 mM Tris/maleate pH 5.0, 37°C [11]) [11]
0.385 <10> (pyridoxal-5'-phosphate, <10> 1 mM $MnCl_2$, 50 mM Tris-HCl pH 7.0 [12]) [12]
0.385 <10> (pyridoxine-5'-phosphate, <10> 1 mM $MnCl_2$, 50 mM Tris-HCl pH 7.0 [12]) [12]
Additional information <4> (<4> kinetic data, effect of pH on K_m for 4-pyridoxic acid 5'-phosphate, pyridoxal 5'-phosphate, pyridoxine 5'-phosphate and pyridoxamine 5'-phosphate [6]; <4> kinetic mechanism, kinetic properties [2]) [2, 6]

K_i-Value (mM)

0.00348 <4> (molybdate) [2]
0.00638 <4> (Ca^{2+}) [2]
0.22 <4> (phosphate) [2]
0.8 <4> (phosphate, <4> pH 7.4, 37°C [5]) [5]
Additional information <4> [2]

pH-Optimum

5 <14> [11]
6-6.5 <4> [5]
6-8 <4> [1, 6, 7]
7.4 <4> (<4> assay at [2,8]) [2, 8]
7.5 <10> [12]
9 <4> [9]
Additional information <4> (<4> alkaline and acid pyridoxal phosphate phosphatase, not identical with alkaline and acid phosphatase [4]) [4]

pH-Range

5.5-9.5 <10> [12]

Temperature optimum (°C)

22 <4> (<4> assay at, inhibition studies [6]) [6]
37 <4, 12> (<4,12> assay at [2,5,8]; <4> assay at, kinetic analysis [6]) [2, 5, 6, 8]

4 Enzyme Structure

Molecular weight

29000 <10> (<10> gel filtration [12]) [12]
32000 <4> (<4> SDS-PAGE [13]) [13]
33000 <1, 2, 4, 5, 6, 7, 8, 9, 13> (<1,2,4,5,6,7,8,9,13> SDS-PAGE [14]) [14]
60000 <12> (<12> gel filtration, recombinant enzyme [8]) [8]
64000 <4> (<4> gel filtration [5]) [1, 5, 6, 7]
90000 <14> (<14> gel filtration [11]) [11]

Subunits

? <11> (<11> x * 31512, sequence calculation [8]) [8]
dimer <4, 12, 14> (<4> 2 * 33000, SDS-PAGE [5]; <4> 2 * 32000 [1,2,7]; <12> 2 * 31698, sequence calculation, 2 * 32000, recombinant enzyme, SDS-PAGE [8]; <14> 2 * 45000, gel filtration compared to deduced molecular weight from sequence [11]) [1, 2, 5, 6, 7, 8, 11]
monomer <10> (<10> 1 * 29000, SDS-PAGE [12]) [12]

5 Isolation/Preparation/Mutation/Application

Source/tissue

Hep-G2 cell <4> [14]
J774A.1 cell <2> [14]
PC-12 cell <2> [14]
SiHa cell <4> [14]
brain <1, 4, 5, 6, 7, 8, 9, 11, 12, 13> (<5> activity measurement over a 6-week period in rats fed pyridoxine-sufficient and pyridoxine-deficient diets [10]; <12> highly abundant in, most highly represented in all the regions of central nerve system except the spinal cord [8]) [8, 9, 10, 14]
cell culture <4, 10, 14> [11, 12, 14]
central nervous system <4> (<4> high expression [13]) [13]
erythrocyte <4> (<4> outdated packed erythrocytes [1,7]) [1, 2, 5, 6, 7]
heart <5> [14]
kidney <4> (<4> high expression [13]) [13]
leukocyte <4> (<4> polymorphonuclear [4]) [4]
liver <4, 5, 12> (<4> high expression [13]; <5> activity measurement over a 6-week period in rats fed pyridoxine-sufficient and pyridoxine-deficient diets [10]; <12> major expression site additional to brain and testis [8]) [8, 10, 13, 14]
lung <4, 5> [14]

muscle <4, 5> [14]
stomach <4, 5> [14]
testis <4, 12> (<4> high expression [13]; <12> major expression site additional to brain and liver [8]) [8, 13]
Additional information <12> (<12> mRNA is differentially expressed in a tissue-specific manner, tissue distribution [8]) [8]

Localization
cytoplasm <10> [12]
soluble <4> [5]
Additional information <4> (<4> subcellular localization in polymorphonuclear leukocytes [4]) [4]

Purification
<4> [1, 2, 7]
<4> (168000fold) [6]
<4> (51000fold) [5]
<4> (70fold) [9]
<4> (9.6fold, recombinant enzyme) [8]
<4> (Ni-affinity chromatography) [13, 14]
<10> (5 chromatography steps to homogeneity) [12]
<14> (one chromatography step to homogeneity) [11]

Cloning
<4> (His-tag fusion, expression in Escherichia coli) [13]
<4> (cDNA, from brain, expression in Escherichia coli M15/pRER4, sequencing, ORF is located on chromosome 22q12.3, genomic organization) [8]
<4> (cloned into plasmid pET15b, expression in Escherichi coli BL21 (DE3) as His-tag fusion protein) [14]
<11> (full-length cDNA, from brain, sequencing, ORF is located on chromosome 15.E1, genomic organization) [8]
<14> (expression in Escherichia coli HMS174(DE3)) [11]

Engineering
D12N <14> (<14> complete loss of activity, the corresponding Asp residue is part of the active site in other HAD superfamily members [11]) [11]

6 Stability

pH-Stability
5-9 <4> (<4> 60 min, no loss of activity [6]) [6]

Organic solvent stability
Ethanol <4> (<4> 10%, no effect on enzyme activity [2]) [2]

General stability information
<4>, purified enzyme is unstable at low protein concentration, 0.002% Triton X-100 stabilizes, enzyme is unstable to freezing in the absence of glycerol [5]

Storage stability

<4>, 4°C, storage at concentrations of 0.3 mg/ml or greater, 30 mM phosphate, at least 6 months, stable [6]

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Phosphoethanolamine/phosphocholine phosphatase 3.1.3.75

1 Nomenclature

EC number
3.1.3.75

Systematic name
phosphoethanolamine phosphohydrolase

Recommended name
phosphoethanolamine/phosphocholine phosphatase

Synonyms
3X11A <1> [1]
PChP <2> [6]
PHOSPHO1 <1, 4> [1, 5]
phosphorylcholine phosphatase <2> [6]
Additional information <1, 3, 4> (<1,3> PHOSPHO1 is a member of the haloacid dehalogenase superfamily of hydrolases [3]; <4> PHOSPHO1 is a member of the haloacid dehalogenase superfamily of magnesium-dependent hydrolases [4]; <1> PHOSPHO1 is a member of the haloacid dehalogenase superfamily of Mg^{2+}-dependent hydrolases [1]; <4> PHOSPHO1 belongs to the haloacid dehalogenase superfamily [5]) [1, 3, 4, 5]

CAS registry number
52227-92-6

2 Source Organism

<1> *Homo sapiens* (no sequence specified) [1, 3]
<2> *Pseudomonas aeruginosa* (no sequence specified) [6]
<3> *Gallus gallus* (UNIPROT accession number: O73884) [2, 3]
<4> *Homo sapiens* (UNIPROT accession number: Q8TCT1) [4, 5]

3 Reaction and Specificity

Catalyzed reaction
O-phosphoethanolamine + H_2O = ethanolamine + phosphate (<1> requires active site Mg^{2+} but also works, to a lesser extend, with Co^{2+} and Mn^{2+}, the enzyme is highly specific for phosphoethanolamine and phosphocholine [1]; <4> residues D32 and D203 of catalytic motifs are essential for catalytic ac-

tivity, residues D43 and D123 are important for substrate binding and specificity [5])
phosphocholine + H_2O = choline + phosphate (<1> requires active site Mg^{2+} but also works, to a lesser extend, with Co^{2+} and Mn^{2+}, the enzyme is highly specific for phosphoethanolamine and phosphocholine [1])

Reaction type
hydrolysis of phosphoric ester

Natural substrates and products
- **S** O-phosphocholine + H_2O <2, 4> (Reversibility: ?) [5, 6]
- **P** choline + phosphate
- **S** O-phosphoethanolamine + H_2O <1, 2, 4> (<1> likely a natural substrate, phosphoethanolamine metabolism, PHOSPHO1 is upregulated in mineralizing cells, enzyme is implicated in the generation of phosphate for matrix mineralization [1]) (Reversibility: ?) [1, 5, 6]
- **P** ethanolamine + phosphate
- **S** phosphocholine + H_2O <1> (<1> likely a natural substrate, phosphocholine metabolism, PHOSPHO1 is upregulated in mineralizing cells, enzyme is implicated in the generation of phosphate for matrix mineralization [1]) (Reversibility: ?) [1]
- **P** choline + phosphate
- **S** Additional information <1, 3, 4> (<3> 3X11A participates in a biochemical pathway that is particularly active in differentiating chondrocytes, it may be involved in the generation of inorganic phosphate during matrix mineralization [2]; <4> PHOSPHO1 expression is upregulated in mineralizing cells and is implicated in the generation of inorganic phosphate for matrix mineralization [4]; <1> PHOSPHO1 may be involved in the mineralization process [3]; <3> PHOSPHO1 may be involved in the mineralization process, plays a role in bone and cartilage matrix mineralization [3]) (Reversibility: ?) [2, 3, 4]
- **P** ?

Substrates and products
- **S** 4-nitrophenyl phosphate + H_2O <2> (Reversibility: ?) [6]
- **P** 4-nitrophenol + phosphate
- **S** O-phosphocholine + H_2O <2, 4> (Reversibility: ?) [5, 6]
- **P** choline + phosphate
- **S** O-phosphoethanolamine + H_2O <1, 2, 4> (<1> likely a natural substrate, phosphoethanolamine metabolism, PHOSPHO1 is upregulated in mineralizing cells, enzyme is implicated in the generation of phosphate for matrix mineralization [1]; <1> PHOSPHO1 exhibits high specific activities toward phosphoethanolamine and phosphocholine, phosphoethanolamine is hydrolyzed 1.5times faster than phosphocholine [1]) (Reversibility: ?) [1, 5, 6]
- **P** ethanolamine + phosphate
- **S** β-glycerol phosphate + H_2O <1> (<1> poor substrate [1]) (Reversibility: ?) [1]

P glycerol + phosphate
S *p*-nitrophenyl phosphate + H_2O <1> (<1> poor substrate [1]) (Reversibility: ?) [1]
P nitrophenol + phosphate
S phospho-L-serine + H_2O <2> (Reversibility: ?) [6]
P L-serine + phosphate
S phosphocholine + H_2O <1> (<1> likely a natural substrate, phosphocholine metabolism, PHOSPHO1 is upregulated in mineralizing cells, enzyme is implicated in the generation of phosphate for matrix mineralization [1]; <1> PHOSPHO1 exhibits high specific activities toward phosphoethanolamine and phosphocholine, phosphocholine is hydrolyzed 1.5times slower than phosphoethanolamine [1]) (Reversibility: ?) [1]
P choline + phosphate
S pyridoxal 5'-phosphate + H_2O <1> (<1> poor substrate [1]) (Reversibility: ?) [1]
P pyridoxal + phosphate
S ribose 5-phosphate + H_2O <1> (<1> poor substrate [1]) (Reversibility: ?) [1]
P ribose + phosphate
S Additional information <1, 3, 4> (<3> 3X11A participates in a biochemical pathway that is particularly active in differentiating chondrocytes, it may be involved in the generation of inorganic phosphate during matrix mineralization [2]; <4> PHOSPHO1 expression is upregulated in mineralizing cells and is implicated in in the generation of inorganic phosphate for matrix mineralization [4]; <1> PHOSPHO1 may be involved in the mineralization process [3]; <3> PHOSPHO1 may be involved in the mineralization process, plays a role in bone and cartilage matrix mineralization [3]; <1> not: diphosphate, phospho-L-serine, glycone phosphate, fructose 6-phosphate, phospho-L-tyrosine, ATP [1]; <4> three-dimensional model of PHOSPHO1, Asp-43 and Asp-123 may contribute to substrate specificity [4]; <4> high substrate specificity of PHOSPHO1, residual activity with β-glycerol phosphate, 4-nitrophenyl phosphate, and ribose 5-phosphate, no activity with phospho-L-serine, diphosphate, fructose 6-phosphate, phospho-L-tyrosine, and ATP, overview [5]) (Reversibility: ?) [1, 2, 3, 4, 5]
P ?

Inhibitors

betaine <2> (<2> 32% inhibition of wild-type enzyme in vivo at 5 mM, at lower concentrations betaine induces the enzyme expression in vivo [6]) [6]
Choline <2> (<2> 12% inhibition of wild-type enzyme in vivo at 5 mM, at lower concentrations choline induces the enzyme expression in vivo [6]) [6]
Additional information <2> (<2> no inhibition by high concentrations of phosphorylcholine [6]) [6]

Activating compounds

Additional information <2> (<2> the enzyme is induced by growth on choline, βine, dimethylglycine, or carnitine, choline and betaine are inhibitory at high concentrations [6]) [6]

Metals, ions

Co^{2+} <1> (<1> stimulates to a lesser extend than Mg^{2+}, higher activity with phosphocholine than with phosphoethanolamine in the presence of Co^{2+} and Mn^{2+} most probably due to an allosteric effect caused by a difference in the metal-binding properties of each enzyme-substrate complex [1]) [1]
Mg^{2+} <1, 2, 4> (<1> high specific Mg^{2+}-dependence, optimum concentration: 2 mM $MgCl_2$ [1]; <4> Mg^{2+}-dependent, binding site of the catalytic Mg^{2+} [4]) [1, 4, 5, 6]
Mn^{2+} <1> (<1> stimulates to a lesser extend than Mg^{2+}, higher activity with phosphocholine than with phosphoethanolamine in the presence of Co^{2+} and Mn^{2+} most probably due to an allosteric effect caused by a difference in the metal-binding properties of each enzyme-substrate complex [1]) [1]
Ni^{2+} <1> (<1> stimulates to a lesser extend than Mg^{2+} [1]) [1]
Additional information <1> (<1> not activated by Ca^{2+} or Zn^{2+} [1]) [1]

Turnover number (min^{-1})

1.98 <1> (phosphocholine, <1> pH 6.7, 37°C, 2 mM Mg^{2+} [1]) [1]
2.27 <1> (O-phosphoethanolamine, <1> pH 6.7, 37°C, 2 mM Mg^{2+} [1]) [1]

Specific activity (U/mg)

0.0176 <1> (<1> pH 7.2, 37°C, hydrolysis of pyridoxal 5'-phosphate [1]) [1]
0.0396 <1> (<1> pH 7.2, 37°C, hydrolysis of β-glycerol phosphate [1]) [1]
0.0645 <1> (<1> pH 7.2, 37°C, hydrolysis of *p*-nitrophenyl phosphate [1]) [1]
0.0748 <1> (<1> pH 7.2, 37°C, hydrolysis of ribose 5-phosphate [1]) [1]
0.092 <4> (<4> purified recombinant mutant D43N enzyme fragment M19-C267, substrate O-phosphoethanolamine [5]) [5]
0.28 <4> (<4> purified recombinant mutant D123N enzyme fragment M19-C267, substrate O-phosphoethanolamine [5]) [5]
2.98 <1> (<1> pH 7.2, 37°C, hydrolysis of phosphocholine [1]) [1]
4.6 <1> (<1> pH 7.2, 37°C, hydrolysis of phosphoethanolamine [1]) [1]
4.96 <4> (<4> purified recombinant wild-type enzyme fragment M19-C267, substrate O-phosphoethanolamine [5]) [5]
Additional information <2> [6]

K_m-Value (mM)

0.003 <1> (O-phosphoethanolamine, <1> pH 6.7, 37°C, 2 mM Mg^{2+} [1]) [1]
0.0114 <1> (phosphocholine, <1> pH 6.7, 37°C, 2 mM Mg^{2+} [1]) [1]

pH-Optimum

5 <2> (<2> assay at, substrates phospho-L-serine and 4-nitrophenyl phosphate [6]) [6]
6.7 <1, 4> (<1> around [1]; <4> assay at [5]) [1, 5]
7.4 <2> (<2> assay at, substrates O-phosphocholine and phospho-L-serine [6]) [6]

pH-Range

6-7.5 <1> (<1> above 70% of maximum activity between pH 6 and 7.2, activity with phosphocholine declines significantly above pH 7.2, activity with phosphoethanolamine declines above pH 7.5 [1]) [1]

Temperature optimum (°C)

37 <1, 4> (<1,4> assay at [1,5]) [1, 5]

4 Enzyme Structure

Subunits

? <1, 3, 4> (<4> x * 29700, sequence calculation [4]; <3> x * 30442, sequence calculation [2]; <1> x * 32000, recombinant PHOSPHO1, SDS-PAGE, Western blot analysis [1]; <3> x * 33000, recombinant PHOSPHO1, SDS-PAGE, x * 28600, x * 30400, alternative transcripts for PHOSPHO1 in growth cartilage, Western blot analysis [3]) [1, 2, 3, 4]

Additional information <4> (<4> structure motif analysis using the amino acid sequence structural modelling and ligand docking [5]) [5]

5 Isolation/Preparation/Mutation/Application

Source/tissue

B-cell <4> (<4> expression of PHOSPHO1 [4]) [4]

SAOS-2 cell <1, 4> (<1> osteoblast-like cells [1]; <1> mineralizing osteoblast-like cells, PHOSPHO1 gene expression profile [3]; <4> osteoblast-like cells, expression of PHOSPHO1 [4]) [1, 3, 4]

bone <3> (<3> from 3 weeks old male chickens, from tibia, diaphyseal cortical and trabecular bone, expression pattern, PHOSPHO1 is specifically expressed at sites of mineralization in bone and cartilage [3]) [3]

brain <4> (<4> expression of PHOSPHO1 [4]) [4]

calvarium <3> (<3> from 17 days old embryonic chickens, PHOSPHO1 gene expression [3]) [3]

cartilage <3> (<3> from 3 weeks old male chickens, from tibia, growth plate cartilage, expression pattern, PHOSPHO1 is specifically expressed at sites of mineralization in bone and cartilage [3]) [3]

cervix <4> (<4> expression of PHOSPHO1 [4]) [4]

chondrocyte <3> (<3> from growth plates of proximal tibiotarsi, 3X11A expression at high levels in differentiating growth plate chondrocytes, 3X11A expression is upregulated 5fold during chondrocyte terminal differentiation and its expression is approximately 100fold higher in hypertrophic chondrocytes than in non-chondrogenic tissues [2]) [2]

colon <4> (<4> expression of PHOSPHO1 [4]) [4]

liver <4> (<4> fetal, expression of PHOSPHO1 [4]) [4]

ovary <4> (<4> expression of PHOSPHO1 [4]) [4]

placenta <4> (<4> expression of PHOSPHO1 [4]) [4]

skin <4> (<4> expression of PHOSPHO1 [4]) [4]
spleen <4> (<4> expression of PHOSPHO1 [4]) [4]
sympathetic trunk <4> (<4> expression of PHOSPHO1 [4]) [4]
Additional information <1, 3> (<3> expression pattern of 3X11A [2]; <1> no PHOSPHO1 gene expression in non-mineralizing MG-63 osteoblast-like cells [3]; <3> not in heart, liver, lung, abdominal fat, kidney, skeletal and heart muscle [3]) [2, 3]

Localization

cytoplasm <4> [4]
periplasm <2> [6]
soluble <4> [4]

Purification

<1> (recombinant PHOSPHO1) [1]
<2> (recombinant enzyme from Escherichia coli by chitin affinity chromatography) [6]
<3> (recombinant PHOSPHO1) [3]
<4> (recombinant His-tagged wild-type and mutant fragments M19-C267 from Escherichia coli by nickel affinity chromatography to homogeneity) [5]

Cloning

<1> (expression in Escherichia coli TOP10) [1]
<2> (gene PA5292, expression in Escherichia coli, expression in and complementation of a Pseudomonas aeruginosa mutant strain JUF8-00) [6]
<3> (from growth plate chondrocytes, expression in Escherichia coli TOP10) [3]
<3> (sequencing) [2]
<4> (expression of His-tagged wild-type and mutant fragment comprising residues M19-C267 in Escherichia coli) [5]

Engineering

D123N <4> (<4> site-directed mutagenesis, the mutant enzyme fragment shows reduced activity compared to the wild-type enzyme fragment [5]) [5]
D203S <4> (<4> site-directed mutagenesis, inactive mutant enzyme fragment [5]) [5]
D32N <4> (<4> site-directed mutagenesis, inactive mutant enzyme fragment [5]) [5]
D43N <4> (<4> site-directed mutagenesis, the mutant enzyme fragment shows reduced activity compared to the wild-type enzyme fragment [5]) [5]
D43N/D123N <4> (<4> site-directed mutagenesis, inactive mutant enzyme fragment [5]) [5]
Additional information <2> (<2> Tn5-751 transposon mutagenesis is used to construct an enzyme-deficient, inactive mutant JUF8-00 of Pseudomonas aeruginosa, the mutant can be complemented by expression of the wild-type enzyme [6]) [6]

Application

medicine <1> (<1> may have implications for the diagnosis of hypophosphatasia and treatment of bone mineralization abnormalities such as osteomalacia and pathological soft-tissue ossification, a process clinically significant in atherosclerosis and heart failure [1]) [1]

References

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Lipid-phosphate phosphatase 3.1.3.76

1 Nomenclature

EC number
3.1.3.76

Systematic name
(9S,10S)-10-hydroxy-9-(phosphonooxy)octadecanoate phosphohydrolase

Recommended name
lipid-phosphate phosphatase

Synonyms
$EPXH_2$ <2> [2, 3, 5]
Nterm-phos <2> [5]
lipid phosphatase <2> [7]
lipid phosphate phosphatase <2> [5]
Additional information <1, 2, 3> (<2,3> c.f. EC 3.3.2.10 [1, 3, 4, 5, 7]; <2> EC 3.1.3.76 belongs to the haloacid dehalogenase superfamily of enzymes, c.f. EC 3.3.2.10 [2,6]; <1,3> EC 3.1.3.76 belongs to the haloacid dehalogenase superfamily of enzymes, see also EC 3.3.2.10 [6]) [1, 2, 3, 4, 5, 6, 7]

CAS registry number
9025-77-8
9048-63-9

2 Source Organism

<1> *Mus musculus* (no sequence specified) [6]
<2> *Homo sapiens* (no sequence specified) [1, 2, 3, 4, 5, 6, 7]
<3> *Rattus norvegicus* (no sequence specified) [4, 6]

3 Reaction and Specificity

Catalyzed reaction
(9S,10S)-10-hydroxy-9-(phosphonooxy)octadecanoate + H_2O = (9S,10S)-9,10-dihydroxyoctadecanoate + phosphate (<1,2,3> the phosphatase activity of the enzyme is located at the N-terminal part, the C-terminal part harbors the epoxide hydrolase activity of EC 3.3.2.10, both catalytic sites act independently [2,3,4,6,7]; <2> the phosphatase activity of the enzyme is located at the N-terminal part, the C-terminal part harbors the epoxide hydrolase activity

of EC 3.3.2.10, both catalytic sites act independently, reaction mechanism [5]; <2> the phosphatase activity of the enzyme is located at the N-terminal part, the C-terminal part harbors the epoxide hydrolase activity of EC 3.3.2.10, both catalytic sites act independently, reaction mechanism, structure-mechanism relationship [1])

Natural substrates and products

- **S** (9E)-12-phosphonooxyoctadec-9-enoic acid + H_2O <2> (Reversibility: ?) [2]
- **P** (9E)-12-hydroxyoctadec-9-enoic acid + phosphate
- **S** (9Z)-12-phosphonooxyoctadec-9-enoic acid + H_2O <2> (Reversibility: ?) [2]
- **P** (9Z)-12-hydroxyoctadec-9-enoic acid + phosphate
- **S** 12-phosphonooxyoctadecanoic acid + H_2O <2> (Reversibility: ?) [2]
- **P** 12-hydroxyoctadecanoic acid + phosphate
- **S** erythro-9,10-phosphonooxy-hydroxy-octadecanoic acid + H_2O <2> (Reversibility: ?) [2]
- **P** ?
- **S** threo-9,10-phosphonooxy-hydroxy-octadecanoic acid + H_2O <2> (Reversibility: ?) [2]
- **P** ?

Substrates and products

- **S** (9E)-12-phosphonooxyoctadec-9-enoic acid + H_2O <2> (Reversibility: ?) [2]
- **P** (9E)-12-hydroxyoctadec-9-enoic acid + phosphate
- **S** (9E)-12-sulfonooxyoctadec-9-enoic acid + H_2O <2> (Reversibility: ?) [5]
- **P** (9E)-12-hydroxyoctadec-9-enoic acid + sulfate
- **S** (9Z)-12-phosphonooxyoctadec-9-enoic acid + H_2O <2> (Reversibility: ?) [2]
- **P** (9Z)-12-hydroxyoctadec-9-enoic acid + phosphate
- **S** (9Z)-12-sulfonooxyoctadec-9-enoic acid + H_2O <2> (Reversibility: ?) [5]
- **P** (9Z)-12-hydroxyoctadec-9-enoic acid + sulfate
- **S** 10-sulfonooxy-octadecanoic acid + H_2O <2> (Reversibility: ?) [5]
- **P** 10-hydroxyoctadecanoic acid + phosphate
- **S** 12-phosphonooxyoctadecanoic acid + H_2O <2> (Reversibility: ?) [2]
- **P** 12-hydroxyoctadecanoic acid + phosphate
- **S** 2'-[2-benzothiazolyl]-6'-hydroxybenzothiazole phosphate + H_2O <2> (<2> i.e. attophos [5]) (Reversibility: ?) [5]
- **P** 2'-[2-benzothiazolyl]-6'-hydroxybenzothiazole + phosphate
- **S** 4-methylumbelliferyl phosphate + H_2O <2, 3> (Reversibility: ?) [5, 6]
- **P** 4-methylumbelliferone + phosphate
- **S** 4-nitrophenyl phosphate + H_2O <1, 2, 3> (Reversibility: ?) [2, 3, 5, 6]
- **P** 4-nitrophenol + phosphate
- **S** erythro-10-phosphonooxy-9-hydroxy-octadecanoic acid + H_2O <2> (Reversibility: ?) [2]
- **P** erythro-9,10-dihydroxyoctadecanoate + phosphate

S erythro-9,10-phosphonooxy-hydroxy-octadecanoic acid + H_2O <2> (Reversibility: ?) [2]
P ?
S erythro-9-hydroxy-10-(phosphonooxy)octadecanoate + H_2O <2> (Reversibility: ?) [7]
P erythro-9,10-dihydroxy octadecanoic acid + phosphate (<2> i.e. dihydroxy elaidic acid [7])
S farnesyl diphosphate + H_2O <2> (Reversibility: ?) [4, 5]
P farnesol + phosphate
S farnesyl phosphate + H_2O <2, 3> (Reversibility: ?) [4, 5]
P farnesol + phosphate
S geranyl diphosphate + H_2O <2> (Reversibility: ?) [4, 5]
P geraniol + phosphate
S geranyl phosphate + H_2O <2, 3> (Reversibility: ?) [4]
P geraniol + phosphate
S geranylgeranyl diphosphate + H_2O <2> (Reversibility: ?) [4, 5]
P geranylgeraniol + phosphate
S geranylgeranyl phosphate + H_2O <2, 3> (Reversibility: ?) [4]
P geranylgeraniol + phosphate
S isopentenyl diphosphate + H_2O <2> (Reversibility: ?) [4]
P isopentenol + phosphate
S isopentenyl phosphate + H_2O <2, 3> (Reversibility: ?) [4]
P isopentenol + phosphate
S threo-10-phosphonooxy-9-hydroxy-octadecanoic acid + H_2O <2> (Reversibility: ?) [2]
P threo-9,10-dihydroxyoctadecanoate + phosphate
S threo-9,10-phosphonooxy-hydroxy-octadecanoic acid + H_2O <2> (Reversibility: ?) [2]
P ?
S threo-9,10-phosphonooxyoctadecanoic acid + H_2O <2> (Reversibility: ?) [5]
P ?
S threo-9-hydroxy-10-(phosphonooxy)octadecanoate + H_2O <2> (<2> preferred substrate [7]) (Reversibility: ?) [7]
P threo-9,10-dihydroxy octadecanoic acid + phosphate (<2> i.e. dihydroxy stearic acid [7])
S Additional information <2> (<2> no activity with 4-nitrophenyl sulfate of the isolated recombinant N-terminal domain [6]; <2> the enzyme acts regioselectively, substrate specificity [7]) (Reversibility: ?) [6, 7]
P ?

Inhibitors

(9E)-12-sulfonooxy-9-octadecenoic acid <2> (<2> IC50 is 0.0097 mM [5]) [5]
(9Z)-12-sulfonooxy-9-octadecenoic acid <2> (<2> IC50 is above 0.1 mM [5]) [5]
10-sulfonooxyoctadecanoic acid <2> (<2> IC50 is 0.0059 mM [5]) [5]
4-nitrophenyl sulfate <2> (<2> IC50 is above 0.1 mM [5]) [5]

4-phenylchalcone oxide <2> (<2> 11% inhibition at 0.1 mM [2]) [2]
4-fluorochalcone oxide <2> (<2> 8% inhibition at 0.1 mM [2]) [2]
9,10-hydroxysulfonooxyoctadecanoic acid <2> (<2> IC50 is 0.0175 mM [5]) [5]
9-octadecanyl sulfate <2> (<2> IC50 is 0.0047 mM [5]) [5]
Cd^{2+} <2> [2]
Cu^{2+} <2> [2]
D-galactose 6-sulfate <2> (<2> IC50 is above 0.1 mM [5]) [5]
EDTA <1, 2, 3> (<1,2,3> complete inhibition at 20 mM [6]) [6]
estrone 3-sulfate <2> (<2> IC50 is above 0.1 mM [5]) [5]
Hg^{2+} <2> [2]
L-ascorbic acid 2-sulfate <2> (<2> IC50 is above 0.1 mM [5]) [5]
N-acetyl-D-galactosamine 4-sulfate <2> (<2> IC50 is above 0.1 mM [5]) [5]
Orthovanadate <2> (<2> 65% inhibition of the phosphatase activity at 0.1 mM [4]) [4]
sodium dodecyl sulfate <2> (<2> IC50 is 0.0052 mM [5]) [5]
sodium fluoride <2> (<2> 10% inhibition at 1.0 mM [2]) [2]
sodium molybdate <2> (<2> 8% inhibition at 1.0 mM [2]) [2]
taurocholic acid <2> (<2> IC50 is above 0.1 mM [5]) [5]
Zn^{2+} <2> [2]
α-hydroxyfarnesyl phosphonic acid <2> (<2> IC50 is 0.073 mM [5]) [5]
α-sulfostearic acid <2> (<2> IC50 is 0.0096 mM [5]) [5]
dibenzyl phosphonate <2> (<2> IC50 is above 0.1 mM [5]) [5]
diethyl 2,2,2-trifluoro-1-hydroxyethyl phosphonate <2> (<2> IC50 is above 0.1 mM [5]) [5]
diethyl 4-methylbenzyl phosphonate <2> (<2> IC50 is above 0.1 mM [5]) [5]
diethyl allyl phosphonate <2> (<2> IC50 is above 0.1 mM [5]) [5]
diethyl benzoylphosphonate <2> (<2> IC50 is above 0.1 mM [5]) [5]
diethyl cyclopropyl methylphosphonate <2> (<2> IC50 is above 0.1 mM [5]) [5]
diethyl ethylthiomethyl phosphonate <2> (<2> IC50 is above 0.1 mM [5]) [5]
diethyl trans-cinnamyl phosphonate <2> (<2> IC50 is above 0.1 mM [5]) [5]
diethyl vinylphosphonate <2> (<2> IC50 is above 0.1 mM [5]) [5]
dimethyl 2-oxoheptyl phosphonate <2> (<2> IC50 is above 0.1 mM [5]) [5]
dioctyl phenyl phosphonate <2> (<2> IC50 is 0.013 mM [5]) [5]
dodecyl phosphonic acid <2> (<2> IC50 is 0.040 mM [5]) [5]
sodium dodecyl sulfonate <2> (<2> IC50 is 0.0037 mM [5]) [5]
taurolithocholic acid 3-sulfate <2> (<2> IC50 is 0.005 mM [5]) [5]
tetraisopropyl methylenediphosphonate <2> (<2> IC50 is above 0.1 mM [5]) [5]
Additional information <2, 3> (<2> inhibition mechanism of sulfates, sulfonates, and phosphonates [5]; <2> no inhibition of the phosphatase activity by 1-cyclohexyl-3-dodecyl urea at 0.1 mM [4]; <3> no inhibition of the phosphatase activity by dicyclohexylurea and 4-fluorochalcone oxide [6]; <2> poor or no inhibition by chalcone oxide, 1-cyclohexyl-3-ethyl urea, 1-cyclohexyl-3-hexyl urea, 1-cyclohexyl-3-dodecyl urea, 1,3-dicyclohexyl urea, okadaic acid, tartaric acid, and sodium orthovanadate [2]) [2, 4, 5, 6]

Metals, ions

Co^{2+} <2> (<2> half as effective as Mg^{2+} [2]) [2]
Mg^{2+} <1, 2, 3> (<2,3> dependent on [4,5,7]; <2> dependent on, binding structure at the N-terminus [1]; <2> dependent on, maximal activity at 0.1 mM [4]; <2> dependent on, optimal at 1 mM $MgCl_2$, one Mg^{2+} per catalytic subunit, dissociation constants, recombinant enzyme [2]; <3> required for phosphatase activity [6]; <2> required for phosphatase activity of the enzyme [3]) [1, 2, 3, 4, 5, 6, 7]
phosphate <2> (<2> binding structure at the N-terminus [1]) [1]

Turnover number (min^{-1})

0.0061 <2> (4-nitrophenyl phosphate, <2> pH 7.8, 37°C, recombinant wild-type enzyme [6]) [6]
0.013 <2> (4-nitrophenyl phosphate, <2> pH 7.8, 37°C, recombinant wild-type N-terminal domain [6]) [6]
0.13 <2> (geranylgeranyl diphosphate, <2> recombinant enzyme [4]) [4]
0.25 <2> (farnesyl diphosphate, <2> recombinant enzyme [4]) [4]
0.51 <2> (geranyl phosphate, <2> recombinant enzyme [4]) [4]
0.71 <2> (farnesyl phosphate, <2> recombinant enzyme [4]) [4]
0.84 <3> (4-nitrophenyl phosphate, <3> pH 7.8, 37°C, native enzyme [6]) [6]
1.61 <3> (4-nitrophenyl phosphate, <3> pH 7.8, 37°C, recombinant enzyme [6]) [6]
1.85 <2> (geranylgeranyl phosphate, <2> recombinant enzyme [4]) [4]

Specific activity (U/mg)

0.007 <2> (<2> purified recombinant mutant R103C/R287Q, substrate 4-nitrophenyl phosphate [3]) [3]
0.015 <2> (<2> purified recombinant mutant K55R, substrate 4-nitrophenyl phosphate [3]) [3]
0.016 <2> (<2> purified recombinant mutant C154Y, substrate 4-nitrophenyl phosphate [3]) [3]
0.017 <2> (<2> purified recombinant mutant R103C, substrate 4-nitrophenyl phosphate [3]) [3]
0.021 <2> (<2> purified recombinant mutant R287Q, substrate 4-nitrophenyl phosphate [3]) [3]
0.029 <2> (<2> purified recombinant wild-type allele MFA enzyme and mutant E470G, substrate 4-nitrophenyl phosphate [3]) [3]
0.03 <2> (<2> purified recombinant enzyme, substrate isopentenyl phosphate [4]; <2> purified recombinant mutant V422A, substrate 4-nitrophenyl phosphate [3]) [3, 4]
0.0954 <2> (<2> purified recombinant enzyme [2]) [2]
0.25 <2> (<2> purified recombinant enzyme, substrate geranyl phosphate [4]) [4]
0.49 <2> (<2> purified recombinant enzyme, substrate farnesyl phosphate [4]) [4]
0.53 <2> (<2> purified recombinant enzyme, substrate geranylgeranyl phosphate [4]) [4]

0.8 <3> (<3> purified recombinant enzyme, substrate geranyl phosphate [4]) [4]
0.85 <3> (<3> purified recombinant enzyme, substrate isopentenyl phosphate [4]) [4]
0.99 <3> (<3> purified recombinant enzyme, substrate farnesyl phosphate [4]) [4]
1.05 <3> (<3> purified recombinant enzyme, substrate geranylgeranyl phosphate [4]) [4]
Additional information <2> (<2> optimization of activity assay conditions with the recombinant enzyme, maximal in 25 mM Tris buffer, pH 7.0, 30°C, 1 mM $MgCl_2$ [2]) [2]

K_m-Value (mM)

0.0036 <2> (2'-[2-benzothiazolyl]-6'-hydroxybenzothiazole phosphate, <2> pH 7.0, 30°C, recombinant enzyme [5]) [5]
0.0036 <2> (farnesyl diphosphate, <2> pH 7.0, 30°C, recombinant enzyme [5]) [5]
0.0036 <2> (farnesyl monophosphate, <2> pH 7.0, 30°C, recombinant enzyme [5]) [5]
0.0036 <2> (geranylgeranyl diphosphate, <2> pH 7.0, 30°C, recombinant enzyme [5]) [5]
0.02 <2> (threo-10-phosphonooxy-9-hydroxy-octadecanoic acid, <2> pH 7.0, 30°C, recombinant enzyme [2]) [2]
0.021 <2> (threo-9,10-phosphonooxyoctadecanoic acid, <2> pH 7.0, 30°C, recombinant enzyme [5]) [5]
0.021 <2> (threo-9-hydroxy-10-(phosphonooxy)octadecanoate, <2> pH 7.8, 37°C, recombinant enzyme [7]) [7]
0.024 <2> (farnesyl phosphate, <2> recombinant enzyme [4]) [4]
0.033 <2> (geranylgeranyl diphosphate, <2> recombinant enzyme [4]) [4]
0.084 <2> (farnesyl diphosphate, <2> recombinant enzyme [4]) [4]
0.124 <2> (geranyl phosphate, <2> recombinant enzyme [4]) [4]
0.21 <2> (4-methylumbelliferyl phosphate, <2> pH 7.0, 30°C, recombinant enzyme [5]) [5]
0.24 <3> (4-nitrophenyl phosphate, <3> pH 7.8, 37°C, native enzyme [6]) [6]
0.37 <3> (4-nitrophenyl phosphate, <3> pH 7.8, 37°C, recombinant enzyme [6]) [6]
1.1 <2> (4-nitrophenyl phosphate, <2> pH 7.4, 37°C, recombinant wild-type allele MFA enzyme and mutant R103C [3]; <2> pH 7.8, 37°C, recombinant wild-type enzyme [6]) [3, 6]
1.2 <2> (4-nitrophenyl phosphate, <2> pH 7.4, 37°C, recombinant mutants K55R and C154Y [3]) [3]
1.3 <2> (4-nitrophenyl phosphate, <2> pH 7.4, 37°C, recombinant mutant R287Q [3]) [3]
1.6 <2> (4-nitrophenyl phosphate, <2> pH 7.0, 30°C, recombinant enzyme [5]) [5]
1.746 <2> (geranylgeranyl phosphate, <2> recombinant enzyme [4]) [4]

2.3 <2> (4-nitrophenyl phosphate, <2> pH 7.8, 37°C, recombinant wild-type N-terminal domain [6]) [6]
2.5 <2> (4-nitrophenyl phosphate, <2> pH 7.4, 37°C, recombinant mutant R103C/R287Q [3]) [3]
Additional information <2, 3> (<2,3> kinetics [5,6]; <2> kinetics, recombinant enzyme [2]) [2, 5, 6]

K_i-Value (mM)
Additional information <2> (<2> inhibition kinetics [5]) [5]

pH-Optimum
7 <2> (<2> recombinant enzyme [2]; <2> assay at [5]) [2, 5]
7.4 <2> (<2> assay at [3]) [3]
7.8 <1, 2, 3> (<1,2,3> assay at [6]) [6]

Temperature optimum (°C)
30 <2> (<2> assay at [5]; <2> recombinant enzyme [2]) [2, 5]
37 <1, 2, 3> (<1,2,3> assay at [3,6]) [3, 6]

4 Enzyme Structure

Subunits
dimer <1, 2, 3> [6]
Additional information <1, 2, 3> (<2> the phosphatase activity of the enzyme is located at N-terminal part, the C-terminal part harbors the epoxide hydrolase activity of EC 3.3.2.10, Arg287 may be involved in formation of the quarternary enzyme structure [3]; <2> the phosphatase activity of the enzyme is located at the 35 kDa N-terminal part which has a α/β-fold, the 25 kDa C-terminal part, with a different hydrolase α/β-fold, harbors the epoxide hydrolase activity of EC 3.3.2.10, quarternary structure analysis, structure-mechanism relationship [1]; <1,2,3> the phosphatase activity of the enzyme is located at the 35 kDa N-terminal part which has a α/β-fold, the 25 kDa C-terminal part, with a different hydrolase α/β-fold, harbors the epoxide hydrolase activity of EC 3.3.2.10, structure analysis [2,6]; <2> the phosphatase activity of the enzyme is located at the N-terminal part, the C-terminal part harbors the epoxide hydrolase activity of EC 3.3.2.10 [5,7]) [1, 2, 3, 5, 6, 7]

5 Isolation/Preparation/Mutation/Application

Source/tissue
liver <1, 2, 3> [4, 6]

Localization
cytosol <1, 2, 3> [4, 6]
soluble <2> [3]

Purification

<2> (recombinant His-tagged wild-type and D9A mutant enzymes and N-terminal domains from Escherichia coli strain BL21-AI by nickel affinity chromatography, recombinant strep-tagged wild-type enzyme from Escherichia coli strain XL1-Blue by streptavidin affinity chromatography) [6]
<2> (recombinant His-tagged wild-type and mutant enzymes from Sf21 insect cells by nickel affinity chromatography) [3]
<2> (recombinant His-tagged wild-type and mutant enzymes from insect cells by nickel affinity chromatography) [4]
<2> (recombinant wild-type and mutant enzymes from insect cells by affinity chromatography) [2]
<3> (from liver to homogeneity by ultracentrifugation and affinity chromatography on a benzyl thio resin using 4-fluorochalcone oxide as eluent) [6]
<3> (recombinant enzyme by S-benzyl affinity chromatography with 4-fluorochalcone oxide as eluent) [4]

Crystallization

<2> (crystal structure analysis) [7]
<2> (purified recombinant enzyme, sitting drop vapour diffusion method, 0.005 ml of 12-16 mg/ml protein in 3 mM DTT, 0.1 M sodium phosphate, pH 7.4, is mixed with 0.005 ml precipitation solution containing 0.1 M Tris, pH 9.0, 30% w/v PEG 4000, and 0.2 M Li_2SO_4, versus 1 ml reservoir of precipitatin solution, 4°C, 10 days, X-ray diffraction structure determination and analysis at 2.6 A resolution, modeling) [1]

Cloning

<2> (arabinose-inducible expression of His-tagged wild-type and D9A mutant enzymes and N-terminal domains from cDNA in Escherichia coli strain BL21-AI, expression of strep-tagged enzyme from full-length gene in Escherichia coli strain XL1-Blue) [6]
<2> (expression of His-tagged wild-type and mutant enzymes in insect cells using the baculovirus infection system) [4]
<2> (expression of wild-type and mutant enzymes in insect cells using the baculovirus infection system) [2]
<2> (gene EPHX2, expression of His-tagged wild-type and mutant enzymes in Spodoptera frugiperda Sf21 cells using the baculovirus infection system) [3]
<3> (arabinose-inducible expression of the enzyme from full-length gene in Escherichia coli strain BL21-AI) [6]
<3> (expression of enzyme in insect cells using the baculovirus infection system) [4]

Engineering

C154Y <2> (<2> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 2.2, compared to the wild-type ratio of 1.0 [3]; <2> the mutant shows similar activity for isoprenoid phosphate compared to the wild-type enzyme [4]) [3, 4]

D9A <2> (<2> site-directed mutagenesis, inactive mutant N-terminal domain [6]) [6]
E470G <2> (<2> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 0.9, compared to the wild-type ratio of 1.0 [3]) [3]
K55R <2> (<2> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 1.9, compared to the wild-type ratio of 1.0 [3]) [3]
R103C <2> (<2> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 1.3, compared to the wild-type ratio of 1.0 [3]; <2> the mutant shows increased activity for isoprenoid phosphate compared to the wild-type enzyme [4]) [3, 4]
R103C/R287Q <2> (<2> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 1.2, compared to the wild-type ratio of 1.0 [3]; <2> the mutant shows similar activity for isoprenoid phosphate compared to the wild-type enzyme [4]) [3, 4]
R287Q <2> (<2> site-directed mutagenesis, the mutant shows the same hydrolase/phosphatase ratio of 1.0 as the wild-type, but reduced stability at 37°C [3]; <2> the mutant shows increased activity for isoprenoid phosphate compared to the wild-type enzyme [4]) [3, 4]
V422A <2> (<2> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 0.8, compared to the wild-type ratio of 1.0 [3]) [3]
Y465F <2> (<2> site-directed mutagenesis, mutant enzyme shows phosphatase, but no epoxide hydrolase activity [2]) [2]
Additional information <2> (<2> the isolated recombinant N-terminal domain is a fully active phosphatase [6]) [6]

6 Stability

Temperature stability

37 <2> (<2> inactivation rate constants for recombinant wild-type and mutant enzymes [3]) [3]

Storage stability

<3>, -70°C, 0.5 mg/ml purified enzyme, completely stable [6]

References

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Acireductone synthase 3.1.3.77

1 Nomenclature

EC number
3.1.3.77

Systematic name
5-(methylthio)-2,3-dioxopentyl-phosphate phosphohydrolase (isomerizing)

Recommended name
acireductone synthase

Synonyms
E-1 <1, 2> [2, 3]
enolase-phosphatase <1, 2> [3]

2 Source Organism

<1> *Klebsiella pneumoniae* (no sequence specified) [1, 2, 3]
<2> *Klebsiella oxytoca* (no sequence specified) [3]

3 Reaction and Specificity

Catalyzed reaction
5-(methylthio)-2,3-dioxopentyl phosphate + H_2O = 1,2-dihydroxy-5-(methylthio)pent-1-en-3-one + phosphate (overall reaction)
5-(methylthio)-2,3-dioxopentyl phosphate = 2-hydroxy-5-(methylthio)-3-oxopent-1-enyl phosphate
2-hydroxy-5-(methylthio)-3-oxopent-1-enyl phosphate + H_2O = 1,2-dihydroxy-5-(methylthio)pent-1-en-3-one + phosphate

Substrates and products
S 2,3-diketo-1-phosphohexane + H_2O <1> (<1> including two intermediates, 2-hydroxy-3-keto-1-phospho-1-hexene and 1,2-dihydroxy-3-keto-1-hexene [2]) (Reversibility: ?) [2]
P 2-ketopentanoate + formate + glyoxylate + butyrate

Metals, ions
Mg^{2+} <1> (<1> required for activity [2]) [2]

Specific activity (U/mg)
0.001 <2> (<2> clone MM294, transformant DIOX1, DIOX3, DIOX4 and non-hybridizing isolate taken as a negative control [3]) [3]
0.00816 <2> (<2> UNF932 strain [3]) [3]
0.179 <2> (<2> clone MM294, transformant DIOX2 [3]) [3]
21 <1> (<1> purified enzyme by Phenyl-Superose column [2]) [2]

K_m-Value (mM)
0.0071 <1> (2-hydroxy-3-keto-1-phospho-1-hexene) [2]
0.077 <1> (2,3-diketo-1-phosphohexane) [2]

4 Enzyme Structure

Molecular weight
25500 <1> (<1> gel filtration [2]) [2]
25620 <1> (<1> deduced from amino acid sequence [3]) [3]
27000 <1> (<1> calculated from sequence [2]) [2]
28300 <1> (<1> SDS-PAGE [2]) [2]

Subunits
monomer <1> [2]

5 Isolation/Preparation/Mutation/Application

Purification
<1> (100fold purified to homogeneity in 10% yield, SDS-PAGE, amino acid sequence analysis) [2]
<1> (gel filtration) [3]
<2> (gel filtration) [3]

6 Stability

Storage stability
<2>, -80°C, 50% glycerol [3]

References

[1] Wray, J.W.; Abeles, R.B.: The methionine salvage pathway in Klebsiella pneumoniae and rat liver. J. Biol. Chem., **270**, 3147-3153 (1995)
[2] Myers, R.W.; Wray, J.W.; Fish, S.; Abeles, R.H.: Purification and characterization of an enzyme involved in oxidative carbon-carbon bond cleavage reactions in the methionine salvage pathway of Klebsiella pneumoniae. J. Biol. Chem., **268**, 24785-24791 (1993)

[3] Balakrishnan, R.; Frohlich, M.; Rahaim, P.T.; Backman, K.; Yocum, R.R.: Appendix. Cloning and sequence of the gene encoding enzyme E-1 from the methionine salvage pathway of Klebsiella oxytoca. J. Biol. Chem., **268**, 24792-24795 (1993)

Cyclic-guanylate-specific phosphodiesterase 3.1.4.52

1 Nomenclature

EC number
3.1.4.52

Systematic name
cyclic bis(3-5')diguanylate 3-guanylylhydrolase

Recommended name
cyclic-guanylate-specific phosphodiesterase

CAS registry number
338732-46-0

3 Reaction and Specificity

Catalyzed reaction
cyclic di-3',5'-guanylate + H_2O = 5'-phosphoguanylyl(3'-5')guanosine

Ribonuclease D 3.1.13.5

1 Nomenclature

EC number
3.1.13.5

Recommended name
ribonuclease D

Synonyms
RNase D <1> [6]

CAS registry number
9073-62-5

2 Source Organism

<1> *Escherichia coli* (no sequence specified) (<1> isozyme SMO2-2 [5]) [1, 2, 3, 4, 5, 6, 7]

3 Reaction and Specificity

Catalyzed reaction
exonucleolytic cleavage that removes extra residues from the 3'-terminus of tRNA to produce 5'-mononucleotides

Natural substrates and products
- **S** tRNA containing extra residues at the 3'-terminus <1> (<1> ribonuclease D is not essential for the normal growth of Escherichia coli or bacteriophage T4 or for the biosynthesis of a T4 suppressor tRNA [4]; <1> the enzyme can act at the 3-terminus of tRNA in vivo. RNase D participates in tRNA metabolism [5]) (Reversibility: ?) [4, 5]
- **P** 5'-mononucleotide + tRNA
- **S** Additional information <1> (<1> enzyme is involved in structured RNA processing [7]; <1> the enzyme may represent an intracellular scavenging mechanism for denatured tRNAs and other inactive RNA molecules [1]) (Reversibility: ?) [1, 7]
- **P** ?

Substrates and products
- **S** structurally altered tRNA <1> (<1> exonuclease activity. RNase D can recognize structurally altered tRNA molecules. The enzyme acts poorly on intact tRNA and is inactive with the synthetic polyribonucleotides,

poly(A), poly(U), or double-stranded poly(A)*poly(U). The enzyme acts on diesterase-treated tRNA, but relatively poorly on intact tRNA. RNase D does not attack ribosomal RNA [1]) (Reversibility: ?) [1]

P 5'-mononucleotide + ?

S tRNA containing extra residues at the 3'-terminus <1> (Reversibility: ?) [5]

P 5'-mononulcotide + tRNA

S tRNA containing extra residues at the 3'-terminus <1> (<1> ribonuclease D is not essential for the normal growth of Escherichia coli or bacteriophage T4 or for the biosynthesis of a T4 suppressor tRNA [4]; <1> the enzyme can act at the 3-terminus of tRNA in vivo. RNase D participates in tRNA metabolism [5]) (Reversibility: ?) [4, 5]

P 5'-mononucleotide + tRNA

S tRNA-C-C-A-Cn + H_2O <1> (Reversibility: ?) [3]

P tRNA-C-C-A-Cn-1 + C

S Additional information <1> (<1> enzyme is involved in structured RNA processing [7]; <1> the enzyme may represent an intracellular scavenging mechanism for denatured tRNAs and other inactive RNA molecules [1]; <1> alteration of the 3-terminal base has no effect on the rate of hydrolysis, whereas modification of the 3-terminal sugar has a major effect. tRNA terminating with a 3-phosphate is completely inactive as a substrate. The rate of hydrolysis of intact tRNA is very slow compared to tRNAs containing extra residues or compared to tRNAs from which part of the -C-C-A sequence has been removed. Oxidation of the terminal sugar, reduction of the dialdehyde with borohydride, or removal of the terminal AMP from intact tRNA increase the activity of the substrate. Addition of a second -C-C-A sequence gives an active substrate indicating that the relative resistance of intact tRNA to RNase D hydrolysis is not due to the sequence per se but to the structural environment of the 3-terminus. The enzyme is an exonuclease which initiates hydrolysis at the 3-terminus and removes 5-mononucleotides in a random fashion [3]) (Reversibility: ?) [1, 3, 7]

P ?

Inhibitors

5,5'-Dithiobis(2-nitrobenzoic acid) <1> (<1> the enzyme is relatively insensitive to sulfhydryl reagents [2]) [2]

EDTA <1> (<1> inhibition can be reversed by Mg^{2+} [1]) [1]

$HgCl_2$ <1> (<1> the enzyme is relatively insensitive to sulfhydryl reagents, increased inhibition after addition of dithiothreitol [2]) [2]

NEM <1> (<1> the enzyme is relatively insensitive to sulfhydryl reagents [2]) [2]

Na^+ <1> (<1> slight [1]) [1]

p-Hydroxymercuribenzoate <1> (<1> the enzyme is relatively insensitive to sulfhydryl reagents [2]) [2]

Activating compounds

NH_4^+ <1> (<1> stimulates [1]) [1]

Metals, ions

Co^{2+} <1> (<1> enzyme requires a divalent cation for activity. This requirement can be satisfied by Mg^{2+}, Mn^{2+} or Co^{2+} [3]) [3]
K^{+} <1> (<1> stimulates [1]) [1]
Mg^{2+} <1> (<1> enzyme requires a divalent cation for activity. This requirement can be satisfied by Mg^{2+}, Mn^{2+} or Co^{2+} [3]; <1> required, optimal concentration is 5 mM [1]) [1, 3]
Mn^{2+} <1> (<1> can partially replace for Mg^{2+} [1]; <1> enzyme requires a divalent cation for activity. This requirement can be satisfied by Mg^{2+}, Mn^{2+} or Co^{2+} [3]) [1, 3]

Specific activity (U/mg)

5 <1> [2]
Additional information <1> [6]

pH-Optimum

7.5-9 <1> (<1> with diesterase-treated tRNA as substrate [1]) [1]
9.1-9.5 <1> [3]

pH-Range

8-10.3 <1> (<1> pH 8.0: about 50% of maximal activity, pH 10.3: about 70% of maximal activity [3]) [3]

4 Enzyme Structure

Molecular weight

40000 <1> (<1> gel filtration [2]) [2]
60000 <1> (<1> gel filtration [1]) [1]

Subunits

monomer <1> (<1> 1 * 40000, SDS-PAGE [2]) [2]

Posttranslational modification

Additional information <1> (<1> the enzyme is devoid of nucleic acid [2]) [2]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [2, 6, 7]
<1> (partial) [1]

Crystallization

<1> (1.6 A resolution crystal structure. Hanging-drop technique was used for subsequent crystal optimizations. Each drop contains equal volumes of protein and reservoir solution. RNase D protein crystallizes in the presence of 2.0-2.5 M ammonium sulfate and various buffer conditions. The crystals used here are grown with a reservoir containing 2.2-2.3 sulfate and 0.1 M HEPES

(pH 7.0). Crystals of RNase D usually appeared overnight under these conditions. Crystals grow to full size in 2-7 days, with a maximum size of over 1 mm in each dimension. Zn^{2+}-containing crystals are grown with 1 mM $ZnSO_4$ included in the reservoir prior to drop setup. Mercury(II) derivatives are prepared by soaking RNase D crystals (without Zn^{2+}) overnight in the mother liquor containing 5 mM $HgSO_4$) [7]

Cloning

<1> [7]

6 Stability

Temperature stability

50 <1> (<1> 3-4 min, 50% loss of the activity against phosphodiesterase-treated tRNA [2]) [2]

Additional information <1> (<1> the enzyme is sensitive to inactivation by elevated temperatures but can be protected by a variety of RNAs [2]) [2]

Storage stability

<1>, -20°C, 6 months, enzyme retains 90% of its activity [2]

References

[1] Ghosh, R.K.; Deutscher, M.P.: Identification of an Escherichia coli nuclease acting on structurally altered transfer RNA molecules. J. Biol. Chem., **253**, 997-1000 (1978)

[2] Cudny, H.; Zaniewski, R.; Deutscher, M.P.: Escherichia coli RNase D. Purification and structural characterization of a putative processing nuclease. J. Biol. Chem., **256**, 5627-5632 (1981)

[3] Cudny, H.; Zaniewski, R.; Deutscher, M.P.: Escherichia coli RNase D. Catalytic properties and substrate specificity. J. Biol. Chem., **256**, 5633-5637 (1981)

[4] Blouin, R.T.; Zaniewski, R.; Deutscher, M.P.: Ribonuclease D is not essential for the normal growth of Escherichia coli or bacteriophage T4 or for the biosynthesis of a T4 suppressor tRNA. J. Biol. Chem., **258**, 1423-1426 (1983)

[5] Zhang, J.R.; Deutscher, M.P.: Transfer RNA is a substrate for RNase D in vivo. J. Biol. Chem., **263**, 17909-17912 (1988)

[6] Zhang, J.R.; Deutscher, M.P.: Escherichia coli RNase D: sequencing of the rnd structural gene and purification of the overexpressed protein. Nucleic Acids Res., **16**, 6265-6278 (1988)

[7] Zuo, Y.; Wang, Y.; Malhotra, A.: Crystal structure of Escherichia coli RNase D, an exoribonuclease involved in structured RNA processing. Structure, **13**, 973-984 (2005)

tRNase Z 3.1.26.11

1 Nomenclature

EC number
3.1.26.11

Recommended name
tRNase Z

Synonyms
(tRNase) Z <6> [27]
3 tRNase
3' tRNase <2, 5, 6, 8, 9, 20, 21, 32> [19, 21, 22, 29, 30]
3'-tRNA processing endoribonuclease
AthTrz1 <35> [21]
AthTrz2 <36> [21]
AthTrz3 <37> [21]
AthTrz4 <14> [21]
BsuTrz <24> [21]
CelTrz <31> [21]
DmeTrz <32> [21]
$ELAC_2$ <2, 32, 34> [21, 30]
EcoTrz <23> [21]
EcoZ <23> [21]
Elac1 <23, 33> [21]
HsaTrz1 <33> [21]
HsaTrz2 <34> [21]
HvoTrz <17, 26, 27> [21]
MjaTrz <25> [21]
PaeTrz <28> [21]
RNase BN <23> [21]
RNase Z <2, 5, 6, 14, 17, 19, 20, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37> [21, 23, 24, 25, 27, 29]
RNaseZ <1, 2, 10, 14, 30> [14, 20, 22, 30, 31]
SceTrz <29> [21]
TM0207 <20> [18]
TRZ1 <14> [26]
TmaTrz <20> [21]
Trz1p <29> [21]
YqiK <24> [21]
YqjK <2> [22]
ZiPD <5, 23> [21, 23]

arylsulfatase <23> [21]
nuclease, transfer ribonucleate maturation 3'-endoribo-(9CI)
pre-tRNA processing endoribonuclease
precursor tRNA 3'-end processing endoribonuclease
tRNA 3 endonuclease
tRNA 3' processing endoribonuclease
tRNA precursor-processing endoribonuclease
tRNase Z <1, 2, 4, 5, 6, 8, 9, 14, 15, 17, 20, 21, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37> [18, 19, 21, 23, 24, 26, 29, 32]
tRNase Z^2 <20> [18]
tRNase Z^L <6> [27]
tRNase Z^S <6> [27]
tRNaseZ <1> [28]
tRnaseZ^L <6, 8, 9> [19]
tRnaseZ^S <6, 21> [19]
transfer RNA maturation endonuclease
zinc phosphodiesterase <5> [23]

CAS registry number
98148-84-6

2 Source Organism

<1> *Drosophila melanogaster* (no sequence specified) [8, 28, 31, 32]
<2> *Bacillus subtilis* (no sequence specified) [18, 19, 22, 29, 30]
<3> *Mus musculus* (no sequence specified) [3, 4]
<4> *Thermus thermophilus* (no sequence specified) [18]
<5> *Escherichia coli* (no sequence specified) [19, 23]
<6> *Homo sapiens* (no sequence specified) [3,7,19,27]
<7> *Rattus norvegicus* (no sequence specified) [7,12,14,15]
<8> *Sus scrofa* (no sequence specified) [2,3,4,5,8,10,11,19]
<9> *Saccharomyces cerevisiae* (no sequence specified) [7,9,19]
<10> *Triticum aestivum* (no sequence specified) [1, 9, 12, 16, 20]
<11> *Aspergillus nidulans* (no sequence specified) [6]
<12> *Spinacia oleracea* (no sequence specified) [9]
<13> *Solanum tuberosum* (no sequence specified) [1, 12, 13, 17]
<14> *Arabidopsis thaliana* (no sequence specified) [14, 16, 20, 21, 26]
<15> *Thermoplasma acidophilum* (no sequence specified) [18]
<16> *Xenopus laevis* (no sequence specified) [12]
<17> *Haloferax volcanii* (no sequence specified) [12, 21]
<18> *Methanococcus jannaschii* (no sequence specified) [16]
<19> *Synechocystis sp.* (no sequence specified) [25]
<20> *Thermotoga maritima* (no sequence specified) [18, 19, 21, 24]
<21> *Pyrobaculum aerophilum* (no sequence specified) [18, 19]
<22> *Physarum sp.* (no sequence specified) [7]
<23> *Escherichia coli* (UNIPROT accession number: P0A8V0) [21]

<24> *Bacillus subtilis* (UNIPROT accession number: P54548) [21]
<25> *Methanococcus jannaschii* (UNIPROT accession number: Q58897) [21]
<26> *Pyrococcus furiosus* (UNIPROT accession number: Q8U182) [21]
<27> *Thermoplasma acidophilum* (UNIPROT accession number: Q9HJ19) [21]
<28> *Pyrobaculum aerophilum* (UNIPROT accession number: Q8ZTJ7) [21]
<29> *Saccharomyces cerevisiae* (UNIPROT accession number: P36159) [21]
<30> *Methanococcus jannaschi* (no sequence specified) [20]
<31> *Caenorhabditis elegans* (UNIPROT accession number: O44476) [21]
<32> *Drosophila melanogaster* (UNIPROT accession number: Q8MKW7) [21]
<33> *Homo sapiens* (UNIPROT accession number: Q9H777) [21]
<34> *Homo sapiens* (UNIPROT accession number: Q9BQ52) [21]
<35> *Arabidopsis thaliana* (UNIPROT accession number: Q8LGU7) [21]
<36> *Arabidopsis thaliana* (UNIPROT accession number: Q8L633) [21]
<37> *Arabidopsis thaliana* (UNIPROT accession number: Q8VYS2) [21]

3 Reaction and Specificity

Catalyzed reaction

endonucleolytic cleavage of RNA, removing extra 3' nucleotides from tRNA precursor, generating 3' termini of tRNAs. A 3'-hydroxy group is left at the tRNA terminus and a 5'-phosphoryl group is left at the trailer molecule

Reaction type

hydrolysis of phosphoric ester

Substrates and products

S 415-nt CAT mRNA + H_2O <8> (<8> 5-half portion of the E. coli chloramphenicol acetyltransferase mRNA [10]) (Reversibility: ?) [10]
P ?
S GENV7-Env + H_2O <3, 8> (<3,8> complex of SPH2 with 5 half $tRNA^{Arg}$ [4]) (Reversibility: ?) [4]
P ?
S GT7H + H_2O <8> (<8> complex of SPH2 with 5 half tRNA [10]) (Reversibility: ?) [10]
P ?
S GT7HM10 + H_2O <8> (<8> complex of SPH2 with 5 half tRNA [10]) (Reversibility: ?) [10]
P ?
S GT7HM20 + H_2O <8> (<8> complex of SPH2 with 5 half tRNA [10]) (Reversibility: ?) [10]
P ?
S GT7HM22 + H_2O <8> (<8> complex of SPH2 with 5 half tRNA [10]) (Reversibility: ?) [10]
P ?
S IM0 + H_2O <10, 14, 30> (<10,14,30> pre-$tRNA^{Ile}$ with CCA 3-trailer sequence [20]) (Reversibility: ?) [20]
P ?

S IM1 + H_2O <10, 14, 30> (<10,14,30> pre-tRNA^{Ile} with CCG 3-trailer sequence [20]) (Reversibility: ?) [20]
P ?
S IM2 + H_2O <10, 14, 30> (<10,14,30> pre-tRNA^{Ile} with CUG 3-trailer sequence [20]) (Reversibility: ?) [20]
P ?
S IM3 + H_2O <10, 14, 30> (<10,14,30> pre-tRNA^{Ile} with UUG 3-trailer sequence [20]) (Reversibility: ?) [20]
P ?
S IT1 + H_2O <10, 14, 30> (<10,14,30> pre-tRNA^{Ile} with CCG and 50 nucleotide 3-trailer sequence [20]) (Reversibility: ?) [20]
P ?
S IT2 + H_2O <10, 14, 30> (<10,14,30> pre-tRNA^{Ile} with CUG and 50 nucleotide 3-trailer sequence [20]) (Reversibility: ?) [20]
P ?
S IT3 + H_2O <10, 14, 30> (<10,14,30> pre-tRNA^{Ile} with UUG and 50 nucleotide 3-trailer sequence [20]) (Reversibility: ?) [20]
P ?
S ITO + H_2O <10, 14, 30> (<10,14,30> pre-tRNA^{Ile} with CCA and 50 nucleotide 3-trailer sequence [20]) (Reversibility: ?) [20]
P ?
S R-A1 + H_2O <3> (<3> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-ACA19 + H_2O <3> (<3> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-ACA3 + H_2O <3> (<3> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-ASA1 + H_2O <8> (<8> pre-tRNA^{Arg} construct with one base-pair addition after the fifth base-pair of the acceptor stem [11]) (Reversibility: ?) [11]
P ?
S R-ASA2 + H_2O <8> (<8> pre-tRNA^{Arg} construct with two base-pair additions after the fifth base-pair of the acceptor stem [11]) (Reversibility: ?) [11]
P ?
S R-ASA3 + H_2O <8> (<8> pre-tRNA^{Arg} construct with three base-pair additions after the fifth base-pair of the acceptor stem [11]) (Reversibility: ?) [11]
P ?
S R-ASD1 + H_2O <8> (<8> pre-tRNA^{Arg} construct with one base-pair deletion after the third base-pair of the acceptor stem [11]) (Reversibility: ?) [11]
P ?
S R-ASD2 + H_2O <8> (<8> pre-tRNA^{Arg} construct with two base-pair deletions after the third base-pair of the acceptor stem [11]) (Reversibility: ?) [11]

P ?
S R-AT12A + H_2O <8> (<8> pre-tRNA^{Arg} construct with one additional base-pair in the acceptor stem and one deleted base-pair in the T stem [11]) (Reversibility: ?) [11]
P ?
S R-AT12B + H_2O <8> (<8> pre-tRNA^{Arg} construct with one deleted base-pair in the acceptor stem and one additional base-pair in the T stem [11]) (Reversibility: ?) [11]
P ?
S R-AT14 + H_2O <8> (<8> pre-tRNA^{Arg} construct with one base-pair addition in both acceptor and T stems [11]) (Reversibility: ?) [11]
P ?
S R-AT16 + H_2O <8> (<8> pre-tRNA^{Arg} construct with two base-pair additions in both acceptor and T stems [11]) (Reversibility: ?) [11]
P ?
S R-ATM1 + H_2O <8> (<8> small pre-tRNA^{Arg} construct [2]) (Reversibility: ?) [2]
P ?
S R-ATM10 + H_2O <8> (<8> small pre-tRNA^{Arg} construct [2]) (Reversibility: ?) [2]
P ?
S R-ATM11 + H_2O <8> (<8> small pre-tRNA^{Arg} construct [2]) (Reversibility: ?) [2]
P ?
S R-ATM2 + H_2O <8> (<8> small pre-tRNA^{Arg} construct [2]) (Reversibility: ?) [2]
P ?
S R-ATM3 + H_2O <8> (<8> small pre-tRNA^{Arg} construct [2]) (Reversibility: ?) [2]
P ?
S R-ATM4 + H_2O <8> (<8> small pre-tRNA^{Arg} construct [2]) (Reversibility: ?) [2]
P ?
S R-ATM5 + H_2O <8> (<8> small pre-tRNA^{Arg} construct [2]) (Reversibility: ?) [2]
P ?
S R-ATM7 + H_2O <8> (<8> small pre-tRNA^{Arg} construct [2]) (Reversibility: ?) [2]
P ?
S R-ATM8 + H_2O <8> (<8> small pre-tRNA^{Arg} construct [2]) (Reversibility: ?) [2]
P ?
S R-ATM9 + H_2O <8> (<8> small pre-tRNA^{Arg} construct [2]) (Reversibility: ?) [2]
P ?
S R-ATW + H_2O <8> (<8> small pre-tRNA^{Arg} [2]) (Reversibility: ?) [2]
P ?

S R-C_1 + H_2O <3, 8> (<8> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-CCA19 + H_2O <3, 8> (<3,8> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-CCA6 + H_2O <8> (<8> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-CCA8 + H_2O <8> (<8> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-CCG + H_2O <3> (<3> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-CUA + H_2O <3> (<3> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-G1 + H_2O <3, 8> (<3,8> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-G10 + H_2O <8> (<8> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-G13 + H_2O <8> (<8> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-G15 + H_2O <8> (<8> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-G19 + H_2O <8> (<8> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-G3 + H_2O <3, 8> (<3,8> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-G8 + H_2O <8> (<8> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-GCA + H_2O <3> (<3> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-L0 + H2o <8> (<8> wild type pre-tRNA^{Arg} [5,11]) (Reversibility: ?) [5, 11]
P ?
S R-L3 + H2o <8> (<8> pre-tRNA^{Arg} construct [5]) (Reversibility: ?) [5]
P ?
S R-L6 + H2o <8> (<8> pre-tRNA^{Arg} construct [5]) (Reversibility: ?) [5]
P ?
S R-TSA1 + H_2O <8> (<8> pre-tRNA^{Arg} construct with one base-pair addition in the T stem [11]) (Reversibility: ?) [11]
P ?
S R-TSA2 + H_2O <8> (<8> pre-tRNA^{Arg} construct with two base-pair additions in the T stem [11]) (Reversibility: ?) [11]
P ?
S R-TSD1 + H_2O <8> (<8> pre-tRNA^{Arg} construct with one base-pair deletion in the T stem [11]) (Reversibility: ?) [11]
P ?

S R-TSD2 + H_2O <8> (<8> pre-tRNA^{Arg} construct with two base-pair deletions in the T stem [11]) (Reversibility: ?) [11]
P ?
S R-U1 + H_2O <3> (<3> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-UCA + H_2O <3> (<3> pre-tRNA^{Arg} construct [3]) (Reversibility: ?) [3]
P ?
S R-UUU + H_2O <8> (<8> small pre-tRNA^{Arg} construct [2]) (Reversibility: ?) [2]
P ?
S Rib3 + H_2O <14> (<14> substrate with mature 5tRNA and 3 unprocessed snoRNA [14]; <14> 5 mature dicistronic tRNA^{Gly}-snoR43.1 precursor RNA [14]) (Reversibility: ?) [14]
P ?
S Rib4 + H_2O <14> (<14> substrate with mature 5tRNA [14]; <14> 5 and 3 mature dicistronic tRNA^{Gly}-snoR43.1 precursor RNA [14]) (Reversibility: ?) [14]
P ?
S S5L + H_2O <10, 14, 30> (<10,14,30> pre-tRNA^{Tyr} construct with 5 base pairs in the acceptor stem [20]) (Reversibility: ?) [20]
P ?
S S6L + H_2O <10, 14, 30> (<10,14,30> pre-tRNA^{Tyr} construct with 6 base pairs in the acceptor stem [20]) (Reversibility: ?) [20]
P ?
S S7L + H_2O <10, 14, 30> (<10,14,30> wild type pre-tRNA^{Ile} [20]) (Reversibility: ?) [20]
P ?
S S8L + H_2O <10, 14, 30> (<10,14,30> pre-tRNA^{Tyr} construct with 8 base pairs in the acceptor stem [20]) (Reversibility: ?) [20]
P ?
S S9L + H_2O <10, 14, 30> (<10,14,30> pre-tRNA^{Tyr} construct with 9 base pairs in the acceptor stem [20]) (Reversibility: ?) [20]
P ?
S T3 + H_2O <13, 17> (<17> tRNA variant without the anticodon arm [12]; <13> without anticodon arm [17]) (Reversibility: ?) [12, 17]
P ?
S T6 + H_2O <13, 17> (<17> tRNA variant without the anticodon arm [12]; <13> without anticodon and variable arm [17]) (Reversibility: ?) [12, 17]
P ?
S T7-5 + H_2O <8> (<8> complex of SPH2 with shorter RNA [10]) (Reversibility: ?) [10]
P ?
S T7M7 + H_2O <8> (<8> complex of SPH2 with RNA heptamer [10]) (Reversibility: ?) [10]
P ?
S T7M7-T3H + H_2O <3, 8> (<3,8> complex of SPH2 with 5 half tRNA^{Arg} [4]) (Reversibility: ?) [4]

P ?
S T7M7-T7HM1 + H_2O <3, 8> (<3,8> complex of SPH_2 with 5 half $tRNA^{Arg}$ [4]) (Reversibility: ?) [4]
P ?
S T7M7-T7HM2 + H_2O <3, 8> (<3,8> complex of SPH_2 with 5 half $tRNA^{Arg}$ [4]) (Reversibility: ?) [4]
P ?
S T7M71 + H_2O <8> (<8> complex of SPH_2 with RNA heptamer [10]) (Reversibility: ?) [10]
P ?
S pTyrI + H_2O <2> (<2> pre-$tRNA^{Tyr}$ from Oenothera berteriana [22]) (Reversibility: ?) [22]
P ?
S pre-tRNA-CAG-trailer + H_2O <12> (<12> 5-, 3-extended chloroplast pre-$tRNA^{Phe}$ [9]) (Reversibility: ?) [9]
P ?
S pre-tRNA-CCA-trailer + H_2O <9, 12> (<9,12> 3-extended yeast pre-$tRNA^{Phe}$ [9]) (Reversibility: ?) [9]
P ?
S pre-tRNA-CCAOH-trailer + H_2O <9, 10, 12> (<9,10,12> 3-mature chloroplast pre-$tRNA^{Phe}$ [9]) (Reversibility: ?) [9]
P ?
S pre-$tRNA^{Arg}$(+L) + H_2O <4, 2> (<20> tRNA from Homo sapiens, efficient cleavage [18]; <4,20> tRNA from Homo sapiens, no cleavage [18]) (Reversibility: ?) [18]
P ?
S pre-$tRNA^{Arg}$(-L) + H_2O <2, 4, 15, 20, 21> (<2,15,20> tRNA from Homo sapiens, efficient cleavage [18]; <4,20,21> tRNA from Homo sapiens, no cleavage [18]) (Reversibility: ?) [18]
P ?
S pre-$tRNA^{Arg}$(CCA) + H_2O <2, 4, 15, 20, 21> (<4> tRNA from Thermotoga maritima, cleavage barely detectable [18]; <20> tRNA from Thermotoga maritima, efficient cleavage [18]; <2,15> tRNA from Thermotoga maritima, no cleavage [18]; <20,21> tRNA from Thermotoga maritima, weak cleavage [18]) (Reversibility: ?) [18]
P ?
S pre-$tRNA^{Arg}$(CCG) + H_2O <20> (<20> tRNA from Thermotoga maritima, cleavage barely detectable [18]; <20> tRNA from Thermotoga maritima, no cleavage [18]) (Reversibility: ?) [18]
P ?
S pre-$tRNA^{Arg}$(CUG) + H_2O <2, 4, 15, 20, 21> (<20> tRNA from Thermotoga maritima, cleavage barely detectable [18]; <2,20> tRNA from Thermotoga maritima, efficient cleavage [18]; <4,21> tRNA from Thermotoga maritima, no cleavage [18]; <15> tRNA from Thermotoga maritima, weak cleavage [18]) (Reversibility: ?) [18]
P ?

S pre-tRNA^{Cys} + H_2O <2, 4, 20> (<2> tRNA from Bacillus subtilis, efficient cleavage in presence of 0.2 mM Mn^{2+} [18]; <4,20> tRNA from Bacillus subtilis, no cleavage [18]) (Reversibility: ?) [18]
P ?
S pre-tRNA^{Glu}-CCAN17 + H_2O <19> (Reversibility: ?) [25]
P ?
S pre-tRNA^{Glu}-CCUN17 + H_2O <19> (Reversibility: ?) [25]
P ?
S pre-tRNA^{Glu}-CUAN17 + H_2O <19> (Reversibility: ?) [25]
P ?
S pre-tRNA^{Glu}-UCAN17 + H_2O <19> (Reversibility: ?) [25]
P ?
S pre-tRNA^{Glu}-UUAN17 + H_2O <19> (Reversibility: ?) [25]
P ?
S pre-tRNA^{Glu}-UUUN17 + H_2O <19> (Reversibility: ?) [25]
P ?
S pre-tRNA^{His} + H_2O <1> (<1> the nuclear tRNA is processed to an mature tRNA of 72 nucleotides and a 3 trailer of 32 nucleotides [31]) (Reversibility: ?) [28, 31, 32]
P ?
S pre-tRNA^{His}-AUG + H_2O <1> (Reversibility: ?) [32]
P ?
S pre-tRNA^{His}-CCA + H_2O <1> (Reversibility: ?) [32]
P ?
S pre-tRNA^{Ile} + H_2O <1> (<1> the mitochondrial tRNA is processed to an mature tRNA of 65 nucleotides and a 3 trailer of 75 nucleotides [31]) (Reversibility: ?) [31]
P ?
S pre-tRNA^{Lys} + H_2O <2, 4, 20> (<2,4> tRNA from Thermus thermophilus, no cleavage [18]; <20> tRNA from Thermus thermophilus, weak cleavage [18]) (Reversibility: ?) [18]
P ?
S pre-tRNA^{Met} + H_2O <4, 20, 21> (<20> tRNA from Pyrobaculum aerophilum, efficient cleavage [18]; <4,21> tRNA from Pyrobaculum aerophilum, no cleavage [18]) (Reversibility: ?) [18]
P ?
S pre-tRNA^{Met}(CCA) + H_2O <20> (<20> tRNA from Thermotoga maritima, efficient cleavage [18]; <20> tRNA from Thermotoga maritima, weak cleavage [18]) (Reversibility: ?) [18]
P ?
S pre-tRNA^{Met}(UAG) + H_2O <4, 2> (<20> tRNA from Thermotoga maritima, efficient cleavage [18]; <4> tRNA from Thermotoga maritima, no cleavage [18]; <20> tRNA from Thermotoga maritima, weak cleavage [18]) (Reversibility: ?) [18]
P ?
S pre-tRNA^{Met}Trp + H_2O <2, 4, 20> (<20> tRNA from Bacillus subtilis, efficient cleavage [18]; <2> tRNA from Bacillus subtilis, no cleavage [18];

<4> tRNA from Bacillus subtilis, weak cleavage [18]) (Reversibility: ?) [18]

P ?

S pre-tRNA^{Phe} + H_2O <4, 15, 20, 21> (<4> tRNA from Escherichia coli, cleavage barely detectable [18]; <20> tRNA from Escherichia coli, efficient cleavage [18]; <15,20,21> tRNA from Escherichia coli, no cleavage [18]; <20> tRNA from Pyrobaculum aerophilum, efficient cleavage [18]; <4,21> tRNA from Pyrobaculum aerophilum, no cleavage [18]; <21> tRNA from Thermoplasma acidophilum, no cleavage [18]; <15,20> tRNA from Thermoplasma acidophilum, weak cleavage [18]) (Reversibility: ?) [18]

P ?

S pre-tRNA^{Phe}(CCA) + H_2O <20> (<20> tRNA from Thermotoga maritima, no cleavage [18]; <20> tRNA from Thermotoga maritima, weak cleavage [18]) (Reversibility: ?) [18]

P ?

S pre-tRNA^{Phe}(CCG) + H_2O <20> (<20> tRNA from Thermotoga maritima, no cleavage [18]) (Reversibility: ?) [18]

P ?

S pre-tRNA^{Phe}(CUA) + H_2O <20> (<20> tRNA from Thermotoga maritima, efficient cleavage [18]; <20> tRNA from Thermotoga maritima, no cleavage [18]) (Reversibility: ?) [18]

P ?

S pre-tRNA^{Phe}(UCA) + H_2O <20> (<20> tRNA from Thermotoga maritima, efficient cleavage [18]; <20> tRNA from Thermotoga maritima, no cleavage [18]) (Reversibility: ?) [18]

P ?

S pre-tRNA^{Ser}(UCN) + H_2O <6> (<6> wild type [27]) (Reversibility: ?) [27]

P ?

S pre-tRNA^{Ser}(UCN)7443C + H_2O <6> (<6> natural tRNA mutant [27]) (Reversibility: ?) [27]

P ?

S pre-tRNA^{Ser}(UCN)7444U + H_2O <6> (<6> natural tRNA mutant [27]) (Reversibility: ?) [27]

P ?

S pre-tRNA^{Ser}(UCN)7445C + H_2O <6> (<6> natural tRNA mutant [27]) (Reversibility: ?) [27]

P ?

S pre-tRNA^{Ser}(UCN)7445G + H_2O <6> (<6> natural tRNA mutant [27]) (Reversibility: ?) [27]

P ?

S pre-tRNA^{Ser}(UCN)7510G + H_2O <6> (<6> natural tRNA mutant [27]) (Reversibility: ?) [27]

P ?

S pre-tRNA^{Ser}(UCN)7511G + H_2O <6> (<6> natural tRNA mutant [27]) (Reversibility: ?) [27]

P ?

S pre-tRNA^{Ser}(UCN)7512G + H_2O <6> (<6> natural tRNA mutant [27]) (Reversibility: ?) [27]
P ?
S pre-tRNA^{Tyr} + H_2O <14> (Reversibility: ?) [26]
P ?
S pre-tRNAarg + H_2O <6> (Reversibility: ?) [27]
P ?
S t-RNA^{His}48 + H_2O <1, 8> (Reversibility: ?) [8]
P ?
S t-RNA^{His}48 3'C + H_2O <1, 8> (Reversibility: ?) [8]
P ?
S t-RNA^{His}48 3'CC + H_2O <1, 8> (Reversibility: ?) [8]
P ?
S tRNA^{Tyr} + H_2O <13> (<13> wild type tRNA precursor [17]) (Reversibility: ?) [17]
P ?
S trnB-THr + H_2O <2> (<2> pre-tRNATHr with a 47 nucleotide 3 trailing sequence and CCA motif, no cleavage [22]) (Reversibility: ?) [22]
P ?
S trnI-Thr + H_2O <2> (<2> pre-tRNA^{Thr} with a 83 nucleotide 3 trailing sequence, cleavage at one or two bases downstream the discriminator base [22]) (Reversibility: ?) [22]
P ?
S trnI-Thr-CAAATG-trailer + H_2O <2> (Reversibility: ?) [22]
P ?
S trnI-Thr-CCAATG-trailer + H_2O <2> (Reversibility: ?) [22]
P ?
S trnI-Thr-TAAATG-trailer + H_2O <2> (<2> native trailer sequence [22]) (Reversibility: ?) [22]
P ?
S trnI-Thr-TCAATG-trailer + H_2O <2> (Reversibility: ?) [22]
P ?
S usRNA1 + H_2O <2, 5, 6, 8, 9, 20, 21> (<2, 5, 6, 8, 9, 20> 24 nt unstructured RNA [19]; <21> 24 nt unstructured RNA, no cleavage observed [19]) (Reversibility: ?) [19]
P ?
S usRNA10 + H_2O <2, 5, 20> (<2, 5, 20> 40 nt unstructured RNA [19]) (Reversibility: ?) [19]
P ?
S usRNA$_2$ + H_2O <2, 5, 20> (<2, 5, 20> 24 nt unstructured RNA [19]) (Reversibility: ?) [19]
P ?
S usRNA3 + H_2O <2, 5, 20> (<2, 5, 20> 28 nt unstructured RNA [19]) (Reversibility: ?) [19]
P ?
S usRNA4 + H_2O <2, 5, 20> (<2, 5, 20> 39 nt unstructured RNA [19]) (Reversibility: ?) [19]

P ?
S usRNA5 + H_2O <2, 5, 20> (<2, 5, 20> 26 nt unstructured RNA [19]) (Reversibility: ?) [19]
P ?
S usRNA6 + H_2O <2, 5, 20> (<2, 5, 20> 26 nt unstructured RNA [19]) (Reversibility: ?) [19]
P ?
S usRNA7 + H_2O <2, 5, 20> (<2, 5, 20> 24 nt unstructured RNA [19]) (Reversibility: ?) [19]
P ?
S usRNA8 + H_2O <2, 5, 20> (<2, 5, 20> 43 nt unstructured RNA [19]) (Reversibility: ?) [19]
P ?
S usRNA9 + H_2O <2, 5, 20> (<2, 5, 20> 22 nt unstructured RNA [19]) (Reversibility: ?) [19]
P ?

Inhibitors

Ca^{2+} <10, 13> (<10,13> more than 20 mM [1,13]) [1, 13]
EDTA <10, 13> (<10,13> 10 mM [1]) [1]
K^+ <10, 13> (<10,13> more than 150 mM [1]) [1, 13]
Mg^{2+} <10, 13> (<10,13> more than 50 mM [1,13]) [1, 13]
NH_4^+ <10, 13> (<10,13> more than 100 mM [1]) [1, 13]
Additional information <1, 8> (<1,8> the product of the 3-tRNase reaction is a competitive inhibitor [8]) [8]

Activating compounds

NH_4^+ <10, 13> (<10,13> optimal processing at 2 mM, nuclear RNase Z [1]) [1]

Metals, ions

Ca^{2+} <10, 13> (<10,13> optimal processing at 2 mM, nuclear RNase Z [1]) [1]
K^+ <7, 10, 13, 16, 17> (<16> optimum at 100 mM [12]; <10> 30 mM, mitochondrial RNase Z [1]; <7> optimum at 10-25 mM [12]; <17> optimum at 5 mM [12]; <13> optimum at 30 mM [12,13]) [1, 12, 13]
Mg^{2+} <1, 7, 8, 10, 13, 16, 17> (<17> optimum at 5 mM [12]; <16> optimum at 1-5 mM [12]; <10,13> 5 mM, mitochondrial RNase Z [1,12,13]; <7> optimum at 0-2 mM [12,14]; <8> highest reaction velocity at 3 mM [8]; <1> highest reaction velocity at 0.3-1 mM [8]) [1, 8, 12, 13, 14]
$MgCl_2$ <2> (<2> most active at 0-0.2 mM, weak activity above 3 mM [19]) [19]
$MnCl_2$ <2, 20> (<20> most active at 0.05 mM [19]; <2> most active at 1-5 mM [19]) [19]

Turnover number (min^{-1})

0.0049 <1> (pre-$tRNA^{His}$-CCA) [32]
0.012 <6> (pre-$tRNA^{Ser}$(UCN)7445C, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]

0.021 <6> (pre-tRNA^{Arg}, <6> 25 mM K-MOPS, pH 6.75, 1 mM $MnCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.05 <6> (pre-tRNA^{Ser}(UCN)7510G, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.05 <6> (pre-tRNA^{Ser}(UCN)7511G, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.05 <6> (pre-tRNA^{Ser}(UCN)7512G, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.07 <6> (pre-tRNA^{Ser}(UCN), <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.083 <1> (pre-tRNA^{His}) [32]
0.14 <19> (pre-tRNA^{Glu}-CCUN17, <19> 40 mM Tris-HCl pH 7.5, 2 mM dithiothreitol [25]) [25]
0.153 <6> (pre-tRNA^{Ser}(UCN)7444U, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.23 <6> (pre-tRNA^{Ser}(UCN)7443C, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.28 <19> (pre-tRNA^{Glu}-CUAN17, <19> 40 mM Tris-HCl pH 7.5, 2 mM dithiothreitol [25]) [25]
0.345 <6> (pre-tRNA^{Ser}(UCN)7445G, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
2.25 <19> (pre-tRNA^{Glu}-UUAN17, <19> 40 mM Tris-HCl pH 7.5, 2 mM dithiothreitol [25]) [25]
6.18 <6> (pre-tRNA^{Arg}, <6> 25 mM K-MOPS, pH 6.75, 2 mM $MgCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]

Specific activity (U/mg)
4.6 <11> [6]

K_m-Value (mM)
0.000004 <6> (pre-tRNA^{Ser}(UCN)7512G, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.0000048 <6> (pre-tRNA^{Ser}(UCN), <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.0000069 <6> (pre-tRNA^{Ser}(UCN)7444U, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]

0.0000076 <6> (pre-tRNA^{Ser}(UCN)7511G, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.0000108 <6> (pre-tRNA^{Ser}(UCN)7510G, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.0000197 <6> (pre-tRNA^{Ser}(UCN)7445C, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.0000198 <1> (pre-tRNA^{His}) [32]
0.00002 <8> (tRNA^{His}48, <8> at 37°C [8]) [8]
0.000022 <6> (pre-tRNA^{Arg}, <6> 25 mM K-MOPS, pH 6.75, 2 mM $MgCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.0000394 <1> (pre-tRNA^{His}-AUG) [32]
0.00004 <8> (tRNA^{His}48, <8> at 28°C [8]) [8]
0.0000401 <1> (pre-tRNA^{His}-CCA) [32]
0.0000633 <6> (pre-tRNA^{Ser}(UCN)7443C, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.00008 <1> (tRNA^{His}48) [8]
0.0000855 <6> (pre-tRNA^{Ser}(UCN)7445G, <6> 25 mM K-MOPS, pH 6.75, 2.5 mM $CaCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.00009 <8> (tRNA^{His}48 3'+ C, <8> C is added to the 3-end of tRNA [8]) [8]
0.00009 <8> (tRNA^{His}48 3'+ CC, <8> CC is added to the 3-end of tRNA [8]) [8]
0.000132 <6> (pre-tRNA^{Arg}, <6> 25 mM K-MOPS, pH 6.75, 1 mM $MnCl_2$, 1 mM dithiothreitol, 5% glycerol, 4 U/ml RNasin, 100 microg/ml bovine serum albumin [27]) [27]
0.000155 <1> (pre-tRNA^{His}, <1> 25 mM Tris-HCl pH 7.0, 1 mM $MgCl_2$, 1 mM DTT, 50 mM KCl at 28°C [28]) [28]
0.00032 <8> (R-ATM2) [2]
0.00041 <8> (R-ATW) [2]
0.00044 <8> (R-ATM1) [2]
0.00047 <19> (pre-tRNA^{Glu}-CCUN17, <19> 40 mM Tris-HCl pH 7.5, 2 mM dithiothreitol [25]) [25]
0.00052 <8> (R-ATM8) [2]
0.00057 <8> (R-ATM5) [2]
0.00057 <8> (R-UUU) [2]
0.00058 <8> (R-ATM7) [2]
0.00058 <2> (trnI-Thr-TCAATG-trailer) [22]
0.00059 <8> (R-ATM3) [2]
0.0006 <8> (R-L6) [5]
0.00063 <8> (R-ATM9) [2]
0.00065 <8> (R-ATM10) [2]
0.00066 <8> (R-ATM4) [2]

0.00068 <8> (R-ATM11) [2]
0.00069 <8> (R-L3) [5]
0.00073 <8> (R-G10) [3]
0.00078 <8> (R-G8) [3]
0.00083 <8> (R-L0) [5]
0.00085 <8> (R-G3) [3]
0.00085 <8> (R-L0) [11]
0.00096 <19> (pre-tRNA^{Glu}-UUAN17, <19> 40 mM Tris-HCl pH 7.5, 2 mM dithiothreitol [25]) [25]
0.00097 <2> (trnI^{-}Thr-TAAATG-trailer, <2> native trailer sequence [22]) [22]
0.001 <8> (R-G15) [3]
0.0011 <8> (R-TASD2) [11]
0.0012 <8> (R-G19) [3]
0.0014 <8> (R-G13) [3]
0.00155 <19> (pre-tRNA^{Glu}-CUAN17, <19> 40 mM Tris-HCl pH 7.5, 2 mM dithiothreitol [25]) [25]
0.0016 <8> (R-TSA2) [11]
0.0019 <8> (R-TSD1) [11]
0.0022 <2> (trnI-Thr-CAAATG-trailer, <2> the first cytosine is the primary anti-determinant for RNaseZ [22]) [22]
0.0024 <2> (trnI-Thr-CCAATG-trailer, <2> CCA motif inhibits RNaseZ cleavage [22]) [22]

pH-Optimum

5.5 <17> [12]
6.5-8.5 <16> [12]
7.2 <7> [12]
7.5 <3, 8> (<3,8> assay at [2,4]) [2, 4]
7.9 <11> (<11> assay at [6]) [6]
8 <10, 13> (<10> nuclear RNase Z [1]) [1, 12, 13]
8.4 <10> (<10> mitochondrial RNase Z [1]) [1, 12]

Temperature optimum (°C)

28 <1> [8]
30 <10, 11, 13> (<11> assay at [6]; <10> nuclear RNase Z [1]) [1, 6, 12, 13]
35 <10> (<10> mitochondrial RNase Z [1]) [1, 12]
37 <3, 7, 8, 16> (<3,8> assay at [2,3,4]) [2, 3, 4, 12]
40 <17> [12]

4 Enzyme Structure

Molecular weight

40000 <6> (<6> SDS-PAGE [27]) [27]
64000 <10> (<10> gel filtration [16]) [16]
79000 <14> (<14> gel filtration [16]) [16]
82920 <1> (<1> calculated from sequence [28]) [28]

83000 <1> (<1> calculated from sequence [31]) [31]
86000 <6> (<6> SDS-PAGE [27]) [27]
90000 <1> (<1> SDS-PAGE [28]) [28]
92000 <1> (<1> SDS-PAGE [31]) [31]
122000 <10> (<10> gel filtration, nuclear RNase Z [1]) [1]
160000 <11> (<11> gel filtration [6]) [6]
165000 <10> (<10> gel filtration, mitochondrial RNase Z [1]) [1]

Subunits

? <10, 18> (<18> x * 45000, SDS-PAGE [16]; <10> x * 43000, mitochondrial RNase Z, SDS-PAGE [1]) [1, 16]
dimer <2, 5, 14, 20> (<2,5> homodimer, crystal structure [23,30]; <20> crystallization [24]; <14> cross-linking, SDS-PAGE [26]) [23, 24, 26, 30]
homodimer <10, 14> (<14> α_2, 2 * 41000, SDS-PAGE [16]; <10> α_2, 2 * 43000, SDS-PAGE [16]) [16]
monomer <1> (<1> 1 * 90000, SDS-PAGE [28]; <1> 1 * 92000, SDS-PAGE [31]) [28, 31]

5 Isolation/Preparation/Mutation/Application

Source/tissue

SF^-9 cell <6> [27]
cell culture <1, 2, 4, 5, 6, 9, 14, 15, 19, 20, 21> [18, 19, 22, 24, 25, 26, 28, 31, 32]
embryo <10> [9]
liver <7, 8> [2, 14, 19]
tuber <13> [13]

Localization

chloroplast <12> [9]
cytosol <3, 8, 10> [3, 20]
mitochondrion <3, 7, 8, 9, 10, 13, 22> [1, 4, 7, 13, 14]
nucleus <3, 8, 10> [1, 3]
soluble <17> [12]

Purification

<1> [32]
<1> (His-tag fusion, Ni-affinity chromatography) [28, 31]
<2> [19]
<2> (His-tag fusion, Ni-affinity chromatography) [22]
<2> (His-tag, Ni-affinity chromatography) [18]
<3> [3]
<4> (His-tag, Ni-affinity chromatography) [18]
<5> [19]
<5> (affinity chromatography, gel filtration) [23]
<6> [3, 19]
<6> (His-tag, Ni-affinity chromatography) [27]

<7> [14]
<8> [2]
<9> [19]
<10> (partial) [1]
<11> [6]
<13> [1, 13]
<14> [26]
<15> (His-tag, Ni-affinity chromatography) [18]
<17> [12]
<19> (nickel affinity chromatography) [25]
<20> [19]
<20> (chromatography steps) [24]
<20> (His-tag, Ni-affinity chromatography) [18]
<21> [19]
<21> (His-tag, Ni-affinity chromatography) [18]

Crystallization

<2> (cocrystallization with $tRNA^{Thr}$) [29]
<2> (the enzyme bears significant similarity to Zn-dependent metallo-β-lactamases, two Zn^{2+} in the active site are complexed by H63, H65, D67, H68, H140, D211 and H269) [30]
<5> (each monomer exhibits the typical metallo-β-lactamase fold, 2 Zn^{2+} ions are complexed at the active site) [23]
<20> [24]

Cloning

<1> [32]
<1> (expression in insect SF9 cells) [28, 31]
<2> (expression in Escherichia coli) [18, 19, 22]
<4> (expression in Escherichia coli) [18]
<5> (expression in Escherichia coli) [19, 23]
<6> (expression in Escherichia coli) [19]
<6> (expression in Escherichia coli BL21(DE3)) [27]
<9> (expression in Escherichia coli) [19]
<14> (expression in Escherichia coli) [14, 16, 20]
<14> (expression in Escherichia coli BL21(DE3)pLys) [26]
<15> (expression in Escherichia coli) [18]
<18> (expression in Escherichia coli) [16]
<19> (expression in Escherichia coli BL21(DE3)pLys) [25]
<20> (expression in Escherichia coli) [18, 19]
<20> (expression in Escherichia coli BL21(DE3)) [24]
<21> (expression in Escherichia coli) [18, 19]
<30> (expression in Escherichia coli) [20]

Engineering

A493T <1> (<1> processing efficiency similar to wild-type tRNase Z [28]) [28]

A501T <1> (<1> processing efficiency similar to wild-type tRNase Z [28]) [28]
C25G <14> (<14> dimerization, RNA binding, 33% activity compared to wild-type [26]) [26]
C40G <14> (<14> dimerization, RNA binding, similar activity compared to wild-type [26]) [26]
C442A <1> (<1> 12fold reduction of processing efficiency compared to wild-type [32]) [32]
C467A <1> (<1> 8fold reduction of processing efficiency compared to wild-type [32]) [32]
D185G <14> (<14> dimerization, RNA binding, 7% activity compared to wild-type [26]) [26]
D466A <1> (<1> 1500fold reduction of processing efficiency compared to wild-type [32]) [32]
D502T <1> (<1> processing efficiency reduced 7420fold compared to wild-type tRNase Z [28]) [28]
D58A <14> (<14> dimerization, RNA binding, no activity [26]) [26]
Deletion A252 <14> (<14> dimerization, RNA binding, no activity [26]) [26]
Δ200-212 <14> (<14> weak dimerization, no RNA binding, no activity [26]) [26]
Δ270-280 <14> (<14> multimerization, no RNA binding, no activity [26]) [26]
Δ49-164 <14> (<14> weak dimerization, no RNA binding, no activity [26]) [26]
Δ51-60 <14> (<14> weak dimerization, no RNA binding, no activity [26]) [26]
E208A <14> (<14> dimerization, RNA binding, 55% activity compared to wild-type [26]) [26]
E469A <1> (<1> 300fold reduction of processing efficiency compared to wild-type [32]) [32]
F51L <14> (<14> dimerization, RNA binding, similar activity compared to wild-type [26]) [26]
G184V <14> (<14> weak dimerization, no RNA binding, no activity [26]) [26]
G438A <1> (<1> 5000fold reduction of processing efficiency compared to wild-type [32]) [32]
G440A <1> (<1> 4fold reduction of processing efficiency compared to wild-type [32]) [32]
G468A <1> (<1> 900fold reduction of processing efficiency compared to wild-type [32]) [32]
G470A <1> (<1> 13fold reduction of processing efficiency compared to wild-type [32]) [32]
G473A <1> (<1> 8fold reduction of processing efficiency compared to wild-type [32]) [32]
G480A <1> (<1> 8fold reduction of processing efficiency compared to wild-type [32]) [32]

G506A <1> (<1> processing efficiency reduced 11fold compared to wild-type tRNase Z [28]) [28]
G62V <14> (<14> dimerization, RNA binding, 26% activity compared to wild-type [26]) [26]
H133L <14> (<14> dimerization, weak RNA binding, no activity [26]) [26]
H226L <14> (<14> dimerization, RNA binding, no activity [26]) [26]
H248L <14> (<14> multimerization, no RNA binding, no activity [26]) [26]
H498A <1> (<1> processing efficiency reduced 1950fold compared to wild-type tRNase Z [28]) [28]
H500A <1> (<1> processing efficiency reduced 3379fold compared to wild-type tRNase Z [28]) [28]
H503A <1> (<1> processing efficiency reduced 817fold compared to wild-type tRNase Z [28]) [28]
H504A <1> (<1> processing efficiency reduced 7fold compared to wild-type tRNase Z [28]) [28]
H54L <14> (<14> dimerization, weak RNA binding, no activity [26]) [26]
H56L <14> (<14> weak dimerization, weak RNA binding, no activity [26]) [26]
H59L <14> (<14> multimerization, no RNA binding, no activity [26]) [26]
H65A <2> (<2> the mutation was introduced at the central histidine to slow down hydrolysis of precursor tRNA during cocrystallization [29]) [29]
I443A <1> (<1> 8fold reduction of processing efficiency compared to wild-type [32]) [32]
I475A <1> (<1> 5fold reduction of processing efficiency compared to wild-type [32]) [32]
I494A <1> (<1> processing efficiency similar to wild-type tRNase Z [28]) [28]
I505A <1> (<1> processing efficiency similar to wild-type tRNase Z [28]) [28]
I508A <1> (<1> processing efficiency reduced 6fold compared to wild-type tRNase Z [28]) [28]
K203I <14> (<14> multimerization, no RNA binding, no activity [26]) [26]
K446A <1> (<1> 25fold reduction of processing efficiency compared to wild-type [32]) [32]
L205I <14> (<14> weak dimerization, RNA binding, 56% activity compared to wild-type [26]) [26]
L437A <1> (<1> 7fold reduction of processing efficiency compared to wild-type [32]) [32]
L464A <1> (<1> 5fold reduction of processing efficiency compared to wild-type [32]) [32]
L465A <1> (<1> 10fold reduction of processing efficiency compared to wild-type [32]) [32]
L478A <1> (<1> 11fold reduction of processing efficiency compared to wild-type [32]) [32]
L491A <1> (<1> processing efficiency reduced 7fold compared to wild-type tRNase Z [28]) [28]

L499A <1> (<1> processing efficiency similar to wild-type tRNase Z [28]) [28]
L507A <1> (<1> processing efficiency reduced 6fold compared to wild-type tRNase Z [28]) [28]
N445A <1> (<1> 5fold reduction of processing efficiency compared to wild-type [32]) [32]
N449A <1> (<1> 80fold reduction of processing efficiency compared to wild-type [32]) [32]
P178A <14> (<14> dimerization, RNA binding, 74% activity compared to wild-type [26]) [26]
P444A <1> (<1> 5fold reduction of processing efficiency compared to wild-type [32]) [32]
P64A <14> (<14> dimerization, RNA binding, similar activity compared to wild-type [26]) [26]
P83L <14> (<14> multimerization, no RNA binding, no activity [26]) [26]
Q474A <1> (<1> 750fold reduction of processing efficiency compared to wild-type [32]) [32]
Q492A <1> (<1> processing efficiency similar to wild-type tRNase Z [28]) [28]
R252G <14> (<14> dimerization, RNA binding, 26% activity compared to wild-type [26]) [26]
R448A <1> (<1> 625fold reduction of processing efficiency compared to wild-type [32]) [32]
R477A <1> (<1> 2600fold reduction of processing efficiency compared to wild-type [32]) [32]
S441A <1> (<1> 9fold reduction of processing efficiency compared to wild-type [32]) [32]
S497A <1> (<1> processing efficiency reduced 495fold compared to wild-type tRNase Z [28]) [28]
T186I <14> (<14> multimerization, no RNA binding, no activity [26]) [26]
T210I <14> (<14> weak dimerization, RNA binding, 85% activity compared to wild-type [26]) [26]
T439A <1> (<1> 2.5fold reduction of processing efficiency compared to wild-type [32]) [32]
T447A <1> (<1> 2fold reduction of processing efficiency compared to wild-type [32]) [32]
T471A <1> (<1> 5fold reduction of processing efficiency compared to wild-type [32]) [32]
V450A <1> (<1> 4fold reduction of processing efficiency compared to wild-type [32]) [32]
V463A <1> (<1> 6fold reduction of processing efficiency compared to wild-type [32]) [32]
V476A <1> (<1> 6fold reduction of processing efficiency compared to wild-type [32]) [32]
V496A <1> (<1> processing efficiency similar to wild-type tRNase Z [28]) [28]

Y140L <14> (<14> weak dimerization, RNA binding, 30% activity compared to wild-type [26]) [26]
Y253S <14> (<14> dimerization, RNA binding, 23% activity compared to wild-type [26]) [26]
Y472A <1> (<1> 4fold reduction of processing efficiency compared to wild-type [32]) [32]
Y479A <1> (<1> 7fold reduction of processing efficiency compared to wild-type [32]) [32]
Y495A <1> (<1> processing efficiency similar to wild-type tRNase Z [28]) [28]

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Oligoxyloglucan reducing-end-specific cellobiohydrolase 3.2.1.150

1 Nomenclature

EC number
3.2.1.150

Systematic name
oligoxyloglucan reducing-end cellobiohydrolase

Recommended name
oligoxyloglucan reducing-end-specific cellobiohydrolase

Synonyms
1,4-β-D-glucan cellobiohydrolase
1,4-β-glucan cellobiohydrolase
1,4-β-glucan cellobiosidase
C_1 cellulase
OXG-RCBH <2> [3, 4]
avicelase II
β-1,4-cellobiopyranosidase
β-1,4-cellobiosidase
β-1,4-glucan cellobiohydrolase
β-D-cellobiosidase
cellobiohydrolase
cellobiohydrolase I
cellobiohydrolase II
cellobiohydrolase, exo-
cellobiosidase
cellobiosidase, 1,4-β-glucan
cellulase, C1
cellulose 1,4-β-cellobiosidase
exo-1,4-β-D-cellobiohydrolase
exo-β-1,4-glucan cellobiohydrolase
exocellobiohydrolase
oligoxyloglucan reducing-end-specific cellobiohydrolase <2> [3, 4]
spezyme CP

CAS registry number
37329-65-0
753502-07-7

2 Source Organism

<1> *Geotrichum sp.* (no sequence specified) [1, 2]
<2> *Geotrichum sp.* (UNIPROT accession number: Q8J0D2) [3, 4]

3 Reaction and Specificity

Catalyzed reaction
hydrolysis of cellobiose from the reducing end of xyloglucans consisting of a β-1,4-linked glucan carrying α-D-xylosyl groups on O-6 of the glucose residues. To be a substrate, the first residue must be unsubstituted, the second residue may bear a xylosyl group, whether further glycosylated or not, and the third residue, which becomes the new terminus by the action of the enzyme, is preferably xylosylated, but this xylose residue must not be further substituted.

Reaction type
hydrolysis of O-glycosyl bond

Natural substrates and products
S oligoxyloglucan <1> (Reversibility: ?) [1, 2]
P ?

Substrates and products
S heptasaccharide XXXG <1> (Reversibility: ?) [1]
P trisaccharide XG + tetrasaccharide XX <1> [1]
S oligoxyloglucan <1> (<1> enzyme recognizes the reducing end of oligoxyloglucan and releases two glucosyl residue segments from the main chain [1,2]) (Reversibility: ?) [1, 2]
P ?
S xyloglucan heptasaccharide <2> (Reversibility: ?) [3]
P ?
S Additional information <2> (<2> no activity with β-1,3/4-glucan and carboxymethyl cellulose (CMC) [3]) (Reversibility: ?) [3]
P ?

Specific activity (U/mg)
11.6 <1> [1]
15 <2> (<2> with xyloglucan heptasaccharide as substrate [3]) [3]

pH-Optimum
3.5-5 <1> [1]

Temperature optimum (°C)
50-60 <1> [1]

4 Enzyme Structure

Molecular weight
97000 <1> (<1> SDS-PAGE [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> [1, 2]
<2> [4]

Renaturation
<1> (recombinant protein is expressed as insoluble inclusion bodies. Purified inclusion bodies were solubilized in 8 M urea. Renaturing by dialysis results in enzymatically active enzyme) [1]

Crystallization
<1> (hanging- and sitting drop vapor diffusion method with polyethylene glycol 3000 and polyethylene glycol 400 as precipitants) [2]
<2> [3]
<2> (at 2.2 A resolution) [4]

Cloning
<1> [1]
<2> (expression in Escherichia coli) [4]

Engineering
D35N <2> (<2> inactive [3,4]) [3, 4]
D465N <2> (<2> inactive [3,4]) [3, 4]
D490N <2> (<2> inactive [4]) [4]
E444Q <2> (<2> inactive [4]) [4]

6 Stability

pH-Stability
4-8 <1> (<1> stable at 45°C [1]) [1]

Temperature stability
50 <1> (<1> 10% loss of activity after 10 min at pH 4 [1]) [1]

References

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Xyloglucan-specific endo-β-1,4-glucanase 3.2.1.151

1 Nomenclature

EC number
3.2.1.151

Systematic name
1,4-β-D-glucan glucanohydrolase

Recommended name
xyloglucan-specific endo-β-1,4-glucanase

Synonyms
CaXTH1 <5> [21]
LeXTH1 <6> [19]
Mt-XTH1 <15> [17]
SIXTH5 <6> [20]
XEG
XEG5 <18> [15]
XEG74 <20> [15]
XG-ase
XH
XTH <6> [20]
endoxyloglucanase
oligoxyloglucan hydrolase
xyloglucan endo-β-1,4-glucanase
xyloglucan endohydrolase
xyloglucan endotransglucosylase/hydrolase <2, 4, 5, 6, 14, 15> [13, 17, 18, 20, 21]
xyloglucan endotransglucosylase/hydrolases <10> [14]
xyloglucan endotransglycosylase/hydrolase <6> [19]
xyloglucan-specific endo 1,4-β-glucanase
xyloglucan-specific endo-β-1,4-glucanase <18, 21> [15, 16]
xyloglucanase
xyloglucanendohydrolase
xyloglycan hydrolase

CAS registry number
76901-10-5

2 Source Organism

<1> *Pisum sativum* (no sequence specified) (<1> hoxH, β-subunit [7]) [2, 7]
<2> *Nicotiana tabacum* (no sequence specified) [18]
<3> *Glycine max* (no sequence specified) (<3> alkB4 gene from Rhodococcus NRRL B-16531 [6]) [6]
<4> *Arabidopsis thaliana* (no sequence specified) [18]
<5> *Cicer arietinum* (no sequence specified) [21]
<6> *Lycopersicon esculentum* (no sequence specified) [9,19,20]
<7> *Tamarindus indica* (no sequence specified) [8]
<8> *Lactuca sativa* (no sequence specified) [6]
<9> *Allium cepa* (no sequence specified) [6]
<10> *Oryza sativa* (no sequence specified) [14]
<11> *Aspergillus japonicus* (no sequence specified) [12]
<12> *Trichoderma viride* (no sequence specified) [11]
<13> *Glomus mosseae* (no sequence specified) [6]
<14> *Vigna angularis* (no sequence specified) [3, 4, 10, 13]
<15> *Medicago truncatula* (no sequence specified) [17]
<16> *Aspergillus niger* (UNIPROT accession number: Q8TFP1) [1]
<17> *Aspergillus aculeatus* (UNIPROT accession number: O94218) [5]
<18> *Paenibacillus sp.* (UNIPROT accession number: Q3MUH8) [15]
<19> *Populus tremulos tremuloides* (no sequence specified) [22]
<20> *Paenibacillus sp.* (UNIPROT accession number: Q3MUH7) [15]
<21> *Geotrichum sp. M128* (no sequence specified) [16]

3 Reaction and Specificity

Catalyzed reaction
xyloglucan + H_2O = xyloglucan oligosaccharides

Reaction type
hydrolysis of O-glycosyl bond

Natural substrates and products
S xyloglucan + H_2O <1, 2, 3, 4, 6, 8, 9, 12, 13, 14, 16, 17> (<2, 4> xyloglucan endotransglucosylase/hydrolase proteins act at the microfibril-matrix interface during cell elongation [18]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 9, 10, 11, 18]
P xyloglucan oligosaccharides <1, 14> [2, 4, 10]
S Additional information <5, 6, 14, 16> (<16> involved in plant cell wall polysaccharide degradation [1]; <6> involved in tissue softening of ripening fruits [9]; <14> increases the capacity of the cell wall to extend [3]; <6> SIXTH5 has no detectable effect on the mechanical properties of the wall [20]; <5> the enzyme encoded by CaXTH1 is involved in the cell expansion process of Cicer arietinum [21]) (Reversibility: ?) [1, 3, 9, 20, 21]
P ?

Substrates and products

- **S** GGXXXG + H_2O <21> (Reversibility: ?) [16]
- **P** GG + XXXG
- **S** GXGXXXG + H_2O <21> (Reversibility: ?) [16]
- **P** GXG + XXXG
- **S** XGXXXG + H_2O <21> (Reversibility: ?) [16]
- **P** XG + XXXG
- **S** XXGXXXG + H_2O <21> (Reversibility: ?) [16]
- **P** XXG + XXXG
- **S** XXXGXX + H_2O <21> (Reversibility: ?) [16]
- **P** XXXG + XX
- **S** XXXGXXX + H_2O <21> (Reversibility: ?) [16]
- **P** XXXG + XX
- **S** XXXGXXXG + H_2O <18> (Reversibility: ?) [15]
- **P** XXXG
- **S** XXXGXXXG + H_2O <20> (Reversibility: ?) [15]
- **P** XXX + XXXG + GXXXG
- **S** XXXXGXXXXG + H_2O <21> (Reversibility: ?) [16]
- **P** XXXXG
- **S** arabinoxyloglucan + H_2O <12> (Reversibility: ?) [11]
- **P** arabinoxyloglucan oligosaccharides
- **S** azo-cellulose + H_2O <16> (Reversibility: ?) [1]
- **P** azo-cellulose oligosaccharides
- **S** β-glucan + H_2O <16> (Reversibility: ?) [1]
- **P** β-glucan oligosaccharides
- **S** carboxymethyl cellulose + H_2O <12> (Reversibility: ?) [11]
- **P** carboxymethyl cellulose oligosaccharides
- **S** fucoxyloglucan + H_2O <12> (Reversibility: ?) [11]
- **P** fucoxyloglucan oligosaccharides
- **S** xyloglucan + H_2O <1, 2, 3, 4, 6, 8, 9, 11, 12, 13, 14, 16, 17, 18, 20, 21> (<16> best substrate [1]; <1> specifically cleaves the 1,4-β-glucosyl linkages of the xyloglucan backbone to yield mainly nona- and heptasaccharides [2]; <6,14> decreases the weight-average molecular weight of xyloglucans [3,9]; <14> enzyme has hardly any effect on xyloglucans of less than 60 kDa [4]; <14> enzyme has no endo-type transglycosylation activity [10]; <2,4> xyloglucan endotransglucosylase/hydrolase proteins act at the microfibril-matrix interface during cell elongation [18]; <11> high specific activity towards tamarind xyloglucan [12]; <21> tamarind and pea xyloglucan. The enzyme has at least four subsites (-2 to -2) and specifically recognizes xylose branching at the +1 and +2 sites, branching at the -1 site decreases the activity [16]; <6> the enzyme depolymerizes xyloglucan more rapidly in the presence than in the absence of xyloglucan oligosaccharides, indicative of a endotransglucosylase activity [20]; <18> XEG5 randomly cleaves the xyloglucan main chain [15]; <20> XEG74 has dual endo-mode and exo-mode activities [15]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 15, 16, 18, 20]

P xyloglucan oligosaccharides <1, 14> (<14> products are fragments of 5 kDa, no production of oligo- or monosaccharides [10]; <1> mainly nona- and heptasaccharides [2]; <14> products are fragments of about 50 kDa, no production of any oligo- or monosaccharides [4]; <11> XXXG, XXLG and XLLG oligosaccharides [12]) [2, 4, 10]

S Additional information <1, 5, 6, 7, 11, 14, 16, 18, 20, 21> (<16> no substrate: xylan [1]; <1> no substrate: swollen cellulose [2]; <1,14> no substrate: (1–3,1–4)-β-glucan [2,4]; <1,7,14> no substrate: carboxymethylcellulose [2,4,8]; <14> no substrate: hydroxyethyl cellulose [4]; <16> involved in plant cell wall polysaccharide degradation [1]; <6> involved in tissue softening of ripening fruits [9]; <14> increases the capacity of the cell wall to extend [3]; <6> SIXTH5 has no detectable effect on the mechanical properties of the wall [20]; <5> the enzyme encoded by CaXTH1 is involved in the cell expansion process of Cicer arietinum [21]; <18,20> no activity towards Avicel, carboxymethylcellulose, barley β-1,3/1,4-glucan or xylan [15]; <11> no activity with β-glucan and carboxymethylcellulose [12]; <21> no activity with carboxymethylcellulose, Avicel and barley 1,3-1,4-β-glucan. NO activity with GXXXG and XXXGG. Low activity with GGGX and XXXGX [16]) (Reversibility: ?) [1, 2, 3, 4, 8, 9, 12, 15, 16, 20, 21]

P ?

Activating compounds

oligosaccharides <14> (<14> xyloglucan oligosaccharides cause cell wall loosening by enhancing xyloglucan endotransglucosylase/hydrolase activity in azuki bean epicotyls [13]) [13]

Turnover number (min^{-1})

3 <20> (xyloglucan) [15]
12.7 <18> (xyloglucan) [15]
53 <11> (xyloglucan) [12]
113 <17> (xyloglucan, <17> from tamarind, pH 5, 37°C [5]) [5]

Specific activity (U/mg)

1.294 <1> [2]
19 <16> (<16> substrate: xyloglucan [1]) [1]
68 <21> [16]
260 <17> [5]
Additional information <18, 20> [15]

K_m-Value (mM)

0.64 <1> (xyloglucan, <1> pH 6.2, 35°C [2]) [2]
1 <14> (xyloglucan, <14> xyloglucans of 54 kDA, pH 6 [4]) [4]
3.6 <17> (xyloglucan, <17> pH 5, 37°C [5]) [5]
Additional information <11, 18, 20> (<11> K_M-value for xyloglucan is 0.67 mg/ml [12]; <20> K_M-value for xyloglucan is 1.2 mg/ml [15]; <18> K_M-value for xyloglucan is 2.0 mg/ml [15]) [12, 15]

pH-Optimum

4.5 <16> [1]
5 <3, 8, 9, 11, 14> (<3,8,9> 2 pH optima: pH 5 and pH 8 [6]) [6, 10, 12]
5.5 <21> [16]
5.5-6.5 <18> [15]
6-6.5 <20> [15]
6.2 <1> [2]
8 <3, 8, 9> (<3,8,9> 2 pH optima: pH 5 and pH 8 [6]) [6]

pH-Range

3-9 <3, 8, 9> (<3,8,9> 2 pH optima: pH 5 and pH 8, activity declines at pH 3,4,6,7, and 9 [6]) [6]
3.8-6.1 <11> (<11> more than 50% of maximal activity in the pH-range 3.8-6.1 [12]) [12]
4.5-8 <1> (<1> active between pH 4.5 and pH 8 [2]) [2]

Temperature optimum (°C)

35 <1> [2]
37-50 <3, 8, 9> [6]
50-55 <18> [15]
55 <21> [16]
60-70 <20> [15]

4 Enzyme Structure

Molecular weight

13000 <7> (<7> gel filtration [8]) [8]
23600 <17> (<17> mass spectrometry [5]) [5]
32000 <14> (<14> SDS-PAGE [4]) [4]
33000 <14> (<14> SDS-PAGE [10]) [10]
34000 <17> (<17> SDS-PAGE [5]) [5]
70000 <1> (<1> gel filtration [2,7]) [2, 7]
77000 <1> (<1> SDS-PAGE [2,7]) [2, 7]
90500 <16> (<16> calculated from amino acid sequence [1]) [1]

Subunits

? <11, 18, 20, 21> (<21> x * 80000, SDS-PAGE [16]; <20> x * 105000, SDS-PAGE [15]; <18> x * 40000, SDS-PAGE [15]; <11> x * 32000, SDS-PAGE [12]; <21> x * 80500, MALDI-TOF [16]) [12, 15, 16]

5 Isolation/Preparation/Mutation/Application

Source/tissue

epicotyl <5, 14> [4, 10, 13, 21]
epidermal cell <2, 4, 5> [18, 21]
epidermis <14> [3]

fruit <6> (<6> activity is highest at early stages of development [9]) [9]
internode <5> (<5> growing internodes of adult stem [21]) [21]
mycelium <13> [6]
mycorrhizal root <15> (<15> the Mt-XTH1 promoter directs expression in cell throughout the root system with significantly higher levels of activity in mycorrhizal roots. Mt-XTH1 expression is elevated not only in the regions of the roots colonized by the fungus, but also at sites distal to the infected regions [17]) [17]
root <3, 8, 9> [6]
root hair <2, 4> (<2,4> high enzyme action over all the length [18]) [18]
spore <13> [6]
stem <1> [2, 7]
vascular tissue <5> [21]

Localization
cell wall <14> [13]

Purification
<1> [7]
<1> (homogeneity) [2]
<7> [8]
<12> (endoI, endoIV and endoV) [11]
<14> [3, 4]
<14> (homogeneity) [10]
<16> [1]
<17> (homogeneity) [5]
<18> (recombinant) [15]
<20> (recombinant) [15]
<21> [16]

Cloning
<6> (expression in Pichia pastoris) [20]
<16> [1]
<17> [5]
<18> (expression in Escherichia coli) [15]
<20> (expression in Escherichia coli) [15]
<21> (expression in Escherichia coli) [16]

6 Stability

pH-Stability
3-3.8 <17> (<17> optimally stable, stability declines sharply below pH 2.8 and above pH 5 [5]) [5]
5 <11> (<11> 40°C, stable for at least 24 h [12]) [12]
5-7.5 <20> (<20> stable [15]) [15]
5-8 <18> (<18> at 45°C, stable [15]) [15]
5.5-6.5 <21> (<21> 45°C, stable [16]) [16]
5.5-8.5 <21> (<21> 25°C, stable [16]) [16]

Temperature stability

30 <16, 17> (<16> 10% loss of activity after 145 h at pH 4.5 [1]; <17> optimally stable below [5]) [1, 5]
40 <11> (<11> pH 5.0, stable for at least 24 h [12]) [12]
50 <11, 17, 21> (<11> half-life: 24 h [12]; <17> 80% loss of activity after 2 h [5]; <21> pH 5.5, 10 min, more than 90% of its activity is retained [16]) [5, 12, 16]
55 <16, 18, 20> (<20> stable up to [15]; <18> 10 min, less than 10% loss of activity [15]; <16> 80% loss of activity after 145 h and 50% loss of activity after 48 h at pH 4.5 [1]) [1, 15]
60 <11> (<11> 28 h, 50% loss of activity [12]) [12]

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Mannosylglycoprotein endo-β-mannosidase 3.2.1.152

1 Nomenclature

EC number
3.2.1.152

Recommended name
mannosylglycoprotein endo-β-mannosidase

Synonyms
endo-β-mannosidase <2, 3, 4> [1, 2, 3, 4]

CAS registry number
141176-95-6

2 Source Organism

<1> *Brassica oleracea* (no sequence specified) [5]
<2> *Lilium longiflorum* (no sequence specified) [1, 2, 4]
<3> *Arabidopsis thaliana* (UNIPROT accession number: Q75W54) [4]
<4> *Lilium longiflorum* (UNIPROT accession number: AB185918) [3]

3 Reaction and Specificity

Catalyzed reaction
Hydrolysis of the α-D-mannosyl-(1-6)-β-D-mannosyl-(1-4)-β-D-N-acetylglucosaminyl-(1-4)-β-D-N-acetylglucosaminyl sequence of glycoprotein to α-D-mannosyl-(1-6)-D-mannose and β-D-N-acetylglucosaminyl-(1-4)-β-D-N-acetylglucosaminyl sequences

Reaction type
hydrolysis

Natural substrates and products
S Man-β-(1-4)GlcNAc + H_2O <2, 3, 4> (<2,3,4> hydrolysis of Man-β-(1-4)GlcNAc linkage in the trimannosyl core structure of N-glycans [3,4]; <2> hydrolysis of Man-β-(1-4)GlcNAc linkage of N-linked sugar chains [1]; <2> hydrolysis of Man-β-(1-4)GlcNAc linkage of N-linked sugar chains, possible role in the catabolism of N-linked sugar chains leading to oligosaccharides [2]) (Reversibility: ir) [1, 2, 3, 4]
P ?

Substrates and products

- **S** (Man)nMan-α-(1-6)-Man-β-(1-4)GlcNAc-β-(1-4)GlcNAc-peptide + pyridylamino-GlcNAc-β-(1-4)GlcNAc <2> (<2> n = 0-2 [1]) (Reversibility: ?) [1]
- **P** pyridylamino-(Man)nMan-α-(1-6)-Man-β-(1-4)GlcNAc-β-(1-4)GlcNAc + GlcNAc-β-(1-4)GlcNAc-peptide
- **S** Man-α(1-6)[Man-α(1-3)]Man-α-(1-6)Man-β-(1-4)GlcNAc-β-(1-4)GlcNAc-pyridylamide + H_2O <1> (Reversibility: ?) [5]
- **P** Man-α(1-6)[Man-α(1-3)]Man-α-(1-6)Man + GlcNAc-β-(1-4)GlcNAc-pyridylamide
- **S** Man-α-(1-6)Man-α(1-6)Man-β-(1-4)GlcNAc-β-(1-4)GlcNAc-pyridylamide + H_2O <1> (Reversibility: ?) [5]
- **P** Man-α-(1-6)Man-α(1-6)Man + GlcNAc-β-(1-4)GlcNAc-pyridylamide
- **S** Man-α-(1-6)Man-β-(1-4)GlcNAc-β-(1-4)GlcNAc-pyridylamide + H_2O <1> (Reversibility: ?) [5]
- **P** Man-α-(1-6)Man + GlcNAc-β-(1-4)GlcNAc-pyridylamide
- **S** Man-α-(1-6)Man-β-(1-4)GlcNAc-β-(1-4)GlcNAc-pyridylamine + H_2O <2, 3, 4> (Reversibility: ir) [1, 2, 3, 4]
- **P** GlcNAc-β-(1-4)GlcNAc-pyridylamine + Man-α-(1-6)Man
- **S** Man-β-(1-4)GlcNAc + H_2O <2, 3, 4> (<2, 3, 4> hydrolysis of Man-β-(1-4)GlcNAc linkage in the trimannosyl core structure of N-glycans [3,4]; <2> hydrolysis of Man-β-(1-4)GlcNAc linkage of N-linked sugar chains [1]; <2> hydrolysis of Man-β-(1-4)GlcNAc linkage of N-linked sugar chains, possible role in the catabolism of N-linked sugar chains leading to oligosaccharides [2]; <2> hydrolysis of Man-β-1-4GlcNAc linkage of N-linked sugar chains [2]) (Reversibility: ir) [1, 2, 3, 4]
- **P** ?
- **S** Man-β-(1-4)GlcNAc-β-(1-4)GlcNAc-peptide + *p*-nitrophenyl-β-D-glucoside <2> (Reversibility: ?) [1]
- **P** Man-β-(1-4)GlcNAc-β-(1-4)Glc-β-1-*p*-nitrophenyl + GlcNAc-peptide
- **S** Man-β-(1-4)GlcNAc-β-(1-4)GlcNAc-peptide + *p*-nitrophenyl-β-D-mannoside <2> (Reversibility: ?) [1]
- **P** Man-β-(1-4)GlcNAc-β-1-*p*-nitrophenyl-β-D-mannoside + GlcNAc-peptide
- **S** Man-β-(1-4)GlcNAc-β-(1-4)GlcNAc-peptide + *p*-nitrophenyl-β-N-acetylglucosaminide <2> (Reversibility: ?) [1]
- **P** Man-β-(1-4)GlcNAc-β-1-*p*-nitrophenyl-β-N-acetylglucosaminide + GlcNAc-peptide
- **S** Man-β-1-4GlcNAc + H_2O <2> (<2> hydrolysis of Man-β-1-4GlcNAc linkage of N-linked sugar chains [1]) (Reversibility: ir) [1]
- **P** ?
- **S** Additional information <2, 3, 4> (<2> enzyme does not hydrolyze β-(1-4)-mannohexaose or *p*-nitrophenyl-β-mannopyranoside [2]; <2, 3, 4> enzyme does not hydrolyze pyridylamino-sugar chains containing Man-α-(1-3)Man-β or Xylose-β-(1-2)Man-β, enzyme does not hydrolyze *p*-nitrophenyl-β-mannoside [3, 4]; <2> *p*-nitrophenyl-β-N-acetylgalactosaminide does not work as acceptor in the transfer reaction [1]) (Reversibility: ?) [1, 2, 3, 4]
- **P** ?

Specific activity (U/mg)
0.004 <2> (<2> purified enzyme, pH 5.0, 37°C [2]) [2]
0.026 <3> (<3> purified enzyme, pH 5.0, 37°C [4]) [4]

K_m-Value (mM)
1.2 <4> (Man-α-(1-6)Man-β-(1-4)GlcNAc-β-(1-4)GlcNAc-pyridylamine, <4> pH 5.0, 37°C [3]) [3]
1.4 <2> (Man-α-(1-6)Man-β-(1-4)GlcNAc-β-(1-4)GlcNAc-pyridylamine, <2> pH 5.0, 37°C [2]) [2]

pH-Optimum
5 <2, 3, 4> [2, 3, 4]

pH-Range
4-6 <4> [3]

4 Enzyme Structure

Molecular weight
78000 <2> (<2> gel filtration [2,4]) [2, 4]
108000 <4> (<4> calculated from the deduced amino acid sequence [3]) [3]
112000 <3> [4]

Subunits
? <2> (<2> three bands on SDS-PAGE of 42000, 31000, and 28000 Da [4]) [4]
trimer <4> (<4> αβγ, 1 * 42000 + 1 * 31000 + 1 * 28000, SDS-PAGE [3]) [3]

Posttranslational modification
proteolytic modification <4> (<4> enzyme is encoded by a single cDNA, translation product is posttranslationally processed by proteolysis [3]) [3]

5 Isolation/Preparation/Mutation/Application

Source/tissue
bulb <4> (<4> highest activity [3]) [3]
flower <2, 3, 4> [1, 2, 3, 4]
leaf <4> [3]
pistil <4> [3]
stamen <4> [3]
stem <4> [3]

Purification
<1> [5]
<2> [1, 4]
<2> (partial) [2]
<3> (partial purification of the recombinant protein) [4]

Cloning

<3> (expressed in Escherichia coli RosettaBlue) [4]
<4> [3]

Engineering

E464A <3> (<3> no activity [4]) [4]
E549A <3> (<3> no activity [4]) [4]

Application

synthesis <2> (<2> possible application in the synthesis of oligosaccharides containing mannosyl-β-1,4-structures [1]) [1]

6 Stability

pH-Stability

3-7 <3> (<3> unstable below pH 3 and above pH 7 [4]) [4]
3-8 <4> (<4> unstable below pH 3 and above pH 8 [3]) [3]
Additional information <2> (<2> unstable at alkaline pH [2]) [2]

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Fructan β-(2,1)-fructosidase 3.2.1.153

1 Nomenclature

EC number
3.2.1.153

Systematic name
β-(2,1)-D-fructan fructohydrolase

Recommended name
fructan β-(2,1)-fructosidase

Synonyms
1-FEH <3> [9]
1-FEH II <4> [7]
1-FEH IIa <5> [8]
1-FEH w1 <1, 4> [5, 7]
1-FEH w2 <1> [5]
1-fructan exohydrolase <4, 5> [2, 7]
1-fructoexohydrolase <3> [9]
β-(2-1) fructan ewxohydrolase <4> [7]
β-(2-1)-D-fructan fructohydrolase <4> [7]
β-(2-1)-linkage specific FEH <4> [7]
β-(2-1)-linkage specific fructan-β-fructosidase <4> [7]
β-(2-1)-linkage-specific fructan-β-fructosidase <4> [7]
fructan 1-exohydrolase <5> [8]
fructan 1-exohydrolase IIa <5> [3, 4]
fructan 1-exohydrolase IIb <5> [4]
fructan 1-exohydrolase w1 <1> [5]
fructan 1-exohydrolase w2 <1> [5]
inulinase <4> [7]

CAS registry number
1000593-08-7
37288-56-5

2 Source Organism

<1> *Triticum aestivum* (no sequence specified) [5]
<2> *Hordeum vulgare* (no sequence specified) [1]
<3> *Allium cepa* (no sequence specified) [9]
<4> *Helianthus tuberosus* (no sequence specified) [7]

<5> *Cichorium intybus* (no sequence specified) [2,3,4,8]
<6> *Lolium rigidum* (no sequence specified) [6]

3 Reaction and Specificity

Catalyzed reaction
hydrolysis of terminal, non-reducing 2,1-linked β-D-fructofuranose residues in fructans

Reaction type
hydrolysis of O-glycosyl bond

Natural substrates and products
S Additional information <1, 5> (<5> the enzyme is involved in fructan breakdown [3]; <1> isoenzyme 1-FEH w2 might play a role as a β-(2,1)-trimmer throughout the period of active graminan biosynthesis [5]; <5> isoform 1-FEH II is expressed when young chicory plants are defoliated, suggesting that this enzyme can be induced at any developmental stage when large energy supplies are necessary [4]; <5> the enzyme is involved in fructan degradation [8]) (Reversibility: ?) [3, 4, 5, 8]
P ?

Substrates and products
S 1,1,1-kestopentaose + H_2O <4, 6> (Reversibility: ?) [6, 7]
P ?
S 1,1-kestotetraose + H_2O <2, 4> (<2> 85% of the activity with 6G,1-kestotetraose [1]) (Reversibility: ?) [1, 7]
P ?
S 1,1-nystose + H_2O <1, 5> (<1> 72% of the activity with 1-kestose, isoenzyme 1-FEH w1 [5]; <1> 83% of the activity with 1-kestose, isoenzyme 1-FEH w2 [5]) (Reversibility: ?) [2, 5]
P ?
S 1-kestose + H_2O <1, 5> (Reversibility: ?) [2, 5]
P ?
S 1-kestotriose + H_2O <2> (<2> 88% of the activity with 6G,1-kestotetraose [1]) (Reversibility: ?) [1]
P ?
S 1G-kestotetraose + H_2O <2> (<2> 96% of the activity with 6G,1-kestotetraose [1]) (Reversibility: ?) [1]
P ?
S 6-kestose + H_2O <1> (<1> 0.7% of the activity with 1-kestose, isoenzyme 1-FEH w1 [5]; <1> 1% of the activity with 1-kestose, isoenzyme 1-FEH w2 [5]) (Reversibility: ?) [5]
P ?
S 6G,1-kestotetraose + H_2O <2> (Reversibility: ?) [1]
P ?

S 6G,6-kestotetraose + H_2O <2> (<2> 4% of the activity with 6G,1-kestotetraose [1]) (Reversibility: ?) [1]
P ?
S 6G-kestotetraose + H_2O <2> (Reversibility: ?) [1]
P ?
S 6G-kestotriose + H_2O <2> (<2> 5% of the activity with 6G,1-kestotetraose [1]) (Reversibility: ?) [1]
P ?
S inulin + H_2O <1> (<1> 22% of the activity with 1-kestose, isoenzyme 1-FEH w2 [5]; <1> 39% of the activity with 1-kestose, isoenzyme 1-FEH w1 [5]) (Reversibility: ?) [5]
P ?
S inulin + H_2O <4, 5> (<4> multichain mechanism [7]) (Reversibility: ?) [2, 7, 8]
P fructose
S levan + H_2O <1> (<1> 3% of the activity with 1-kestose, isoenzyme 1-FEH w2 [5]; <1> 4% of the activity with 1-kestose, isoenzyme 1-FEH w1 [5]) (Reversibility: ?) [5]
P ?
S neokestose + H_2O <1> (<1> 3% of the activity with 1-kestose, isoenzyme 1-FEH w2 [5]; <1> 4% of the activity with 1-kestose, isoenzyme 1-FEH w1 [5]) (Reversibility: ?) [5]
P ?
S sucrose + H_2O <2> (<2> 3% of the activity with 6G,1-kestotetraose [1]) (Reversibility: ?) [1]
P ?
S Additional information <1, 4, 5> (<5> the enzyme is involved in fructan breakdown [3]; <1> isoenzyme 1-FEH w2 might play a role as a β-(2,1)-trimmer throughout the period of active graminan biosynthesis [5]; <5> isoform 1-FEH II is expressed when young chicory plants are defoliated, suggesting that this enzyme can be induced at any developmental stage when large energy supplies are necessary [4]; <5> the enzyme is involved in fructan degradation [8]; <4> the enzyme hydrolyzes terminal β-(2-1)-fructosylfructose-linkages in linear and branched fructan oligomers. β-(2-6)-linkages are hardly hydrolyzed [7]; <5> the enzyme hydrolyzes the β(2-1) linkage in inulin, 1-kestose and 1,1-nystose at rates at least 5fold faster than the β(2-6) linkages in levan oligosaccharides and levanbiose [2]) (Reversibility: ?) [2, 3, 4, 5, 7, 8]
P ?

Inhibitors

$CaCl_2$ <5> (<5> 1 mM, partial [2]) [2]
Na-EDTA <5> (<5> 1 mM, partial [2]) [2]
sucrose <2, 4, 5, 6> (<5> strong [2]; <2> mixed-type inhibitor [1]; <4> inhibition by sucrose is a mechanism for controlling fructan degradation in plants [7]) [1, 2, 6, 7]

Specific activity (U/mg)
12.2 <1> (<1> isoenzyme 1-FEH w2 [5]) [5]
14.6 <1> (<1> isoenzyme 1-FEH w1 [5]) [5]

K_m-Value (mM)
7 <1> (1-kestose, <1> isoenzyme1-FeH w1 [5]; <1> isoenzyme1-FeH w2 [5]) [5]
8.3 <4> (1,1-kestotetraose) [7]
12 <4> (1,1,1-kestopentaose) [7]
58 <5> (1-kestose) [2]
64 <5> (1,1-nystose) [2]

K_i-Value (mM)
5.9 <5> (Sucrose) [2]

pH-Optimum
5-5.5 <5> [2]
5.2 <4> [7]

Temperature optimum (°C)
35 <5> [2]

4 Enzyme Structure

Molecular weight
33000 <2> [1]
60000 <5> (<5> gel filtration [2]) [2]
75000 <4> (<4> gel filtration [7]) [7]

Subunits
? <1> (<1> x * 70000, isoenzyme 1-FEH w1, SDS-PAGE [5]; <1> x * 70000, isoenzyme 1-FEH w2, SDS-PAGE [5]) [5]
monomer <4, 5> (<4> 1 * 75000, SDS-PAGE [7]; <5> 1 * 64000, SDS-PAGE [2]) [2, 7]

Posttranslational modification
Glycoprotein <1> (<1> glycosylation on at least two of four potential N-glycosylation sites, isoenzyme 1-FEH w1 [5]; <1> glycosylation on at least two of four potential N-glycosylation sites, isoenzyme 1-FEH w2 [5]) [5]

5 Isolation/Preparation/Mutation/Application

Source/tissue
bulb <3> [9]
root <5> [2, 4]
stem <1, 2> [1, 5]
tuber <4> (<4> activity is low in growing tubers but increases during dormancy and sprouting [7]) [7]

Purification

<1> (isoenzyme 1-FEH w1) [5]
<1> (isoenzyme 1-FEH w2) [5]
<2> [1]
<4> [7]
<5> [2]
<5> (fructan 1-exohydrolase IIa and fructan 1-exohydrolase IIb) [4]
<6> (partial, enzyme contains three activities that hydrolyze β-2,6-glycosidic linkages faster than β-2,1-glycosidic linkages and two activities hydrolyze β-2,1-glycosidic linkages faster than β-2,6-glycosidic linkages) [6]

Crystallization

<5> (hanging drop vapour diffusion method of recombinant enzyme heterologously expressed in Pichia pastoris. The structure of the isoenzyme 1-FEH IIa is determined at a resolution of 2.35 A. The structure consists of an N-terminal fivefold β-propeller domain) [8]
<5> (hanging-drop vapour-diffusion method at 4°C. The crystals are tetragonal belonging to space group P4(1)2(1)2 or P4(3)2(1)2, with unit-cell parameters a = 139.83, b = 139.83, c = 181.94 A) [3]

Cloning

<1> (isoenzyme 1-FEH w1) [5]
<1> (isoenzyme 1-FEH w2) [5]
<5> (fructan 1-exohydrolase IIa and fructan 1-exohydrolase IIb) [4]

6 Stability

Temperature stability

40 <4> (<4> 1 h, enzyme retains full activity [7]) [7]

General stability information

<3>, during storage at 15°C, 1-FEH in onion bulbs is low but peaks abruptly after 12 and 16 weeks, respectively, after which it decreases to levels higher to that observed at the beginning of the storage [9]

Storage stability

<3>, during storage at 15°C, 1-FEH in onion bulbs is low but peaks abruptly after 12 and 16 weeks, respectively, after which it decreases to levels higher to that observed at the beginning of the storage [9]

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Fructan β-(2,6)-fructosidase 3.2.1.154

1 Nomenclature

EC number
3.2.1.154

Systematic name
β-(2,6)-D-fructan fructohydrolase

Recommended name
fructan β-(2,6)-fructosidase

Synonyms
6-FEH <1, 4, 10> [4, 5, 7]
AtcwINV3 <3> [11]
β-(2-6)-fructan exohydrolase <10> [7]
β-(2-6)-linkage specific fructan-β-fructosidase <6> [8]
fructan 6-exohydrolases <1> [5]
levanase <9, 10> [1, 7]

CAS registry number
1000597-62-5
37288-56-5

2 Source Organism

<1> *Beta vulgaris* (no sequence specified) [5]
<2> *Triticum aestivum* (no sequence specified) [10]
<3> *Arabidopsis thaliana* (no sequence specified) [11]
<4> *Avena sativa* (no sequence specified) [4]
<5> *Lactobacillus pentosus* (no sequence specified) [6]
<6> *Lolium perenne* (no sequence specified) [8]
<7> *Lactobacillus paracasei* (no sequence specified) [9]
<8> *Streptococcus mutans* (no sequence specified) [2,3]
<9> *Actinomyces viscosus* (no sequence specified) [1]
<10> *Lolium rigidum* (no sequence specified) [7]

3 Reaction and Specificity

Catalyzed reaction

hydrolysis of terminal, non-reducing 2,6-linked β-D-fructofuranose residues in fructans

Reaction type

hydrolysis of O-glycosyl bond

Substrates and products

S 1,1,1-kestose + H_2O <5> (<5> 45% of the activity with levan [6]) (Reversibility: ?) [6]
P ?
S 1,1-kestose + H_2O <5> (<5> 39% of the activity with levan [6]) (Reversibility: ?) [6]
P ?
S 1,1-kestotetraose + H_2O <6> (<6> 5fold lower activity than with 6,6-kestotetraose [8]) (Reversibility: ?) [8]
P ?
S 1,6G-kestotatraose + H_2O <4> (Reversibility: ?) [4]
P 1-kestotriose + fructose
S 1-kestose + H_2O <1, 5> (<5> 12% of the activity with levan [6]; <1> native enzyme shows 12% of the activity with levan [5]) (Reversibility: ?) [5, 6]
P ?
S 6,6,6-kestopentaose + H_2O <10> (Reversibility: ?) [7]
P ?
S 6,6-kestotetraose + H_2O <6> (Reversibility: ?) [8]
P ?
S 6-kestose + H_2O <1> (<1> native enzyme shows 77% of the activity with levan [5]) (Reversibility: ?) [5]
P ?
S 6-kestose + H_2O <2> (<2> as active as levan with native enzyme, 85% of the activity with levan with the heterologous enzyme [10]) (Reversibility: ?) [10]
P sucrose + D-fructose
S 6G,1,6-kestopentaose + H_2O <4> (Reversibility: ?) [4]
P fructose + 6G-kestotriose + ?
S 6G,6-kestotetraoase + H_2O <4> (Reversibility: ?) [4]
P fructose + 6G-kestotriose
S 6G-kestotriose + H_2O <4> (Reversibility: ?) [4]
P sucrose + fructose
S bacterial levan + H_2O <2> (Reversibility: ?) [10]
P ?
S garlic fructan + H_2O <5> (<5> 60% of the activity with levan [6]) (Reversibility: ?) [6]
P ?

S inulin + H_2O <4, 5> (<4> 11.9% of the activity with 6G,6-kestotetraoase [4]; <5> 9% of the activity with levan [6]) (Reversibility: ?) [4, 6]
P ?
S inulin + H_2O <7, 8> (<8> 13% of the activity with levan [3]; <8> 34% of the activity with levan [2]) (Reversibility: ?) [2, 3, 9]
P fructose
S levan + H_2O <1, 4, 9> (<4> 8.5% of the activity with 6G,6-kestotetraoase [4]) (Reversibility: ?) [1, 4, 5]
P ?
S levan + H_2O <5, 8> (<5> best substrate [6]; <8> exo-type mechanism [3]) (Reversibility: ?) [2, 3, 6]
P fructose
S levanbiose + H_2O <1> (<1> native enzyme shows 63% of the activity with levan [5]) (Reversibility: ?) [5]
P ?
S levanbiose + H_2O <2> (<2> i.e. O-β-D-fructofuranosyl-(2-6)-β-D-fructofuranoside. 63% of the activity with levan with the native enzyme, 57% of the activity with levan with the heterologous enzyme [10]) (Reversibility: ?) [10]
P D-fructose
S neokestin + H_2O <1, 4> (<1> native enzyme shows 25% of the activity with levan [5]) (Reversibility: ?) [4, 5]
P ?
S neokestose + H_2O <1> (<1> native enzyme shows 93% of the activity with levan [5]) (Reversibility: ?) [5]
P ?
S phlein + H_2O <1, 2> (<1> native enzyme shows 84% of the activity with levan [5]; <2> 95% of the activity levan with native enzyme, 120% of the activity with levan with the heterologous enzyme [10]) (Reversibility: ?) [5, 1]
P ?
S raffinose + H_2O <8> (<8> 12% of the activity with levan [2]; <8> 5% of the activity with levan [3]) (Reversibility: ?) [2, 3]
P ?
S sucrose + H_2O <5, 8> (<8> 21% of the activity with levan [2]; <5> 3% of the activity with levan [6]; <8> 9% of the activity with levan [3]) (Reversibility: ?) [2, 3, 6]
P ?
S Additional information <1, 4, 7, 9> (<4> no activity with sucrose [4]; <7> low ability to hydrolyze sucrose [9]; <1> no activity with sucrose, 1,1-nystose and inulin [5]; <9> no cleavage of inulin, dextran, sucrsoe, raffinose and melezitose [1]) (Reversibility: ?) [1, 4, 5, 9]
P ?

Inhibitors

Ag^+ <8> (<8> 1 mM, almost complete inhibition [2]) [2]
$BaCl_2$ <9> (<9> 1 mM, 64% inhibition [1]) [1]

Co^{2+} <5> (<5> 4 mM, 32% inhibition [6]) [6]
Cu^{2+} <5, 8> (<8> 1 mM, about 20% inhibition [2]; <5> 4 mM, 39% inhibition [6]) [2, 6]
EDTA <5, 9> (<9> 1 mM, complete inhibition [1]; <5> 4 mM, 60% inhibition [6]) [1, 6]
Fe^{2+} <5> (<5> 4 mM, 94% inhibition [6]) [6]
Fe^{3+} <8> (<8> 1 mM, 90% inhibition [3]) [3]
$FeCl_3$ <9> (<9> 1 mM, 70% inhibition [1]) [1]
Hg^{2+} <8> (<8> 1 mM, complete inhibition [3]; <8> 0.01 mM, almost complete inhibition [2]) [2, 3]
$HgCl_2$ <9> (<9> 1 mM, complete inhibition [1]) [1]
Mg^{2+} <5, 8> (<8> 1 mM, about 20% inhibition [2]; <5> 4 mM, 42% inhibition [6]) [2, 6]
$MnCl_2$ <9> (<9> 1 mM, 19% inhibition [1]) [1]
PCMB <5> (<5> 4 mM, complete inhibition [6]) [6]
sucrose <1, 6, 10> [5, 7, 8]
Zn^{2+} <5, 8> (<8> 1 mM, 70% inhibition [3]; <5> 4 mM, 33% inhibition [6]) [3, 6]
$ZnCl_2$ <9> (<9> 1 mM, 93% inhibition [1]) [1]
Additional information <2, 8> (<8> no inhibition by F^- or Tris. No evidence of product inhibition by fructose [2]; <2> not inhibited by sucrose [10]) [2, 10]

Metals, ions
Co^{2+} <8> (<8> slight stimulation [2]) [2]
Mn^{2+} <8> (<8> slight stimulation [2]) [2]

Specific activity (U/mg)
12.5 <1> [5]
13.9 <8> [3]
66.4 <8> (<8> enzyme from Streptococcus mutans [2]) [2]
204.5 <8> (<8> recombinant enzyme expressed in Escherichia coli [2]) [2]
260.9 <5> [6]
851.2 <9> [1]

K_m-Value (mM)
77 <1> (6-kestose) [5]
Additional information <4> (<4> the K_M-value for neokestin is 2.8% w/v. The K_m-value for 6G,6-kestotetraose is 5.6% w/v [4]) [4]

K_i-Value (mM)
134 <1> (sucrose) [5]

pH-Optimum
4.5 <8> (<8> sucrose degradation [2]) [2]
4.5-5 <4> (<4> hydrolysis of neokestin [4]) [4]
5 <1, 8> (<8> hydrolysis of sucrose [3]) [3, 5]
5.1-5.6 <6> [8]

5.5 <5, 8> (<8> hydrolysis of levan [3]; <8> reaction with levan or inulin [2]) [2, 3, 6]
6 <8, 9> (<8> hydrolysis of inulin [3]) [1, 3]

pH-Range

3.5-6.5 <4> (<4> pH 3.5: about 70% of maximal activity, pH 6.5: about 55% of maximal activity, hydrolysis of neokestin [4]) [4]
3.5-7 <5> (<5> pH 3.5: about 55% of maximal activity, pH 7.0: about 60% of maximal activity [6]) [6]
4-6 <8> (<8> pH 4.0: about 85% of maximal activity, pH 6.0: about 70% of maximal activity, reaction with sucrose [2]) [2]
4-7 <8> (<8> pH 4.0: about 40% of maximal activity, pH 7.0: about 50% of maximal activity, reaction with levan or inulin [2]) [2]
4.3-6.8 <1> (<1> pH 4.3: about 65% of maximal activity, pH 6.8: about 40% of maximal activity [5]) [5]
5-7.5 <9> (<9> pH 5.0: about 50% of maximal activity, pH 7.5: about 60% of maximal activity [1]) [1]

Temperature optimum (°C)

25 <5> [6]
30 <1> [5]
45 <9> [1]
55 <8> (<8> hydrolysis of levan [3]) [3]

Temperature range (°C)

10-40 <5> (<5> 10°C: 60% of maximal activity, 40°C: about 50% of maximal activity [6]) [6]
18-38 <1> (<1> 18°C: about 45% of maximal activity, 38°C: about 20% of maximal activity [5]) [5]
35-50 <9> (<9> 35°C: about 50% of maximal activity, 50°C: about 80% of maximal activity [1]) [1]
55-60 <8> (<8> 55°C: maximal activity, 60°C: 61% of maximal activity [3]) [3]

4 Enzyme Structure

Molecular weight

65000 <6> (<6> gel filtration [8]) [8]
126000 <5> (<5> gel filtration [6]) [6]

Subunits

? <4, 7, 8, 9> (<7> x * 42000, SDS-PAGE [9]; <4> x * 43000, SDS-PAGE [4]; <8> x * 140000, SDS-PAGE [2]; <9> x * 89000, SDS-PAGE [1]; <8> x * 127000, SDS-PAGE [3]) [1, 2, 3, 4, 9]
monomer <2, 5, 6> (<6> 1 * 69000, SDS-PAGE [8]; <5> 1 * 125000, SDS-PAGE [6]; <2> 1 * 70000, SDS-PAGE [10]) [6, 8, 10]

Posttranslational modification
glycoprotein <6> [8]

5 Isolation/Preparation/Mutation/Application

Source/tissue
culture filtrate <7> [9]
culture supernatant <8, 9> [1, 3]
leaf <1> [5]
petiole <1> [5]
root <1> [5]
spike <2> [10]
stem <6> (<6> enzyme is induced after defoliation [8]) [8]

Localization
cytoplasm <8> [2]
cytoplasmic membrane <8> [2]
extracellular <5, 7, 8> [3, 6, 9]

Purification
<1> [5]
<2> [10]
<4> [4]
<5> [6]
<6> [8]
<7> [9]
<8> [2]
<9> [1]
<10> (partial, enzyme contains three activities that hydrolyze β-2,6-glycosidic linkages faster than β-2,1-glycosidic linkages and two activities hydrolyze β-2,1-glycosidic linkages faster than β-2,6-glycosidic linkages) [7]

Cloning
<1> (heterologous expression in Pichia pastoris) [5]
<2> (expression in Pichia pastoris) [10]
<3> (expression in Pichia pastoris) [11]
<8> (fruA is expressed under its own control in Escherichia coli JM83(pFRU1)) [2]

6 Stability

Temperature stability
Additional information <6> (<6> temperature stability is increased in the presence of 6,6-kestotetraose [8]) [8]

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Xyloglucan-specific exo-β-1,4-glucanase 3.2.1.155

1 Nomenclature

EC number
3.2.1.155

Systematic name
[(16)-α-D-xylo]-(14)-β-D-glucan exo-glucanohydrolase

Recommended name
xyloglucan-specific exo-β-1,4-glucanase

Synonyms
Cel74A <4> [1]
XEG74 <5> [2]
XG <1, 3, 4> [1]
xyloglucanase <1, 3, 4> [1]
xyloglucanase Xgh74A <2, 6> [3]

CAS registry number
1000598-79-7
76901-10-5

2 Source Organism

<1> *Aspergillus japonicus* (no sequence specified) [1]
<2> *Clostridium thermocellum* (no sequence specified) [3]
<3> *Chrysosporium lucknowense* (no sequence specified) [1]
<4> *Trichoderma reesei* (UNIPROT accession number: Q7Z9M8) [1]
<5> *Paenibacillus sp.* (UNIPROT accession number: Q3MUH7) [2]
<6> *Clostridium thermocellum* (UNIPROT accession number: Q70DK5) [3]

3 Reaction and Specificity

Catalyzed reaction
xyloglucan + H_2O = xyloglucan oligosaccharides (exohydrolysis of 1,4-β-D-glucosidic linkages in xyloglucan) (<1> endo-type reaction mode, overview [1]; <3,4> exo-type reaction mode, overview [1])

Natural substrates and products
S xyloglucan + H_2O <1, 3, 4> (Reversibility: ?) [1]
P xyloglucan oligosaccharides

S xyloglucan + H_2O <2> (<2> Xgh74 contributes to the in vivo degradation of the hemicellulose xyloglucan and xylan by the cellulosome of Clostridium thermocellum [3]) (Reversibility: ?) [3]
P ?

Substrates and products

S 4-nitrophenyl xyloglucan derivatives + H_2O <4> (<4> artificial substrate [1]) (Reversibility: ?) [1]
P oligosaccharides
S XXXGXXXG + H_2O <5> (Reversibility: ?) [2]
P XXX + XXXG + GXXXG
S β-glucan + H_2O <3, 4, 6> (<3,4,6> 8% of the activity with xyloglucan [1,3]) (Reversibility: ?) [1, 3]
P β-glucan oligosaccharides
S carboxymethyl cellulose + H_2O <3, 4, 6> (<3,6> 5% of the activity with xyloglucan [1,3]; <4> 6% of the activity with xyloglucan [1]) (Reversibility: ?) [1, 3]
P carboxymethyl cellulose oligosaccharides
S tamarind xyloglucan + H_2O <1, 3> (<1> highly specific for, endo-type reaction [1]; <3> highly specific for, exo-type reaction [1]) (Reversibility: ?) [1]
P oligosacchrides (<1,3> with end products XXXG, XXLG/XLXG, and XLLG [1])
S tamarind xyloglucan + H_2O <4> (<4> highly specific for, exo-type reaction [1]) (Reversibility: ?) [1]
P oligosaccharides (<4> with end products XXXG, XXLG/XLXG, and XLLG [1])
S xyloglucan + H_2O <2> (<2> Xgh74 contributes to the in vivo degradation of the hemicellulose xyloglucan and xylan by the cellulosome of Clostridium thermocellum [3]) (Reversibility: ?) [3]
P ?
S xyloglucan + H_2O <2> (<2> tamarind xyloglucan. Xgh74A hydrolyzes every fourth β-1,4-glucan bond in the xyloglucan backbone, thus producing decorated cellotetraose units [3]) (Reversibility: ?) [3]
P cellotetraose
S xyloglucan + H_2O <1, 3, 4, 5, 6> (<3,6> the enzyme is a true endo-glucanase when it acts on linear substrates without bulky substituents in the polymer backbone. When the enzyme acts on polymeric substrate with bulky side chains, its mode of action is switched to exo-like [1,3]; <5> XEG74 has dual endo-mode and exo-mode activities [2]) (Reversibility: ?) [1, 2, 3]
P xyloglucan oligosaccharides (<3,4,6> XXXG, XXLG and XLLG oligosaccharides [1,3])
S Additional information <1, 2, 3, 4, 5> (<1,3,4> substrate specificity, carboxymethylcellulose and barley β-glucan are poor or no substrates [1]; <2> low activity on carboxymethyl cellulose and on glucuronoxylan. Lack of activity on amorphous cellulose [3]; <5> no activity towards Avicel,

carboxymethylcellulose, barley β-1,3/1,4-glucan or xylan [2]) (Reversibility: ?) [1, 2, 3]

P ?

Turnover number (min^{-1})

3 <5> (xyloglucan) [2]
12 <4> (β-glucan) [1]
30 <3> (β-glucan) [1]
46 <4> (carboxymethylcellulose) [1]
55 <4> (xyloglucan) [1]
99 <3> (xyloglucan) [1]

Specific activity (U/mg)

Additional information <5> [2]

K_m-Value (mM)

Additional information <3, 4, 5> (<4> K_M-value for xyloglucan is 0.3 mg/ml, K_M-value for β-glucan is 8 mg/ml, K_M-value for carboxymethylcellulose is above 51 mg/mL [1]; <3> K_M-value for xyloglucan is 0.31 mg/ml, K_M-value for β-glucan is 18 mg/ml, K_M-value for carboxymethylcellulose is above 50 mg/mL [1]; <5> K_M-value for xyloglucan is 1.2 mg/ml [2]) [1, 2]

pH-Optimum

5-6 <1, 3, 4> [1]
5.3 <4> [1]
6 <3> [1]
6-6.5 <5> [2]
6.4 <2, 6> (<2> hydrolysis of tamarid xyloglucan [3]; <6> hydrolysis of tamarind seed xyloglucan [3]) [3]

pH-Range

3.3-6.9 <4> (<4> more than 50% of maximal activity in the pH-range 4.5-7.2 [1]) [1]
4.5-7.2 <3> (<3> more than 50% of maximal activity in the pH-range 4.5-7.2 [1]) [1]
5.7-7.8 <2, 6> (<2> 50% of maximal activity at pH 5.7 and at pH 7.8 [3]; <6> more than 50% of maximal activity in the pH-range 5.7-7.8, hydrolysis of tamarind seed xyloglucan [3]) [3]

Temperature optimum (°C)

40 <4> (<4> assay at [1]) [1]
60-70 <5> [2]
75 <2, 6> (<6> hydrolysis of tamarind seed xyloglucan [3]) [3]

4 Enzyme Structure

Molecular weight

81800 <6> [3]

Subunits

? <1, 3, 4, 5> (<3> x * 78000, SDS-PAGE [1]; <5> x * 105000, SDS-PAGE [2]; <1> x * 32000, SDS-PAGE [1]; <4> x * 75000-105000, several enzyme forms, SDS-PAGE [1]; <4> x * 75000-105000, isoelectrofocusing, there may be different forms of the enzyme resulting from O-glycosylation, SDS-PAGE [1]) [1, 2]
Additional information <4> (<4> peptide fingerprinting [1]) [1]

Posttranslational modification

Glycoprotein <4> (<4> 2 putative N-glycosylation sites in Cel74A [1]; <4> enzyme contains two putative N-glycosylation sites. There may be different forms of the enzyme resulting from O-glycosylation [1]) [1]
Additional information <4> (<4> Cel74A contains a signal peptide [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue

commercial preparation <3, 4> [1]

Localization

cellulosome <2, 6> [3]

Purification

<1> (by ion exchange chromatography, to homogeneity) [1]
<2> (recombinant) [3]
<3> (further purification of the commercial preparation, by ion exchange chromatography, to homogeneity) [1]
<4> (further purification of the commercial preparation, by ion exchange chromatography, to homogeneity) [1]
<5> (recombinant) [2]

Cloning

<5> (expression in Escherichia coli) [2]

6 Stability

pH-Stability

5 <3, 4> (<3,4> 40°C, stable for at least 24 h [1]) [1]
5-7.5 <5> (<5> stable [2]) [2]

Temperature stability

40 <1, 3, 4> (<1,3,4> pH 5.0, stable for at least 24 h [1]) [1]
50 <1, 3, 4> (<3,4> half-life: 24 h [1]; <1,3,4> pH 5.0, half-life below 24 h [1]) [1]
55 <5> (<5> stable up to [2]) [2]

60 <1, 3, 4> (<4> half-life: 15 min [1]; <3> half-life: 18 min [1]; <4> pH 5.0, half-life 15 min [1]; <3> pH 5.0, half-life 18 min [1]; <1> pH 5.0, half-life 28 h [1]) [1]
75 <2> (<2> stable up to [3]) [3]

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Oligosaccharide reducing-end xylanase 3.2.1.156

1 Nomenclature

EC number
3.2.1.156

Systematic name
β-D-xylopyranosyl-(14)-β-D-xylopyranosyl-(14)-β-D-xylopyranose reducing-end xylanase

Recommended name
oligosaccharide reducing-end xylanase

Synonyms
Rex <1> [2]
reducing end xylose-releasing exo-oligoxylanase <1> [2, 3]
reducing-end xylose-releasing exo-oligoxylanase <1> [1]

CAS registry number
55126-95-9
879497-03-7

2 Source Organism

<1> *Bacillus halodurans* (no sequence specified) [1, 2, 3, 4]

3 Reaction and Specificity

Catalyzed reaction
hydrolysis of 1,4-β-D-xylose residues from the reducing end of oligosaccharides

Reaction type
hydrolysis of O-glycosyl bond

Natural substrates and products
S Additional information <1> (<1> the enzyme may play a role as a key enzyme in intracellular xylan metabolism by cleaving xylooligosaccharides that are produced by the action of other intracellular enzymes from the arabino/glucurono-xylooligosaccharides [2]) (Reversibility: ?) [2]
P ?

Substrates and products

- **S** α-xylobiosylfluoride + H_2O + xylose <1> (Reversibility: ?) [4]
- **P** α-xylobiose + HF + xylose (<1> presence of xylose is required, some mutants in D263 also produce xylotriose [4])
- **S** β-D-glucopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose + H_2O <1> (<1> 1.1% of the activity with β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose [2]) (Reversibility: ?) [2]
- **P** ?
- **S** β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-deoxyxylopyranose + H_2O <1> (<1> 3.2% of the activity with β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose [2]) (Reversibility: ?) [2]
- **P** ?
- **S** β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-glucopyranose + H_2O <1> (<1> 0.5% of the activity with β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose [2]) (Reversibility: ?) [2]
- **P** ?
- **S** β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose + ? <1> (Reversibility: ?) [3]
- **P** ?
- **S** β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose + H_2O <1> (<1> the enzyme acts rapidly on the β-anomer of β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose, leaving the new reducing end in the α configuration. It also acts on longer oligosaccharides that have this structure at their reducing ends. The penulimate residue must be xylose, but replacing either of the other two residues with glucose merely slows the rate greatly [2]) (Reversibility: ?) [2]
- **P** β-D-xylopyranosyl-(1-4)-α-D-xylopyranose
- **S** β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose + H_2O <1> (Reversibility: ?) [2]
- **P** ?
- **S** β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose + H_2O <1> (Reversibility: ?) [2]
- **P** ?
- **S** β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose + H_2O <1> (Reversibility: ?) [2]
- **P** ?
- **S** Additional information <1> (<1> the enzyme may play a role as a key enzyme in intracellular xylan metabolism by cleaving xylooligosaccharides that are produced by the action of other intracellular enzymes from the arabino/glucurono-xylooligosaccharides [2]; <1> the enzyme hydrolyzes xylooligosaccharides whoses degree of polymerization is greater than or equal to 3, releasing the xylose unit at the reducing end [3]) (Reversibility: ?) [2, 3]
- **P** ?

Turnover number (min^{-1})

21 <1> (β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose, <1> mutant enzyme H319A [3]) [3]
73 <1> (β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose) [2]
162 <1> (β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose) [2]
163 <1> (β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose) [2]
175 <1> (β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose) [2]

K_m-Value (mM)

0.0024 <1> (β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose) [2]
0.0044 <1> (β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose) [2]
0.005 <1> (β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose) [2]
0.0185 <1> (β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose) [2]
0.0344 <1> (β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose, <1> mutant enzyme H319A [3]) [3]

pH-Optimum

6.2-7.3 <1> [2]

pH-Range

5.3-8.2 <1> (<1> pH 5.3: about 60% of maximal activity, pH 8.2: about 50% of maximal activity [2]) [2]

Temperature optimum (°C)

50 <1> [2]

Temperature range (°C)

35-55 <1> (<1> about 50% of maximal activity at 35°C and at 55°C [2]) [2]

5 Isolation/Preparation/Mutation/Application

Localization

intracellular <1> [2]

Purification

<1> [2]

Crystallization

<1> (hanging-drop vapour-diffusion method using 13.6 mg/ml Rex, 5.6% v/v polyethylene glycol 4000, 70 mM sodium acetate pH 4.6 and 30% v/v glycerol. The crystals belong to the space group P2(1)2(1)2(1), with unit-cell parameters a = 52.69, b = 86.02, c = 87.92 A) [1]
<1> (xylose-enzyme complex is prepared by cocrystallization using a reservoir solution containing 10 mM xylose. The xylobiose complex of the E70A mutant enzyme is prepared by cocrystallization using a reservoir solution containing 10 mM xylotriose) [3]

Cloning

<1> (expression in Escherichia coli) [2]

Engineering

D128A <1> (<1> specific activity is 290fold lower than that of the wild-type enzyme [2]) [2]
D263A <1> (<1> specific activity is 4421fold lower than that of the wild-type enzyme [2]; <1> conversion of glycosynthase from converting enzyme, synthesis of some xylotriose from xylobiose + HF+ xylose [4]) [2, 4]
D263C <1> (<1> conversion of glycosynthase from converting enzyme, synthesis of xylotriose from xylobiose + HF+ xylose, highest glycosynthase activity of mutants tested [4]) [4]
D263G <1> (<1> conversion of glycosynthase from converting enzyme, synthesis of some xylotriose from xylobiose + HF+ xylose [4]) [4]
D263L <1> (<1> conversion of glycosynthase from converting enzyme, synthesis of some xylotriose from xylobiose + HF+ xylose [4]) [4]
D263N <1> (<1> conversion of glycosynthase from converting enzyme, synthesis of xylotriose from xylobiose + HF+ xylose, second highest glycosynthase activity of mutants tested [4]) [4]
D263P <1> (<1> very little glycosynthase or converting activity [4]) [4]
D263S <1> (<1> very little glycosynthase or converting activity [4]) [4]
D263T <1> (<1> conversion of glycosynthase from converting enzyme, synthesis of some xylotriose from xylobiose + HF+ xylose [4]) [4]
D263V <1> (<1> conversion of glycosynthase from converting enzyme, synthesis of some xylotriose from xylobiose + HF+ xylose [4]) [4]
D70A <1> (<1> specific activity is 1545fold lower than that of the wild-type enzyme [2]) [2]
H319A <1> (<1> mutation causes a drastic decrease in activity towards the substrate β-D-xylopyranosyl-(1-4)-β-D-xylopyranosyl-(1-4)-β-D-xylopyranose [3]) [3]

6 Stability

pH-Stability

5-9.8 <1> (<1> 30°C, 30 min, stable [2]) [2]

Temperature stability

40 <1> (<1> pH 7.1, 30 min, stable at temperatures up to [2]) [2]

References

[1] Honda, Y.; Fushinobu, S.; Hidaka, M.; Wakagi, T.; Shoun, H.; Kitaoka, M.: Crystallization and preliminary X-ray analysis of reducing-end xylose-releasing exo-oligoxylanase from Bacillus halodurans C-125. Acta Crystallogr. Sect. F, **61**, 291-292 (2005)

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ι-carrageenase 3.2.1.157

1 Nomenclature

EC number
3.2.1.157

Systematic name
ι-carrageenan 4-β-D-glycanohydrolase (configuration-inverting)

Recommended name
ι-carrageenase

Synonyms
ι-carrageenase <2> [3]

CAS registry number
74191-25-6

2 Source Organism

<1> *Zobellia galactanivorans* (no sequence specified) [2]
<2> *Alteromonas fortis* (no sequence specified) [1, 3, 4]

3 Reaction and Specificity

Catalyzed reaction
endohydrolysis of 1,4-β-D-linkages between D-galactose 4-sulfate and 3,6-anhydro-D-galactose-2-sulfate in iota-carrageenans

Substrates and products
- **S** ι-carrageenan + H_2O <1> (<1> endohydrolytic cleavage, the enzyme proceeds with an overall inversion of the anomeric configuration [2]) (Reversibility: ?) [2]
- **P** ι-neocarratetraose sulfate + iota-neocarrahexaose sulfate (<1> major end products [2])
- **S** ι-carrageenan + H_2O <2> (<2> from an open conformation which allows the initial endo-attack of ι-carrageenan chains, the enzyme switches to a closed-tunnel form, consistent with its highly processive character [4]) (Reversibility: ?) [4]
- **P** hydrolyzed ι-carrageenan
- **S** Additional information <1> (<1> the enzyme shows no activity with κ-carrageenan, λ-carrageenan, agar or agarose [2]) (Reversibility: ?) [2]

P ?

Metals, ions

Ca^{2+} <2> (<2> enzyme contains three calcium binding sites involved in stabilizing the enzyme structure [3]) [3]

Na^{+} <2> (<2> the protein contains one sodium and one chloride binding site [3]) [3]

4 Enzyme Structure

Molecular weight

50000 <1> (<1> gel filtration [2]) [2]

Subunits

monomer <1> (<1> 1 * 50000, SDS-PAGE [2]) [2]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [2]

<2> [1, 3, 4]

Crystallization

<2> (concentration to 6 mg/ml using a dialyzing concentrator. The structure of ι-carrageenase bound to iota-carrageenan fragments is solved at 2.0 A resolution) [4]

<2> (hanging-drop vapour-diffusion method using polyethylene glycol (MW = 6000) as a precipitant. Crystals belong to space group P2(1), with unit-cell parameters a = 56.75 A, b = 91.04 A, c = 125.01 A, β = 93.4°. The unit cell contains two molecules in the asymmetric unit related by a non-crystallographic twofold axis. Crystals diffracted to 2.0 A resolution on a synchrotron beamline) [1]

<2> (native and selenomethionyl-ι-carrageenase. Single crystals are obtained with polyethylene glycol, and the presence of Ca^{2+} appears to be crucial for crystallization. High quality crystals, typically 0.25 * 0.25 * 0.15 mM in dimension are grown with 0.1 M sodium cacodylate at pH 6.5, 15-17% polyethylene glycol and 200 mM calcium acetate. Crystallization of Se-Met-ι-carrageenase is performed under similar conditions except for the addition of 1 mM dithiothreitol and the replacement of sodium cacodylate by imidazole to avoid the reaction between cacodylate and dithiothreitol. Crystal structure at 1.6 A resolution) [3]

Cloning

<2> (gene encoding ι-carrageenase flanked by a C-terminal hexahistidine tag and a N-terminal PelB signal peptide for targeting the gene product into Escherichia coli periplasm. The recombinant plasmid, referred to as pETIAf, is used to transform Escherichia coli BL21(DE3) strain harbouring pLysS plasmid) [1]

6 Stability

General stability information

<2>, enzyme contains three calcium binding sites involved in stabilizing the enzyme structure [3]

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α-Agarase 3.2.1.158

1 Nomenclature

EC number
3.2.1.158

Systematic name
agarose 3-glycanohydrolase

Recommended name
α-agarase

Synonyms
agaraseA33 <2> [2]

CAS registry number
63952-00-1

2 Source Organism

<1> *Alteromonas agarlytica* (no sequence specified) [3]
<2> *Thalassomonas sp.* (no sequence specified) [2]
<3> *Alteromonas agarlyticus* (no sequence specified) [1]

3 Reaction and Specificity

Catalyzed reaction
endohydrolysis of 1,3-α-L-galactosidic linkages in agarose, yielding agarotetraose as the major product

Substrates and products
S agarohexaose + H_2O <2> (Reversibility: ?) [2]
P agarotetraose + agarobiose
S agarose + H_2O <3> (Reversibility: ?) [1]
P agarotetraose + agarohexaose
S agarose + H_2O <1, 2> (Reversibility: ?) [2, 3]
P agarotetraose
S neoagarohexaose + H_2O <2> (Reversibility: ?) [2]
P agarotetraose
S porphyran + H_2O <2> (<2> at 95% of the rate with agarose [2]) (Reversibility: ?) [2]
P ?

S Additional information <2, 3> (<2> no substrate: κ-, or λ-carrageenans, *p*-nitrophenyl-galactopyranosides [2]; <3> no substrate: neoagarobiose, neoagarotetraose [1]) (Reversibility: ?) [1, 2]
P ?

Activating compounds
Ca^{2+} <3> [1]

Metals, ions
Ca^{2+} <1, 2> (<1> required [3]; <2> required, about 15% residual activity in absence of Ca^{2+} [2]) [2, 3]

Specific activity (U/mg)
40.7 <2> (<2> pH 8.5, 45°C [2]) [2]
6423 <3> (<3> pH 7.2, 42°C [1]) [1]

pH-Optimum
7.2 <1, 3> (<1> MOPS or Tris buffer [3]) [1, 3]
8.5 <2> [2]

pH-Range
4.5-9.5 <2> [2]
6-9 <1> [3]

Temperature optimum (°C)
45 <2> [2]

4 Enzyme Structure

Molecular weight
85000 <2> (<2> gel filtration [2]) [2]
360000 <1> (<1> gel filtration [3]) [3]

Subunits
dimer <1> (<1> 2 * 180000, SDS-PAGE [3]) [3]
monomer <2> (<2> 1 * 85000, SDS-PAGE [2]) [2]

5 Isolation/Preparation/Mutation/Application

Source/tissue
culture supernatant <1> [3]

Purification
<1> [3]
<2> [2]
<3> [1]

6 Stability

pH-Stability

6-11 <2> [2]
6.5 <1> (<1> prolonged treatment below, inactivation [3]) [3]

Temperature stability

40 <2> (<2> stable for at least 30 min [2]) [2]
45 <1> (<1> inactivation above [3]) [3]

References

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α-Neoagaro-oligosaccharide hydrolase 3.2.1.159

1 Nomenclature

EC number
3.2.1.159

Systematic name
α-neoagaro-oligosaccharide 3-glycohydrolase

Recommended name
α-neoagaro-oligosaccharide hydrolase

CAS registry number
60063-77-6

2 Source Organism

<1> *Vibrio sp.* (no sequence specified) [1]
<2> *Bacillus sp.* (UNIPROT accession number: AB062678) [2]

3 Reaction and Specificity

Catalyzed reaction
hydrolysis of the 1,3-α-L-galactosidic linkages of neoagaro-oligosaccharides that are smaller than a hexamer, yielding 3,6-anhydro-L-galactose and D-galactose

Substrates and products
- **S** α-1,3-neoagarobiose + H_2O <2> (<2> 40% hydrolysis within 30 min [2]) (Reversibility: ?) [2]
- **P** 3,6-anhydro-L-galactose + D-galactose
- **S** α-1,3-neoagarooligosaccharide + H_2O <1> (Reversibility: ?) [1]
- **P** agaropentaose + agarotriose + agarobiose + D-galactose
- **S** neoagarobiose + H20 <1> (Reversibility: ?) [1]
- **P** ?
- **S** neoagarotetraose + H_2O <2> (<2> complete hydrolysis within 30 min [2]) (Reversibility: ?) [2]
- **P** 3,6-anhydro-L-galactose + agaropentaose
- **S** neoagarotetraose + H_2O <2> (<2> complete hydrolysis within 30 min [2]) (Reversibility: ?) [2]
- **P** 3,6-anhydro-L-galactose + agarotriose

Inhibitors
$AgNO_3$ <2> (<2> 2 mM, 9% residual activity [2]) [2]
$CuCl_2$ <2> (<2> 2 mM, 7% residual activity [2]) [2]
EDTA <2> (<2> 2 mM, 47% residual activity [2]) [2]
$HgCl_2$ <2> (<2> 2 mM, no residual activity [2]) [2]
$NiCl_2$ <2> (<2> 2 mM, 10% residual activity [2]) [2]
SDS <2> (<2> 2 mM, 53% residual activity [2]) [2]
$SrCl_2$ <2> (<2> 2 mM, 43% residual activity [2]) [2]
p-chloromercuribenzoate <2> (<2> 0.2 mM 60% residual activity [2]) [2]

Metals, ions
$MgCl_2$ <2> (<2> 2 mM, 163% of initial activity [2]) [2]
$SnCl_2$ <2> (<2> 2 mM, 121% of initial activity [2]) [2]
$ZnCl_2$ <2> (<2> 2 mM, 128% of initial activity [2]) [2]

Specific activity (U/mg)
22.2 <2> (<2> pH 7.0, 30°C [2]) [2]
92 <1> (<1> pH 7.7, 30°C [1]) [1]

K_m-Value (mM)
5.37 <1> (neoagarobiose, <1> pH 7.7, 30°C [1]) [1]

pH-Optimum
6.1 <2> [2]
7.7 <1> [1]

pH-Range
6.2-8.9 <1> (<1> drastical reduction in activity below or above [1]) [1]

Temperature optimum (°C)
30 <1, 2> [1, 2]

4 Enzyme Structure

Molecular weight
84000 <1> (<1> gel filtration [1]) [1]
320000 <2> (<2> gel filtration [2]) [2]

Subunits
dimer <1> (<1> 2 * 42000, SDS-PAGE [1]) [1]
octamer <2> (<2> 8 * 42000, SDS-PAGE [2]) [2]
Additional information <1, 2> (<1,2> N-terminal amino acid sequence [1,2]) [1, 2]

5 Isolation/Preparation/Mutation/Application

Source/tissue
culture fluid <2> [2]

Purification
<1> [1]

6 Stability

pH-Stability
6.8-8.6 <2> (<2> stable within this range [2]) [2]

Temperature stability
30 <1, 2> (<1> 120 min, stable [1]; <2> stable up to, 30 min, 96% residual activity [2]) [1, 2]
40 <1> (<1> rapid loss of activity [1]) [1]

Storage stability
<1>, -20°C, phosphate buffer pH 7.8, 30% glycerol, 2 months, 60% residual activity [1]
<1>, 4°C, phosphate buffer pH 7.8, 3 weeks, 60% residual activity, 2 months, 30% residual activity [1]

References

[1] Sugano, Y.; Kodama, H.; Terada, I.; Yamazaki, Y.; Noma, M.: Purification and characterization of a novel enzyme, α-neoagarooligosaccharide hydrolase (α-NAOS hydrolase), from a marine bacterium, Vibrio sp. strain JT0107. J. Bacteriol., **176**, 6812-6818 (1994)
[2] Suzuki, H.; Sawai, Y.; Suzuki, T.; Kawai, K.: Purification and characterization of an extracellular α-neoagarooligosaccharide hydrolase from Bacillus sp. MK03. J. Biosci. Bioeng., **93**, 456-463 (2002)

Xyloglucan-specific exo-β-1,4-glucanase 3.2.1.160

1 Nomenclature

EC number
3.2.1.160 (deleted, the enzyme was shown to be identical to EC 3.2.1.155)

Recommended name
xyloglucan-specific exo-β-1,4-glucanase

β-Apiosyl-β-glucosidase 3.2.1.161

1 Nomenclature

EC number
3.2.1.161

Systematic name
7-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyloxy]isoflavonoid β-D-apiofuranosyl-(1→6)-D-glucohydrolase

Recommended name
β-apiosyl-β-glucosidase

Synonyms
furcatin hydrolase <2> [2]
isoflavonoid 7-O-β-apiosyl-glucoside β-glucosidase <2> [2]
isoflavonoid-7-O-β[D-apiofuranosyl-(1→6)-β-D-glucoside] disaccharidase <2> [2]

CAS registry number
1000598-83-3
39346-29-7

2 Source Organism

<1> *Cicer arietinum* (no sequence specified) [1]
<2> *Viburnum furcatum* (UNIPROT accession number: Q75W17) [2]
<3> *Dalbergia nigrescens* (no sequence specified) [3]

3 Reaction and Specificity

Catalyzed reaction
7-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyloxy]isoflavonoid + H_2O = a 7-hydroxyisoflavonoid + β-D-apiofuranosyl-(1→6)-D-glucose

Reaction type
hydrolysis of O-glycosyl bond

Natural substrates and products
S biochanin A 7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside] + H_2O <1> (Reversibility: ir) [1]
P biochanin A + β-D-apiofuranosyl-(1-6)-β-D-glucopyranose

S daidzin + H_2O <3> (Reversibility: ir) [3]
P daidzein + β-D-glucopyranose
S dalcochinin-8'-O-β-D-glucopyranoside + H_2O <3> (Reversibility: ir) [3]
P dalcochinin + β-D-glucopyranose
S dalpaniculin-7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside] + H_2O <3> (Reversibility: ir) [3]
P dalpaniculin + β-D-apiofuranosyl-(1-6)-β-D-glucopyranose
S dalpatein-7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside] + H_2O <3> (Reversibility: ir) [3]
P dalpatein + β-D-apiofuranosyl-(1-6)-β-D-glucopyranose
S genistin + H_2O <3> (Reversibility: ir) [3]
P genistein + β-D-glucopyranose

Substrates and products

S 2-methyl-4'-nitro-isoflavone 7-O-β-D-glucopyranoside + H_2O <1> (Reversibility: ir) [1]
P 2-methyl-4'-nitro-isoflavone + β-D-glucopyranose
S 2-nitrophenyl-β-D-glucopyranoside + H_2O <1> (Reversibility: ir) [1]
P 2-nitrophenol + β-D-glucopyranose
S 2-phenylethyl β-primeveroside + H_2O <2> (<2> 14.7% of the activity with furcatin [2]) (Reversibility: ?) [2]
P 2-phenylethanol + 6-O-β-D-xylopyranosyl-β-D-glucopyranose
S 4-methylumbelliferyl β-D-glucoside + H_2O <1> (Reversibility: ir) [1]
P 4-methylumbelliferol + β-D-glucopyranose
S 4-nitrophenyl-α-L-arabinopyranoside + H_2O <3> (Reversibility: ir) [3]
P 4-nitrophenol + α-L-arabinopyranose
S 4-nitrophenyl-β-D-fucoside + H_2O <3> (Reversibility: ir) [3]
P 4-nitrophenol + β-D-fucose
S 4-nitrophenyl-β-D-galactopyranoside + H_2O <1, 3> (Reversibility: ir) [1, 3]
P 4-nitrophenol + β-D-galactopyranose
S 4-nitrophenyl-β-D-glucopyranoside + H_2O <1, 3> (Reversibility: ir) [1, 3]
P 4-nitrophenol + β-D-glucopyranose
S 4-nitrophenyl-β-D-mannopyranoside + H_2O <3> (Reversibility: ir) [3]
P 4-nitrophenol + β-D-mannopyranose
S 4-nitrophenyl-β-D-thioglucopyranoside + H_2O <3> (Reversibility: ir) [3]
P 4-nitrophenol + β-D-thioglucopyranose
S 4-nitrophenyl-β-D-xylopyranoside + H_2O <3> (Reversibility: ir) [3]
P 4-nitrophenol + β-D-xylopyranose
S apigenin 7-O-β-D-glucopyranoside + H_2O <1> (Reversibility: ir) [1]
P apigenin + β-D-glucopyranose
S β-allylphenyl-β-D-glucopyranoside + H_2O <2> (<2> 43.3% of the activity with furcatin [2]) (Reversibility: ?) [2]
P 4-allylphenol + β-D-glucopyranose
S biochanin A 7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside] + H_2O <1> (Reversibility: ir) [1]
P biochanin A + β-D-apiofuranosyl-(1-6)-β-D-glucopyranose

S biochanin A 7-O-β-D-glucopyranoside + H_2O <1> (Reversibility: ir) [1]
P biochanin A + β-D-glucopyranose
S daidzin + H_2O <3> (Reversibility: ir) [3]
P daidzein + β-D-glucopyranose
S dalcochinin-8'-O-β-D-glucopyranoside + H_2O <3> (Reversibility: ir) [3]
P dalcochinin + β-D-glucopyranose
S dalpaniculin-7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside] + H_2O <3> (Reversibility: ir) [3]
P dalpaniculin + β-D-apiofuranosyl-(1-6)-β-D-glucopyranose
S dalpatein-7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside] + H_2O <3> (Reversibility: ir) [3]
P dalpatein + β-D-apiofuranosyl-(1-6)-β-D-glucopyranose
S formononetein 7-O-β-D-glucopyranoside + H_2O <1> (Reversibility: ?) [1]
P formononetein + β-D-glucopyranose
S furcatin + H_2O <2> (Reversibility: ?) [2]
P 4-allylphenol + β-D-apiofuranosyl-(1-6)-β-D-glucopyranose
S genistin + H_2O <3> (Reversibility: ir) [3]
P genistein + β-D-glucopyranose
S *p*-nitrophenyl β-D-glucopyranoside + H_2O <2> (<2> 3.33% of the activity with furcatin [2]) (Reversibility: ?) [2]
P 4-nitrophenol + β-D-glucopyranose
S *p*-nitrophenyl β-gentiobioside + H_2O <2> (<2> 4.11% of the activity with furcatin [2]) (Reversibility: ?) [2]
P 4-nitrophenol + β-gentiobiose
S *p*-nitrophenyl β-primeveroside + H_2O <2> (<2> 87.3% of the activity with furcatin [2]) (Reversibility: ?) [2]
P 4-nitrophenol + 6-O-β-D-xylopyranosyl-β-D-glucopyranose
S vicianin + H_2O <2> (<2> 4.99% of the activity with furcatin [2]) (Reversibility: ?) [2]
P mandelonitrile + 6-O-α-L-arabinopyranosyl-β-D-glucopyranose

Inhibitors

4-nitrophenol <1> (<1> 1 mM, 25% inhibition [1]) [1]
Ag^+ <1> (<1> 1 mM, 80% inhibition [1]) [1]
D-glucono-1,5-lactone <1> (<1> 1 mM, 50% inhibition [1]) [1]
glycerol <1> (<1> weak [1]) [1]
Hg^{2+} <1> (<1> strong [1]) [1]
ethyleneglycol monomethyl ether <1> (<1> weak [1]) [1]
Additional information <1, 3> (<3> EDTA has no effect on the activity [3]; <1> no inhibition: glucose, PCMB, Cu^{2+} [1]) [1, 3]

Metals, ions

Additional information <3> (<3> $FeSO_4$, $CaCl_2$, $HgCl_2$, $MnCl_2$, $ZnSO_4$ have no effect on the activity [3]) [3]

Turnover number (min^{-1})

7 <3> (4-nitrophenyl-β-D-fucoside) [3]
10.4 <3> (4-nitrophenyl-β-D-glucopyranoside) [3]

334 <3> (dalpaniculin-7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside]) [3]
465 <3> (dalpatein-7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside]) [3]
480 <3> (daidzin) [3]
500 <3> (genistin) [3]

Specific activity (U/mg)

9.2 <3> [3]
42.2 <2> [2]

K_m-Value (mM)

0.02 <1> (biochanin A 7-O-β-D-glucopyranoside) [1]
0.03 <1> (formononetein 7-O-β-D-glucopyranoside) [1]
0.05 <1> (apigenin 7-O-β-D-glucopyranoside, <1> isoenzyme R3, isoenzyme L1 [1]) [1]
0.07 <1> (2-methyl-4'-nitro-isoflavone 7-O-β-D-glucopyranoside, <1> isoenzyme R3 [1]) [1]
0.09 <3> (genistin) [3]
0.11 <3> (daidzin) [3]
0.15 <1> (biochanin A 7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside], <1> isoenzyme R3, isoenzyme L1 [1]) [1]
0.17 <1> (4-methylumbelliferyl β-D-glucoside, <1> isoenzyme R3, isoenzyme L1 [1]) [1]
0.2 <1> (4-methylumbelliferyl β-D-glucoside, <1> isoenzyme H1 [1]) [1]
0.2 <1> (biochanin A 7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside], <1> isoenzyme H1 [1]) [1]
0.5 <3> (dalpatein-7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside]) [3]
0.7 <1> (2-nitrophenyl-β-D-glucopyranoside, <1> isoenzyme R3, isoenzyme L1 [1]) [1]
0.7 <3> (dalpaniculin-7-O-[β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside]) [3]
1 <1> (2-nitrophenyl-β-D-glucopyranoside, <1> isoenzyme H1 [1]) [1]
1.5 <1> (4-nitrophenyl-β-D-glucopyranoside, <1> isoenzyme R3, isoenzyme L1 [1]) [1]
1.8 <3> (4-nitrophenyl-β-D-fucoside) [3]
2 <1> (4-nitrophenyl-β-D-glucopyranoside, <1> isoenzyme H1 [1]) [1]
2 <1> (4-nitrophenyl-β-D-galactopyranoside, <1> isoenzyme R3, isoenzyme L1 [1]) [1]
2.2 <2> (furcatin, <2> pH 6.0, 37°C [2]) [2]
3 <1> (4-nitrophenyl-β-D-galactopyranoside, <1> isoenzyme H1 [1]) [1]
5.1 <2> (*p*-nitrophenyl β-primeveroside, <2> pH 6.0, 37°C [2]) [2]
14.7 <3> (4-nitrophenyl-β-D-glucopyranoside) [3]

pH-Optimum

4.5-5 <1> (<1> and a second optimum at pH 7-7.5 [1]) [1]
5 <2> [2]
5-6 <3> [3]
7-7.5 <1> (<1> and a second optimum at pH 4.5-5 [1]) [1]

pH-Range
3.5-6.5 <3> (<3> less than half-maximal activity above and below [3]) [3]
4-10 <2> (<2> recombinant enzyme is active over the range [2]) [2]

Temperature optimum (°C)
40 <2> [2]
65 <3> [3]

4 Enzyme Structure

Molecular weight
125000-135000 <1> (<1> gel filtration, sucrose density gradient centrifugation [1]) [1]
240000 <3> (<3> gel filtration [3]) [3]

Subunits
dimer <1> (<1> 2 * 68000, SDS-PAGE [1]) [1]
tetramer <3> (<3> 4 * 62000-63000, SDS-PAGE [3]) [3]

Posttranslational modification
Glycoprotein <1> [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue
hypocotyl <1> [1]
leaf <1, 2> [1, 2]
root <1> [1]
seed <3> [3]

Localization
chloroplast <2> [2]

Purification
<1> [1]
<2> [2]
<3> (DEAE anion exchange chromatograpy, gel filtration) [3]

Cloning
<2> (expression in Escherichia coli) [2]

6 Stability

Temperature stability
50 <2> (<2> stable below [2]) [2]

References

[1] Hosel, W.; Barz, W.: β-Glucosidases from Cicer arietinum L. Purification and properties of isoflavone-7-O-glucoside-specific β-glucosidases. Eur. J. Biochem., **57**, 607-616 (1975)
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λ-carrageenase 3.2.1.162

1 Nomenclature

EC number
3.2.1.162

Recommended name
λ-carrageenase

Synonyms
CglA hydrolase <4> [1]
endo-β-1,4-carrageenose 2,6,2'-trisulfate-hydrolase <3> [4]

CAS registry number
5093637-3

2 Source Organism

<1> *Pseudomonas carrageenovora* (no sequence specified) [2]
<2> *Pseudoalteromonas carrageenovora* (no sequence specified) [3]
<3> *Pseudoalteromonas sp.* (UNIPROT accession number: Q05JY7) [4]
<4> *Pseudoalteromonas carrageenovora* (UNIPROT accession number: Q0JRK4) [1]

3 Reaction and Specificity

Catalyzed reaction
endohydrolysis of β-1,4-linkages in the backbone of λ-carrageenan, resulting in the tetrasaccharide α-D-Galp2,6S-(1-3)-β-D-Galp2S-(1-4)-α-D-Galp2,6S-(1-3)-D-Galp2S
preferential cleavage at Gly-Ser-Arg373-/- of glycoprotein gp43 in Xenopus laevis coelemic egg envelope to yield gp41

Substrates and products
S λ-carrageenan + H_2O <2> (Reversibility: ?) [3]
P neo-carrabiose oligosaccharides
S λ-carrageenan + H_2O <3> (Reversibility: ?) [4]
P α-D-Galp2,6S-(1-3)-β-D-Galp2S-(1-4)-α-D-Galp2,6S-(1-3)-D-Galp2S + ?
S λ-carrageenan + H_2O <4> (<4> CglA hydrolase proceeds according to an endolytic mode of action and a mechanism of inversion of the anomeric configuration [1]) (Reversibility: ?) [1]
P neo-λ-carratetraose + neo-λ-carrahexaose (<4> neo-λ-carratetraose is the main degradation product [1])

Inhibitors
EDTA <1> (<1> 0.0001 mM [2]) [2]
Hg^{2+} <1> [2]

Metals, ions
CsCl <3> (<3> maximal activity in presence of 0.4 M NaCl. CsCl is able to compensate for KCl [4]) [4]
KCl <3> (<3> maximal activity in presence of 0.4 M NaCl. KCl is able to compensate for KCl [4]) [4]
LiCl <3> (<3> maximal activity in presence of 0.4 M NaCl. LiCl is able to compensate for KCl [4]) [4]
NaCl <3> (<3> maximal activity in presence of 0.4 M NaCl [4]) [4]
RbCl <3> (<3> maximal activity in presence of 0.4 M NaCl. RbCl is able to compensate for KCl [4]) [4]

pH-Optimum
7 <3> [4]

pH-Range
6-8.5 <3> (<3> pH 6.0: about 40% of maximal activity, pH 8.5: about 45% of maximal activity [4]) [4]

Temperature optimum (°C)
35 <3> [4]

Temperature range (°C)
20-40 <3> (<3> 20°C: about 40% of maximal activity, 40°C: about 65% of maximal activity [4]) [4]

4 Enzyme Structure

Molecular weight
100000 <3> (<3> gel filtration [4]) [4]

Subunits
monomer <3, 4> (<3> 1 * 100000, SDS-PAGE [4]; <4> 1 * 97000, SDS-PAGE [1]) [1, 4]

5 Isolation/Preparation/Mutation/Application

Source/tissue
cell culture <3> (<3> containing λ-carrageenan as a carbon source [4]) [4]
culture medium <1, 4> [1, 2]

Localization
extracellular <4> [1]

Purification
<3> [4]
<4> [1]

Cloning
<3> (expression in Escherichia coli) [4]
<4> (expression in Escherichia coli as insoluble inclusion bodies. These inclusion bodies are purified and solubilized in 8 M urea) [1]

6 Stability

Temperature stability
25 <3> (<3> 120 min, stable [4]) [4]
30 <3> (<3> 0.5 M sorbitol, 120 min, enzyme is stable up to [4]) [4]
35 <1> (<1> 30 min, inactivated [2]) [2]

General stability information
<1>, inactivated by freezing, by dialysis against distilled water [2]
<3>, sorbitol, sucrose and trehalose improve stability [4]

References

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[4] Ohta, Y.; Hatada, Y.: A novel enzyme, λ-carrageenase, isolated from a deep-sea bacterium. J. Biochem., **140**, 475-481 (2006)

1,6-α-D-Mannosidase 3.2.1.163

1 Nomenclature

EC number
3.2.1.163

Systematic name
(1-6)-α-mannosyl α-D-mannohydrolase

Recommended name
1,6-α-D-mannosidase

Synonyms
α 1,6-mannosidase <1> [5]
α-1,6-linkage-specific mannosidase <3> [3]
core-specific α1,6-mannosidase <1> [5]
core-specific lysosomal α (1-6)-mannosidase <2> [1]
core-specific lysosomal α 1,6-mannosidase <1> [5]
lysosomal α (1-6)-mannosidase <1> [4]
lysosomal α 1,6-mannosidase <2> [1]
Additional information <1> (<1> the enzyme belongs to the glycosylhydrolase family 38 [5]) [5]

CAS registry number
90910-03-5

2 Source Organism

<1> *Homo sapiens* (no sequence specified) [4, 5]
<2> *Rattus norvegicus* (no sequence specified) [1]
<3> *Aspergillus phoenicis* (no sequence specified) [2, 3, 6]

3 Reaction and Specificity

Catalyzed reaction
hydrolysis of the 1,6-linked α-D-mannose residues in α-D-Manp-(1-6)-D-Manp

Natural substrates and products
S Additional information <1, 2> (<1> the core-specific lysosomal enzyme is involved in N-glycan catabolism and degradation of $Man_3GlcNAc_2$ N-glycan structures, requiring for chitobiase action before α1,6-mannosi-

dase activity [5]; <2> the core-specific lysosomal enzyme is involved in N-glycan catabolism, Man-α(l-6)linkage hydrolysis depends on the action of endoglycosidases, namely aspartyl-N-acetyl-β-D-glucosaminidase and endo-N-acetyl-β-D-glucosaminidase, overview [1]; <1> the enzyme is active on the branched trimannosyl core of complex glycans cleaving αl-6-linked mannose residues from branched Man_3Glc-NAc to form Manαl-3Manβ1-4GlcNAc, overview, activities of α-mannosidases in fibroblasts, overview [4]) (Reversibility: ?) [1, 4, 5]

P ?

Substrates and products

S Man-α(1-3)[Man-α(l-6)]Man-β(1-4)GlcNAc-β(1-4)GlcNAc-β(1-N)Asn + H_2O <2> (<2> i.e. Man3GlcNAc2Asn, degradation in concert with endoglycosidases, low activity, overview [1]) (Reversibility: ?) [1]

P ?

S Man-α(1-6)Man-α(1-4)GlcNAc-β(1-4)GlcNAc-β(1-N)Asn + H_2O <2> (<2> i.e. $Man_2GlcNAc_2Asn$, degradation in concert with endoglycosidases, low activity, overview [1]) (Reversibility: ?) [1]

P ?

S α-D-Manp-(1,6)-D-Manp + H_2O <3> (<3> the enzyme cleaves specifically (1-6)-linked mannobiose and has no activity towards α-D-Manp-1,2-D-Manp or α-D-Manp-1,3-D-Manp [6]; <3> the enzyme cleaves specifically (1-6)-linked mannobiose and has no activity towards any other linkages [2]) (Reversibility: ?) [2, 6]

P α-D-mannose

S α-D-mannopyranosyl-(1,6)-D-mannose + H_2O <3> (Reversibility: ?) [3]

P ?

S Additional information <1, 2, 3> (<1> the core-specific lysosomal enzyme is involved in N-glycan catabolism and degradation of $Man_3GlcNAc_2$ N-glycan structures, requiring for chitobiase action before α1,6-mannosidase activity [5]; <2> the core-specific lysosomal enzyme is involved in N-glycan catabolism, Man-α(1-6)linkage hydrolysis depends on the action of endoglycosidases, namely aspartyl-N-acetyl-β-D-glucosaminidase and endo-N-acetyl-β-D-glucosaminidase, overview [1]; <1> the enzyme is active on the branched trimannosyl core of complex glycans cleaving α1-6-linked mannose residues from branched Man_3Glc-NAc to form Manαl-3Manβ1-4GlcNAc, overview, activities of α-mannosidases in fibroblasts, overview [4]; <3> no activity with *p*-nitrophenyl-α-D-mannopyranoside or bakers yeast mannan [2]; <3> regioselective synthesis of mannobiose and mannotriose by reverse hydrolysis of commercially available manno-oligosaccharide [6]; <1> substrate specificity, the enzyme cleaves α1-6-linked mannose residues from branched Man_3Glc-NAc to form Manα1-3Manβ1-4GlcNAc, but shows little activity on artificial substrates 4-methylumbelliferyl α-mannoside and 4-nitrophenyl α-mannoside, overview [4]; <1> the core-specific enzyme is specific for cleaving only the α1-6mannose residue from Man_3GlcNAc but not Man_3GlcNAc2

or other larger high mannose oligosaccharides, NMR analysis of substrates and products, overview [5]) (Reversibility: ?) [1, 2, 4, 5, 6]
P ?

Inhibitors
1,4-dideoxy-1,4-imino-D-mannitol <1> [5]
Mg^{2+} <3> [2]
Mn^{2+} <3> (<3> strong inhibition [2]) [2]
Swainsonine <1, 2> [1, 4, 5]
Additional information <3> (<3> no inhibition by EDTA at 10 mM [2]) [2]

Metals, ions
Co^{2+} <1, 2> (<1,2> stimulates [1,5]) [1, 5]
Zn^{2+} <1, 2> (<1,2> stimulates [1,5]) [1, 5]
Additional information <3> (<3> Ca^{2+} is not required for activity [2]) [2]

Specific activity (U/mg)
48 <3> (<3> purified enzyme [2]) [2]

K_m-Value (mM)
14 <3> (α-D-Manp-(1-6)-D-Manp, <3> pH 4.0, 60°C [2]) [2]
Additional information <2> (<2> kinetics [1]) [1]

pH-Optimum
4 <1> (<1> acidic pH optimum [5]) [4, 5]
4-4.5 <3> [2]
4.5 <3> [6]
5 <2, 3> (<2,3> assay at [1,3]) [1, 3]

pH-Range
3-5 <3> (<3> 36% of maximal activity at pH 3.0, rapid inactivation above pH 5.0 [2]) [2]
3.5-6 <3> [6]

Temperature optimum (°C)
30 <3> (<3> assay at [3]) [3]
37 <1, 2> (<1,2> assay at [1,4,5]) [1, 4, 5]
55 <3> [6]
60 <3> [2]

Temperature range (°C)
45-70 <3> (<3> 21% of maximal activity at 45°C, 16% at 70°C [6]) [6]

4 Enzyme Structure

Molecular weight
81000 <3> (<3> gel filtration [2]) [2]
180000 <1> (<1> recombinant enzyme, gel filtration [5]) [5]

Subunits
monomer <3> (<3> 1 * 74000, SDS-PAGE [2]) [2]

5 Isolation/Preparation/Mutation/Application

Source/tissue
fibroblast <1> [4]
liver <2> [1]
Additional information <1> (<1> the enzyme is expressed in all tissues [5]) [5]

Localization
lysosome <1, 2> [1, 4, 5]

Purification
<1> (native enzyme partially by affinity chromatography) [4]
<1> (recombinant His- or HA-tagged enzyme) [5]
<3> (native enzyme 370fold by anion-exchange chromatography, chromatofocussing, and gel filtration) [2]

Cloning
<1> (DNA and amino acid sequence determination and analysis, phylogenetic analysis, expression as His- or HA-tagged protein) [5]

Application
synthesis <3> (<3> regioselective synthesis of mannobiose and mannotriose by reverse hydrolysis using the 1,6-α-D-mannosidase from Aspergillus phoenicis, method optimization, overview [6]; <3> synthesis of FimH receptor-active manno-oligosaccharides by reverse hydrolysis using α-mannosidases from Penicillium citrinum, Aspergillus phoenicis and almond in a sequential reaction process, method development and optimization, overview [3]; <3> the organism is employed in regioselective synthesis of manno-oligosaccharides involving the enzyme [2]) [2, 3, 6]

6 Stability

Temperature stability
30 <3> (<3> purified enzyme, stable for 24 h, rapid inactivation above [2]) [2]

General stability information
<3>, freeze and thaw cycles and freeze drying have a detrimental effect on the purified enzyme with loss of 60% activity [2]

Storage stability
<3>, 23°C, room temperature, purified enzyme, over 95% remaining activity after 7 days [2]
<3>, 4°C, purified enzyme, over 95% remaining activity after 3 months [2]

References

[1] Haeuw, J.F.; Grard, T.; Alonso, C.; Strecker, G.; Michalski, J.C.: The core-specific lysosomal α(1-6)-mannosidase activity depends on aspartamidohydrolase activity. Biochem. J., **297**, 463-466 (1994)
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[6] Athanasopoulos, V.I.; Niranjan, K.; Rastall, R.A.: Regioselective synthesis of mannobiose and mannotriose by reverse hydrolysis using a novel 1,6-α-D-mannosidase from Aspergillus phoenicis. J. Mol. Catal. B, **27**, 215-219 (2004)

Galactan endo-1,6-β-galactosidase 3.2.1.164

1 Nomenclature

EC number
3.2.1.164

Recommended name
galactan endo-1,6-β-galactosidase

Synonyms
GAL1 <2> [3]
Tv6GAL <4> [4]
β-1,6-galactanase <2> [3]
β-D-galactanase <1> [1]
endo-β-(1-6)-D-galactanase <1> [2]
endo-β-(1-6)-galactanase <3, 4> [4, 5]
endo-β-1,6-D-galactanase <1> [1]
type II arabinogalactan-degrading enzyme <2> [3]
Additional information <2> (<2> the enzyme belongs to the glycoside hydrolase family 5 [3]) [3]

2 Source Organism

<1> *Aspergillus niger* (no sequence specified) [1, 2]
<2> *Fusarium oxysporum* (no sequence specified) [3]
<3> *Trichoderma viride* (no sequence specified) [5]
<4> *Trichoderma viride* (UNIPROT accession number: Q76FP5) [4]

3 Reaction and Specificity

Catalyzed reaction
endohydrolysis of 1,6-β-D-galactosidic linkages in arabinogalactan proteins and 1,3:1,6-β-galactans to yield galactose and β-(1-6)-galactobiose as the final products (<3,4> mode of action, overview [4,5])

Natural substrates and products
S Additional information <1> (<1> activity with galactans from different sources, overview [1]) (Reversibility: ?) [1]
P ?

Substrates and products

- **S** β-galactan + H_2O <3> (<3> galactan from Prototheca zopfii, which has a high proportion of β-1,6-linked galactosyl residues, the extent of hydrolysis of the carbohydrate portion of a radish root arabinogalactan-protein AGP increases when α-L-arabinofuranosyl residues attached to β-1,6-linked galactosyl side chains of the AGP are removed in advance, the enzyme releases galactose, β-1,6-galactobiose, and 4-O-methyl-β-glucuronosyl-1,6-galactose as major hydrolysis products when allowed to act exhaustively on the modified AGP, overview [5]) (Reversibility: ?) [5]
- **P** ?
- **S** desarabinosylated grape 3,6-galactan-protein + H_2O <1> (<1> the enzyme liberates β-1,6-D-galactobiose and galactose [2]) (Reversibility: ?) [2]
- **P** ?
- **S** type II arabinogalactans + H_2O <2> (<2> the enzyme preferentially debranches β-1,6-galactobiose from the substrate, type II arabinogalactans from Western larch [3]) (Reversibility: ?) [3]
- **P** ?
- **S** Additional information <1, 3, 4> (<4> substrate specificity, overview [4]; <1> activity with galactans from different sources, overview [1]; <3> substrate preparations and specificity, the endo-galactanase hydrolyzes specifically β-1,6-galactooligosaccharides with a degree of polymerization higher than 3 and their acidic derivatives with 4-O-methyl-glucosyluronic or glucosyluronic groups at the nonreducing terminals, oveview [5]; <1> the β-1,6-galactanase liberates galactose and 1,6-galactobiose from acid-treated larchwood and Norway spruce arabinogalactans [1]; <1> the enzyme does not cleave β-D-1,3-galactosidic bonds [2]) (Reversibility: ?) [1, 2, 4, 5]
- **P** ?

Inhibitors

Ag^+ <2> (<2> 1 mM, 50% inhibition [3]) [3]
Cd^{2+} <2> (<2> 1 mM, 45% inhibition [3]) [3]
Cu^{2+} <2> (<2> 1 mM, 45% inhibition [3]) [3]
Fe^{2+} <2> (<2> 1 mM, 65% inhibition [3]) [3]
Hg^{2+} <2> (<2> 1 mM, 80% inhibition [3]) [3]
Zn^{2+} <2> (<2> 1 mM, 60% inhibition [3]) [3]
Additional information <2> (<2> no inhibition by EDTA [3]) [3]

Metals, ions

Additional information <2> (<2> the enzyme activity does not require metal ions and is not affected by the presence of Ba^{2+}, Ca^{2+}, Co^{2+}, Fe^{3+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, or Ni^{2+}, overview [3]) [3]

Specific activity (U/mg)

1.55 <2> (<2> purified native enzyme [3]) [3]
25.29 <1> (<1> purified enzyme [2]) [2]

K_m-Value (mM)
Additional information <1, 2> [2, 3]

pH-Optimum
3.5 <1> [2]
4.3 <3> [5]
5 <2> [3]

pH-Range
2-7.5 <1> (<1> activity range, 85% of maximal activity at pH 2.5 [2]) [2]

Temperature optimum (°C)
30 <3> (<3> assay at [5]) [5]
50 <2> [3]
60 <1> [2]

Temperature range (°C)
20-75 <1> (<1> activity range [2]) [2]

4 Enzyme Structure

Molecular weight
17000 <1, 3> (<3> gel filtration [5]; <1> gel filtration, the enzyme shows unusual behaviour in gel filtration [1]) [1, 5]
29000 <1> (<1> gel filtration [2]) [2]
47000 <3> (<3> native PAGE [5]) [5]
60000 <1> (<1> native PAGE [2]) [2]

Subunits
? <2, 4> (<2> x * 47000, SDS-PAGE [3]; <4> x * 50488, sequence calculation, x * 64000, recombinant enzyme, SDS-PAGE, x * 50687, detagged recombinant enzyme, mass spectrometry, x * 68296, recombinant His6-tagged thioredoxin fusion enzyme, sequence calculation [4]) [3, 4]
monomer <1> (<1> 1 * 58000, SDS-PAGE [1]; <1> 1 * 60000, SDS-PAGE [2]) [1, 2]

5 Isolation/Preparation/Mutation/Application

Source/tissue
commercial preparation <1, 3> (<3> commercial cellulase preparation Onozuka R-10 [5]; <1> commercial pectinase preparation [1]; <1> crude enzyme extract [2]) [1, 2, 5]

Purification
<1> (native enzyme 26.2fold to homogeneity by several chromatographic steps, overview) [2]
<1> (native enzyme from commercial pectinase preparation by anion and cation exchange chromatography, ultrafiltration, and gel filtration) [1]

<2> (recombinant maltose-binding-protein fusion enzyme from Escherichia coli strain DH5α, by cation exchange and amylose affinity chromatography, followed by hydrophobic interaction chromatography, native enzyme 88fold from strain S12 culture filtrate by anion exchange and hydrophobic interaction chromatography, followed by cation exchange chromatography, dialysis and gel filtration) [3]
<3> (native enzyme form a commercial cellulase preparation, 57fold to homogeneity by ammonium sulfate fractionation, two steps of ion exchange chromatography, and gel filtration) [5]
<4> (recombinant His6-tagged enzyme from Escherichia coli strain Bl21 by two steps of metal chelating chromatography, the thioredoxin tag is cleaved off) [4]

Cloning

<2> (gene Fogal1, DNA and amino acid sequence determination and analysis, expression in Escherichia coli strain DH5α as maltose-binding-protein fusion enzyme) [3]
<4> (DNA and maino acid sequence determination and analysis, sequence comparison, expression in Escherichia coli strain BL21 as His6-tagged enzyme fused to thioredoxin) [4]

6 Stability

pH-Stability

2-7.5 <1> (<1> 2 h, 40°C, 50% remaining activity at pH 2.0 and at pH 7.5 [2]) [2]
4-10 <2> (<2> 16 h, purified native enzyme, over 80% remaining activity [3]) [3]

Temperature stability

30 <1> (<1> 4 h, purified enzyme, 1 M acetate buffer, pH 4.2, completely stable [2]) [2]
45 <1> (<1> 4 h, purified enzyme, 1 M acetate buffer, pH 4.2, 50% remaining activity [2]) [2]
60 <1, 2> (<2> 1 h, purified native enzyme, over 80% remaining activity [3]; <1> 4 h, purified enzyme, 1 M acetate buffer, pH 4.2, 17% remaining activity [2]) [2, 3]

References

[1] Luonteri, E.; Laine, C.; Uusitalo, S.; Teleman, A.; Siika-aho, M.; Tenkanen, M.: Purification and characterization of Aspergillus β-D-galactanases acting on β-1,4- and β-1,3/6-linked arabinogalactans. Carbohydr. Polym., **53**, 155-168 (2003)

[2] Brillouet, J.M.; Williams, P.; Moutounet, M.: Purification and some properties of a novel endo-β-(1-6)-D-galactanase from Aspergillus niger. Agric. Biol. Chem., **55**, 1565-1571 (1991)
[3] Sakamoto, T.; Taniguchi, Y.; Suzuki, S.; Ihara, H.; Kawasaki, H.: Characterization of Fusarium oxysporum β-1,6-galactanase, an enzyme that hydrolyzes larch wood arabinogalactan. Appl. Environ. Microbiol., **73**, 3109-3112 (2007)
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[5] Okemoto, K.; Uekita, T.; Tsumuraya, Y.; Hashimoto, Y.; Kasama, T.: Purification and characterization of an endo-β-(1-6)-galactanase from Trichoderma viride. Carbohydr. Res., **338**, 219-230 (2003)

N-Methyl nucleosidase 3.2.2.25

1 Nomenclature

EC number
3.2.2.25

Systematic name
7-methylxanthosine ribohydrolase

Recommended name
N-methyl nucleosidase

Synonyms
7-methyl-N^9-nucleoside hydrolase <2> [2]
N-MeNase <1> [1]

CAS registry number
72270-62-3

2 Source Organism

<1> *Camellia sinensis* (no sequence specified) [1]
<2> *Coffea arabica* (no sequence specified) [2]

3 Reaction and Specificity

Catalyzed reaction
7-methylxanthosine + H_2O = 7-methylxanthine + D-ribose

Natural substrates and products
- **S** 7-methylxanthosine + H_2O <1, 2> (<1> second step in caffeine biosynthesis, pathway overview [1]; <2> step in caffeine biosynthesis, feeding experiments, overview [2]) (Reversibility: ir) [1, 2]
- **P** 7-methylxanthine + D-ribose

Substrates and products
- **S** 1-methyladenosine + H_2O <1> (<1> 22% of the activity with 7-methylxanthosine, substrate as 7-methyladenosine perchlorate [1]) (Reversibility: ir) [1]
- **P** 1-methyladenine + D-ribose
- **S** 1-methylinosine + H_2O <1> (<1> 9% of the activity with 7-methylxanthosine [1]) (Reversibility: ir) [1]

P 1-methylhypoxanthine + D-ribose
S 1-methylxanthosine + H_2O <1> (<1> 12% of the activity with 7-methylxanthosine [1]) (Reversibility: ir) [1]
P 1-methylxanthine + D-ribose
S 3-methyladenosine + H_2O <1> (<1> 178% of the activity with 7-methylxanthosine, substrate as 3-methyladenosine *p*-toluenesulfonate [1]) (Reversibility: ir) [1]
P 3-methyladenine + D-ribose
S 3-methylguanosine + H_2O <1> (<1> 38% of the activity with 7-methylxanthosine [1]) (Reversibility: ir) [1]
P 3-methylguanine + D-ribose
S 3-methylinosine + H_2O <1> (<1> 128% of the activity with 7-methylxanthosine [1]) (Reversibility: ir) [1]
P 3-methylhypoxanthine + D-ribose
S 3-methylxanthosine + H_2O <1> (<1> 198% of the activity with 7-methylxanthosine [1]) (Reversibility: ir) [1]
P 3-methylxanthine + D-ribose
S 7-methyladenosine + H_2O <1> (<1> 13% of the activity with 7-methylxanthosine [1]) (Reversibility: ir) [1]
P 7-methyladenine + D-ribose
S 7-methylguanosine + H_2O <1> (<1> 189% of the activity with 7-methylxanthosine [1]) (Reversibility: ir) [1]
P 7-methylguanine + D-ribose
S 7-methylinosine + H_2O <1> (<1> 121% of the activity with 7-methylxanthosine [1]) (Reversibility: ir) [1]
P 7-methylhypoxanthine + D-ribose
S 7-methylxanthosine + H_2O <1, 2> (<1> second step in caffeine biosynthesis, pathway overview [1]; <2> step in caffeine biosynthesis, feeding experiments, overview [2]; <1> the reverse reaction is catalyzed by a nucleoside phosphorylase using ribose-1-phosphate, overview [1]) (Reversibility: ir) [1, 2]
P 7-methylxanthine + D-ribose
S Additional information <1> (<1> substrate specificity, overview, no activity with 1-methylguanosine and guanosine, poor activity with inosine and adenosine [1]) (Reversibility: ?) [1]
P ?

Inhibitors

7-methylhypoxanthine <1> (<1> 50% inhibition at 5 mM, no inhibition at 1 mM [1]) [1]
7-methylxanthine <1> (<1> 40% inhibition at 5 mM, no inhibition at 1 mM [1]) [1]
Ca^{2+} <1> (<1> slight inhibition [1]) [1]
Cu^{2+} <1> (<1> slight inhibition [1]) [1]
EDTA <1> (<1> 90 inhibition at 1 mM [1]) [1]
PCMB <1> (<1> 80% inhibition at 0.5 mM [1]) [1]

Zn^{2+} <1> (<1> slight inhibition [1]) [1]
Additional information <1> (<1> no inhibitin by Mg^{2+}, Fe^{2+}, Co^{2+}, NaF, and iodoacetate [1]) [1]

Metals, ions
Mg^{2+} <2> (<2> slightly stimulating [2]) [2]

Specific activity (U/mg)
0.00656 <1> (<1> partially purified enzyme, after anion exchange chromatography [1]) [1]
0.0294 <1> (<1> purified enzyme [1]) [1]

pH-Optimum
8-8.5 <1> [1]
8.5 <2> (<2> assay at [2]) [2]

Temperature optimum (°C)
30 <2> (<2> assay at [2]) [2]
37 <1> (<1> assay at [1]) [1]

4 Enzyme Structure

Molecular weight
55000 <1> (<1> gel filtration [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue
fruit <2> (<2> immature and ripe [2]) [2]
leaf <1> [1]
seedling <2> [2]

Purification
<1> (native enzyme from leaves, separated from adenosine nucleosidase, EC 3.2.2.7, by anion exchange chromatography, followed by hydroxylapatite chromatography and gel filtration, to homogeneity) [1]

6 Stability

pH-Stability
Additional information <1> (<1> the purified enzyme is unstable in acidic medium [1]) [1]

References

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Microsomal epoxide hydrolase 3.3.2.9

1 Nomenclature

EC number
3.3.2.9

Systematic name
cis-stilbene-oxide hydrolase

Recommended name
microsomal epoxide hydrolase

Synonyms
EC 3.3.2.3
EHb <5, 9> [48]
EPHX1 <4> [55, 56]
JH epoxide hydrolase <24> [32]
JHEH <24> [32]
PNSO hydrolase <4> [22]
SEH <5, 8, 10, 11, 12, 13, 21, 23, 30> [36]
XEHase <3> [43]
juvenile hormone epoxide hydrolase <24> [32]
mEH <3, 4, 5, 9, 27> [35, 40, 41, 44, 46, 47, 49, 50, 51, 52, 54, 55, 56, 59]
mEH-like protein <27> [54]
microsomal epoxide hydrolase-like protein <27> [54]
microsomal xenobiotic epoxide hydrolase <5> [42]
styrene-epoxide hydrolase <5> [53]
xenobiotic epoxide hydrolase <3, 5> [42, 43, 53]
Additional information <5, 13> (<13> see also 3.1.3.76 [36]; <5> the enzyme belongs to the family of C-X bond hydrolase enzymes [49]) [36, 49]

CAS registry number
9048-63-9

2 Source Organism

<1> *Cavia porcellus* (no sequence specified) [4, 5, 6, 7, 8, 23, 28, 57]
<2> *Drosophila melanogaster* (no sequence specified) [39]
<3> *Mus musculus* (no sequence specified) [1, 4, 5, 13, 23, 24, 25, 28, 41, 43, 44, 56, 57, 59]
<4> *Homo sapiens* (no sequence specified) [1, 3, 5, 8, 17, 19, 22, 23, 27, 28, 30, 41, 46, 47, 51, 52, 55, 56, 58, 59]

<5> *Rattus norvegicus* (no sequence specified) [1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 14, 16, 17, 18, 19, 20, 21, 23, 26, 30, 31, 35, 36, 40, 41, 42, 45, 48, 49, 51, 53, 56, 57, 59]
<6> *Sus scrofa* (no sequence specified) [4]
<7> *Bos taurus* (no sequence specified) [23]
<8> *Triticum aestivum* (no sequence specified) [36]
<9> *Oryctolagus cuniculus* (no sequence specified) [1, 2, 4, 5, 28, 48, 50]
<10> *Zea mays* (no sequence specified) [36]
<11> *Solanum tuberosum* (no sequence specified) [36]
<12> *Nicotiana tabacum* (no sequence specified) [36]
<13> *Glycine max* (no sequence specified) [36]
<14> *Pseudomonas sp.* (no sequence specified) [37]
<15> *Pigeon* (no sequence specified) [4]
<16> *Canis familiaris* (no sequence specified) [33]
<17> *Rhodotorula glutinis* (no sequence specified) [38]
<18> *Trypanosoma cruzi* (no sequence specified) [15]
<19> *Felis catus* (no sequence specified) [4]
<20> *Monkey* (no sequence specified) [28]
<21> *Apium graveolens* (no sequence specified) [36]
<22> *Macaca mulatta* (no sequence specified) [1, 5, 29]
<23> *Oryza sativa* (no sequence specified) [36]
<24> *Manduca sexta* (no sequence specified) [32, 34]
<25> *toad* (no sequence specified) [4]
<26> *trout* (no sequence specified) [4]
<27> *Danio rerio* (no sequence specified) [54]
<28> *hamster* (no sequence specified) [28]
<29> *quail* (no sequence specified) [4]
<30> *Musa paradisiaca* (no sequence specified) [36]

3 Reaction and Specificity

Catalyzed reaction

cis-stilbene oxide + H_2O = (+)-(1R,2R)-1,2-diphenylethane-1,2-diol (<4> active site structure, quantitative structure-activity relationships, molecular modelling [58]; <9> formation of threo-1,2-diphenyl-1,2-ethanediols from all epoxides involving anti-stereospecific opening of the oxirane ring with about 90% preference for the (S)-carbon to give nearly optically pure (R,R)-diols [50]; <5, 8, 10, 11, 12, 13, 21, 23, 30> reaction mechanism, catalytic triad [36]; <27> the catalytic triad is Asp223-Glu402-His429, the active site includes an oxyanion hole [54]; <5> the catalytic triad is Asp226-His431-Asp352, catalytic mechanism involving an ester intermediate formed by alkylation of an active site carboxyl group [49]; <3> two step mechanism with formation of of a hydroxyl-alkyl-enzyme intermediate, catalytic triad [41]; <4> two step mechanism with formation of of a hydroxyl-alkyl-enzyme intermediate, the catalytic triad is Asp226-His431-Glu404 with polarizing residues Tyr299 and Tyr374 [41])

cis-stilbene oxide + H_2O = (+)-(1R,2R)-1,2.diphenylethane-1,2-diol (<5> two step mechanism with formation of of a hydroxyl-alkyl-enzyme intermediate, catalytic triad [41])

Natural substrates and products

- **S** androstene oxide + H_2O <3, 4, 5> (<3,4,5> high activity [41]) (Reversibility: ?) [41]
- **P** androstenediol
- **S** cis-stilbene oxide + H_2O <3, 4, 5> (Reversibility: ?) [45, 55, 59]
- **P** (+)-(1R,2R)-1,2-diphenylethane-1,2-diol
- **S** Additional information <1, 2, 3, 4, 5, 18, 24> (<1,3,4,5> enzyme is induced by a number of xenobiotics, the enzyme is thought to play a detoxifying role by preventing epoxides from reacting irreversibly with critical cellular macromolecules. Some evidence exists to support the hypothesis that microsomal epoxide hydrolase is closely associated with at least certain forms of cytochrome P-450, such association may influence the functional role of the microsomal epoxide hydrolase in the various pathways of bioactivation [23]; <24> the enzyme may play a significant role in the secondary metabolism of juvenile hormone acid generated by juvenile hormone esterase [34]; <3> the enzyme plays an important role in the metabolism of various xenobiotic compounds including the polycyclic aromatic hydrocarbon carcinogens. It is specifically involved in the formation of the ultimate carcinogen of benzopyrene [25]; <18> induced about threefold by 5 mM phenobarbital [15]; <4> involved in the biotransformation of epoxides produced from either xeno- or endobiotics. The diols produced are generally detoxification products but in several instances, such as 9,10-epoxybenzopyrene-7,8-diol, they are very potent mutagens and possible carcinogens. The enzyme is induced by phenobarbital [30]; <4> the enzyme is expected to play a purely protective role with respect to epoxides metabolically produced from pharmaceutical drugs [5]; <5> role in benzopyrene-induced mutagenesis and carcinogenesis [3]; <5> enzyme is involved in the metabolism of steroids, the microsomal enzyme plays a central role in both the inactivation of primary mutagenic and carcinogenic metabolites of polycyclic aromatic hydrocarbons, and in activating these metabolites to even more toxic or mutagenic secondary products, enzyme production is induced by 3-methylcholanthrene, phenobarbital, trans-stilbene oxide, 2(3)-tert-butyl-4-hydroxyanisol or 2-acetylaminofluorene [2]; <5> potent and selective induction by trans-stilbene oxide [4]; <3> probably represents an important factor in the control of reactive epoxides [24]; <2> the enzyme in Drosophila melanogaster is involved in xenobiotic biotransformation, but not in juvenile hormone metabolism [39]; <4> genetic polymorphisms of the enzyme are responsible for varying susceptibility of cigarette smoking humans to chronic obstructive pulmonary disease, COPD [52]; <4> key enzyme in the metabolism of environmental contaminants being responsible for xenobiotic transformations, regulation of the enzyme occurs at transcriptional, translational, and post-translational level, the enzyme is also in-

volved in cytoprotection and steroid metabolism, as well as in cellular responses to glucose metabolism and in Na^+-dependent bile acid transport, the enzyme is part of a multi-transport system at the cell surface [56]; <3> key enzyme in the metabolism of environmental contaminants being responsible for xenobiotic transformations, regulation of the enzyme occurs at transcriptional, translational, and post-translational levelthe enzyme is also involved in cytoprotection and steroid metabolism, as well as in cellular responses to glucose metabolism and in Na^+-dependent bile acid transport, the enzyme is part of a multi-transport system at the cell surface [56]; <5> the enzyme activity in diabetic and in fasted rats is reduced by 60-71%, key enzyme in the metabolism of environmental contaminants being responsible for xenobiotic transformations, regulation of the enzyme occurs at transcriptional, translational, and post-translational level, the enzyme is also involved in cytoprotection and steroid metabolism, as well as in cellular responses to glucose metabolism and in Na^+-dependent bile acid transport, the enzyme is part of a multi-transport system at the cell surface [56]; <4> the enzyme is a phase II biotransformation enzyme which detoxifies epoxides, including carcinogens such as polycyclic aromatic hydrocarbons found in cigarette smoke and cooked meats, enzyme polymorphisms are not associated with colon cancer risk [46]; <4,5> the enzyme is critical for biotransformations in xenobiotic metabolism and detoxification [51]; <5> the enzyme is involved in xenobiotic metabolism and detoxification, e.g. of 1,3-butadiene oxide, styrene oxide, and benzo[a]pyrene-4,5-oxide [41]; <4> the enzyme is involved in xenobiotic metabolism and detoxification, e.g. of 1,3-butadiene oxide, styrene oxide, and benzo[a]pyrene-4,5-oxide, enzyme deficiency leads to acute and severe phenytoin toxicity in vivo, the enzyme is involved in transport of bile acids in the liver [41]; <3> the enzyme is involved in xenobiotic metabolism and detoxification, e.g. of 1,3-butadiene oxide, styrene oxide, and benzo[a]pyrene-4,5-oxide, the enzyme is important in sexual development [41]; <5> the enzyme metabolizes xenobiotic epoxides [42]) (Reversibility: ?) [2, 3, 4, 5, 15, 23, 24, 25, 30, 34, 39, 41, 42, 46, 51, 52, 56]

P ?

Substrates and products

S (R)-1,2-epoxyhexane + H_2O <17> (Reversibility: ?) [38]
P n-2-hydroxyhexanol
S (R)-styrene oxide + H_2O <17> (Reversibility: ?) [38]
P 1,2-diphenylethane-1,2-diol
S (S)-1,2-epoxyhexane + H_2O <17> (Reversibility: ?) [38]
P n-2-hydroxyhexanol
S (S)-styrene oxide + H_2O <17> (Reversibility: ?) [38]
P 1,2-diphenylethane-1,2-diol
S (exo)-2,3-epoxynorbornane + H_2O <5> (Reversibility: ?) [23]
P ?
S 1,10-phenanthroline 5,6-oxide + H_2O <5> (Reversibility: ?) [31]

P ?
S 1,2,3,4,9,9-hexachloro-6,7-epoxy-1,4,41,5,6,7,8,8a-octahydro-1,4-methanonaphthalene + H_2O <1, 3, 5, 6, 9, 15, 19, 25, 26, 29> (Reversibility: ?) [2, 4]
P ?
S 1,2-epoxybutane + H_2O <5> (Reversibility: ?) [23]
P 2-hydroxy-n-butanol
S 1-methyl-1-phenyloxirane + H_2O <4> (Reversibility: ?) [8]
P ?
S 2,3-epoxypropyl ether + H_2O <1> (Reversibility: ?) [7]
P ?
S 2,3-epoxypropyl-*p*-methoxyphenyl ether + H_2O <5> (Reversibility: ?) [23]
P ?
S 2-bromoethylene oxide + H_2O <5> (Reversibility: ?) [23]
P ?
S 2-chloroethylene oxide + H_2O <4, 5> (Reversibility: ?) [19, 23]
P ?
S 3,3,3-trichloropropylene oxide + H_2O <5> (Reversibility: ?) [23]
P ?
S 3-methylcholanthrene 11,12-oxide + H_2O <4, 5> (Reversibility: ?) [14, 19, 23]
P ?
S 4-chlorophenyl 2,3-epoxypropyl ether + H_2O <1, 5> (Reversibility: ?) [7, 23]
P ?
S 4-nitrostyrene 7,8-oxide + H_2O <4> (<4> i.e. PNSO [22]) (Reversibility: ?) [22]
P 1-(4-nitro)phenyl-ethane-1,2-diol
S 4-vinylcyclohexene dioxide + H_2O <5> (<5> low activity [23]) (Reversibility: ?) [23]
P ?
S 7-glycidooxycoumarin + H_2O <3> (<3> substrate is a stable epoxide [43]) (Reversibility: ?) [43]
P 7-(1',2'-dihydroxy-3'-propoxy)coumarin
S 7-glycidoxycoumarin + H_2O <16> (Reversibility: ?) [33]
P ?
S 7-methylbenzanthracene-5,6-oxide + H_2O <5> (Reversibility: ?) [23]
P ?
S 7-methylbenzoanthracene + H_2O <5> (Reversibility: ?) [3]
P ?
S N-2,3-epoxypropyl-*p*-methoxyphenyl ether + H_2O <5> (Reversibility: ?) [23]
P ?
S allylbenzene + H_2O <1, 3, 5> (Reversibility: ?) [57]
P 3-phenylpropane-1,2-diol
S androstene oxide + H_2O <4, 5> (Reversibility: ?) [2, 21, 27]
P ?

S androstene oxide + H_2O <3, 4, 5> (<3,4,5> high activity [41]) (Reversibility: ?) [41]
P androstenediol
S benz[a]anthracene 5,6-oxide + H_2O <5> (Reversibility: ?) [42]
P 5,6-dihydro-chrysene-5,6-diol
S benz[a]pyrene 4,5-oxide + H_2O <4> (Reversibility: ?) [22]
P 4,5-dihydro-benzo[def]chrysene-4,5-diol
S benzene oxide + H_2O <5> (Reversibility: ?) [2]
P ?
S benzoanthracene 5,6-oxide + H_2O <4, 5> (Reversibility: ?) [2, 14, 17, 19, 23]
P ?
S benzoanthracene-9,10-oxide + H_2O <5> (Reversibility: ?) [18]
P ?
S benzopyrene 11,12-oxide + H_2O <5> (Reversibility: ?) [2, 14, 17, 18]
P ?
S benzopyrene 4,5-oxide + H_2O <1, 3, 4, 5, 6, 9, 15, 19, 25, 26, 29> (Reversibility: ?) [2, 3, 4, 10, 14, 16, 17, 18, 19, 21, 23, 24]
P (-)benzopyrene 4,5-dihydrodiol <4> [3]
S benzopyrene 7,8-oxide + H_2O <4, 5> (Reversibility: ?) [2, 14, 17, 18, 19, 23]
P ?
S benzopyrene 9,10-oxide + H_2O <5> (Reversibility: ?) [2, 14, 18, 23]
P ?
S bisnorsqualene oxide + H_2O <1> (Reversibility: ?) [7]
P ?
S butadiene monoxide + H_2O <5> (<5> low activity [23]) (Reversibility: ?) [23]
P ?
S cis-(9R,10S)-epoxystearic acid + H_2O <5, 1> (Reversibility: ?) [36]
P threo-(9R,10R)-dihydroxystearic acid + threo-(9S,10S)-dihydroxystearic acid (<5> enantioselective production of 80% (9R,10R)-diol and 20% (9S,10S)-diol [36]; <10> enantioselective production of 84% (9R,10R)-diol and 16% (9S,10S)-diol [36])
S cis-(9R,10S)-epoxystearic acid methyl ester + H_2O <5, 1> (Reversibility: ?) [36]
P threo-(9R,10R)-dihydroxystearic acid methyl ester + threo-(9S,10S)-dihydroxystearic acid methyl ester (<5> enantioselective production of 79% (9R,10R)-diol and 21% (9S,10S)-diol [36]; <10> enantioselective production of 83% (9R,10R)-diol and 17% (9S,10S)-diol [36])
S cis-(9S,10R)-epoxystearic acid + H_2O <5, 1> (Reversibility: ?) [36]
P threo-(9R,10R)-dihydroxystearic acid + threo-(9S,10S)-dihydroxystearic acid (<5> enantioselective production of 39% (9R,10R)-diol and 61% (9S,10S)-diol [36]; <10> enantioselective production of 52% (9R,10R)-diol and 48% (9S,10S)-diol [36])

S cis-(9S,10R)-epoxystearic acid methyl ester + H_2O <5, 1> (Reversibility: ?) [36]
P threo-(9R,10R)-dihydroxystearic acid methyl ester + threo-(9S,10S)-dihydroxystearic acid methyl ester (<5> enantioselective production of 48% (9R,10R)-diol and 52% (9S,10S)-diol [36]; <10> enantioselective production of 68% (9R,10R)-diol and 32% (9S,10S)-diol [36])
S cis-4,4'-dichlorostilbene oxide + H_2O <9> (Reversibility: ?) [50]
P (1R,2R)-1,2-bis(4-chlorophenyl)ethane-1,2-diol (<9> product identification [50])
S cis-4,4'-diethylstilbene oxide + H_2O <9> (Reversibility: ?) [50]
P (1R,2R)-1,2-bis(4-ethylphenyl)ethane-1,2-diol (<9> product identification [50])
S cis-4,4'-diisopropylstilbene oxide + H_2O <9> (Reversibility: ?) [50]
P (1R,2R)-1,2-bis(4-isopropylphenyl)ethane-1,2-diol (<9> product identification [50])
S cis-4,4'-dimethylstilbene oxide + H_2O <9> (Reversibility: ?) [50]
P (1R,2R)-1,2-bis(4-methylphenyl)ethane-1,2-diol (<9> product identification [50])
S cis-8-ethylstyrene 7,8-oxide + H_2O <4> (Reversibility: ?) [22]
P ?
S cis-stilbene oxide + H_2O <1, 3, 4, 5, 9, 22, 28> (Reversibility: ?) [2, 27, 28, 29]
P ?
S cis-stilbene oxide + H_2O <2> (Reversibility: ?) [39]
P 1,2-diphenylethane-1,2-diol
S cis-stilbene oxide + H_2O <5> (Reversibility: ?) [41]
P (+)-(1R,2R)-1,2.diphenylethane-1,2-diol
S cis-stilbene oxide + H_2O <3, 4, 5, 9, 22, 27> (<27> the recombinant enzyme expressed in Escherichia coli acts stereospecifically on the the (R)-styrene [54]) (Reversibility: ?) [1, 40, 41, 45, 50, 54, 55, 59]
P (+)-(1R,2R)-1,2-diphenylethane-1,2-diol
S clofibrate + H_2O <3, 4, 5, 9, 22> (Reversibility: ?) [1]
P ?
S cyclododecene-1,2-oxide + H_2O <5> (Reversibility: ?) [23]
P ?
S cyclohexene oxide + H_2O <4> (Reversibility: ?) [8]
P ?
S cyclohexene-1,2-oxide + H_2O <5> (Reversibility: ?) [23]
P ?
S di(2-ethylhexyl)phthalate + H_2O <3, 4, 5, 9, 22> (Reversibility: ?) [1]
P ?
S dibenzanthracene 5,6-oxide + H_2O <4, 5> (Reversibility: ?) [14, 17, 19, 23]
P ?
S dibenzoanthacene + H_2O <5> (Reversibility: ?) [2, 3]
P ?

S dl-1,3-butadiene diepoxide + H_2O <5> (<5> low activity [23]) (Reversibility: ?) [23]
P ?
S epibromhydrin + H_2O <5> (Reversibility: ?) [23]
P ?
S epichlorhydrin + H_2O <5> (Reversibility: ?) [23]
P 3-chloro-1,2-propanediol
S epifluorohydrin + H_2O <5> (Reversibility: ?) [23]
P ?
S epoxy-stearic acid + H_2O <4> (<4> highly enantiospecific reaction, low activity [56]) (Reversibility: ?) [56]
P ?
S estroxide + H_2O <4, 5> (<5> 16α,17α-epoxyestratrienol [21]) (Reversibility: ?) [2, 16, 21, 27]
P ?
S glycidyl methacrylate + H_2O <5> (<5> low activity [23]) (Reversibility: ?) [23]
P ?
S indene 1,2-oxide + H_2O <4> (Reversibility: ?) [8]
P ?
S indene-1,2-oxide + H_2O <1> (Reversibility: ?) [7]
P ?
S juvenile hormone I + H_2O <24> (Reversibility: ?) [34]
P ?
S juvenile hormone II + H_2O <24> (Reversibility: ?) [34]
P ?
S juvenile hormone III + H_2O <24> (Reversibility: ?) [34]
P ?
S naphthalene 1,2-oxide + H_2O <4> (Reversibility: ?) [22]
P naphthalene 1,2-diol
S naphthalene-1,2-oxide + H_2O <1, 4, 5> (Reversibility: ?) [2, 7, 14, 23]
P ?
S octene 1,2-oxide + H_2O <4, 5> (Reversibility: ?) [2, 3, 14, 17, 18, 23]
P ?
S octene 1,2-oxide + H_2O <5> (Reversibility: ?) [42]
P octan-1,2-diol
S octene-1,2-oxide + H_2O <1> (Reversibility: ?) [7]
P ?
S *p*-chlorophenyl-2,3-epoxypropylether + H_2O <5> (Reversibility: ?) [10]
P ?
S *p*-nitrostyrene 7,8-oxide + H_2O <4, 5> (Reversibility: ?) [23]
P ?
S phenanthrene 9,10-oxide + H_2O <3, 4, 5> (Reversibility: ?) [17, 18, 23, 31]
P ?
S phenanthrene-9,10-oxide + H_2O <1, 5> (Reversibility: ?) [2, 7, 14]
P ?

S propylene oxide + H_2O <5> (<5> low activity [23]) (Reversibility: ?) [23]
P ?
S racemic cis-9,10-epoxystearic acid + H_2O <8, 11, 12, 13, 21, 23, 30> (<21> 30% enantioselective (9S,10S)-cis-diol production [36]; <8> 59% enantioselective (9S,10S)-cis-diol production [36]; <30> 63.5% enantioselective (9S,10S)-cis-diol production [36]; <23> 74% enantioselective (9S,10S)-cis-diol production [36]; <11> 90% enantioselective (9S,10S)-cis-diol production by the constitutive enzyme, 86% by the pathogen-induced enzyme [36]; <12,13> 94% enantioselective (9S,10S)-cis-diol production [36]) (Reversibility: ?) [36]
P threo-(9R,10R)-dihydroxystearic acid + threo-(9S,10S)-dihydroxystearic acid
S racemic ethyl 3-phenylglycidate + H_2O <14> (Reversibility: ?) [37]
P (2R,3S)-ethyl 3-phenylglycidate (<14> production of (2R,3S)-ethyl 3-phenylglycidate with 95% enantiomeric excess and 26% yield in 12 h from 0.2% (w/v) of the racemat [37])
S styrene 7,8-oxide + H_2O <4, 5> (Reversibility: ?) [2, 3, 14, 17, 18, 19, 20, 23, 26, 27]
P styrene glycol <5> [20, 26]
S styrene 7,8-oxide + H_2O <4, 5> (Reversibility: ?) [22, 42]
P 1-phenyl-ethane-1,2-diol
S styrene epoxide + H_2O <5> (Reversibility: ?) [53]
P 1-phenyl-ethane-1,2-diol
S styrene oxide + H_2O <4, 5, 9, 22> (Reversibility: ?) [1]
P 1-phenyl-ethane-1,2-diol
S styrene oxide + H_2O <1, 3, 5> (Reversibility: ?) [1, 57]
P 1-phenylethane-1,2-diol
S styrene oxide + H_2O <1, 3, 4, 5, 6, 9, 15, 18, 19, 25, 26, 29> (Reversibility: ?) [4, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 25, 26]
P styrene glycol <3, 5, 18> [10, 11, 13, 14, 15, 16]
S styrene oxide + H_2O <13> (<13> recombinant enzyme, low activity [36]) (Reversibility: ?) [36]
P 1,2-diphenylethane-1,2-diol
S trans-8-ethylstyrene 7,8-oxide + H_2O <4> (<4> i.e. TESO [22]) (Reversibility: ?) [22]
P ?
S trans-9,10-epoxystearate + H_2O <3, 4, 5, 9, 22> (Reversibility: ?) [1]
P 9,10-dihydroxy-octadecanoic acid
S trans-ethyl styrene oxide + H_2O <3, 5, 9> (<3> trans-β-ethyl styrene oxide [24]) (Reversibility: ?) [2, 24]
P ?
S trans-stilbene oxide + H_2O <2> (Reversibility: ?) [39]
P 1,2-diphenylethane-1,2-diol
S trans-stilbene oxide + H_2O <1, 3, 4, 9, 22, 28> (<22> cis-stilbene oxide hydrolase and trans-stilbene hydrolase isoenzymes are not structurally identical [29]) (Reversibility: ?) [22, 24, 28, 29]
P ?

S Additional information <1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 13, 18, 21, 22, 23, 24, 30> (<4> substrate specificity [22]; <1,3,4,5> enzyme is induced by a number of xenobiotics, the enzyme is thought to play a detoxifying role by preventing epoxides from reacting irreversibly with critical cellular macromolecules. Some evidence exists to support the hypothesis that microsomal epoxide hydrolase is closely associated with at least certain forms of cytochrome P-450, such association may influence the functional role of the microsomal epoxide hydrolase in the various pathways of bioactivation [23]; <24> the enzyme may play a significant role in the secondary metabolism of juvenile hormone acid generated by juvenile hormone esterase [34]; <3> the enzyme plays an important role in the metabolism of various xenobiotic compounds including the polycyclic aromatic hydrocarbon carcinogens. It is specifically involved in the formation of the ultimate carcinogen of benzopyrene [25]; <18> induced about threefold by 5 mM phenobarbital [15]; <4> involved in the biotransformation of epoxides produced from either xeno- or endobiotics. The diols produced are generally detoxification products but in several instances, such as 9,10-epoxybenzopyrene-7,8-diol, they are very potent mutagens and possible carcinogens. The enzyme is induced by phenobarbital [30]; <4> the enzyme is expected to play a purely protective role with respect to epoxides metabolically produced from pharmaceutical drugs [5]; <5> role in benzopyrene-induced mutagenesis and carcinogenesis [3]; <5> enzyme is involved in the metabolism of steroids, the microsomal enzyme plays a central role in both the inactivation of primary mutagenic and carcinogenic metabolites of polycyclic aromatic hydrocarbons, and in activating these metabolites to even more toxic or mutagenic secondary products, enzyme production is induced by 3-methylcholanthrene, phenobarbital, trans-stilbene oxide, 2(3)-tert-butyl-4-hydroxyanisol or 2-acetylaminofluorene [2]; <5> potent and selective induction by trans-stilbene oxide [4]; <3> probably represents an important factor in the control of reactive epoxides [24]; <2> the enzyme in Drosophila melanogaster is involved in xenobiotic biotransformation, but not in juvenile hormone metabolism [39]; <4> genetic polymorphisms of the enzyme are responsible for varying susceptibility of cigarette smoking humans to chronic obstructive pulmonary disease, COPD [52]; <4> key enzyme in the metabolism of environmental contaminants being responsible for xenobiotic transformations, regulation of the enzyme occurs at transcriptional, translational, and post-translational level, the enzyme is also involved in cytoprotection and steroid metabolism, as well as in cellular responses to glucose metabolism and in Na^+-dependent bile acid transport, the enzyme is part of a multi-transport system at the cell surface [56]; <3> key enzyme in the metabolism of environmental contaminants being responsible for xenobiotic transformations, regulation of the enzyme occurs at transcriptional, translational, and post-translational levelthe enzyme is also involved in cytoprotection and steroid metabolism, as well as in cellular responses to glucose metabolism and in Na^+-dependent bile acid transport, the enzyme is part of a multi-transport system at

the cell surface [56]; <5> the enzyme activity in diabetic and in fasted rats is reduced by 60-71%, key enzyme in the metabolism of environmental contaminants being responsible for xenobiotic transformations, regulation of the enzyme occurs at transcriptional, translational, and post-translational level, the enzyme is also involved in cytoprotection and steroid metabolism, as well as in cellular responses to glucose metabolism and in Na^+-dependent bile acid transport, the enzyme is part of a multi-transport system at the cell surface [56]; <4> the enzyme is a phase II biotransformation enzyme which detoxifies epoxides, including carcinogens such as polycyclic aromatic hydrocarbons found in cigarette smoke and cooked meats, enzyme polymorphisms are not associated with colon cancer risk [46]; <4,5> the enzyme is critical for biotransformations in xenobiotic metabolism and detoxification [51]; <5> the enzyme is involved in xenobiotic metabolism and detoxification, e.g. of 1,3-butadiene oxide, styrene oxide, and benzo[a]pyrene-4,5-oxide [41]; <4> the enzyme is involved in xenobiotic metabolism and detoxification, e.g. of 1,3-butadiene oxide, styrene oxide, and benzo[a]pyrene-4,5-oxide, enzyme deficiency leads to acute and severe phenytoin toxicity in vivo, the enzyme is involved in transport of bile acids in the liver [41]; <3> the enzyme is involved in xenobiotic metabolism and detoxification, e.g. of 1,3-butadiene oxide, styrene oxide, and benzo[a]pyrene-4,5-oxide, the enzyme is important in sexual development [41]; <5> the enzyme metabolizes xenobiotic epoxides [42]; <5,8,10,11,12,13,21,23,30> enantioselectivity with fatty acid epoxide substrates [36]; <4> mEH prefers mono- and cis-substituted epoxides, low but highly enantioselective activity with epoxy fatty acids [41]; <3,5> mEH prefers mono- and cis-substituted epoxides, low but highly enantioselective activity with epoxy fatty acids, the enzyme acts as an antiestrogen binding site subunit [41]; <5> no activity with 5,6α-epoxy-5α-cholestan-3β-ol and 5,6β-epoxy-5α-cholestan-3β-ol [53]; <22> substrate specificity, no activity with 1,2,3,4,9,9-hexachloro-6,7-epoxy-1,4,41,5,6,7,8,8a-octahydro-1,4-methanonaphthalene,i.e. HEOM, 2-acetylaminofluorene, arochlor 1254, and benzopyrene 4,5-oxide, the cytosolic enzyme hydrates epoxides on cyclic systems very slowly, but hydrates a wide variety of aliphatic epoxides rapidly, trisubstituted terpenoid epoxides show low K_m-valus, fatty acid and ester epoxides are hydrated very quickly [1]; <3, 4, 5, 9> substrate specificity, no activity with 1,2,3,4,9,9-hexachloro-6,7-epoxy-1,4,41,5,6,7,8,8a-octahydro-1,4-methanonaphthalene,i.e. HEOM, 2-acetylaminofluorene, arochlor 1254, and benzopyrene 4,5-oxide, the cytosolic enzyme hydrates epoxides on cyclic sytems very slowly, but hydrates a wide variety of aliphatic epoxides rapidly, trisubstituted terpenoid epoxides show low K_m-values, fatty acid and ester epoxides are hydrated very quickly [1]; <5> the enzyme is distinct from the microsomal epoxide hydrolase EC 3.3.2.11, which specifically utilizes 5,6α-epoxy-5α-cholestan-3β-ol, that is no substrate for EC 3.3.2.9 [42]; <5,9> the enzyme shows a broad substrate specificity, all epoxides with a trisubstituted oxirane ring are no or poor substrates, no activity with 5,6α-epoxy-5α-cholestan-3β-ol [48]; <4> wide substrate specificity, epox-

ide-containing glycerol-phospholipids are poor substrates, the enzyme prefers mono- and cis-1,2-disubstituted epoxides as substrates, while gem-di-, trans-di-, tri- and tetra-substituted epoxides are either low turnover substrates or inhibitors, the enzyme has a tamaoxifen binding site [56]; <3,5> wide substrate specificity, the enzyme prefers mono- and cis-1,2-disubstituted epoxides as substrates, while gem-di-, trans-di-, tri- and tetra-substituted epoxides are either low turnover substrates or inhibitors [56]) (Reversibility: ?) [1, 2, 3, 4, 5, 15, 22, 23, 24, 25, 30, 34, 36, 39, 41, 42, 46, 48, 51, 52, 53, 56]

P ?

Inhibitors

(1R,2R)-1,2-epoxy-1-phenyl-1-propane <3> (<3> 88% inhibition at 0.8 mM [44]) [44]
(1S,2S)-1,2-epoxy-1-phenyl-1-propane <3> (<3> 89% inhibition at 0.8 mM [44]) [44]
(2R,3R)-1-benzyloxy-2,3-epoxy-3-(4-nitrophenyl)propane <3> (<3> 9.0% inhibition at 0.1 mM [44]) [44]
(2R,3R)-3-(4-nitrophenyl)glycidol <3> (<3> 8.2% inhibition at 0.2 mM [44]) [44]
(2S,3S)-1-benzyloxy-2,3-epoxy-3-(4-nitrophenyl)propane <3> (<3> 11% inhibition at 0.1 mM [44]) [44]
(2S,3S)-3-(4-nitrophenyl)glycidol <3> (<3> 17% inhibition at 0.2 mM [44]) [44]
(2S,3S)-*p*-nitrophenyl-glycidol <24> [32]
1,1,1-trichloropropene 2,3-oxide <3, 4, 5, 9, 16, 22> [1, 27, 33]
1,1,1-trichloropropene oxide <5, 9> [48]
1,1,1-trifluoro-2-propanone <24> [34]
1,1-diphenyloxirane <1, 4, 5> [8]
1-chloro-2,3-epoxypropane <18> (<18> non-competitive inhibitor [15]) [15]
1-isopropyl-1-phenyloxirane <1, 4, 5> [8]
1-methyl-1-phenyloxirane <1, 4, 5> [8]
1-methylcyclohexene oxide <1, 4> [8]
1-trichloropropene oxide <22> [29]
2-Bromo-4'-nitroacetophenone <3, 4, 5, 9, 16, 22> [1, 17, 33]
3,3,3-trichloropropene oxide <1, 4, 5> [5, 8]
3,3,3-trifluoropropene oxide <1, 4, 5> [8]
3,3-dimethylbutene oxide <1, 4, 5> [8]
3-methylbutene 1,2-oxide <1, 4, 5> [8]
3-methylcyclohexene oxide <1, 4, 5> [8]
3-phenylpropene 1,2-oxide <1, 4, 5> [8]
4-chlorophenyl-2,3-epoxypropyl ether <18> (<18> competitive [15]) [15]
4-phenylchalcone oxide <22, 24> [29, 32]
5,5'-dithiobis(2-nitrobenzoic acid) <24> [32, 34]
Al^{3+} <3, 4> (<3,4> slight inhibition at 1 mM [59]) [59]

Ba^{2+} <3, 4, 5> (<5> slight inhibition at 1 mM [59]; <3,4> 15% inhibition at 1 mM [59]) [59]
Ca^{2+} <3, 4, 5> (<3> 25% inhibition at 1 mM [59]; <4> 20% inhibition at 1 mM [59]; <5> slight inhibition at 1 mM [59]) [59]
Cd^{2+} <3, 4, 5> (<4,5> 20% inhibition at 1 mM [59]; <3> 70% inhibition at 1 mM [59]) [59]
chalcone oxide <24> [32]
cis-stilbene oxide <1, 4, 5> [8]
Co^{2+} <4, 5> (<5> 25% inhibition at 1 mM [59]; <4> 20% inhibition at 1 mM [59]) [59]
Cu^{2+} <3, 4, 5, 9, 22> (<3> 25% inhibition at 1 mM [59]; <4,5> slight inhibition at 1 mM [59]) [1, 59]
cyclohexene oxide <1, 4, 5, 16> (<5> inhibits hydration of estroxide, androstene oxide and styrene 7,8-oxide [21]) [8, 17, 21, 27, 33]
Emulgen 911 <3, 5> (<3> slight inhibition [43]; <5> 0.1%, 53% inhibition [14]) [14, 43]
Hg^{2+} <3, 4, 5> (<5> 70% inhibition at 1 mM [59]; <4> 90% inhibition at 1 mM [59]; <3> over 90% inhibition at 1 mM [59]) [59]
iodoacetamide <5> (<5> 0.1 mM, 15% inhibition [9]) [9, 14]
lipid <5> (<5> above the critrical micelle concentration gives kinetic pattern that mimics competitive inhibition [3]) [3]
Lubrol <5, 18, 22> (<18> 0.025% [15]; <5,22> Lubrol PX [26,29]; <5> phospholipid reverses the inhibition [26]) [15, 26, 29]
mersalyl <1> (<1> 0.09 mM, 13% inhibition [6]) [6]
metyrapone <4> (<4> strong inhibition of hydration of benzopyrene 11,12-oxide and dibenzoanthracene 5,6-oxide [17]) [17]
Mg^{2+} <3, 4, 5> (<4> 30% inhibition at 1 mM [59]; <3,5> 10% inhibition at 1 mM [59]) [59]
Mn^{2+} <3, 4, 5> (<3> 20% inhibition at 1 mM [59]; <4,5> 10% inhibition at 1 mM [59]) [59]
Ni^{2+} <3, 4, 5> (<3,4,5> 30% inhibition at 1 mM [59]) [59]
Pb^{2+} <3, 4, 5> (<3> 30% inhibition at 1 mM [59]; <4,5> 10% inhibition at 1 mM [59]) [59]
sodium cholate <5> (<5> inhibits hydration of estroxide and androstene oxide [21]) [21]
sodium deoxycholate <5> (<5> inhibits hydration of estroxide and androstene oxide [21]) [21]
styrene 7,8-oxide <5> (<5> inhibits hydration of estroxide and androstene oxide [21]) [21]
styrene oxide <1, 4, 5> [8]
trans-stilbene oxide <5> (<5> inhibits hydration of estroxide, androstene oxide and styrene 7,8-oxide [21]) [21]
Tween-80 <5, 18> (<18> 0.025% [15]; <5> inhibits hydration of estroxide and androstene oxide [21]) [15, 21]
Zn^{2+} <3, 4, 5> (<4,5> 80% inhibition at 1 mM [59]; <3> over 90% inhibition at 1 mM [59]) [59]

androstene oxide <5> (<5> inhibits hydration of estroxide and styrene 7,8-oxide [21]) [21]
cis-2-methyl-1-phenyloxirane <1, 4, 5> [8]
cyclohexane oxide <3, 5> (<3> IC50 is 0.0022 mM [44]) [44, 53]
elaidamide <3, 4, 5> (<3,4,5> mixed inhibition type [41]) [41]
estroxide <5> (<5> inhibits hydration of androstene oxide and styrenen 7,8-oxide [21]) [21]
indene 1,2-oxide <1, 4, 5> [8]
juvenile hormone I <18> (<18> competitive [15]) [15]
m-bromostyrene oxide <1, 4, 5> [8]
m-nitrostyrene oxide <1, 4, 5> [8]
methyl-*p*-epoxymethylbenzoate <1, 4, 5> [8]
n-octene 1,2-oxide <1, 4, 5> [8]
naphthalene 1,2-oxide <1, 4, 5> [8]
ω-bromo-nitro-acetophenone <24> [32]
p-hydroxymercuribenzoate <5> (<5> 0.1 mM, 15% inhibition [9]) [9, 14]
p-bromostyrene oxide <1, 4, 5> [8]
p-chlorophenyl 2,3-epoxypropyl ether <1, 4, 5> [8]
p-nitrostyrene oxide <1, 4, 5> [8]
p-phenylstyrene oxide <1, 4, 5> [8]
propene oxide <1, 4, 5> [8]
trichloropropene oxide <3, 4, 5, 24> (<5> inhibits hydration of estroxide, androstene oxide and styrene 7,8-oxide [21]) [21, 32, 41]
urea derivatives <3, 4, 5> [41]
Additional information <3, 4, 5, 9> (<5> 5,6α-epoxy-5α-cholestan-3β-ol causes down-regulation of the enzyme, no effect by cholestyramine and clofibrate [42]; <4> HNF3 negatively regulates the transactivation of E1 promoter by GATA-4 in hepatoma cells [55]; <4,5> metal chelators like 1,10-phenanthroline, 1,7-phenanthroline, EDTA, EGTA, and dipicolinic acid preserve enzyme activity in presence of metal ions [59]; <3> no inhibition by 3-phenylglycidol enantiomers, inhibitor synthesis, overview, inhibition mechanisms, overview [44]; <5,9> no inhibition by 5,6α-imino-5α-cholestane-3β-ol [48]; <3> no inhibition by Co^{2+} at 1 mM, metal chelators like 1,10-phenanthroline, 1,7-phenanthroline, EDTA, EGTA, and dipicolinic acid preserve enzyme activity in presence of metal ions [59]; <5> no inhibition by isoquinoline, 7-oxocholesterol, 7-oxocholestanol, and 6-oxocholestanol [53]; <4> quantitative structure-activity relationships in epoxides inhibiting EH activity, molecular modelling, kinetics, overview [58]; <5> suppression of enzyme expression by dexamethasone, gadolinium chloride, acriflavine, lipopolysaccharide [56]; <4> suppression of enzyme expression by glucocorticoid, interacting with the 5-flanking sequence, and by dexamethasone, gadolinium chloride, acriflavine, lipopolysaccharide, gem-di-, trans-di-, tri- and tetra-substituted epoxides are either low turnover substrates or inhibitors [56]) [42, 44, 48, 53, 55, 56, 58, 59]

Cofactors/prosthetic groups

Additional information <1> (<1> no cofactos required [6]) [6]

Activating compounds

1(2-naphthyl)-2-phenylethanedione <5> (<5> stimulates [2]) [2]
1-(2-isopropylphenyl)-imidazole <1, 4, 5> (<5> stimulates [9,14]; <1,4,5> 2 mM, stimulates [8]) [8, 9, 14]
1-(2-cyanophenyl)-imidazole <1, 4, 5> (<1,4,5> 2 mM, stimulates [8]) [8]
3-methylcholanthrene <5> (<5> enhances nuclear hydration of phenanthrene-9,10-oxide, benzopyrene-11,12-oxide and octene-1,2-oxide, no significant effect on microsomal hydration [18]) [18]
CHAPS <17> (<17> stimulates, increases the enantiomeric ratio of activity with R-enantiomer to S-enantiomer of 1,2-epoxyhexane and styrene oxide [38]) [38]
clotrimazole <4, 5> (<4,5> stimulates [2,27]) [2, 27]
cytochrome P450 <5> (<5> activation of microsomal epoxide hydrolase by interaction with cytochromes P450 [35]) [35]
digitonin <17> (<17> stimulates, increases the enantiomeric ratio of activity with R-enantiomer to S-enantiomer of 1,2-epoxyhexane and styrene oxide [38]) [38]
dodecyl maltoside <17> (<17> stimulates, increases the enantiomeric ratio of activity with R-enantiomer to S-enantiomer of 1,2-epoxyhexane and styrene oxide [38]) [38]
ellipticine <5> (<5> stimulates [2]) [2]
emulgen 108 <3> (<3> 2fold activation [43]) [43]
ethanol <1, 4, 5> (<1,4,5> 2.7 M, stimulates [8]) [8]
flavone <5> (<5> stimulates [2]) [2]
harmane <5> (<5> stimulates [2]) [2]
isoquinoline <5, 16> (<5,16> stimulates [2,33]) [2, 33]
lubrol Px <3> (<3> 2fold activation [43]) [43]
metyrapone <1, 4, 5, 9, 16> (<4,5,16> stimulates [2,9,14,18,19,33]; <1,4,5> 2 mM, stimulates [8]; <4> great stimulation of hydration of styrene 7,8-oxide and octene 1,2-oxide, slight stimulation of hydration of benzopyrane 4,5-oxide [17]) [2, 8, 9, 14, 17, 18, 19, 33]
Non-idet P40 <17> (<17> stimulates [38]) [38]
phenobarbital <5> (<5> enhances nuclear and microsomal hydration of phenanthrene-9,10-oxide, benzopyrene-11,12-oxide and octene-1,2-oxide [18]) [18]
phospholipids <4> (<4> the enzyme is tightly associated with phospholipids [56]) [56]
sodium cholate <3> (<3> slight activation [43]) [43]
Thesit <17> (<17> stimulates, increases the enantiomeric ratio of activity with R-enantiomer to S-enantiomer of 1,2-epoxyhexane and styrene oxide [38]) [38]
Triton WR-1339 <3> (<3> nearly 2fold activation [43]) [43]
Triton X-100 <17> (<17> stimulates [38]) [38]
Tween 80 <3> (<3> slight activation [43]) [43]

Zwittergent 3-12 <17> (<17> stimulates, increases the enantiomeric ratio of activity with R-enantiomer to S-enantiomer of 1,2-epoxyhexane and styrene oxide [38]) [38]
benzil <4, 5> (<4,5> stimulates [2,27]) [2, 27]
chalcone epoxide <5> (<5> stimulates [2]) [2]
nitroanisil <5> (<5> stimulates [2]) [2]
norharmane <5> (<5> stimulates [2]) [2]
octyl-glucoside <17> (<17> stimulates, increases the enantiomeric ratio of activity with R-enantiomer to S-enantiomer of 1,2-epoxyhexane and styrene oxide [38]) [38]
p-anisil <5> (<5> stimulates [2]) [2]
sucrose monolaurate <17> (<17> stimulates, increases the enantiomeric ratio of activity with R-enantiomer to S-enantiomer of 1,2-epoxyhexane and styrene oxide [38]) [38]
Additional information <3, 4, 5, 9, 11, 14, 22> (<3> clofibrate induces the enzyme [44]; <4> enzyme expression is induced by exogenous β-amyloid and trimethyl-tin, a neurotoxic agent, β-amyloid aggregation induces enzyme expression in astrocytes [51]; <5> enzyme expression is induced by neurotoxic agent trimethyl-tin in hippocampus and entorhinal cortex [51]; <3,4,5,9,22> induction of the enzyme by clofibrate and di(2-ethylhexyl)phthalate [1]; <5> insulin increases enzyme activity in hepatocyte cell culture [56]; <14> maximal activity with dimethyl formamide as co-solvent, effects of solvents on enzyme activity, overview [37]; <5> no effect by cholestyramine and clofibrate, phenobarbital and isosafrole induce the enzyme expression, it is also induced by trans-stilbene oxide, γ-chlordane, Aroclor 1254, and 2-acetylamineo-fluorene in liver microsomes [42]; <11> pathogens induce enzyme expression [36]) [1, 36, 37, 42, 44, 51, 56]

Metals, ions

Al^{3+} <3, 4> (<3,4> slight inhibition at 1 mM [59]) [59]
Ba^{2+} <3, 4, 5> (<3,4> 15% inhibition at 1 mM [59]; <5> slight inhibition at 1 mM [59]) [59]
Ca^{2+} <3, 4, 5> (<4> 20% inhibition at 1 mM [59]; <3> 25% inhibition at 1 mM [59]; <5> slight inhibition at 1 mM [59]) [59]
Cd^{2+} <3, 4, 5> (<4,5> 20% inhibition at 1 mM [59]; <3> 70% inhibition at 1 mM [59]) [59]
Co^{2+} <4, 5> (<4> 20% inhibition at 1 mM [59]; <5> 25% inhibition at 1 mM [59]) [59]
Cu^{2+} <3, 4, 5> (<3> 25% inhibition at 1 mM [59]; <4,5> slight inhibition at 1 mM [59]) [59]
Fe^{2+} <3, 4, 5> (<5> 10% activation at 1 mM [59]; <3> 15%% activation at 1 mM [59]; <4> 40% activation at 1 mM [59]) [59]
Fe^{3+} <3, 4, 5> (<5> 20% activation at 1 mM [59]; <3> 30%% activation at 1 mM [59]; <4> 50% activation at 1 mM [59]) [59]
Hg^{2+} <3, 4, 5> (<5> 70% inhibition at 1 mM [59]; <4> 90% inhibition at 1 mM [59]; <3> over 90% inhibition at 1 mM [59]) [59]

Mg^{2+} <3, 4, 5> (<3,5> 10% inhibition at 1 mM [59]; <4> 30% inhibition at 1 mM [59]) [59]
Mn^{2+} <3, 4, 5> (<4,5> 10% inhibition at 1 mM [59]; <3> 20% inhibition at 1 mM [59]) [59]
Ni^{2+} <3, 4, 5> (<3,4,5> 30% inhibition at 1 mM [59]) [59]
Pb^{2+} <3, 4, 5> (<4,5> 10% inhibition at 1 mM [59]; <3> 30% inhibition at 1 mM [59]) [59]
Zn^{2+} <3, 4, 5> (<4,5> 80% inhibition at 1 mM [59]; <3> over 90% inhibition at 1 mM [59]) [59]
Additional information <5> (<5> no effect by 1 mM Al^{3+} [59]) [59]

Turnover number (min^{-1})

0.08 <24> (juvenile hormone III) [34]
0.5 <5> (phenanthrene 9,10-oxide) [31]
53 <5> (1,10-phenanthroline 5,6-oxide) [31]

Specific activity (U/mg)

0.000085 <3> (<3> liver microsomes, substrate styrene oxide [57]) [57]
0.000124 <5> (<5> liver microsomes, substrate styrene oxide [57]) [57]
0.00019 <3, 5> (<5> epididymis microsomes [40]; <3> liver microsomes, substrate allylbenzene oxide [57]) [40, 57]
0.00035 <5> (<5> liver microsomes, substrate allylbenzene oxide [57]) [57]
0.00043 <1> (<1> liver microsomes, substrate styrene oxide [57]) [57]
0.00051 <5> (<5> testis microsomes [40]) [40]
0.0036 <1> (<1> liver microsomes, substrate allylbenzene oxide [57]) [57]
0.0045 <24> (<24> enzyme form cytosol [32]) [32]
0.0057 <5> (<5> liver microsomes [40]) [40]
0.0064 <3> (<3> liver microsomes [43]) [43]
0.0083 <24> [34]
0.0272 <4> [30]
0.0559 <5> [30]
0.118 <4> [8]
0.121 <4> [27]
0.196 <17> [38]
0.479 <5> [11]
0.516 <5> [5, 10]
0.596 <5> [14]
0.618 <4> [17]
0.659 <24> (<24> enzyme from microsomes [32]) [32]
0.735 <5> [26]
0.76 <5> [20]
0.842 <1> [6]
1.98 <16> [33]
11.4 <27> [54]
524 <5> [31]
Additional information <3, 4, 5, 9, 22> (<5> highly sensitive and rapid radiometric assay [16]; <3,4,5,9,22> several analytical and activity assay methods [1]) [1, 4, 16]

K_m-Value (mM)

0.00028 <24> (juvenile hormone III) [34]
0.00055 <24> (juvenile hormone II) [34]
0.00061 <24> (juvenile hormone I) [34]
0.0024 <5> (cis-stilbene oxide, <5> pH 7.4, 37°C [59]) [59]
0.004 <4> (cis-stilbene oxide, <4> pH 7.4, 37°C [59]) [59]
0.0055 <5> (phenanthrene 9,10-oxide) [31]
0.01 <3> (trans-stilbene oxide) [24]
0.015 <3> (cis-stilbene oxide, <3> pH 7.4, 37°C [59]) [59]
0.17 <16> (7-glycidoxycoumarin) [33]
0.35 <3> (trans-β-ethylstyrene oxide) [23]
0.53 <1, 18> (styrene oxide) [6, 15]
0.59 <5> (1,10-phenanthroline 5,6-oxide) [31]
0.67 <5> (styrene oxide) [5, 12]
0.98 <4> (styrene 7,8-oxide) [27]
Additional information <3, 4, 5, 8, 9, 10, 11, 12, 13, 21, 23, 30> (<3, 4, 5, 8, 9, 10, 11, 12, 13, 21, 23, 30> kinetics [36, 41, 50]) [3, 36, 41, 50]

K_i-Value (mM)

0.00007 <4> (elaidamide, <4> pH 7.4 [41]) [41]
0.0076 <3> (Zn^{2+}, <3> pH 7.4, 37°C, mixed inhibition type [59]) [59]
0.012 <5> (Zn^{2+}, <5> pH 7.4, 37°C, competitive inhibition type [59]) [59]
0.0154 <5> (cyclohexane oxide, <5> pH 7.4, 37°C [53]) [53]
0.061 <4> (Zn^{2+}, <4> pH 7.4, 37°C, competitive inhibition type [59]) [59]

pH-Optimum

5.5-8 <13> (<13> broad maximum, recombinant enzyme [36]) [36]
6.7 <3> (<3> cytosolic and microsomal enzyme, hydration of trans-β-ethylstyrene oxide [24]) [24]
7 <3, 5> (<5> assay at [45]) [24, 45]
7.2 <3> (<3> cytosolic enzyme, hydration of trans-stilbene oxide [24]) [24]
7.4 <3, 4, 5, 8, 9, 10, 11, 12, 21, 23, 30> (<3, 4, 5, 8, 9, 10, 11, 12, 21, 23, 30> assay at [36, 43, 50, 53, 59]; <5> hydration of benzopyrene 4,5-oxide [4]) [4, 36, 43, 50, 53, 59]
7.4-9 <1, 3, 5> (<1,3,5> assay at [57]) [57]
7.5-8.5 <24> [34]
8 <14, 16> (<14> assay at [37]) [33, 37]
8-9 <1, 4> [6, 41]
8.5 <18> (<18> hydration of styrene oxide [15]) [15]
8.5-9 <24> [32]
8.9 <5> (<5> hydration of styrene oxide, Tris buffer [4,5,12]; <5> hydration of benzopyrene 7,8-oxide and benzopyrene 9,10-oxide [4]) [4, 5, 12]
8.9-9.4 <5> (<5> hydration of styrene 7,8-oxide [2]) [2]
9 <3, 5> (<3> assay at [44]; <5> hydration of styrene oxide in sodium phosphate buffer [4,5,12]; <3> benzopyrene 4,5-oxide, microsomal enzyme [24]) [4, 5, 12, 24, 42, 44]

9.2 <4> [27]
9.4 <5> (<5> somewhat higher than, hydration of styrene oxide in glycine buffer [4,12]) [4, 12]

pH-Range

6.5-9.8 <1> (<1> pH 6.5: about 50% of maximal activity, pH 9.8: about 55% of maximal activity [6]) [6]
7-9.5 <24> (<24> pH 7.0: about 35% of maximal activity, pH 9.5: about 70% of maximal activity [32]) [32]
7-10 <16> (<16> pH 7.0: about 60% of maximal activity, pH 10.0: about 25% of maximal activity [33]) [33]
7.4-9 <1, 3, 5> [57]
Additional information <3> [24]

Temperature optimum (°C)

26 <5, 8, 10, 11, 12, 13, 21, 23, 30> (<5, 8, 10, 11, 12, 13, 21, 23, 30> assay at [36]) [36]
30 <14> (<14> assay at [37]) [37]
37 <3, 4, 5, 9> (<3, 4, 5, 9> assay at [42, 43, 44, 45, 50, 53, 59]) [42, 43, 44, 45, 50, 53, 59]

4 Enzyme Structure

Molecular weight

31000 <21> (<21> gel filtration, 2 peaks [36]) [36]
62000 <21> (<21> gel filtration, 2 peaks [36]) [36]
64000 <10> (<10> gel filtration [36]) [36]
130000 <3, 4> (<3,4> gel filtration [23]) [23]
600000 <5> (<5> enzyme aggregate formed in absence of amphiphathic detergents, gel filtration [23]) [23]
800000 <5> (<5> enzyme aggregate formed in absence of amphiphathic detergents, equilibrium sedimentation [23]) [23]
Additional information <3, 4, 5> (<5> enzyme aggregates in absence of amphipathic detergents [23]) [23]

Subunits

? <1, 3, 4, 5, 24> (<4, 5, 24> x * 50000, SDS-PAGE [19, 22, 26, 32, 34, 40]; <4> x * 58000, SDS-PAGE [23]; <4> x * 51000, SDS-PAGE [27]; <4,5> x * 49000, SDS-PAGE [4, 5, 10, 12, 17, 18, 20, 23]; <5> x * 53000-54000, SDS-PAGE [9]; <3, 5> x * 49500 SDS-PAGE [11, 13]; <4> x * 50000, about [56]) [4, 5, 9, 10, 11, 12, 13, 17, 18, 19, 20, 22, 23, 26, 27, 32, 34, 40, 56]
dimer <13> (<13> mainly, in solution, recombinant enzyme, mass spectrometry [36]) [36]
Additional information <3, 4, 5, 21> (<21> monomer or dimer [36]; <4> peptide mapping [22]; <5> structure analysis, amino acid sequence analysis and comparisons, the enzyme forms an α/β hydrolase fold [49]; <3, 4, 5> the enzyme possesses a long N-terminal transmembrane domain of 20 amino

acids which acts as an anchor for cellular membranes [41]; <4> the enzyme possesses an N-terminal strong hydrophobic transmembrane anchor [56]) [22, 36, 41, 49, 56]

5 Isolation/Preparation/Mutation/Application

Source/tissue

Huh-7 cell <4> (<4> hepatoma cell line [55]) [55]
adrenal gland <1, 4, 5> (<5> adrenalectomy elevates enzyme levels [56]) [4, 5, 16, 21, 27, 56]
astrocyte <4> (<4> patchy distribution [51]) [51]
bladder <5> [4, 5]
brain <1, 3, 4, 5> (<5> very low activity [45]) [4, 5, 45, 51, 56]
bronchus <4> [56]
cecum <5> [4, 5]
cell culture <4> (<4> numerous cancer cell lines and primary cells [56]) [56]
colon <5> [4, 5]
culture condition:styrene oxide-grown cell <14> [37]
culture condition:styrene-grown cell <14> [37]
dermis <5> [4, 5]
egg <24> [34]
entorhinal area <5> [51]
epidermis <5> [4, 5]
epididymis <5> (<5> caput, corpus, cauda [40]) [40]
epimastigote <18> [15]
epithelium <4> (<4> bronchial [56]) [56]
esophagus <5> [4, 5]
fruit pulp <30> [36]
glial cell <3, 4, 5> (<3,4,5> high enzyme content [56]) [51, 56]
heart <3, 5> [4, 5, 56]
hepatocyte <5> [56]
hippocampus <4, 5> (<4> glial cell culture, enzyme expression is elevated in hippocampus and associated cortex of patients with Alzheimers disease [51]) [51]
hypothalamus <5> [56]
intestine <5> (<5> epithelium, very low activity [45]) [45]
kidney <3, 4, 5, 9, 22> (<5> low activity [45]; <3, 4, 5, 9, 22> high activity [1]) [1, 5, 16, 40, 45]
larva <2> [39]
leaf <12> (<12> harvested 7 days after inoculation with Erypsiphe graminis [36]) [36]
liver <1, 3, 4, 5, 9, 22> (<3, 4, 5, 9, 22> high activity [1]; <5> highest enzyme activity, specific for [45]; <5> the liver enzyme is controlled by the pituitary gland [56]; <4> tissue-specific expression of the enzyme in liver [55]) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 29, 30, 31, 35, 36, 40, 41, 42, 43, 44, 45, 48, 50, 53, 55, 56, 57, 59]
lung <3, 4, 5, 9> (<5> low activity [45]) [4, 5, 16, 21, 45, 56]

lymphocyte <3, 4> (<3> intra-epithelial, cells show down-regulated enzyme expression [56]) [56]
monocyte <4> [56]
muscle <5> (<5> triceps [4,5]) [4, 5]
neuron <3, 4, 5> (<4> pyramidal [51]; <3,4,5> low enzyme content [56]) [51, 56]
ovary <3, 5> [4, 5, 16, 21, 56]
pituitary gland <5> (<5> hypophysectomy induces the liver enzyme in females and males [56]) [56]
prostate gland <5> [4, 5]
root <21> [36]
seedling <8, 10, 23> [36]
small intestine <1, 5> [4, 5]
spermatid <5> [40]
spleen <5> (<5> very low activity [45]) [4, 5, 45]
stomach <5> (<5> membranous and glandular [4,5]) [4, 5]
subcutis <5> [4, 5]
submandibular gland <5> [4, 5]
synovial tissue <4> [56]
testis <3, 4, 5, 9, 22> (<3, 4, 5, 9, 22> low activity [1, 45]) [1, 4, 5, 16, 21, 40, 45, 56]
thymus <5> [4, 5]
tongue <5> [4, 5]
trachea <5> [4, 5]
tuber <11> [36]
Additional information <3, 4, 5> (<3, 4, 5> wide tissue distribution [41]; <5> tissue distribution analysis [45]; <3,4> wide tissue distribution in mammalian tissue, enzyme levels vary with environmental exposure, sex, and age [56]; <5> wide tissue distribution in mammalian tissue, enzyme levels vary with environmental exposure, sex, and age, growth hormones are involved in sexually dimorphic liver enzyme expression [56]) [41, 45, 56]

Localization

Golgi apparatus <5> [45]
cytosol <4, 5, 24> (<5> no activity detected [21]; <4> in neoplastic livers [56]) [21, 32, 34, 36, 56]
endoplasmic reticulum <4> [27]
membrane <3, 4, 5, 17, 24> (<3,4,5> orientation of the enzyme depends on the membrane type [41]) [34, 38, 40, 41]
microsome <1, 2, 3, 4, 5, 7, 9, 19, 22, 24, 27, 28> (<3> integral microsomal protein [24]; <5> slightly higher enzyme activity in smooth compared to rough microsomes [45]) [2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 34, 35, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 59]
mitochondrion <5> (<5> very low activity [21]) [21]
nucleus <5> [18]
plasma membrane <3, 4, 5> (<3,4,5> in liver [41]; <4> in liver, the catalytic site facing the extracellular medium [56]) [41, 56]

rough endoplasmic reticulum <5> [21]
smooth endoplasmic reticulum <3, 4, 5> (<3,4,5> in liver [41]; <4> in liver, the catalytic site facing the cytosol [56]) [21, 41, 56]
soluble <3, 4, 5, 8, 9, 10, 11, 12, 13, 21, 22, 23, 30> [1, 36]
Additional information <4, 5> (<5> subcellular distribution analysis [45]; <4> the enzyme shows strong hydrophobic interaction with the membrane, deletion of the N-terminal membrane anchor does not render the enzyme soluble [56]) [45, 56]

Purification

<1> [6, 7]
<3> [13, 25]
<3> (from liver microsomes) [44]
<3> (partially 2.4fold from liver using PEG precipitation, and to homogeneity by ion exchange, hydrophobic interaction chromatography, and hydroxylapatite chromatography) [1]
<3> (partially from liver by micosome preparation, two steps of gel filtration, separation from the cholesterol epoxide hydrolase, EC 3.3.2.11) [43]
<4> [8, 17]
<4> (hydrophobic chromatography: a one-step method) [30]
<4> (multiple enzyme forms) [19]
<4> (partial) [27]
<4> (partially from liver) [1]
<5> [4, 5, 10, 11, 14, 20, 21, 26, 31]
<5> (multiple enzyme forms) [19]
<5> (phenobarbital-treated animals) [30]
<5> (preparation of liver microsomes, separation from the cholesterol 5,6-oxide hydrolase, EC 3.3.2.11, by immunoprecipitation) [48]
<5> (to homogeneity from liver of phenobarbital- and isosafrole-treated rats, separation from the cholesterol 5,6-oxide hydrolase, EC 3.3.2.11, by immunoprecipitation) [42]
<8> (preparation of cytosolic fraction by ultracentrifugation) [36]
<9> (partially by microsome preparation) [50]
<9> (partially from liver) [1]
<9> (preparation of liver microsomes, separation from the cholesterol 5,6-oxide hydrolase, EC 3.3.2.11, by immunoprecipitation) [48]
<10> (to homogeneity from seedling cytosol) [36]
<11> (preparation of cytosolic fraction by ultracentrifugation) [36]
<12> (preparation of cytosolic fraction by ultracentrifugation) [36]
<13> (recombinant His-tagged enzyme from Saccharomyces cerevisiae strain WA6 by nickel affinity chromatography) [36]
<21> (to homogeneity from root cytosol) [36]
<22> [29]
<23> (preparation of cytosolic fraction by ultracentrifugation) [36]
<24> [34]
<30> (preparation of cytosolic fraction by ultracentrifugation) [36]

Crystallization

<4> (molecular modelling of human enzyme by homology with the crystal structure of the Aspergillus niger enzyme at 1.8 A resolution, structure-activity relationships in epoxides inhibiting EH activity) [58]

Cloning

<2> (expression using a baculovirus expression system) [39]
<4> (gene EPHX1 is located on chromosome 1, DNA and amino acid sequence determination and analysis, expression of liver enzyme in Schizosaccharomyces pombe, expression in Cos-1 cells, expression using the baculovirus system) [56]
<4> (genotyping analysis of several humans of different origin) [52]
<4> (genotyping of 3553 humans) [46]
<4> (genotyping of 497 humans) [47]
<4> (the E1-b promoter functions as the primary driver of EPHX1 expression in human tissues, E1 promoter directs expression only in the liver, analysis of promoters and regulation enzyme expression in tissues, expression in Hep-G2 cells and 293A cells, co-expression of GATA-4, overview) [55]
<5> [56]
<5> (baculovirus expression system) [31]
<13> (expression of His-tagged enzyme in Saccharomyces cerevisiae strain WA6, the N-terminal His-tag reduces enzyme activity by 75%, therefore a C-terminal His-tag is used) [36]
<24> (baculovirus expression system) [32]
<27> (functional expression in Escherichia coli) [54]

Engineering

E404D <4> (<4> mutation of the catalytic triad residue leads to increased activity compared to the wild-type enzyme [56]) [56]
H139R <4> (<4> mutant shows increased activity compared to the wild-type enzyme, molecular modelling [58]; <4> natural genotype, frequency of the polymorphism, the mutant enzyme shows 25% increased activity compared to the wild-type enzyme [46]; <4> natural genotype, frequency of the polymorphism, the mutant enzyme shows increased activity compared to the wild-type enzyme [47]) [46, 47, 58]
R43T <4> (<4> mutant shows reduced activity compared to the wild-type enzyme, molecular modelling [58]) [58]
T275A <4> (<4> mutant shows similar activity compared to the wild-type enzyme, molecular modelling [58]) [58]
Y113H <4> (<4> mutant shows similar activity compared to the wild-type enzyme, molecular modelling [58]; <4> natural genotype, frequency of the polymorphism, the mutant enzyme shows 40% reduced activity compared to the wild-type enzyme [46]; <4> natural genotype, frequency of the polymorphism, the mutant enzyme shows reduced activity compared to the wild-type enzyme [47]) [46, 47, 58]
Additional information <3, 4> (<4> genotyping of 3553 humans, enzyme polymorphisms are not associated with colon cancer [46]; <4> genotyping of 497 humans, enzyme polymorphisms are associated with esophageal squa-

mous-cell-carcinoma, ESCC, and smoking or areca chewing, higher enzyme activity can contribute to lower the risk of cancer development, overview [47]; <3> mEH knockout mice are less sensitive to the carcinogenic activity of 7,12-dimethylbenz[a]anthracene compared to wild-type mice, but show no altered phenotype [41]; <4> naturally occurring genetic polymorphisms of the enzyme are responsible for varying susceptibility of cigarette smoking humans to chronic obstructive pulmonary disease, COPD [52]; <4> polymorphisms associated with the onset of diseases [56]) [41, 46, 47, 52, 56]

Application

drug development <3, 4, 5> (<3,4,5> the enzyme is a target for inhibitor design [41]) [41]

synthesis <14, 27> (<14> the enantioselective enzyme is useful in production of chiral substances, e.g. production of (2R,3S)-ethyl 3-phenylglycidate with 95% enantiomeric excess and 26% yield in 12 h from 0.2% (w/v) of the racemat by whole cells of Pseudomonas sp. strain BZS21, maximal activity with dimethyl formamide as co-solvent [37]; <27> the purified recombinant enzyme can be used as biocatalyst for kinetic resolution of racemic styrene oxide with the result of over 99% enantiopure (S)-styrene oxide in 23,5% yield [54]) [37, 54]

6 Stability

Temperature stability

25 <5> (<5> pH 7.0, 5 h, stable [5,10]) [5, 10]

37 <3, 4, 5> (<5> pH 7.0, 24 h, less than 10% inactivation [5,10]) [5, 10]

90 <24> (<24> 1 min, complete loss of activity [34]) [34]

Storage stability

<5>, -70°C, stable for months [20]

<5>, 0°C, 50 mM sodium phosphate buffer, pH 7.0, 2 months, stable [2, 5, 10]

<5>, 4°C, stable for up to 8 weeks [20]

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Soluble epoxide hydrolase 3.3.2.10

1 Nomenclature

EC number
3.3.2.10

Systematic name
epoxide hydrolase

Recommended name
soluble epoxide hydrolase

Synonyms
AnEH <32> [35]
BNSEH1 <17> [29]
CEH <3, 4, 5> [42, 46, 50]
Cterm-EH <4> [47]
Cytosolic epoxide hydrolase <2, 3, 4, 5> [42, 46, 50, 54, 56, 65]
EC 3.3.2.3
EH <32> [45]
EPXH1 <4> [41]
EPXH2 <4> [33, 41, 63]
EPXH2B <34> [63]
PNSO hydrolase <4> [3]
PsEH <12> [23]
SEH <1, 3, 4, 5, 7, 10, 11, 12, 13, 14, 15, 17, 19, 22, 24, 25, 30, 31, 34, 35, 36, 37> [29, 33, 37, 38, 39, 40, 41, 43, 44, 48, 49, 53, 55, 57, 58, 60, 61, 62, 63, 64, 66]
TESO hydrolase <4> [3]
TSO hydrolase <3> [19]
epoxide hydrolase 1 <15> [34]
mEH <5> [25]
soluble-type epoxide hydrolase <33> [51]
Additional information <3, 4, 5> (<4> see also EC 3.1.3.76 [27,33,44,57]; <3> see also EC 3.3.2.6 [56]; <3,4,5> the enzyme belongs to the α/β-hydrolase fold family of proteins [41]; <5> the enzyme belongs to the family of C-X bond hydrolase enzymes [48]) [27, 33, 41, 44, 48, 56, 57]

CAS registry number
9048-63-9

2 Source Organism

<1> *Vicia sativa* (no sequence specified) [63]
<2> *Cavia porcellus* (no sequence specified) [13, 63, 65]
<3> *Mus musculus* (no sequence specified) [4, 5, 6, 7, 8, 9, 10, 15, 19, 20, 21, 22, 23, 40, 41, 42, 43, 46, 50, 53, 54, 55, 56, 58, 61, 62, 65, 66]
<4> *Homo sapiens* (no sequence specified) [1, 3, 9, 12, 22, 23, 24, 27, 33, 38, 40, 41, 43, 44, 47, 50, 57, 58, 61, 63, 64, 66]
<5> *Rattus norvegicus* (no sequence specified) [1, 3, 9, 11, 14, 23, 24, 25, 37, 41, 42, 48, 49, 52, 53, 61, 63, 65, 66]
<6> *Sus scrofa* (no sequence specified) [63]
<7> *Triticum aestivum* (no sequence specified) [63]
<8> *Oryctolagus cuniculus* (no sequence specified) [1,2,9,63]
<9> *Aspergillus niger* (no sequence specified) [17,31,36]
<10> *Spinacia oleracea* (no sequence specified) [63]
<11> *Zea mays* (no sequence specified) [63]
<12> *Solanum tuberosum* (no sequence specified) [23, 59, 63, 66]
<13> *Nicotiana tabacum* (no sequence specified) [63]
<14> *Glycine max* (no sequence specified) [16, 63]
<15> *Arabidopsis thaliana* (no sequence specified) [23, 34, 63]
<16> *Pseudomonas sp.* (no sequence specified) [28]
<17> *Brassica napus* (no sequence specified) [29, 63]
<18> *Corynebacterium sp.* (no sequence specified) [18]
<19> *Ricinus communis* (no sequence specified) [63]
<20> *Mesocricetus auratus* (no sequence specified) [63]
<21> *Equus caballus* (no sequence specified) [63]
<22> *Apium graveolens* (no sequence specified) [63]
<23> *Macaca mulatta* (no sequence specified) [1, 63]
<24> *Oryza sativa* (no sequence specified) [63]
<25> *Ananas comosus* (no sequence specified) [63]
<26> *Oryzias latipes* (no sequence specified) [63]
<27> *Agrobacterium radiobacter* (no sequence specified) [26, 30]
<28> *Salmo gairdneri* (no sequence specified) [63]
<29> *cress* (no sequence specified) [23]
<30> *Papio sp.* (no sequence specified) [63]
<31> *Euphorbia lagascae* (no sequence specified) [32, 63]
<32> *Aspergillus niger* (UNIPROT accession number: Q9UR30) [35, 45]
<33> *Glycine max* (UNIPROT accession number: O49857) [51]
<34> *Mus musculus* (UNIPROT accession number: P34914) [39, 63]
<35> *Gallus gallus* (UNIPROT accession number: Q45QT1) [60]
<36> *Citrus jambhiri* (no sequence specified) [63]
<37> *Malus pumila* (no sequence specified) [63]
<38> *Pimphales promelas* (no sequence specified) [63]
<39> *Stenotomus chrysops* (no sequence specified) [63]
<40> *Mus msuculus* (no sequence specified) [1]
<41> no activity in *Trichoplusia ni* [59]

3 Reaction and Specificity

Catalyzed reaction

an epoxide + H_2O = a glycol (<3> detailed reaction mechanism, molecular dynamics simulations, detailed analysis of substrate binding to the active site and determination of the preferred conformation, involving Tyr381, Tyr465, and His523 [55]; <5> reaction mechanism, catalytic triad [25]; <3, 4, 5> the C-terminal part harbors the epoxide hydrolase activity, the phosphatase activity of the enzyme is located at the N-terminal part of EC 3.1.3.76, both catalytic sites act independently [33, 47, 57, 61]; <4> the C-terminal part harbors the epoxide hydrolase activity, the phosphatase activity of the enzyme is located at the N-terminal part of EC 3.1.3.76, both catalytic sites act independently, the epoxide hydrolase reaction proceeds though an alkyl-enzyme intermediate involving the catalytic Asp333, and hydrogen bonds with Tyr381 and Tyr465, reaction mechanism, structure-mechanism relationship [27]; <5> the catalytic triad is Asp333-His523-Asp495 [48]; <35> the catalytic triad is Asp334-His523-Asp495 [60]; <33> the catalytic triad is formed by Asp126, Asp285, and His320 [51]; <32> the catalytic triad is formed by Asp192, Asp348, and His374 [45]; <3> two step mechanism with formation of of a hydroxyl-alkyl-enzyme intermediate, catalytic triad is Asp333-His523-Asp495 with polarizing residues Tyr381 and Tyr465, catalytic cycle [41]; <5> two step mechanism with formation of of a hydroxyl-alkyl-enzyme intermediate, the catalytic triad is Asp333-His523-Asp495, catalytic cycle [41]; <4> two step mechanism with formation of of a hydroxyl-alkyl-enzyme intermediate, the catalytic triad is Asp334-His523-Asp495 with polarizing residues Tyr382 and Tyr465, catalytic cycle [41])

Natural substrates and products

- **S** 11,12-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [37]
- **P** 11,12-dihydroxyeicosatrienoic acid
- **S** 14,15-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [37]
- **P** 14,15-dihydroxyeicosatrienoic acid
- **S** 5,6-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [37]
- **P** 5,6-dihydroxyeicosatrienoic acid
- **S** 8,9-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [37]
- **P** 8,9-dihydroxyeicosatrienoic acid
- **S** 9,10-epoxy-18-hydroxy octadeca-(12Z)-eneoic acid + H_2O <12, 36, 37> (<37> step in cutin biosynthesis [63]) (Reversibility: ?) [63]
- **P** ?
- **S** cis-11,12-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [49]
- **P** ?
- **S** cis-14,15-epoxyeicosatrienoic acid + H_2O <5> (<5> formation of 65% R,R-diol product, and 35% S,S-diol [42]) (Reversibility: ?) [42, 49]
- **P** ?
- **S** cis-5,6-epoxyeicosatrienoic acid + H_2O <5> (<5> formation of 38% R,R-diol product, and 62% S,S-diol [42]) (Reversibility: ?) [42]
- **P** ?

S cis-8,9-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [49]
P ?
S epoxyeicosatrienoic acid + H_2O <4, 5> (<4> elimination of the biological effects of the substrate, involved in regulation of renal eicosanoid levels and blood pressure, mechanism [38]; <5> physiological function and regulation of the reaction, overview [52]) (Reversibility: ?) [38, 52]
P dihydroxyeicosatrienoic acid
S leukotriene A_4 + H_2O <3> (Reversibility: ?) [56]
P 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acid
S Additional information <1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 26, 28, 30, 31, 33, 34, 36, 37, 38, 39> (<5> induced by xenobiotics [9]; <3> induced by parental exposure to N-ethyl-N-nitrosourea [21]; <3> inducers [9]; <3> oxygenated lipids may be endogenous substrates for the cytosolic epoxide hydrolase [20]; <31> plays an important role during germination of seeds [32]; <3> enzyme inhibition decreases plasma levels of proinflammatory cytokines and nitric oxide metabolites while promoting the formation of lipoxins, thus supporting inflammatory resolution [62]; <5> enzyme inhibition in vivo leads to increased blood pressure and heart rate due to an increase in epoxyeicosatrienoic acid-mediated generation of reactive oxygen species [52]; <4, 26, 28, 38, 39> enzyme regulation, overview [63]; <5> metabolism of epoxyeicosatrienoic acids, no or poor activity with cis-11,12-epoxyeicosatrienoic acid and cis-8,9-epoxyeicosatrienoic acid in vivo [42]; <1, 7, 10, 11, 13, 14, 15, 17, 19, 22, 24, 25, 31> preferred endogenous substrates are epoxides containing fatty acids, e.g. epoxides of stearic and linoleic acids, and hepoxilins [63]; <37> synthesis of anti-fungal substances in fruits, the enzyme is involved in host-defense and cutin biosynthesis, preferred endogenous substrates are epoxides containing fatty acids, e.g. epoxides of stearic and linoleic acids, and hepoxilins [63]; <5> the activity for epoxyeicosatrienoic acids is elevated 5 to 54fold in renal cortical S9 fraction from the spontaneously hypertensive rats compared to normotensive Wistar-Kyoto rats, the enzyme is involved in regulation of blood pressure [49]; <36> the enzyme is involved in host-defense and cutin biosynthesis, preferred endogenous substrates are epoxides containing fatty acids, e.g. epoxides of stearic and linoleic acids, and hepoxilins [63]; <12> the enzyme is involved in host-defense and cutin biosynthesis, synthesis of (9S,10S,11R)-trihydroxy-12(Z)-octadecenoic and (9S,12S,13S)-trihydroxy-10(E)-octadecenoic acids with potent anti-fungal properties, preferred endogenous substrates are epoxides containing fatty acids, e.g. epoxides of stearic and linoleic acids, and hepoxilins [63]; <2, 5, 6, 8, 20, 21, 23> the enzyme is involved in metabolism of epoxide lipids in blood pressure, inflammation, reproduction and in lidpi/carbohydrate metabolism, enzyme regulation, overview [63]; <30> the enzyme is involved in metabolism of epoxide lipids in blood pressure, inflammation, reproduction and in lipid/carbohydrate metabolism, enzyme regulation, overview [63]; <3,4,5> the enzyme is involved in regulation of blood pressure and inflammation [41]; <34> the enzyme is involved in synthesis of tetrahydrofuran diol and tri-

hydroxy furanyl lipids, enzyme regulation, overview [63]; <5> the enzyme is involved in the arachidonic acid metabolic pathway [37]; <33> the soluble-type epoxide hydrolase may play a crucial role in the self-defense system of the plant [51]) (Reversibility: ?) [9, 20, 21, 32, 37, 41, 42, 49, 51, 52, 62, 63]

P ?

Substrates and products

S (11S,12S)-epoxy-5,14-cis-7,9-trans-eicosatetraenoic acid + H_2O <2> (<2> i.e. 11,12-leukotriene A_4 [13]) (Reversibility: ?) [13]
P (11R,12S)-dihydroxy-5,14-cis-7,9-trans-eicosatetraenoic acid + H_2O <2> [13]
S (1S,2S)-trans-methylstyrene oxide + H_2O <3> (<3> a hydrogen bond from Tyr465 to the substrate oxygen is essential for controlling the regioselectivity of the reaction [55]) (Reversibility: r) [55]
P ?
S (R)-1-chloro-2-(2,4-difluorophenyl)-2,3-epoxypropane + H_2O <9> (<9> in a racemic mixture only the (R)-enantiomeric epoxide is converted to the (R)-enantiomeric diol [36]) (Reversibility: ?) [36]
P (R)-3-chloro-2-(2,4-difluoro-phenyl)-propane-1,2-diol
S (R)-4-nitrostyrene oxide + H_2O <32> (Reversibility: ?) [45]
P ?
S (R)-*p*-nitrostyrene oxide + H_2O <27> (Reversibility: ?) [26]
P 1-(4-nitrophenyl)ethane-1,2-diol
S (R)-styrene oxide + H_2O <27> (Reversibility: ?) [26]
P 1-phenylethane-1,2-diol
S (S)-*p*-nitrostyrene oxide + H_2O <27> (Reversibility: ?) [26]
P 1-(4-nitrophenyl)ethane-1,2-diol
S (S)-styrene oxide + H_2O <27> (Reversibility: ?) [26]
P ?
S 1,2,3,4,9,9-hexachloro-6,7-epoxy-1,4,41,5,6,7,8,8a-octahydro-1,4-methanonaphthalene + H_2O <4, 5, 8, 23, 40> (<4, 5, 8, 23, 40> i.e. HEOM [1]) (Reversibility: ?) [1]
P ?
S 1,3-trans-diphenylpropene oxide + H_2O <15> (<15> little to no selectivity for cis-isomer or trans-isomer [23]) (Reversibility: ?) [23]
P 1,3-diphenylpropane-1,2-diol
S 11,12-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [37]
P 11,12-dihydroxyeicosatrienoic acid
S 11,12-leukotriene A_4 + H_2O <2> (Reversibility: ?) [63]
P ?
S 11R,12S-epoxyeicosatrienoic acid + H_2O <34> (Reversibility: ?) [63]
P 11R,12S-dihydroxyeicosatrienoic acid
S 11S,12R-epoxyeicosatrienoic acid + H_2O <34> (Reversibility: ?) [63]
P 11S,12R-hydroxyeicosatrienoic acid
S 12,13-epoxy octadeca-(9Z)-eneoic acid + H_2O <1, 7, 10, 11, 12, 13, 14, 15, 17, 19, 22, 24, 25, 31, 36, 37> (Reversibility: ?) [63]

P ?
S 12-phosphonooxyoctadec-9E-enoic acid + H_2O <4> (Reversibility: ?) [33]
P (9E)-octadecenoic acid + phosphate
S 12-phosphonooxyoctadec-9Z-enoic acid + H_2O <4> (Reversibility: ?) [33]
P (9Z)-octadecenoic acid + phosphate
S 12-phosphonooxyoctadecanoic acid + H_2O <4> (Reversibility: ?) [33]
P octadecanoic acid + phosphate
S 14,15-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [37]
P 14,15-dihydroxyeicosatrienoic acid
S 14,15-leukotriene A_4 + H_2O <2> (Reversibility: ?) [13, 63]
P ?
S 14R,15S-epoxyeicosatrienoic acid + H_2O <34> (Reversibility: ?) [63]
P 14R,15S-dihydroxyeicosatrienoic acid
S 14S,15R-epoxyeicosatrienoic acid + H_2O <34> (Reversibility: ?) [63]
P 14S,15R-dihydroxyeicosatrienoic acid
S 14S,15S-trans-epoxy-(5Z,8Z,10E,12E)-eicosatrienoic acid + H_2O <34> (Reversibility: ?) [63]
P 14S,15R-dihydroxyeicosatrienoic acid
S 2,2-dimethylstyrene oxide + H_2O <4> (Reversibility: ?) [12]
P ?
S 2,3-epoxy-1,3-diphenyl-propane + H_2O <3, 4, 5, 12, 15> (Reversibility: ?) [23]
P ?
S 2,3-squalene oxide + H_2O <4, 5, 8, 23, 40> (Reversibility: ?) [1]
P ?
S 2-acetylaminofluorene + H_2O <4, 5, 8, 23, 40> (Reversibility: ?) [1]
P ?
S 4-nitrophenyl (2R,3R)-2,3-epoxy-3-(4-nitrophenyl)propyl carbonate + H_2O <3> (Reversibility: ?) [54]
P ?
S 4-nitrophenyl (2R,3R)-2,3-epoxy-3-phenylpropyl carbonate + H_2O <3, 4, 5> (<4> 14.6% of the activity with 2,3-epoxy-1,3-diphenyl-propane, no activity with the 2S,3S-enantiomer [23]; <5> 5.1% of the activity with 2,3-epoxy-1,3-diphenyl-propane, no activity with the 2S,3S-enantiomer [23]) (Reversibility: ?) [23, 54]
P ?
S 4-nitrophenyl (2S,3S)-2,3-epoxy-3-(4-nitrophenyl)propyl carbonate + H_2O <3> (<3> preferred phenylpropyl carbonate substrate [54]) (Reversibility: ?) [54]
P ?
S 4-nitrophenyl (2S,3S)-2,3-epoxy-3-phenylpropyl carbonate + H_2O <3> (Reversibility: ?) [54]
P ?
S 4-nitrophenyl [(2R,3R)-3-phenyloxiran-2-yl]methyl carbonate + H_2O <15> (<15> 15.2% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]
P ?

S 4-nitrophenyl [(2S,3S)-3-phenyloxiran-2-yl]methyl carbonate + H_2O <15> (<15> 38% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]
P ?
S 4-nitrophenyl-trans-2,3-epoxy-3-phenylpropyl carbonate + H_2O <3, 4> (Reversibility: ?) [40, 43, 47]
P ?
S 4-nitrostyrene 7,8-oxide + H_2O <4, 5> (<4,5> i.e. PNSO [3]) (Reversibility: ?) [3]
P ?
S 4-nitrostyrene oxide + H_2O <4, 5, 8, 23, 40> (Reversibility: ?) [1]
P ?
S 5,6-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [37]
P 5,6-dihydroxyeicosatrienoic acid
S 5,6-leukotriene A_4 + H_2O <2> (Reversibility: ?) [63]
P ?
S 5α-cholest-2-ene β-oxide + H_2O <3> (Reversibility: ?) [9]
P ?
S 8,9-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [37]
P 8,9-dihydroxyeicosatrienoic acid
S 8R,9S-epoxyeicosatrienoic acid + H_2O <34> (Reversibility: ?) [63]
P 8R,9S-dihydroxyeicosatrienoic acid
S 8S,9R-epoxyeicosatrienoic acid + H_2O <34> (Reversibility: ?) [63]
P 8S,9R-dihydroxyeicosatrienoic acid
S 9(10),12(13)-diepoxy octadecanoic acid + H_2O <34> (Reversibility: ?) [63]
P 9,10,12,13-tetrahydroxy octadecanoic acid
S 9,10-epoxy octadeca-(12Z)-eneoic acid + H_2O <1, 7, 10, 11, 12, 13, 14, 15, 17, 19, 22, 24, 25, 31, 36, 37> (<7, 11, 24> no enantioselectivity [63]; <13> no enantioselectivity of infection-induced isozymes, conversion of the (S)-carbon to the corresponding threo-(R,R)-diol in over 85% [63]; <22> the enzyme prefers the (9R,10S)-enantiomer [63]; <12> the enzyme prefers the (9R,10S)-enantiomer, conversion of the (S)-carbon to the corresponding threo-(R,R)-diol in over 85% [63]; <14> the enzyme strongly prefers the (9R,10S)-enantiomer, conversion of the (S)-carbon to the corresponding threo-(R,R)-diol in over 85% [63]) (Reversibility: ?) [63]
P ?
S 9,10-epoxy-18-hydroxy octadeca-(12Z)-eneoic acid + H_2O <1, 7, 10, 11, 12, 13, 14, 15, 17, 19, 22, 24, 25, 31, 36, 37> (<37> step in cutin biosynthesis [63]) (Reversibility: ?) [63]
P ?
S 9-hydroxy-10,11-epoxy octadeca-(12Z)-eneoic acid + H_2O <1, 7, 10, 11, 12, 13, 14, 15, 17, 19, 22, 24, 25, 31, 36, 37> (Reversibility: ?) [63]
P ?
S 9-hydroxy-12,13-epoxy octadeca-(11E)-eneoic acid + H_2O <1, 7, 10, 11, 12, 13, 14, 15, 17, 19, 22, 24, 25, 31, 36, 37> (Reversibility: ?) [63]
P ?

S [3-(4-chlorophenyl)oxiran-2-yl]methyl cyano(6-methoxy-2-naphthyl)-methyl carbonate + H_2O <3, 4> (Reversibility: ?) [40]
P 6-methoxy-2-naphthaldehyde + CN^- + ?
S allylbenzene + H_2O <2, 3, 5> (Reversibility: ?) [65]
P ?
S arachidonic acid 11,12-oxide + H_2O <3> (Reversibility: ?) [9]
P 11,12-dihydroxyarachidonic acid
S arachidonic acid 14,15-oxide + H_2O <3> (Reversibility: ?) [9]
P 14,15-dihydroxyarachidonic acid
S arachidonic acid 5,6-oxide + H_2O <3> (Reversibility: ?) [9]
P 5,6-dihydroxyarachidonic acid
S arachidonic acid 8,9-oxide + H_2O <3> (Reversibility: ?) [9]
P 8,9-dihydroxyarachidonic acid
S arochlor 1254 + H_2O <4, 5, 8, 23, 40> (Reversibility: ?) [1]
P ?
S benzopyrene 4,5-oxide + H_2O <3, 4, 5, 8, 23, 40> (Reversibility: ?) [1, 8, 9]
P (-)benzopyrene 4,5-dihydrodiol
S chalcone oxides + H_2O <3, 4, 5> (Reversibility: ?) [41]
P ?
S cholesterol 5,6-α-oxide + H_2O <3> (<3> very weak activity [9]) (Reversibility: ?) [9]
P ?
S cis-(11R,12S)-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(11R,12S)-epoxyeicosatrienoic acid methyl ester + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(11R,12S)-hexahydro-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(11S,12R)-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(11S,12R)-epoxyeicosatrienoic acid methyl ester + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(11S,12R)-hexahydro-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(14R,15S)-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(14R,15S)-epoxyeicosatrienoic acid methyl ester + H_2O <3, 5> (Reversibility: ?) [42]
P ?

S cis-(14R,15S)-hexahydro-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(14S,15R)-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(14S,15R)-epoxyeicosatrienoic acid methyl ester + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(14S,15R)-hexahydro-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(8R,9S)-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(8R,9S)-epoxyeicosatrienoic acid methyl ester + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(8R,9S)-hexahydro-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(8S,9R)-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(8S,9R)-epoxyeicosatrienoic acid methyl ester + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(8S,9R)-hexahydro-epoxyeicosatrienoic acid + H_2O <3, 5> (Reversibility: ?) [42]
P ?
S cis-(9R,10S)-epoxysteric acid + H_2O <5> (Reversibility: ?) [25]
P threo-(9R,10R)-dihydroxystearic acid + threo-(9S,10S)-dihydroxystearic acid (<5> enantioselective, low production rate of the (9R,10R)-diol by wild-type and E404D mutant [25])
S cis-(9S,10R)-epoxysteric acid + H_2O <5> (Reversibility: ?) [25]
P threo-(9R,10R)-dihydroxystearic acid + threo-(9S,10S)-dihydroxystearic acid (<5> enantioselective, low production rate of the (9R,10R)-diol by wild-type and E404D mutant [25])
S cis-1,2-dimethylstyrene oxide + H_2O <4> (Reversibility: ?) [12]
P ?
S cis-1,3-diphenylpropene oxide + H_2O <12, 15> (<12> little to no selectivity for cis-isomer or trans-isomer, 44% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <15> little to no selectivity for cis-isomer or trans-isomer, 99% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]
P 1,3-diphenylpropane-1,2-diol
S cis-1,3-diphenylpropene oxide + H_2O <3, 4> (<4> 18.1% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <3> 3.0% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]

P ?
S cis-1,3-diphenylpropene oxide + H_2O <5> (<5> 2.1% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]
P 1,2-diphenylethane-1,2-diol
S cis-11,12-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [49]
P ?
S cis-11,12-epoxyeicosatrienoic acid + H_2O <35> (Reversibility: ?) [60]
P cis-11,12-dihydroxyeicosatrienoic acid
S cis-14,15-epoxyeicosatrienoic acid + H_2O <35> (<35> preferred eicosatrienoic acid-substrate [60]) (Reversibility: ?) [60]
P cis-14,15-dihydroxyeicosatrienoic acid
S cis-14,15-epoxyeicosatrienoic acid + H_2O <4, 5> (<5> formation of 65% R,R-diol product, and 35% S,S-diol [42]; <4> simulation of substrate binding, modeling [64]) (Reversibility: ?) [42, 49, 64]
P ?
S cis-2-methylstyrene oxide + H_2O <4> (Reversibility: ?) [12]
P ?
S cis-5,6-epoxyeicosatrienoic acid + H_2O <5> (<5> formation of 38% R,R-diol product, and 62% S,S-diol [42]) (Reversibility: ?) [42]
P ?
S cis-8,9-epoxyeicosatrienoic acid + H_2O <5> (Reversibility: ?) [49]
P ?
S cis-8,9-epoxyeicosatrienoic acid + H_2O <35> (Reversibility: ?) [60]
P cis-8,9-dihydroxyeicosatrienoic acid
S cis-8-ethylstyrene 7,8-oxide + H_2O <4, 5> (Reversibility: ?) [3]
P ?
S cis-9,10-epoxy-12-octadecenoate methyl ester + H_2O <12> (<12> 12% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]
P 9,10-dihydroxystearic acid methyl ester
S cis-9,10-epoxy-12-octadecenoate methyl ester + H_2O <15> (<15> 59% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]
P 9,10-dihydroxystearic acid methyl ester
S cis-9,10-epoxy-12-octadecenoate methyl ester + H_2O <4> (<4> 5.2% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]
P ?
S cis-9,10-epoxy-12-octadecenoate methyl ester + H_2O <3, 5> (<5> 3.5% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <3> 6.5% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]
P 9,10-dihydroxystearixc acid methyl ester
S cis-9,10-epoxystearate + H_2O <14> (Reversibility: ?) [16]
P threo-9,10-dihydroxystearate <14> [16]
S cis-9,10-epoxystearic acid + H_2O <3, 5, 12, 15> (<12> 13.4% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <15> 30% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <5> 4.1% of the activity with

2,3-epoxy-1,3-diphenyl-propane [23]; <3> 6.7% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]
P 9,10-dihydroxystearic acid
S cis-9,10-epoxystearic acid + H_2O <4> (<4> 7.9% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]
P ?
S cis-stilbene oxide + H_2O <2, 3, 4, 5, 8, 12, 23, 29, 40> (<12, 29> greater selectivity for trans-stilbene oxide versus cis-stilbene oxide [23]) (Reversibility: ?) [1, 7, 8, 9, 13, 19, 23, 24]
P ?
S cis-stilbene oxide + H_2O <3, 12, 15, 17> (<17> low activity [29]; <3> 0.4% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <15> activity is 50fold lower than with trans-stilbene oxide [34]; <12> far greater selectivity for trans-stilbene oxide versus cis-stilbene oxide. 0.06% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <15> far greater selectivity for trans-stilbene oxide versus cis-stilbene oxide. 0.2% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23, 29, 34]
P 1,2-diphenylethane-1,2-diol
S clofibrate + H_2O <4, 5, 8, 23, 40> (Reversibility: ?) [1]
P ?
S cyano(2-methoxy-naphthalen-6-yl)methyl trans-2-(3-propyloxiran-2-yl) acetate + H_2O <3, 4> (Reversibility: ?) [40]
P 6-methoxy-2-naphthaldehyde + CN^- + ?
S cyano(6-methoxy-2-naphthyl)methyl (3,3-dimethyloxiran-2-yl)methyl carbonate + H_2O <3, 4> (Reversibility: ?) [40]
P 6-methoxy-2-naphthaldehyde + CN^- + ?
S cyano(6-methoxy-2-naphthyl)methyl (3-ethyloxiran-2-yl)methyl carbonate + H_2O <3, 4> (Reversibility: ?) [40]
P 6-methoxy-2-naphthaldehyde + CN^- + ?
S cyano(6-methoxy-2-naphthyl)methyl (3-phenyloxiran-2-yl)acetate + H_2O <3, 4> (Reversibility: ?) [40]
P 6-methoxy-2-naphthaldehyde + CN^- + ?
S cyano(6-methoxy-2-naphthyl)methyl (3-phenyloxiran-2-yl)methyl carbonate + H_2O <3, 4> (<4> preferred fluorogenic substrate [40]) (Reversibility: ?) [40]
P 6-methoxy-2-naphthaldehyde + CN^- + ?
S cyano(6-methoxy-2-naphthyl)methyl (3-propyloxiran-2-yl)methyl carbonate + H_2O <3, 4> (Reversibility: ?) [40]
P 6-methoxy-2-naphthaldehyde + CN^- + ?
S cyano(6-methoxy-2-naphthyl)methyl [3-(4-nitrophenyl)oxiran-2-yl]-methyl carbonate + H_2O <3, 4> (Reversibility: ?) [40]
P 6-methoxy-2-naphthaldehyde + CN^- + ?
S cyclohexene oxide + H_2O <3, 18> (Reversibility: ?) [7, 18]
P ?
S di(2-ethylhexyl)phthalate + H_2O <4, 5, 8, 23, 40> (Reversibility: ?) [1]
P ?

S epoxy stearic acid + H_2O <3, 4> (Reversibility: ?) [43]
P ?
S epoxyeicosatrienoic acid + H_2O <4, 5> (<4> elimination of the biological effects of the substrate, involved in regulation of renal eicosanoid levels and blood pressure, mechanism [38]; <5> physiological function and regulation of the reaction, overview [52]) (Reversibility: ?) [38, 52]
P dihydroxyeicosatrienoic acid
S erythro-10-hydroxy-9-phosphonooxy-octadecanoic acid + H_2O <4> (Reversibility: ?) [33]
P 10-hydroxy-octadecanoic acid + phosphate
S glycidyl phenyl ether + H_2O <9> (<9> the selectivity factor E reflecting the relative rate of the reaction of the two enantiomers is only 4.6 for the wild-type enzyme, in slight favor of the (S)-product, a value of 10.8 is observed with the natural EH variant IS002B1 with the three amino acid exchanges A217V, K332E and A390E. A value of 7.4 is observed with the natural variant IR003B1 with the amino acid exchange R219G, a value of 6.6 is observed with the natural variant IB001C2 with the amino acid exchange F340Y, a value of 6.4 is observed with the natural variant IE001H6 with the amino acid exchange A327V, a value of 6.2 is observed with the natural variant IS001H8 with the amino acid exchange A327V and a value of 5.5 is observed with the natural variant IL001D4 with the amino acid exchange P222S [31]) (Reversibility: ?) [31]
P ?
S juvenile hormone III + H_2O <3, 4, 5, 12, 15> (<15> 11.9% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <5> 3.5% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <3, 4> 6.3% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <12> 7.1% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23]
P ?
S lanosterol + H_2O <3> (Reversibility: ?) [9]
P ?
S leukotriene A_4 + H_2O <3> (Reversibility: ?) [56]
P 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acid (<3> i.e. compound D, product identification by GC-MS [56])
S leukotriene A_4 + H_2O <2, 3> (Reversibility: ?) [9, 13]
P ?
S naphthalene 1,2-oxide + H_2O <4, 5> (Reversibility: ?) [3]
P ?
S *p*-nitrophenyl phosphate + H_2O <4> (Reversibility: ?) [33]
P ?
S *p*-nitrostyrene oxide + H_2O <9> (Reversibility: ?) [17]
P ?
S phenanthrene 9,10-oxide + H_2O <4, 5, 8, 23, 40> (Reversibility: ?) [1]
P ?
S phenobarbital + H_2O <4, 5, 8, 23, 40> (Reversibility: ?) [1]
P ?

S racemic 4-nitrophenyl-trans-2,3-epoxy-3-phenylpropyl carbonate + H_2O <3, 4> (Reversibility: ?) [58]
P ?
S racemic ethyl 3-phenylglycidate + H_2O <16> (<16> production of (2R,3S)-ethyl 3-phenylglycidate with 95% enantiomeric excess and 26% yield in 12 h from 0.2% (w/v) of the racemat [28]) (Reversibility: ?) [28]
P
S racemic trans-1,3-diphenylpropene oxide + H_2O <4> (Reversibility: ?) [47]
P ?
S squalene 2,3-23,24-dioxide + H_2O <3> (Reversibility: ?) [9]
P ?
S squalene diepoxide + H_2O <34> (Reversibility: ?) [63]
P ?
S squalene-2,3-epoxide + H_2O <34> (Reversibility: ?) [63]
P ?
S stearic acid epoxide + H_2O <4> (Reversibility: ?) [41]
P ?
S styrene 7,8-oxide + H_2O <4, 5> (Reversibility: ?) [11, 12]
P styrene glycol
S styrene 7,8-oxide + H_2O <4, 5> (Reversibility: ?) [3]
P ?
S styrene oxide + H_2O <4, 5, 8, 23, 33, 40> (Reversibility: ?) [1, 51]
P ?
S styrene oxide + H_2O <2, 3, 4, 5, 8> (Reversibility: ?) [2, 7, 9, 13, 24]
P styrene glycol <8> [2]
S threo-10-hydroxy-9-phosphonooxy-octadecanoic acid + H_2O <4> (Reversibility: ?) [33]
P 10-hydroxy-octadecanoic acid + phosphate
S trans-1,2-dimethylstyrene oxide + H_2O <4> (<4> hydration by microsomal enzyme, no activity with cytosolic enzyme [12]) (Reversibility: ?) [12]
P ?
S trans-1,3-diphenylpropene oxide + H_2O <12> (<12> little to no selectivity for cis-isomer or trans-isomer [23]) (Reversibility: ?) [23]
P 1,3-diphenylpropane-1,2-diol
S trans-1,3-diphenylpropene oxide + H_2O <5, 12, 29, 35> (<35> the His-tag of the recombinant enzyme probably interfers with the enzyme activity [60]) (Reversibility: ?) [23, 49, 60]
P ?
S trans-2-methylstyrene oxide + H_2O <2, 4> (Reversibility: ?) [12, 13]
P ?
S trans-8-ethylstyrene 7,8-oxide + H_2O <4, 5> (<4, 5> i.e. TESO [3]) (Reversibility: ?) [3]
P ?
S trans-9,10-epoxystearate + H_2O <4, 5, 8, 23, 35, 40> (Reversibility: ?) [1, 6]
P ?

S trans-9,10-epoxystearate + H_2O <14> (Reversibility: ?) [16]
P threo-9,10-dihydroxystearate <14> [16]
S trans-β-methyl-styrene oxide + H_2O <2, 5> (Reversibility: ?) [65]
P ?
S trans-β-methylstyrene oxide + H_2O <2> (Reversibility: ?) [13]
P ?
S trans-β-propylstyrene oxide + H_2O <3> (Reversibility: ?) [7, 9]
P ?
S trans-bety-methyl-styrene oxide + H_2O <3> (Reversibility: ?) [65]
P ?
S trans-diphenyl propene oxide + H_2O <4> (Reversibility: ?) [33]
P ?
S trans-diphenylpropene oxide + H_2O <3, 4, 5, 12> (<3,12> recombinant enzyme [66]) (Reversibility: ?) [37, 38, 40, 43, 66]
P ?
S trans-ethyl styrene oxide + H_2O <2, 3, 4, 5, 8, 23, 40> (<5> trans-β-ethyl styrene oxide [11]) (Reversibility: ?) [1, 7, 9, 11, 13]
P ?
S trans-stilbene oxide + H_2O <12> (Reversibility: ?) [59]
P trans-1,2-diphenylethane-1,2-diol
S trans-stilbene oxide + H_2O <2, 3, 4, 5, 8, 12, 23, 29, 40> (<12,29> greater selectivity for trans-stilbene oxide versus cis-stilbene oxide [23]; <4> highly selective for the trans-enantiomer. 1.2% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [1, 3, 5, 6, 7, 8, 9, 11, 13, 14, 15, 19, 23, 24, 43, 44, 61]
P ?
S trans-stilbene oxide + H_2O <3, 5, 12, 15, 17> (<3> 2.8% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <12> far greater selectivity for trans-stilbene oxide versus cis-stilbene oxide. 5.6% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <15> far greater selectivity for trans-stilbene oxide versus cis-stilbene oxide. 84% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]; <5> highly selective for the trans-enantiomer. 1% of the activity with 2,3-epoxy-1,3-diphenyl-propane [23]) (Reversibility: ?) [23, 29, 34]
P 1,2-diphenylethane-1,2-diol
S Additional information <1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 26, 28, 30, 31, 33, 34, 36, 37, 38, 39, 40> (<4> microsomal enzyme shows highest activity with trans-2-methylstyrane oxide, followed by styrene 7,8-oxide, cis-2-methylstyrene oxide, cis-1,2-dimethylstyrene oxide, trans-1,2-dimethylstyrene oxide and 2,2-dimethylstyrene oxide. With the cytosolic enzyme the same order is obtained for the first three substrates, whereas activity with 2,2-dimethylstyrene oxide is higher than with cis-1,2-dimethylstyrene oxide and no hydration occurs with trans-1,2-dimethylstyrene oxide [12]; <5> Asp333, Asp495 and His523 form the catalytic triad [14]; <3> involvement of the Asp333-His523 pair in the catalytic mechanism [15]; <5> induced by xenobiotics [9]; <3> induced by parental exposure to N-ethyl-N-nitro-

sourea [21]; <3> inducers [9]; <3> oxygenated lipids may be endogenous substrates for the cytosolic epoxide hydrolase [20]; <31> plays an important role during germination of seeds [32]; <4> the N-terminal domain of the enzyme is a functional phosphatase unaffected by a number of classic phosphatase inhibitors. The phosphatase domain has high specificity for lipophilic phosphates [33]; <3> enzyme inhibition decreases plasma levels of proinflammatory cytokines and nitric oxide metabolites while promoting the formation of lipoxins, thus supporting inflammatory resolution [62]; <5> enzyme inhibition in vivo leads to increased blood pressure and heart rate due to an increase in epoxyeicosatrienoic acid-mediated generation of reactive oxygen species [52]; <4, 26, 28, 38, 39> enzyme regulation, overview [63]; <5> metabolism of epoxyeicosatrienoic acids, no or poor activity with cis-11,12-epoxyeicosatrienoic acid and cis-8,9-epoxyeicosatrienoic acid in vivo [42]; <1, 7, 10, 11, 13, 14, 15, 17, 19, 22, 24, 25, 31> preferred endogenous substrates are epoxides containing fatty acids, e.g. epoxides of stearic and linoleic acids, and hepoxilins [63]; <37> synthesis of anti-fungal substances in fruits, the enzyme is involved in host-defense and cutin biosynthesis, preferred endogenous substrates are epoxides containing fatty acids, e.g. epoxides of stearic and linoleic acids, and hepoxilins [63]; <5> the activity for epoxyeicosatrienoic acids is elevated 5 to 54fold in renal cortical S9 fraction from the spontaneously hypertensive rats compared to normotensive Wistar-Kyoto rats, the enzyme is involved in regulation of blood pressure [49]; <36> the enzyme is involved in host-defense and cutin biosynthesis, preferred endogenous substrates are epoxides containing fatty acids, e.g. epoxides of stearic and linoleic acids, and hepoxilins [63]; <12> the enzyme is involved in host-defense and cutin biosynthesis, synthesis of (9S,10S,11R)-trihydroxy-12(Z)-octadecenoic and (9S,12S,13S)-trihydroxy-10(E)-octadecenoic acids with potent anti-fungal properties, preferred endogenous substrates are epoxides containing fatty acids, e.g. epoxides of stearic and linoleic acids, and hepoxilins [63]; <2, 5, 6, 8, 20, 21, 23> the enzyme is involved in metabolism of epoxide lipids in blood pressure, inflammation, reproduction and in lidpi/carbohydrate metabolism, enzyme regulation, overview [63]; <30> the enzyme is involved in metabolism of epoxide lipids in blood pressure, inflammation, reproduction and in lipid/carbohydrate metabolism, enzyme regulation, overview [63]; <3, 4, 5> the enzyme is involved in regulation of blood pressure and inflammation [41]; <34> the enzyme is involved in synthesis of tetrahydrofuran diol and trihydroxy furanyl lipids, enzyme regulation, overview [63]; <5> the enzyme is involved in the arachidonic acid metabolic pathway [37]; <33> the soluble-type epoxide hydrolase may play a crucial role in the self-defense system of the plant [51]; <3, 5> chiral product analysis, regio- and/or stereochemistry of water addition is dependent on the substrate regioisomer, effects of methylation and hydrogenation on chirality of the products from epoxyeicosatrienoic acid substrates, overview [42]; <5> enantioselectivity with fatty acid epoxide substrates [25]; <1, 7, 10, 11, 12, 13, 14, 15, 17, 19, 22, 24, 25, 31,

36, 37> plant enzymes prefer trans- over cis-epoxides of sterically hindered substrates like stilbene oxides [63]; <3, 4> reaction mechanism with flurogenic substrates [40]; <3,5> sEH prefers gem-di-, trans-di-, cis-di-, tri-, and tetra-substituted epoxides [41]; <4> substrate specificity, 2 enzymes with different specificities termed cytosolic TESO hydrolase and cytosolic PNSO hydrolase, no activity with benz[a]pyrene 4,5-oxide [3]; <5> substrate specificity, no activity with benz[a]pyrene 4,5-oxide [3]; <34> substrate specificity, the enzyme prefers trans- over cis-epoxides of sterically hindered substrates like stilbene oxides, the C-terminal domain catalyzes epoxy fatty acid hydrolysis, the N-terminal catalytic domain has also phosphatase activity with specificity for fatty acid diol phosphates, except for the isozyme EPXH2B, overview [63]; <4, 5, 8, 23, 40> substrate specificity, the microsomal enzyme rapidly hydrolyzes epoxides on cyclic systems as well as mono, 1,1-di and cis-1,2-disubstituted epoxides [1]; <4> the enzyme also shows phosphatase activity, EC 3.1.3.76, sEH prefers gem-di-, trans-di-, cis-di-, tri-, and tetra-substituted epoxides [41]; <2, 6, 8, 20, 21, 23, 30> the enzyme prefers trans- over cis-epoxides of sterically hindered substrates like stilbene oxides, the C-terminal domain catalyzes epoxy fatty acid hydrolysis, the N-terminal catalytic domain has also phosphatase activity with specificity for fatty acid diol phosphates [63]; <4,5> the enzyme prefers trans-over cis-epoxides of sterically hindered substrates like stilbene oxides, the C-terminal domain catalyzes epoxy fatty acid hydrolysis, the N-terminal catalytic domain has also phosphatase activity with specificity for fatty acid diol phosphates, overview [63]) (Reversibility: ?) [1, 3, 9, 12, 14, 15, 20, 21, 25, 32, 33, 37, 40, 41, 42, 49, 51, 52, 62, 63]

P ?

Inhibitors

(1R,2R)-1,2-epoxy-1-phenyl-1-propane <3> (<3> weak inhibition, IC50 is 1.1 mM [46]) [46]

(1S,2S)-1,2-epoxy-1-phenyl-1-propane <3> (<3> weak inhibition, IC50 is 2.4 mM, preincubation of enzyme with inhibitor does not influence the inhibitory effect [46]) [46]

(2R,3R)-1-acetoxy-2,3-epoxy-3-(4-nitrophenyl)propane <3> (<3> IC50 is 0.069 mM [46]) [46]

(2R,3R)-1-benzyloxy-2,3-epoxy-3-(4-nitrophenyl)propane <3> (<3> IC50 is 0.024 mM [46]) [46]

(2R,3R)-1-ethoxy-2,3-epoxy-3-(4-nitrophenyl)propane <3> (<3> IC50 is 0.069 mM [46]) [46]

(2R,3R)-2,3-epoxy-3-(4-nitrophenyl)glycidol <3> (<3> IC50 is 1.2 mM, the S-enantiomer is a 750fold better inhibitor compared to the R-isomer [46]) [46]

(2R,3R)-3-(4-bromophenyl)glycidol <3> (<3> preincubation of enzyme with inhibitor increases the inhibitory effect [46]) [46]

(2R,3R)-3-(4-nitrophenyl)glycidol <3> [46]

(2S,3S)-1-acetoxy-2,3-epoxy-3-(4-nitrophenyl)propane <3> (<3> IC50 is 0.012 mM, preincubation of enzyme with inhibitor decreases the inhibitory effect [46]) [46]
(2S,3S)-1-benzyloxy-2,3-epoxy-3-(4-nitrophenyl)propane <3> (<3> IC50 is 0.039 mM [46]) [46]
(2S,3S)-1-ethoxy-2,3-epoxy-3-(4-nitrophenyl)propane <3> (<3> IC50 is 0.012 mM, preincubation of enzyme with inhibitor decreases the inhibitory effect [46]) [46]
(2S,3S)-2,3-epoxy-3-(4-nitrophenyl)glycidol <3> (<3> IC50 is 0.0016 mM, the S-enantiomer is a 750fold better inhibitor compared to the R-isomer [46]) [46]
(2S,3S)-3-(4-bromophenyl)glycidol <3> [46]
(2S,3S)-3-(4-nitrophenyl)glycidol <3> (<3> preincubation of enzyme with inhibitor increases the inhibitory effect [46]) [46]
(3-[4-(allyloxy)phenyl]oxiran-2-yl)(phenyl)methanone <3, 4> (<4> IC50 is 0.00029 mM [43]; <3> IC50 is 0.0021 mM [43]) [43]
(3-[4-(benzyloxy)phenyl]oxiran-2-yl)(phenyl)methanone <3, 4> (<4> IC50 is 0.00023 mM [43]; <3> IC50 is 0.00028 mM [43]) [43]
(4-bromophenyl)(3-phenyloxiran-2-yl)methanone <3, 4> (<4> IC50 is 0.00022 mM [43]; <3> IC50 is 0.0006 mM [43]) [43]
(4-bromophenyl)[3-(2-naphthyl)oxiran-2-yl]methanone <3, 4> (<3> IC50 is 0.00014 mM [43]; <4> IC50 is 0.00018 mM [43]) [43]
(4-fluorophenyl)(3-phenyloxiran-2-yl)methanone <3, 4> (<4> IC50 is 0.00039 mM [43]; <3> IC50 is 0.0018 mM [43]) [43]
(4-iodophenyl)(3-phenyloxiran-2-yl)methanone <3, 4> (<4> IC50 is 0.00042 mM [43]; <3> IC50 is 0.0014 mM [43]) [43]
(4-methoxyphenyl)(3-phenyloxiran-2-yl)methanone <3, 4> (<4> IC50 is 0.00020 mM [43]; <3> IC50 is 0.00032 mM [43]) [43]
(4-methylphenyl)(3-phenyloxiran-2-yl)methanone <3, 4> (<4> IC50 is 0.00023 mM [43]; <3> IC50 is 0.0017 mM [43]) [43]
(4-methylphenyl)[3-(2-naphthyl)oxiran-2-yl]methanol <3, 4> (<3> IC50 is 0.00009 mM [43]; <4> IC50 is 0.00015 mM [43]) [43]
(4-methylphenyl)[3-(2-naphthyl)oxiran-2-yl]methanone <3, 4> (<3> IC50 is 0.00010 mM [43]; <4> IC50 is 0.00019 mM [43]) [43]
(4-nitrophenyl)(3-phenyloxiran-2-yl)methanone <3, 4> (<4> IC50 is 0.00025 mM [43]; <3> IC50 is 0.0015 mM [43]) [43]
(E)-[3-(2-naphthyl)oxiran-2-yl](phenyl)methanone oxime <3, 4> (<4> IC50 is 0.035 mM [43]; <3> IC50 is 0.042 mM [43]) [43]
(E)-phenyl(3-phenyloxiran-2-yl)methanone oxime <3, 4> (<4> IC50 is 0.00029 mM [43]; <3> IC50 is 0.0035 mM [43]) [43]
1,1,1-trichloropropene 2,3-oxide <4, 5, 8, 23, 40> [1]
1,3-dicyclohexyl urea <4> (<4> complete inhibition at 0.1 mM [33]; <4> 0.1 mM, complete inhibition of epoxide hydrolase activity, no inhibition of phosphatase activity [33]) [33]
1,3-disubstituted amides <3, 4, 5> (<3,4,5> potent and stable inhibition, Ki in the nanomolar range, mechanism [41]) [41]

1,3-disubstituted carbamate derivatives <3, 4, 5> (<3,4,5> potent and stable inhibition, K_i in the nanomolar range, mechanism [41]) [41]
1,3-disubstituted urea derivatives <3, 4, 5> (<3,4,5> potent and stable inhibition, K_i in the nanomolar range, mechanism [41]) [41]
1-benzylimidazole <4> (<4> microsomal activity with cis-stilbene oxide as substrate [24]) [24]
1-[3-(4-nitrophenyl)oxiran-2-yl]ethanol <3, 4> (<3> IC50 is 0.0023 mM [43]; <4> IC50 is 0.0187 mM [43]) [43]
1-[3-(4-nitrophenyl)oxiran-2-yl]ethanone <3, 4> (<3> IC50 is 0.163 mM [43]; <4> IC50 is 0.269 mM [43]) [43]
1-adamantan-3-(5-[2-(2-ethylethoxy)ethoxy]pentyl)urea <3> (<3> i.e. compound 950 [62]) [62]
1-cyclohexyl-3-dodecyl urea <4> (<4> complete inhibition at 0.1 mM [33]; <4> 0.1 mM, complete inhibition of epoxide hydrolase activity, no inhibition of phosphatase activity [33]) [33]
1-cyclohexyl-3-ethyl urea <4> (<4> 54% inhibition at 0.1 mM [33]; <4> 0.1 mM, 54% inhibition of epoxide hydrolase activity, no inhibition of phosphatase activity [33]) [33]
1-cyclohexyl-3-hexyl urea <4> (<4> complete inhibition at 0.1 mM [33]; <4> 0.1 mM, complete inhibition of epoxide hydrolase activity, 4% inhibition of phosphatase activity [33]) [33]
1-trichloropropene oxide <4> [24]
10-sulfonooxyoctadecanoic acid <4> (<4> IC50 is 0.028 mM [47]) [47]
12-(3-adamantan-1-yl-ureido)-dodecanoic acid butyl ester <3> (<3> i.e. AUDA-BE, physiologic consequences of enzyme inhibition in vivo [62]) [62]
12-sulfonoxy-cis-9-octadecenoic acid <4> (<4> IC50 is above 0.1 mM [47]) [47]
12-sulfonoxy-trans-9-octadecenoic acid <4> (<4> IC50 is 0.016 mM [47]) [47]
2,2'-dithiopyridine <3, 4> (<3> 66% inhibition at 1 mM at pH 7.4 [50]; <4> 73% inhibition at 1 mM at pH 7.4 [50]) [50]
2,3-epoxy-1,3-diphenyl-propan-1-one <3, 12, 15> (<15> IC50: 0.023 mM [23]; <12> IC50: 0.0034 mM [23]; <3> IC50: 0.0029 mM [23]) [23]
2,3-epoxy-1-(4-bromophenyl)-3-phenyl-propan-1-one <3, 12, 15> (<15> IC50: 0.013 mM [23]; <12> IC50: 0.00089 mM [23]; <3> IC50: 0.0006 mM [23]) [23]
2,3-epoxy-1-(4-ethylphenyl)-3-phenyl-propan-1-one <3, 12, 15> (<12> IC50: 0.0014 mM [23]; <15> IC50: 0.03 mM [23]; <3> IC50: 0.0017 mM [23]) [23]
2,3-epoxy-1-(4-fluorophenyl)-3-phenyl-propan-1-one <3, 12, 15> (<15> IC50: 0.022 mM [23]; <3> IC50: 0.0018 mM [23]; <12> IC50: 0.0012 mM [23]) [23]
2,3-epoxy-1-(4-methoxyphenyl)-3-phenyl-propan-1-one <3, 12, 15> (<12> IC50: 0.00021 mM [23]; <3> IC50: 0.00032 mM [23]; <15> IC50: 0.0054 mM [23]) [23]
2,3-epoxy-1-(4-nitroxyphenyl)-3-phenyl-propan-1-one <3, 12, 15> (<15> IC50: 0.016 mM [23]; <3> IC50: 0.0015 mM [23]; <12> IC50: 0.019 mM [23]) [23]

2,3-epoxy-1-(4-phenylphenyl)-3-phenyl-propan-1-one <3, 12> (<12> IC50: 0.0025 mM [23]; <3> IC50: 0.00137 mM [23]) [23]
2,3-epoxy-3-(4-bromophenyl)-1-phenyl-propan-1-one <3, 12, 15> (<12> IC50: 0.00012 mM [23]; <15> IC50: 0.0022 mM [23]; <3> IC50: 0.0007 mM [23]) [23]
2,3-epoxy-3-(4-ethylphenyl)-1-phenyl-propan-1-one <3, 12, 15> (<3> IC50: 0.0019 mM [23]; <12> IC50: 0.00016 mM [23]; <15> IC50: 0.0056 mM [23]) [23]
2,3-epoxy-3-(4-fluorophenyl)-1-phenyl-propan-1-one <3, 12, 15> (<3> IC50: 0.0013 mM [23]; <12> IC50: 0.00048 mM [23]; <15> IC50: 0.0047 mM [23]) [23]
2,3-epoxy-3-(4-methoxyphenyl)-1-phenyl-propan-1-one <3, 12, 15> (<3> IC50: 0.0002 mM [23]; <15> IC50: 0.0028 mM [23]; <12> IC50: 0.00019 mM [23]) [23]
2,3-epoxy-3-(4-n-butylphenyl)-1-phenyl-propan-1-one <3, 15> (<3> IC50: 0.00015 mM [23]; <15> IC50: 0.0017 mM [23]) [23]
2,3-epoxy-3-(4-nitrophenyl)-1-phenyl-propan-1-one <3, 12, 15> (<15> IC50: 0.011 mM [23]; <3> IC50: 0.00018 mM [23]; <12> IC50: 0.00038 mM [23]) [23]
2,3-epoxy-3-(4-phenylphenyl)-1-phenyl-propan-1-one <3> (<3> IC50: 0.00014 mM [23]) [23]
2-(2-naphthyl)-3-(phenylsulfinyl)oxirane <3, 4> (<4> IC50 is 0.070 mM [43]; <3> IC50 is 0.073 mM [43]) [43]
2-bromo-4'-nitroacetophenone <3, 4, 5, 8, 23, 40> (<3> 81% inhibition at 1 mM at pH 7.4 [50]; <4> complete inhibition at 1 mM at pH 7.4 [50]) [1, 50]
2-[methoxy(phenyl)methyl]-3-(2-naphthyl)oxirane <3, 4> (<3> IC50 is 0.00048 mM [43]; <4> IC50 is 0.00134 mM [43]) [43]
2-cyclohexa-1,5-dien-1-yl-3-(phenylsulfinyl)oxirane <3, 4> (<4> IC50 is 0.00031 mM [43]; <3> IC50 is 0.0023 mM [43]) [43]
2-cyclohexa-1,5-dien-1-yl-3-[methoxy(phenyl)methyl]oxirane <3, 4> (<4> IC50 is 0.034 mM [43]; <3> IC50 is 0.103 mM [43]) [43]
2-methylglycidyl 4-nitrobenzoate <3> (<3> IC50 for the S-enantiomer is 0.717 mM, the R-enantiomer shows 25% inhibition at 0.2 mM [54]) [54]
2-naphthyl(3-phenyloxiran-2-yl)methanone <3, 4> (<4> IC50 is 0.00028 mM [43]; <3> IC50 is 0.0014 mM [43]) [43]
3,3-dimethylglycidyl 4-nitrobenzoate <3> (<3> IC50 for the S-enantiomer is 0.012 mM, 23% inhibition at 5 mM of the R-enantiomer [54]) [54]
4'-azidochalcone oxide <3> [8]
4'-fluorochalcone oxide <3> [19]
4'-methylchalcone oxide <3> [19]
4'-phenylchalcone oxide <3> [8]
4-(3-adamantan-1-yl-ureido) butyric acid <4> [64]
4-(3-adamantan-1-yl-ureido) butyric acid methyl ester <4> [64]
4-(3-benzoyloxiran-2-yl)benzoic acid <3, 4> (<3,4> IC50 is above 0.5 mM [43]) [43]
4-(3-cyclohexylureido)-butyric acid <4> (<4> binding structure analysis [64]) [64]

4-(3-cyclohexylureido)-butyric acid methyl ester <4> [64]
4-(3-cyclohexylureido)-ethanoic acid <4> (<4> binding structure analysis [64]) [64]
4-(3-cyclohexylureido)-ethanoic acid methyl ester <4> [64]
4-(3-cyclohexylureido)-heptanoic acid <4> (<4> binding structure analysis [64]) [64]
4-(3-cyclohexylureido)-hexanoic acid <4> (<4> binding structure analysis [64]) [64]
4-(3-cyclohexylureido)-hexanoic acid methyl ester <4> [64]
4-([3-(2-naphthyl)oxiran-2-yl]carbonyl)benzoic acid <3, 4> (<3> IC50 is 0.00008 mM [43]; <4> IC50 is 0.00016 mM [43]) [43]
4-([3-(4-fluorophenyl)oxiran-2-yl]carbonyl)benzoic acid <3, 4> (<3> IC50 is 0.103 mM [43]; <4> IC50 is 0.113 mM [43]) [43]
4-hydroxymercuribenzoate <9> [17]
4-nitrophenyl sulfate <4> (<4> IC50 is above 0.1 mM [47]) [47]
4-phenylchalcone oxide <3, 4> (<4> complete inhibition at 0.1 mM, competitive substrate [33]) [7, 8, 33]
4-[(3-phenyloxiran-2-yl)carbonyl]benzoic acid <3, 4> (<3> IC50 is 0.073 mM [43]; <4> IC50 is 0.144 mM [43]) [43]
4-[3-(4-fluorobenzoyl)oxiran-2-yl]benzoic acid <3, 4> (<3,4> IC50 is above 0.5 mM [43]) [43]
4-azidochalcone oxide <3> [6, 8]
4-benzyloxychalcone oxide <3> [19]
4-bromo-4'-methoxychalcone <3, 12, 15> (<15> IC50: 0.0048 mM [23]; <12> IC50: 0.00021 mM [23]; <3> IC50: 0.00027 mM [23]) [23]
4-fluoro-chalcone oxide <4> (<4> 0.1 mM, 87% inhibition of epoxide hydrolase activity, 8% inhibition of phosphatase activity [33]) [33]
4-fluorochalcone oxide <3, 4, 5, 12> (<4> 87% inhibition at 0.1 mM, competitive substrate [33]; <12> complete inhibition of recombinant and native enzyme at 0.057 mM [59]; <3> IC50 is 0.026 mM [46]; <5> inhibition of the epoxide hydrolase activity [61]) [8, 14, 19, 33, 46, 59, 61]
4-methylchalcone oxide <3> [19]
4-nitrobenzenesulfonyl fluoride <3, 4> (<3> 47% inhibition at 1 mM at pH 7.4 [50]; <4> 94% inhibition at 1 mM at pH 7.4 [50]) [50]
4-phenyl-chalcone oxide <4> (<4> 0.1 mM, complete inhibition of epoxide hydrolase activity, 11% inhibition of phosphatase activity [33]) [33]
5,5'-dithiobis(2-nitrobenzoic acid) <3> [7]
7,8-styrene oxide <4> [12]
9,10-hydroxysulfonooxyoctadecanoic acid <4> (<4> IC50 is 0.09 mM [47]) [47]
9-octadecanyl sulfate <4> (<4> IC50 is 0.021 mM [47]) [47]
ACU <35> (<35> i.e. N-adamantyl-N-cyclohexylurea, strong inhibition [60]) [60]
AUDA <3, 4, 5, 35> (<3,4> i.e. 12-(3-adamantan-1-yl-ureido) dodecanoic acid [40]; <5> i.e. 12-(3-adamantan-1-yl-ureido) dodecanoic acid, inhibition in vivo by intracerebroventricular delivery [52]; <35> i.e. 12-(3-adamantan-1-yl-ureido) dodecanoic acid, strong inhibition [60]) [40, 52, 60]

acetamide <27> [26]
acetone <5> (<5> hydration of trans-stilbene oxide [11]) [11]
acetonitrile <3, 5> (<5> hydration of trans-stilbene oxide [11]; <3> 89% loss of activity at 5% acetonitrile [19]) [11, 19]
Al^{3+} <3, 4, 5> (<4,5> 20% inhibition at 1 mM [66]; <3> 10% inhibition at 1 mM [66]) [66]
Ba^{2+} <3, 4, 12> (<12> 30% inhibition at 1 mM [66]; <4> 20% inhibition at 1 mM [66]; <3> 40% inhibition at 1 mM [66]) [66]
Benzamide <27> [26]
CDU <3, 4, 35> (<3,4> i.e. 1-cyclohexyl-3-dodecyl-urea [40]; <35> i.e. 1-cyclohexyl-3-dodecyl-urea, strong inhibition [60]) [40, 60]
CEU <3, 4, 35> (<3,4,35> i.e. N-cyclohexyl-N-ethylurea [40,60]) [40, 60]
CHU <3, 4> (<3,4> i.e. N-cyclohexyl-N-hexylurea [40]) [40]
Ca^{2+} <3, 4, 12> (<12> 30% inhibition at 1 mM [66]; <4> 25% inhibition at 1 mM [66]; <3> 20% inhibition at 1 mM [66]; <4> inhibition of Mg^{2+}-dependent acrtivity [33]) [33, 66]
Cd^{2+} <3, 4, 5, 12> (<3,4> complete inhibition at 1 mM [66]; <12> 60% inhibition at 1 mM [66]; <5> nearly complete inhibition at 1 mM [66]; <4> inhibition of phosphatase activity and epoxide hydrolase activity [33]) [33, 41, 66]
Ce3+ <4> (<4> inhibition of Mg^{2+}-dependent acrtivity [33]) [33]
chalcone <3, 4> (<4> microsomal activity with cis-stilbene oxide as substrate, cytosolic enzyme form cEHCSO and cEHTSO [24]) [19, 24]
chalcone oxide <2, 3, 4, 12, 17, 29> (<17> 1 mM, 98% inhibition [29]; <2> inhibits hydration of (11S,12S)-epoxy-5,14-cis-7,9-trans-eicosatetraenoic acid [13]; <4> 0.1 mM, 80% inhibition of epoxide hydrolase activity, no inhibition of phosphatase activity [33]; <4> 80% inhibition at 0.1 mM, competitive substrate [33]; <3> IC50 is 0.055 mM [46]) [6, 7, 13, 19, 23, 29, 33, 46]
chloroacetamide <27> [26]
clofibrate <3> (<3> 2 mM, 70-100% inhibition [19]; <3> slight inhibition of enzyme from untreated mice, slight activation of the enzyme from clofibrate-treated mice [7]) [7, 19]
Co^{2+} <3, 4, 5, 12> (<4,12> 30% inhibition at 1 mM [66]; <3> 45% inhibition at 1 mM [66]; <5> 15% inhibition at 1 mM [66]; <4> inhibition of Mg^{2+}-dependent acrtivity [33]) [33, 66]
Cu^{+} <4> (<4> inhibition of phosphatase activity and epoxide hydrolase activity [33]) [33]
Cu^{2+} <3, 4, 5, 12> (<12> 30% inhibition at 1 mM [66]; <3> complete inhibition at 1 mM [66]; <4> nearly complete inhibition at 1 mM [66]; <4> inhibition of phosphatase activity and epoxide hydrolase activity [33]; <5> over 95% inhibition at 1 mM [66]) [33, 41, 66]
cyclohexene oxide <4, 5> [9]
D-galactose 6-sulfate <4> (<4> IC50 is above 0.1 mM [47]) [47]
DCU <3, 4, 35> (<3,4,35> i.e. N,N-dicyclohexylurea [40,60]) [40, 60]

DTNB <3, 4> (<3> 59% inhibition at 1 mM at pH 7.4 [50]; <4> 86% inhibition at 1 mM at pH 7.4 [50]) [50]
diethyldicarbonate <3, 4> (<4> 72% inhibition at 1 mM at pH 7.4 [50]; <3> 80% inhibition at 1 mM at pH 7.4 [50]) [50]
diisopropyl fluorophosphate <3> (<3> 16% inhibition at 1 mM at pH 7.4 [50]) [50]
dimethylsulfoxide <3, 17> [7, 29]
estrone 3-sulfate <4> (<4> IC50 is above 0.1 mM [47]) [47]
ethanol <3, 5> (<3> 3% inhibition at 3% ethanol [19]; <5> hydration of trans-stilbene oxide [11]) [11, 19]
Fe^{2+} <4, 5> (<4> 20% inhibition at 1 mM [66]; <5> 10% inhibition at 1 mM [66]; <4> inhibition of Mg^{2+}-dependent acrtivity [33]) [33, 66]
Fe^{3+} <4, 5> (<5> 20% inhibition at 1 mM [66]; <4> 15% inhibition at 1 mM [66]; <4> inhibition of Mg^{2+}-dependent acrtivity [33]) [33, 66]
H_2O_2 <9> [17]
Hg^{2+} <3, 4, 5, 12> (<3,4,5> complete inhibition at 1 mM [66]; <12> 70% inhibition at 1 mM [66]; <4> inhibition of phosphatase activity and epoxide hydrolase activity [33]) [33, 41, 66]
$HgCl_2$ <9> [17]
iodoacetamide <9> [17]
isopropanol <5> (<5> hydration of trans-stilbene oxide [11]) [11]
L-ascorbic acid 2-sulfate <4> (<4> IC50 is above 0.1 mM [47]) [47]
methanol <5> (<5> hydration of trans-stilbene oxide [11]) [11]
methyl methanethiosulfonate <3, 4> (<3,4> 89% inhibition at 1 mM at pH 7.4 [50]) [50]
Mg^{2+} <3, 4, 12> (<3> 30% inhibition at 1 mM [66]; <4> 20% inhibition at 1 mM [66]; <12> 40% inhibition at 1 mM [66]) [66]
Mn^{2+} <3, 4, 12> (<4> 30% inhibition at 1 mM [66]; <3,12> 20% inhibition at 1 mM [66]; <4> inhibition of Mg^{2+}-dependent activity [33]) [33, 66]
N,N'-adamantyl urea <4> [64]
N,N'-bis-(3,4-dichlorophenyl)urea <3, 4> [58]
N,N'-dicyclohexylcarbodiimide <17> (<17> 0.01 mM, complete inhibition [29]) [29]
N,N'-dicyclohexylurea <17> (<17> 0.01 mM, 30% inhibition [29]) [29]
N,N'-dicyclohexylurea <5> (<5> specific inhibitor, decreases the enzyme activity and blood pressure in spontaneously hypertensive rats, IC50 with different substrates, overview [49]) [49]
N,N-dimethyl formamide <5> (<5> hydration of trans-stilbene oxide [11]) [11]
N-(4-[(3-phenyloxiran-2-yl)carbonyl]phenyl)acetamide <3, 4> (<4> IC50 is 0.00014 mM [43]; <3> IC50 is 0.0017 mM [43]) [43]
N-ethylmaleimide <3, 4> (<3> 21% inhibition at 1 mM at pH 7.4 [50]; <4> 98% inhibition at 1 mM at pH 7.4 [50]) [50]
N-phenylmaleimide <3, 4> (<3> 60% inhibition at 1 mM at pH 7.4 [50]; <4> 97% inhibition at 1 mM at pH 7.4 [50]) [50]
N-[4-(3-benzoyloxiran-2-yl)phenyl]acetamide <3, 4> (<3> IC50 is 0.00022 mM [43]; <4> IC50 is 0.00027 mM [43]) [43]

N-acetyl imidazole <3> (<3> 12% inhibition at 1 mM at pH 7.4 [50]) [50]
N-acetyl-D-galactosamine 4-sulfate <4> (<4> IC50 is above 0.1 mM [47]) [47]
N-bromosuccimide <3> (<3> 98% inhibition at 1 mM at pH 7.4 [50]) [50]
N-cyclohexyl-N'-(3-phenyl)propyl urea <4> [64]
N-cyclohexyl-N'-(iodophenyl)urea <4> (<4> binding structure at the C-terminus [27]) [27]
N-cyclohexyl-N'-4-chlorophenylurea <3, 4> [58]
N-cyclohexyl-N'-decylurea <3> [41]
N-cyclohexyl-N'-dodecylurea <5> (<5> weak inhibition [49]) [49]
N-cyclohexyl-N'-iodophenyl urea <4> (<4> binding structure analysis [64]) [64]
N-cyclopentyl-N'-dodecylurea <3, 4> [58]
NEM <3> [7]
NODA <5> (<5> i.e. 11-nonyloxy-undec-8-enoic acid [52]) [52]
Ni^{2+} <3, 4, 5, 12> (<12> 30% inhibition at 1 mM [66]; <4> 25% inhibition at 1 mM [66]; <5> 20% inhibition at 1 mM [66]; <3> 40% inhibition at 1 mM [66]) [66]
ω-bromo-4-nitroacetophenone <3> [7]
PMSF <3> (<3> 27% inhibition at 1 mM at pH 7.4 [50]) [50]
Pb^{2+} <3, 4, 5, 12> (<3> 30% inhibition at 1 mM [66]; <12> 60% inhibition at 1 mM [66]; <5> 25% inhibition at 1 mM [66]; <4> 20% inhibition at 1 mM [66]; <4> inhibition of Mg^{2+}-dependent acrtivity [33]) [33, 66]
phenylacetamide <27> [26]
phenylcarbamate <27> [26]
phenylglyoxal <3, 4> (<4> 19% inhibition at 1 mM at pH 7.4 [50]; <3> 22% inhibition at 1 mM at pH 7.4 [50]) [50]
quercetin <17> (<17> 1 mM, 75% inhibition [29]) [29]
aodium dodecyl sulfate <4> (<4> IC50 is above 0.1 mM [47]) [47]
atyrene oxide <3, 4, 5, 8, 14, 23, 40> (<14> inhibits hydration of trans-9,10-epoxystearate [16]) [1, 16, 19, 24]
taurocholic acid <4> (<4> IC50 is 0.09 mM [47]) [47]
tetrahydrofuran <3, 5, 17> (<3> 73% loss of activity with 1% tetrahydrofuran, 98% loss of activity with 5% tetrahydrofuran [19]; <5> hydration of trans-stilbene oxide [11]) [7, 11, 19, 29]
trans-9,10-epoxystearate <14> (<14> 1 mM, 20% inhibition of hydration of 0.025 mM cis-9,10-epoxystearate [16]) [16]
trans-stilbene oxide <14> (<14> inhibits hydration of trans-9,10-epoxystearate [16]) [16]
urea <3, 27> [26, 55]
Zn^{2+} <3, 4, 5, 12> (<3,4> complete inhibition at 1 mM [66]; <12> 40% inhibition at 1 mM [66]; <4> inhibition of phosphatase activity and epoxide hydrolase activity [33]; <4> noncompetitive, inhibits epoxide hydrolase and phosphatase activities, allosteric mechanism [41]; <5> over 95% inhibition at 1 mM [66]) [33, 41, 66]
[3-(2-naphthyl)oxiran-2-yl](4-nitrophenyl)methanone <3, 4> (<3> IC50 is 0.00013 mM [43]; <4> IC50 is 0.00016 mM [43]) [43]

[3-(2-naphthyl)oxiran-2-yl](phenyl)methanol <3, 4> (<3> IC50 is 0.00051 mM [43]; <4> IC50 is 0.00072 mM [43]) [43]
[3-(2-naphthyl)oxiran-2-yl](phenyl)methanone <3, 4> (<3> IC50 is 0.00014 mM [43]; <4> IC50 is 0.0002 mM [43]; <3> IC50 is 0.00049 mM [43]; <4> IC50 is 0.00085 mM [43]) [43]
[3-(4-bromophenyl)oxiran-2-yl](phenyl)methanone <3, 4> (<4> IC50 is 0.0002 mM [43]; <3> IC50 is 0.0007 mM [43]) [43]
[3-(4-butylphenyl)oxiran-2-yl](phenyl)methanone <3, 4> (<3, 4> IC50 is 0.00015 mM [43]) [43]
[3-(4-fluorophenyl)oxiran-2-yl](phenyl)methanol <3, 4> (<4> IC50 is 0.018 mM [43]; <3> IC50 is 0.072 mM [43]) [43]
[3-(4-fluorophenyl)oxiran-2-yl](phenyl)methanone <3, 4> (<4> IC50 is 0.0003 mM [43]; <3> IC50 is 0.0013 mM [43]) [43]
[3-(4-heptylphenyl)oxiran-2-yl](phenyl)methanone <3, 4> (<4> IC50 is 0.00048 mM [43]; <3> IC50 is 0.00065 mM [43]) [43]
[3-(4-isopropylphenyl)oxiran-2-yl](phenyl)methanone <3, 4> (<3> IC50 is 0.00047 mM [43]; <4> IC50 is 0.00048 mM [43]) [43]
[3-(4-methoxyphenyl)oxiran-2-yl](phenyl)methanone <3, 4> (<4> IC50 is 0.00011 mM [43]; <3> IC50 is 0.0002 mM [43]) [43]
[3-(4-methylphenyl)oxiran-2-yl](phenyl)methanone <3, 4> (<4> IC50 is 0.00036 mM [43]; <3> IC50 is 0.0019 mM [43]) [43]
[3-(4-nitrophenyl)oxiran-2-yl](phenyl)methanol <3, 4> (<3> IC50 is 0.0037 mM [43]; <4> IC50 is 0.028 mM [43]) [43]
[3-(4-nitrophenyl)oxiran-2-yl](phenyl)methanone <3, 4> (<4> IC50 is 0.00063 mM [43]; <3> IC50 is 0.0018 mM [43]) [43]
[3-(4-phenoxycyclohexa-1,5-dien-1-yl)oxiran-2-yl](phenyl)methanone <3, 4> (<3> IC50 is 0.00014 mM [43]; <4> IC50 is 0.00051 mM [43]) [43]
[4-(allyloxy)phenyl](3-phenyloxiran-2-yl)methanone <3, 4> (<4> IC50 is 0.00014 mM [43]; <3> IC50 is 0.0014 mM [43]) [43]
[4-(bromomethyl)phenyl][3-(2-naphthyl)oxiran-2-yl]methanone <3, 4> (<3> IC50 is 0.00011 mM [43]; <4> IC50 is 0.00016 mM [43]) [43]
acetaldehyde/borohydride <3> (<3> 13% inhibition at 1 mM at pH 7.4 [50]) [50]
α-naphthoflavone <3> [7]
α-hydroxyfarnesyl phosphonic acid <4> (<4> IC50 is 0.073 mM [47]) [47]
α-sulfostearic acid <4> (<4> IC50 is above 0.1 mM [47]) [47]
benzaldehyde/borohydride <3> (<3> 16% inhibition at 1 mM at pH 7.4 [50]) [50]
benzil <4> (<4> inhibition of microsomal enzyme with cis-stilbene oxide, and of cytosolic enzyme forms cEHTSO and cEHCSO [24]) [24]
benzyl phenylmetanethiosulfonate <4> (<4> complete inhibition at 1 mM at pH 7.4 [50]) [50]
benzyl phenylmethanethiosulfonate <3> (<3> 94% inhibition at 1 mM at pH 7.4 [50]) [50]
β-naphthoflavone <3, 17> (<17> 1 mM, 50% inhibition [29]) [7, 19, 29]

carbodiimide/glycine methyl ester <3, 4> (<4> 48% inhibition at 1 mM at pH 5.2, no inhibition at pH 7.4 [50]; <3> 65% inhibition at 1 mM at pH 5.2, 17% at pH 7.4 [50]) [50]
cis-limonene epoxide <9> (<9> weak [17]) [17]
cyclohexane 1,2-oxide <4, 5, 8, 23, 40> [1]
dibenzyl phosphonate <4> (<4> IC50 is above 0.1 mM [47]) [47]
dicyclohexylurea <5> (<5> inhibition of the epoxide hydrolase activity [61]) [61]
diethyl 2,2,2-trifluoro-1-hydroxyethyl phosphonate <4> (<4> IC50 is above 0.1 mM [47]) [47]
diethyl 4-methylbenzyl phosphonate <4> (<4> IC50 is above 0.1 mM [47]) [47]
diethyl allyl phosphonate <4> (<4> IC50 is above 0.1 mM [47]) [47]
diethyl benzoylphosphonate <4> (<4> IC50 is above 0.1 mM [47]) [47]
diethyl cyclopropyl methylphosphonate <4> (<4> IC50 is above 0.1 mM [47]) [47]
diethyl ethylthiomethyl phosphonate <4> (<4> IC50 is above 0.1 mM [47]) [47]
diethyl trans-cinnamyl phosphonate <4> (<4> IC50 is above 0.1 mM [47]) [47]
diethyl vinylphosphonate <4> (<4> IC50 is above 0.1 mM [47]) [47]
dimethyl 2-oxoheptyl phosphonate <4> (<4> IC50 is above 0.1 mM [47]) [47]
dioctyl phenyl phosphonate <4> (<4> IC50 is 0.092 mM [47]) [47]
dithionitrobenzene <9> [17]
dodecyl phosphonic acid <4> (<4> IC50 is 0.040 mM [47]) [47]
epichlorhydrin <9> (<9> weak [17]) [17]
geraniol derivatives <9> (<9> weak [17]) [17]
glycidyl 4-nitrobenzoate <3> (<3> IC50 for the S-enantiomer is 0.14 mM, and for the R-enantiomer 0.51 mM [54]) [54]
m-chloroperbenzoic acid <9, 14> [16, 17]
ninhydrin <3, 4> (<3> 12% inhibition at 1 mM at pH 7.4 [50]; <4> 13% inhibition at 1 mM at pH 7.4 [50]) [50]
okadaic acid <4> (<4> 12% inhibition at 0.1 mM [33]) [33]
ω-bromo-nitro-acetophenone <9> [17]
p-hydroxymercuribenzoate <17> [29]
p-hydroxymercuriphenylsulfonate <3, 4, 5, 8, 23, 40> [1, 7]
p-chlorobenzamide <27> [26]
p-nitrobenzamide <27> [26]
phenoxyacetamide <27> [26]
phenyl(3-phenyloxiran-2-yl)methanol <3, 4> (<3> IC50 is 0.0126 mM [43]; <4> IC50 is 0.022 mM [43]) [43]
phenyl(3-phenyloxiran-2-yl)methanone <3, 4> (<4> IC50 is 0.0003 mM [43]; <3> IC50 is 0.0029 mM [43]) [43]
phenylthioisocyanate <3, 4> (<3> 34% inhibition at 1 mM at pH 7.4 [50]; <4> 54% inhibition at 1 mM at pH 7.4 [50]) [50]
racemic 2,3-epoxy-1,3-diphenyl-1-propanol <3> (<3> IC50 is 0.029 mM [46]) [46]

racemic 2,3-epoxy-3-(4-fluorophenyl)-1-phenyl-1-propanol <3> (<3> IC50 is 0.032 mM [46]) [46]
racemic 2,3-epoxy-3-(4-nitrophenyl)-1-phenyl-1-propanol <3> (<3> IC50 is 0.0017 mM [46]) [46]
racemic 3,4-epoxy-4-(4-nitrophenyl)-1-butanol <3> (<3> IC50 is 0.012 mM [46]) [46]
racemic 3-(4-nitrophenyl)glycidol <3> (<3> IC50 is 0.005 mM [46]) [46]
sodium dodecyl sulfonate <4> (<4> IC50 is 0.05 mM [47]) [47]
sodium orthovanadate <4> (<4> 9% inhibition at 0.1 mM [33]) [33]
substituted chalcone oxides <4, 5, 8, 23, 40> [1]
taurolithocholic acid 3-sulfate <4> (<4> IC50 is above 0.1 mM [47]) [47]
tetraisopropyl methylenediphosphonate <4> (<4> IC50 is above 0.1 mM [47]) [47]
trans-(2R,3R)-3-phenylglycidol <3, 12> (<12> 23% inhibition of recombinant and of native enzyme at 0.057 mM [59]; <3> IC50 for the R-enantiomer is about 3.9 mM [54]) [54, 59]
trans-(2S,3S)-3-phenylglycidol <3, 12> (<12> 98% inhibition of recombinant and 85% inhibition of native enzyme at 0.057 mM [59]; <3> IC50 for the S-enantiomer is about 2.2 mM [54]) [54, 59]
trans-1-phenylpropylene oxide <3> (<3> IC50 for the S-enantiomer is 3.47 mM, and for the R-enantiomer 2.98 mM [54]) [54]
trans-2-methyl-3-phenylglycidol <3> (<3> IC50 for the S-enantiomer is 1.52 mM, and for the R-enantiomer 2.0 mM [54]) [54]
trans-3-(4-bromophenyl)glycidol <3> (<3> IC50 for the S-enantiomer is 0.12 mM, and for the R-enantiomer 0.77 mM [54]) [54]
trans-3-(4-nitrophenyl)glycidol <3> (<3> IC50 for the S-enantiomer is 0.013 mM, and for the R-enantiomer 4.24 mM [54]) [54]
trans-3-(4-nitrophenyl)glycidyl acetate <3> (<3> IC50 for the S-enantiomer is 0.232 mM, and for the R-enantiomer 0.39 mM [54]) [54]
trans-3-(4-nitrophenyl)glycidyl benzoate <3> (<3> IC50 for the S-enantiomer is 0.229 mM, and for the R-enantiomer 0.10 mM [54]) [54]
trans-3-methylglycidyl 4-nitrobenzoate <3> (<3> the S-enantiomer shows 20% inhibition at 0.8 mM, the R-enantiomer 16.5% inhibition at 0.8 mM [54]) [54]
trans-limonene epoxide <9> (<9> weak [17]) [17]
trichloropropene oxide <4, 9> [12, 17]
trichloropropylene oxide <3> [7]
Additional information <3, 4, 5, 12> (<3> cyclohexandione is a poor inhibitor, no inhibition by amino-, guanido-, or activated serine-selective reagents [50]; <3,4> IC50 values with different substrates and structure-activity relationships of enzyme inhibitors, development of an in vitro inhibition assay using fluorogenic substrates [40]; <4> inhibition mechanism of sulfates, sulfonates, and phosphonates [47]; <3> inhibitor synthesis, overview, inhibition mechanisms, overview [46]; <3,4> irreversible inhibition mechanism of chalcone oxide derivatives, quantitative structure-activity relationship, QSAR, overview [43]; <4,12> metal chelators like 1,10-phenanthroline, 1,7-phenanthroline, EDTA, EGTA, and dipicolinic acid preserve enzyme activity in pres-

ence of metal ions [66]; <4> most enzyme inhibitors do not influence the enzymes phosphatase activity [41]; <4> N-acetyl imidazole, N-bromosuccimide, cyclohexandione, acetaldehyde/borohydride and benzaldehyde/borohydride are poor inhibitors, no inhibition by PMSF and diisopropyl fluorophosphate, no inhibition by amino-, guanido-, or activated serine-selective reagents [50]; <3> no inhibition by Fe^{2+} and Fe^{3+} at 1 mM, metal chelators like 1,10-phenanthroline, 1,7-phenanthroline, EDTA, EGTA, and dipicolinic acid preserve enzyme activity in presence of metal ions [66]; <5> no inhibition by Mn^{2+}, Ba^{2+}, Mg^{2+}, and Ca^{2+} at 1 mM, metal chelators like 1,10-phenanthroline, 1,7-phenanthroline, EDTA, EGTA, and dipicolinic acid preserve enzyme activity in presence of metal ions [66]; <4> poor or no inhibition by tartaric acid, sodium fluoride, and sodium molybdate [33]; <4> QSAR and classification in a seven-discriptor model of enzyme inhibition by 348 urea-like compounds, IC50 ranging from 60 nM to 0.5 mM, overview [58]; <3> quantitative structure-activity relationship, QSAR, and classification in a five-discriptor model of enzyme inhibition by 348 urea-like compounds, IC50 ranging from 60 nM to 0.5 mM, overview [58]; <3> sterical differences play a role in inhibitory potency [54]) [33, 40, 41, 43, 46, 47, 50, 54, 58, 66]

Activating compounds

1-(2-isopropylphenyl)-imidazole <4, 5, 8, 23, 40> [1]
1-(2-cyanophenyl)-imidazole <4, 5, 8, 23, 40> [1]
1-benzylimidazole <4> (<4> activates mirosomal activity with styrene oxide as substrate [24]) [24]
4-azafluorene <4, 5, 8, 23, 40> [1]
4-phenylpyridine <4, 5, 8, 23, 40> [1]
5,6-benzoflavone <4, 5, 8, 23, 40> [1]
7,8-benzoflavone <4, 5, 8, 23, 40> [1]
9-fluorenone <4, 5, 8, 23, 40> [1]
aminotriazole <3> (<3> slight activation [7]) [7]
chalcone <4, 5, 8, 23, 40> [1]
chalcone oxide <3, 4, 5, 8, 23, 40> (<3> substituted chalcone oxides, potent reversible inhibitors [8]) [1, 8]
clofibrate <3> (<3> slight inhibition of enzyme from untreated mice, slight activation of the enzyme from clofibrate-treated mice [7]) [7]
ellipticine <3, 4, 5, 8, 23, 40> [1, 7]
ethanol <4, 5, 8, 23, 40> [1]
flavone <4, 5, 8, 23, 40> [1]
harmane <3> [7]
iodoacetamide <3> [7]
isoquinoline <4, 5, 8, 23, 40> [1]
metyrapone <4, 5, 8, 23, 40> [1]
quinoline <4, 5, 8, 23, 40> [1]
benzil <3, 4, 5, 8, 23, 40> (<4> activates mirosomal activity with styrene oxide as substrate [24]) [1, 7, 24]
benzophenone <4, 5, 8, 23, 40> [1]
benzyl phenyl ketone <4, 5, 8, 23, 40> [1]

cyclobenzaprine <4, 5, 8, 23, 40> [1]
cyclobenzarpine epoxide <4, 5, 8, 23, 40> [1]
diphenylcyclopropenone <4, 5, 8, 23, 40> [1]
harman <4, 5, 8, 23, 40> [1]
norharman <4, 5, 8, 23, 40> [1]
propyl phenyl ketone <4, 5, 8, 23, 40> [1]
Additional information <1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25, 30, 31, 33, 34, 36, 37, 40> (<3> clofibrate induces the enzyme [46, 54, 56]; <3> clofibrate induces the liver enzyme [53]; <33> ethylene induces the enzyme expression in stem and seeds, not in leaves [51]; <1, 7, 10, 17, 19, 22, 24, 25, 31, 37> inducible isozymes can be induced by e.g. fruit ripening, germination, host-defense, exogenous exposure to hormones, growth [63]; <14> inducible isozymes can be induced by e.g. fruit ripening, germination, host-defense, exogenous exposure to hormones, growth, ethylene induces the enzyme in germinating seeds [63]; <36> inducible isozymes can be induced by e.g. fruit ripening, germination, host-defense, exogenous exposure to hormones, growth, pathogenic fungus infection induces the enzyme in leaves [63]; <13> inducible isozymes can be induced by e.g. fruit ripening, germination, host-defense, exogenous exposure to hormones, growth, viral infection of the aerial body and the plant induces the enzyme [63]; <12> inducible isozymes can be induced by e.g. fruit ripening, germination, host-defense, growth, the enzyme is not affected by cytokinin, abscisic acid, 6-benzylaminopurine, or gibberellin, but highly by methyl jasmonate, auxin, and ethylene, wounding induces the enzyme in leaves [63]; <15> inducible isozymes can be induced by e.g. fruit ripening, germination, host-defense, growth, the enzyme is not affected by cytokinin, abscisic acid, 6-benzylaminopurine, or gibberellin, while auxin, 2,4-dichlorophenoxy acetic acid, and naphthalene acetic acid induce the enzyme in stem and leaves, drought stress slightly induces the enzmye in stem and leaves [63]; <16> maximal activity with dimethyl formamide as co-solvent, effects of solvents on enzyme activity, overview [28]; <2, 4, 5, 6, 8, 20, 21, 23, 30, 34> the enzyme is induced by clofibrate [63]; <5> the enzyme is induced by clofibrate in liver not in epididymis [37]; <4, 5, 8, 23, 40> the enzyme is induced by several substances, e.g. 2-acetylfluorene, α-1-acetylmethadol, butylated hydroxyanisole, butylated hydroxytoluene, chalcone, γ-chlordane, 1,2-dibromo-3-chloropropane, ethoxyquin, isosafrole, 3-methylcholanthrene, α-naphthoflavone, phenobarbital, polychlorinated and/or polybrominated biphenyls, pregnenolone-16α-carbonitrile, and trans-stilbene oxide [1]; <12> wounding and methyl jasmonate induce enzyme expression in leaves, while abscisic acid does not [59]) [1, 28, 37, 46, 51, 53, 54, 56, 59, 63]

Metals, ions

Al^{3+} <3, 4, 5, 12> (<3> 10% inhibition at 1 mM [66]; <4,5> 20% inhibition at 1 mM [66]; <12> 35%% activation at 1 mM [66]) [66]
Ba^{2+} <3, 4, 12> (<4> 20% inhibition at 1 mM [66]; <12> 30% inhibition at 1 mM [66]; <3> 40% inhibition at 1 mM [66]) [66]

Ca^{2+} <3, 4, 12> (<3> 20% inhibition at 1 mM [66]; <4> 25% inhibition at 1 mM [66]; <12> 30% inhibition at 1 mM [66]) [66]
Cd^{2+} <3, 4, 5, 12> (<3,4> complete inhibition at 1 mM [66]; <12> 60% inhibition at 1 mM [66]; <5> nearly complete inhibition at 1 mM [66]) [66]
Ce^{3+} <4> (<4> enhances activity [33]) [33]
Co^{2+} <3, 4, 5, 12> (<4> 50% of the activation with Mg^{2+} [33]; <5> 15% inhibition at 1 mM [66]; <4,12> 30% inhibition at 1 mM [66]; <3> 45% inhibition at 1 mM [66]) [33, 66]
Cu^{2+} <3, 4, 5, 12> (<3> complete inhibition at 1 mM [66]; <12> 30% inhibition at 1 mM [66]; <4> nearly complete inhibition at 1 mM [66]; <5> over 95% inhibition at 1 mM [66]) [66]
Fe^{2+} <3, 4, 5, 12> (<3> 10% activation at 1 mM [66]; <5> 10% inhibition at 1 mM [66]; <12> 15%% activation at 1 mM [66]; <4> 20% inhibition at 1 mM [66]) [66]
Fe^{3+} <4, 5, 12> (<4> 15% inhibition at 1 mM [66]; <5> 20% inhibition at 1 mM [66]; <12> 30%% activation at 1 mM [66]) [66]
Hg^{2+} <3, 4, 5, 12> (<3,4,5> complete inhibition at 1 mM [66]; <12> 70% inhibition at 1 mM [66]) [66]
Mg^{2+} <3, 4, 12> (<4> enhances activity [33]; <4> 20% inhibition at 1 mM [66]; <3> 30% inhibition at 1 mM [66]; <12> 40% inhibition at 1 mM [66]) [33, 66]
Mn^{2+} <3, 4, 12> (<3,12> 20% inhibition at 1 mM [66]; <4> 30% inhibition at 1 mM [66]) [66]
Ni^{2+} <3, 4, 5, 12> (<4> enhances activity [33]; <5> 20% inhibition at 1 mM [66]; <4> 25% inhibition at 1 mM [66]; <12> 30% inhibition at 1 mM [66]; <3> 40% inhibition at 1 mM [66]) [33, 66]
Pb^{2+} <3, 4, 5, 12> (<4> 20% inhibition at 1 mM [66]; <5> 25% inhibition at 1 mM [66]; <3> 30% inhibition at 1 mM [66]; <12> 60% inhibition at 1 mM [66]) [66]
Zn^{2+} <3, 4, 5, 12> (<3,4> complete inhibition at 1 mM [66]; <12> 40% inhibition at 1 mM [66]; <5> over 95% inhibition at 1 mM [66]) [66]
Additional information <3, 5> (<3> no effect on activity by 1 mM Fe^{3+} [66]; <5> no effect on activity by 1 mM Mg^{2+}, Ca^{2+}, and Ba^{2+} [66]) [66]

Turnover number (min^{-1})

0.06 <4> (*p*-nitrophenyl phosphate) [33]
0.3 <17> (trans-stilbene oxide, <17> pH 7.0, 30°C [29]) [29]
0.35 <4> (threo-10-hydroxy-9-phosphonooxy-octadecanoic acid) [33]
0.5 <5> (trans-stilbene oxide, <5> native enzyme [61]) [61]
0.6 <27> ((S)-styrene oxide, <27> pH 9.0, 30°C, mutant enzyme Y215A [26]) [26]
0.8 <27> ((R)-styrene oxide, <27> pH 9.0, 30°C, mutant enzyme Q134A [26]) [26]
0.9 <35> (trans-1,3-diphenylpropene oxide, <35> pH 7.4, 30°C, purified recombinant enzyme [60]) [60]
1.3 <15> (trans-stilbene oxide, <15> pH 7.0, 30°C [34]) [34]

1.4 <27> ((S)-styrene oxide, <27> pH 9.0, 30°C, mutant enzyme Q134A [26]) [26]
1.64 <15> (2,3-epoxy-1,3-diphenyl-propane, <15> 30°C [23]) [23]
1.7 <27> ((R)-styrene oxide, <27> pH 9.0, 30°C, mutant enzyme Y215A [26]) [26]
1.7 <9> ((S)-*p*-nitrostyrene oxide) [17]
2.2 <27> ((R)-*p*-nitrostyrene oxide, <27> pH 9.0, 30°C, mutant enzyme Q134A [26]) [26]
4.3 <4> (2,3-epoxy-1,3-diphenyl-propane, <4> 30°C [23]) [23]
4.76 <12> (2,3-epoxy-1,3-diphenyl-propane, <12> 30°C [23]) [23]
4.8 <3> (4-nitrophenyl (2R,3R)-2,3-epoxy-3-(4-nitrophenyl)propyl carbonate, <3> pH 7.4, 25°C [54]) [54]
7.7 <3> (4-nitrophenyl (2S,3S)-2,3-epoxy-3-(4-nitrophenyl)propyl carbonate, <3> pH 7.4, 25°C [54]) [54]
12 <5> (2,3-epoxy-1,3-diphenyl-propane, <5> 30°C [23]) [23]
13.9 <9> ((R,S)-*p*-nitrostyrene oxide) [17]
15.8 <3> (4-nitrophenyl (2S,3S)-2,3-epoxy-3-phenylpropyl carbonate, <3> pH 7.4, 25°C [54]) [54]
16.4 <9> ((R)-*p*-nitrostyrene oxide) [17]
18 <3> (2,3-epoxy-1,3-diphenyl-propane, <3> 30°C [23]) [23]
26.7 <3> (4-nitrophenyl (2R,3R)-2,3-epoxy-3-phenylpropyl carbonate, <3> pH 7.4, 25°C [54]) [54]
Additional information <3, 4, 12, 29> [23, 43]

Specific activity (U/mg)

0.00017 <5> (<5> liver cytosol, substrate trans-β-methyl-styrene oxide [65]) [65]
0.00049 <2> (<2> liver cytosol, substrate trans-β-methyl-styrene oxide [65]) [65]
0.00051 <5> (<5> epididymis cytosol [37]) [37]
0.00062 <4> (<4> diseased kidney, cell-free enzyme extract [38]) [38]
0.001 <5> (<5> liver cytosol [37]) [37]
0.00122 <4> (<4> healthy kidney, cell-free enzyme extract [38]) [38]
0.0014 <12> (<12> crude potato enzyme extract [59]) [59]
0.00157 <5> (<5> liver cytosol, substrate allylbenzene oxide [65]) [65]
0.00189 <3> (<3> liver cytosol, substrate trans-bety-methyl-styrene oxide [65]) [65]
0.00264 <2> (<2> liver cytosol, substrate allylbenzene oxide [65]) [65]
0.0027 <5> (<5> kidney cytosol [37]) [37]
0.003 <35> (<35> purified recombinant enzyme, substrate trans-9,10-epoxy-stearate [60]) [60]
0.0064 <4> (<4> epoxide hydrolysis [33]) [33]
0.007 <3> (<3> liver cytosol, substrate allylbenzene oxide [65]) [65]
0.0078 <4> (<4> purified enzyme, substrate 4-nitrostyrene 7,8-oxide [3]) [3]
0.01 <3> (<3> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl (3,3-dimethyloxiran-2-yl)methyl carbonate [40]) [40]

0.012 <35> (<35> purified recombinant enzyme, substrate cis-8,9-epoxyeicosatrienoic acid or cis-11,12-epoxyeicosatrienoic acid [60]) [60]
0.025 <35> (<35> purified recombinant enzyme, substrate cis-14,15-epoxyeicosatrienoic acid [60]) [60]
0.051 <4> (<4> purified enzyme, substrate trans-8-ethylstyrene 7,8-oxide [3]) [3]
0.063 <12> (<12> recombinant enzyme in crude enzyme extract [59]) [59]
0.102 <9> [17]
0.12 <4> (<4> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl (3,3-dimethyloxiran-2-yl)methyl carbonate [40]) [40]
0.13 <3> (<3> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl (3-ethyloxiran-2-yl)methyl carbonate [40]) [40]
0.145 <4> [12]
0.167 <3> [19]
0.173 <5> [11]
0.21 <3> (<3> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl (3-phenyloxiran-2-yl)acetate [40]) [40]
0.26 <3> (<3> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl (3-propyloxiran-2-yl)methyl carbonate [40]) [40]
0.28 <3> (<3> purified recombinant enzyme, substrate cyano(2-methoxynaphthalen-6-yl)methyl trans-2-(3-propyloxiran-2-yl) acetate [40]) [40]
0.36 <4> (<4> purified recombinant enzyme, substrate [3-(4-chlorophenyl)oxiran-2-yl]methyl cyano(6-methoxy-2-naphthyl)methyl carbonate [40]) [40]
0.38 <4> (<4> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl [3-(4-nitrophenyl)oxiran-2-yl]methyl carbonate [40]) [40]
0.41 <4> (<4> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl (3-ethyloxiran-2-yl)methyl carbonate [40]) [40]
0.53 <4> (<4> purified recombinant enzyme, substrate cyano(2-methoxynaphthalen-6-yl)methyl trans-2-(3-propyloxiran-2-yl) acetate [40]) [40]
0.574 <3> [6]
0.71 <4> (<4> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl (3-phenyloxiran-2-yl)acetate [40]) [40]
0.78 <3> (<3> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl (3-phenyloxiran-2-yl)methyl carbonate [40]) [40]
0.8 <3> (<3> purified enzyme, substrate cis-8,9-hexahydro-epoxyeicosatrienoic acid [42]) [42]
0.823 <35> (<35> purified recombinant enzyme, substrate trans-1,3-diphenylpropene oxide [60]) [60]
0.86 <3> (<3> enzyme from untreated mice [5]; <3> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl [3-(4-nitrophenyl)oxiran-2-yl]methyl carbonate [40]) [5, 40]
1 <3> (<3> purified enzyme, substrate cis-8,9-epoxyeicosatrienoic acid methyl ester [42]) [42]
1.02 <4> (<4> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl (3-propyloxiran-2-yl)methyl carbonate [40]) [40]

1.04 <3> (<3> purified recombinant enzyme, substrate [3-(4-chlorophenyl)-oxiran-2-yl]methyl cyano(6-methoxy-2-naphthyl)methyl carbonate [40]) [40]
1.3 <3> (<3> purified enzyme, substrate cis-14,15-hexahydro-epoxyeicosatrienoic acid [42]) [42]
1.33 <3> (<3> enzyme from clofibrate-treated mice [5]) [5]
1.38 <8> [2]
1.41 <18> [18]
1.46 <3> [10]
1.5 <3> (<3> purified enzyme, substrate cis-8,9-epoxyeicosatrienoic acid [42]) [4, 42]
1.7 <3> (<3> purified enzyme, substrate cis-11,12-hexahydro-epoxyeicosatrienoic acid [42]) [42]
2.67 <12> [23]
2.69 <4> (<4> purified recombinant enzyme, substrate cyano(6-methoxy-2-naphthyl)methyl (3-phenyloxiran-2-yl)methyl carbonate [40]) [40]
3.8 <3> (<3> purified enzyme, substrate cis-11,12-epoxyeicosatrienoic acid [42]) [42]
3.9 <15> [23]
3.91 <29> [23]
5.6 <3> (<3> purified enzyme, substrate cis-14,15-epoxyeicosatrienoic acid [42]) [42]
Additional information <4, 5, 8, 23, 40> (<5> substrate specificity [3]; <4,5,8,23,40> several analytical and activity assay methods [1]; <4> substrate specificity, 2 enzymes with different specificities termed cytosolic TESO hydrolase and cytosolic PNSO hydrolase [3]) [1, 3]

K_m-Value (mM)

0.001 <27> ((R)-styrene oxide, <27> pH 9.0, 30°C, mutant enzyme Q134A [26]) [26]
0.0017 <5> (trans-stilbene oxide) [11, 24]
0.003 <5> (trans-stilbene oxide, <5> native enzyme [61]) [61]
0.003 <3> (cis-(11R,12S)-epoxyeicosatrienoic acid, <3> pH 7.4, 30°C [42]) [42]
0.004 <3> (cis-(11S,12R)-epoxyeicosatrienoic acid, <3> pH 7.4, 30°C [42]) [42]
0.004 <3> (cis-(14R,15S)-epoxyeicosatrienoic acid, <3> pH 7.4, 30°C [42]) [42]
0.0042 <3> (trans-stilbene oxide, <3> enzyme from clofibrate-treated animals [7]) [7]
0.0043 <3> (2,3-epoxy-1,3-diphenyl-propane, <3> 30°C [23]) [23]
0.0049 <3> (trans-stilbene oxide, <3> enzyme from untreated animals [7]) [7]

0.005 <3> (cis-(14S,15R)-epoxyeicosatrienoic acid, <3> pH 7.4, 30°C [42]) [42]
0.005 <3> (cis-(8R,9S)-epoxyeicosatrienoic acid, <3> pH 7.4, 30°C [42]) [42]
0.0052 <3, 15> (trans-stilbene oxide, <3> cytosolic enzyme [19]; <15> pH 7.0, 30°C [34]) [19, 34]
0.006 <3, 5> (trans-stilbene oxide, <3> peroxisomal enzyme [19]; <5> mutant enzyme W540L [14]) [14, 19]
0.0062 <4> (2,3-epoxy-1,3-diphenyl-propane, <4> 30°C [23]) [23]
0.0069 <5> (2,3-epoxy-1,3-diphenyl-propane, <5> 30°C [23]) [23]
0.007 <15> (2,3-epoxy-1,3-diphenyl-propane, <15> 30°C [23]) [23]
0.007 <5> (trans-stilbene oxide, <5> native enzyme [14]) [14]
0.0078 <3> (trans-diphenylpropene oxide, <3> pH 7.4, 37°C [66]) [66]
0.008 <3> (4-nitrophenyl (2S,3S)-2,3-epoxy-3-(4-nitrophenyl)propyl carbonate, <3> pH 7.4, 25°C [54]) [54]
0.008 <5> (trans-stilbene oxide, <5> recombinant fusion protein expressed in E. coli [14]) [14]
0.0081 <4> (trans-diphenylpropene oxide, <4> pH 7.4, 37°C [66]) [66]
0.0083 <12> (2,3-epoxy-1,3-diphenyl-propane, <12> 30°C [23]) [23]
0.0085 <5> (trans-stilbene oxide, <5> mutant enzyme H332Q [14]) [14]
0.009 <27> ((R)-*p*-nitrostyrene oxide, <27> pH 9.0, 30°C, mutant enzyme Q134A [26]) [26]
0.01 <5> (trans-diphenylpropene oxide, <5> pH 7.4, 37°C [66]) [66]
0.011 <17> (trans-stilbene oxide, <17> pH 7.0, 30°C [29]) [29]
0.015 <3> (4-nitrophenyl (2R,3R)-2,3-epoxy-3-(4-nitrophenyl)propyl carbonate, <3> pH 7.4, 25°C [54]) [54]
0.018 <2> ((11S,12S)-epoxy-5,14-cis-7,9-trans-eicosatetraenoic acid) [13]
0.0209 <4> (threo-10-hydroxy-9-phosphonooxy-octadecanoic acid) [33]
0.0253 <35> (trans-1,3-diphenylpropene oxide, <35> pH 7.4, 30°C, purified recombinant enzyme [60]) [60]
0.026 <14> (cis-9,10-epoxystearate, <14> microsomal enzyme [16]) [16]
0.027 <3> (cis-stilbene oxide, <3> peroxisomal enzyme [19]) [19]
0.033 <3> (4-nitrophenyl (2S,3S)-2,3-epoxy-3-phenylpropyl carbonate, <3> pH 7.4, 25°C [54]) [54]
0.041 <3> (cis-(8S,9R)-epoxyeicosatrienoic acid, <3> pH 7.4, 30°C [42]) [42]
0.045 <14> (cis-9,10-epoxystearate, <14> soluble enzyme [16]) [16]
0.055 <4> (cis-2-methylstyrene oxide, <4> cytosolic enzyme [12]) [12]
0.066 <3> (cis-stilbene oxide, <3> enzyme from clofibrate-treated animals [7]) [7]
0.068 <3> (4-nitrophenyl (2R,3R)-2,3-epoxy-3-phenylpropyl carbonate, <3> pH 7.4, 25°C [54]) [54]
0.07 <27> ((S)-styrene oxide, <27> pH 9.0, 30°C, mutant enzyme Q134A [26]) [26]
0.073 <3> (cis-stilbene oxide, <3> enzyme from untreated animals [7]) [7]
0.076 <3> (cis-stilbene oxide, <3> cytosolic enzyme [19]) [19]
0.083 <4> (cis-1,2-dimethylstyrene oxide, <4> microsomal enzyme [12]) [12]
0.089 <4> (cis-1,2-dimethylstyrene oxide, <4> cytosolic enzyme [12]) [12]
0.143 <4> (7,8-styrene oxide, <4> microsomal enzyme [12]) [12]

0.229 <4> (2,2-dimethylstyrene oxide, <4> microsomal enzyme [12]) [12]
0.319 <4> (cis-2-methylstyrene oxide, <4> microsomal enzyme [12]) [12]
0.358 <4> (2,2-dimethylstyrene oxide, <4> cytosolic enzyme [12]) [12]
0.473 <4> (trans-1,2-dimethylstyrene oxide, <4> microsomal enzyme [12]) [12]
0.517 <4> (7,8-styrene oxide, <4> cytosolic enzyme [12]) [12]
0.6 <9> ((R)-*p*-nitrostyrene oxide) [17]
0.6 <27> ((R)-styrene oxide, <27> pH 9.0, 30°C, mutant enzyme Y215A [26]) [26]
1 <9> ((R,S)-*p*-nitrostyrene oxide) [17]
1.36 <3> (styrene oxide, <3> enzyme from clofibbrate-treated animals [7]) [7]
1.6 <4> (*p*-Nitrophenyl phosphate) [33]
1.98 <3> (styrene oxide, <3> enzyme from untreated animals [7]) [7]
2.4 <27> ((S)-styrene oxide, <27> pH 9.0, 30°C, mutant enzyme Y215A [26]) [26]
3.4 <8> (styrene oxide) [2]
3.7 <9> ((S)-*p*-nitrostyrene oxide) [17]
Additional information <3, 4, 5, 12, 29> (<3,4,5> kinetics [25,42,43,54]; <3,4,5> kinetics, the hydrolysis of the intermediate is the rate-limiting step [41]) [23, 25, 41, 42, 43, 54]

K_i-Value (mM)

0.00003 <5> (dicyclohexylurea, <5> native enzyme [61]) [61]
0.0005 <5> (4-fluorochalcone oxide, <5> native enzyme [61]) [61]
0.00058 <3> (Zn^{2+}, <3> pH 7.4, 37°C, recombinant enzyme, uncompetitive inhibition type [66]) [66]
0.02 <4> (Zn^{2+}) [41]
0.023 <4> (Zn^{2+}, <4> pH 7.4, 37°C, noncompetitive inhibition type [66]) [66]
0.026 <5> (N,N'-dicyclohexylurea, <5> purified recombinant enzyme [49]) [49]
0.03 <27> (phenylacetamide, <27> pH 9.0, 30°C, wild-type enzyme [26]) [26]
0.2 <27> (phenylacetamide, <27> pH 9.0, 30°C, mutant enzyme Q134A [26]) [26]
0.27 <27> (phenylacetamide, <27> pH 9.0, 30°C, mutant enzyme Y215A [26]) [26]
0.6 <27> (phenoxyacetamide, <27> pH 9.0, 30°C, wild-type enzyme [26]) [26]
0.62 <12> (Zn^{2+}, <12> pH 7.4, 37°C, recombinant enzyme, noncompetitive inhibition type [66]) [66]
0.75 <27> (phenylacetamide, <27> pH 9.0, 30°C, mutant enzyme Y215F [26]) [26]
1.1 <27> (benzamide, <27> pH 9.0, 30°C, wild-type enzyme [26]) [26]
1.6 <27> (phenylcarbamate, <27> pH 9.0, 30°C, wild-type enzyme [26]) [26]
2 <27> (phenylacetamide, <27> pH 9.0, 30°C, mutant enzyme Y152F [26]) [26]
2 <27> (*p*-nitrobenzamide, <27> pH 9.0, 30°C, wild-type enzyme [26]) [26]

3 <27> (*p*-chlorobenzamide, <27> pH 9.0, 30°C, wild-type enzyme [26]) [26]
3.5 <27> (chloroacetamide, <27> pH 9.0, 30°C, wild-type enzyme [26]) [26]
5 <27> (urea, <27> pH 9.0, 30°C, wild-type enzyme [26]) [26]
37 <27> (acetamide, <27> pH 9.0, 30°C, wild-type enzyme [26]) [26]
Additional information <3, 4> (<3,4> inhibition kinetics [43]) [43]

pH-Optimum

5.2-8.3 <3, 4> (<3,4> assay at pH 5.2, 7.4, and 8.3 [50]) [50]
6-7 <17> (<17> reaction with trans-stilbene oxide [29]) [29]
6-10 <27> (<27> reaction with (R)-*p*-nitrostyrene oxide, wild-type and mutant enzymes Y215F and Y152F [26]) [26]
6.5-7.4 <3> [7]
6.8 <3, 8> (<8> hydration of styrene oxide [2]) [2, 19]
7 <3, 4, 9, 15> (<3,4> assay at [40]; <15> activity towards trans-stilbene oxide [34]) [17, 34, 40]
7-7.5 <4> [41]
7.4 <3, 4, 5, 12, 32, 35> (<4,5> cytosolic enzyme [24]; <3,4,5,12,32> assay at [25,37,42,44,45,47,54,58,66]; <3> assay at, cytosolic fraction [56]) [11, 24, 25, 37, 42, 44, 45, 47, 54, 56, 58, 60, 66]
7.4-7.6 <3, 4> [43]
7.4-9 <2, 3, 5> (<2,3,5> assay at [65]) [65]
7.5 <14> [16]
8 <3, 16> (<16> assay at [28]; <3> assay at, purified enzyme [56]) [28, 56]
9 <3, 4, 5> (<3> assay at [46]; <4> microsomal enzyme [24]) [24, 46]

pH-Range

4.5-8 <17> (<17> pH 4.5: about 40% of maximal activity, pH 8.0: about 50% of maximal activity, reaction with trans-stilbene oxide [29]) [29]
4.5-10 <15> (<15> pH 4.5: about 90% of maximal activity, pH 5.0: about 50% of maximal activity, 6.0: about 60% of maximal activity, pH 8.5: about 90% of maximal acticity, pH 9.0: about 70% of maximal activity, pH 10.0: about 35% of maximal activity [34]) [34]
5.5-8 <9> (<9> pH 5.5: about 35% of maximal activity, pH 8.0: about 45% of maximal activity [17]) [17]
5.5-9.5 <14> (<14> pH 5.5: about 30% of maximal activity, pH 9.5: about 25% of maximal activity [16]) [16]
6-9 <5> (<5> pH 6.0: about 35% of maximal activity, pH 9.0: about 60% of maximal activity [11]) [11]
6.2-8.1 <8> (<8> pH 6.2: about 80% of maximal activity, pH 8.1: about 35% of maximal activity [2]) [2]
7.4-9 <2, 3, 4, 5> (<4> cytosolic enzyme shows higher activity at pH 7.4, microsomal enzyme shows higher activity at pH 9.0 [12]) [12, 65]
Additional information <3> [7]

Temperature optimum (°C)

25 <3> (<3> assay at [54]) [54]
26 <5> (<5> assay at [25]) [25]

30 <3, 4, 5, 12, 16, 35> (<3,4,5,12,16,35> assay at [28,37,40,42,43,47,58,60,66]) [28, 37, 40, 42, 43, 47, 58, 60, 66]
37 <3, 4, 5, 32> (<3,4,5,32> assay at [44,45,46,50,56,66]; <3> inhibition assay at [54]) [44, 45, 46, 50, 54, 56, 66]
40 <9> [17]
50 <3> [7]
51 <3> [19]
54 <15> (<15> activity towards trans-stilbene oxide [34]) [34]
55 <17> (<17> reaction with trans-stilbene oxide [29]) [29]

Temperature range (°C)

15-45 <9> (<9> 15°C: about 50% of maximal activity, 45°C: about 65% of maximal activity [17]) [17]
30-55 <17> (<17> 30°C: about 35% of maximal activity, 55°C: maximum, 65°C: no activity, reaction with trans-stilbene oxide [29]) [29]
37-51 <3> (<3> 37°C: about 60% of maximal activity, 51°C: optimum [19]) [19]
37-55 <3> (<3> 37°C: about 60% of maximal activity, 55°C: about 50% of maximal activity of the enzyme from clofibrate-treated animals, about 80% of maximal activity of the enzyme from untreated animals [7]) [7]

4 Enzyme Structure

Molecular weight

40000 <12, 15, 29> (<12,29> gel filtration [23]; <12,15> about, gel filtration [23]) [23]
45000 <17> (<17> gel filtration [29]) [29]
55000 <3> (<3> gel filtration [8]) [8]
57000 <3> (<3> gel filtration [19]) [19]
57900 <4> (<4> gel filtration [22]) [22]
58000 <4> (<4> gel filtration, monomeric or dimeric enzyme, dependent on conditions [3]) [3]
60700 <3> (<3> uninduced murine liver, gel filtration [22]) [22]
62600 <3> (<3> induced murine liver, gel filtration [22]) [22]
64200 <14> (<14> gel filtration [16]) [16]
100000 <32> (<32> recombinant enzyme, gel filtration [45]) [45]
110000 <8> (<8> equilibrium sedimentation [2]) [2]
120000 <3, 5> (<5> gel filtration [11,24]) [5, 11, 24]
130000 <3, 4, 5> (<3,4> gel filtration [4,6,24]; <3,4,5> about, gel filtration [23]) [4, 6, 23, 24]
140000 <4> (<4> gel filtration, monomeric or dimeric enzyme, dependent on conditions [3]) [3]
185000 <9> (<9> gel filtration [17]) [17]
340000 <18> (<18> gel filtration [18]) [18]
Additional information <3, 4, 5, 8> [9]

Subunits

? <2, 3, 4, 5, 12, 15, 18, 34, 35> (<2, 3> x * 60000, SDS-PAGE [9,13]; <5> x * 50000, SDS-PAGE [3]; <5> x * 65000, SDS-PAGE [37]; <15> x * 37000, SDS-PAGE [34]; <4,5> x * 49000, SDS-PAGE [24]; <18> x * 32140, calculation from nucleotide sequence [18]; <15> x * 38061, MALDI-TOF [34]; <12> x * 38000, recombinant enzyme from insect cells, SDS-PAGE [59]; <34> x * 61500, SDS-PAGE [39]; <35> x * 63000, recombinant enzyme, SDS-PAGE [60]) [3, 9, 13, 18, 24, 34, 37, 39, 59, 60]

dimer <2, 3, 4, 5, 6, 8, 14, 20, 21, 23, 30, 34> (<3> 2 * 58000, SDS-PAGE [6]; <3, 4, 5> 2 * 60000, SDS-PAGE [5, 11, 23, 24]; <8> 2 * 57000, SDS-PAGE [2]; <3> 2 * 59000, SDS-PAGE [4]; <14> 2 * 32000, SDS-PAGE [16]; <2, 4, 5, 6, 8, 20, 21, 23, 30, 34> 2 * 62000, about [63]) [2, 4, 5, 6, 11, 16, 23, 24, 61, 63]

monomer <3, 4, 12, 15, 17, 29, 33> (<3> 1 * 59000, SDS-PAGE [19]; <12> 1 * 39000, SDS-PAGE [23]; <15, 29> 1 * 36000, SDS-PAGE [23]; <17> 1 * 37000, SDS-PAGE [29]; <3> 1 * 62600, induced murine liver, SDS-PAGE [22]; <3> 1 * 60700, uninduced murine liver, SDS-PAGE [22]; <4> 1 * 57900, SDS-PAGE [22]; <3> 1 * 59000, monomeric and dimeric enzyme forms retain catalytic activity, SDS-PAGE [8]; <33> 1 * 33000, recombinant enzyme, SDS-PAGE, 1 * 36171, recombinant and native enzymes, mass spectrometry, 1 * 36000, native enzyme, sedimentation equilibrium analysis [51]; <4> 1 * 62500 [41]) [8, 19, 22, 23, 29, 41, 51]

monomer or dimer <1, 4, 7, 10, 12, 13, 14, 15, 17, 19, 22, 24, 25, 31, 36, 37> (<12, 14, 15, 19> n * 35000, about [63]; <4> n * 58000, SDS-PAGE [3]; <1, 7, 10, 13, 17, 22, 24, 25, 31, 36, 37> x * 35000, about [63]) [3, 63]

tetramer <9> (<9> 4 * 45000, SDS-PAGE [17]) [17]

Additional information <2, 3, 4, 5, 6, 8, 20, 21, 23, 30, 32, 33, 34> (<3, 5> quarternary structure [41]; <3> structure determination and analysis [55]; <4, 5> peptide mapping [3]; <4> quarternary structure, monomers interact in a head-to-tail manner, the enzyme contains the DXDX(T/V) motif [41]; <5> structure analysis, amino acid sequence analysis and comparisons, the enzyme forms an α/β hydrolase fold [48]; <3,4,5> the 25 kDa C-terminal part, with a different hydrolase α/β-fold, harbors the epoxide hydrolase activity, the enzymes phosphatase activity of EC 3.1.3.76 is located at the 35 kDa N-terminal part which has a α/β-fold, structure analysis [61]; <4> the 25 kDa C-terminal part, with a hydrolase α/β-fold, harbors the epoxide hydrolase activity, the enzymes phosphatase activity of EC 3.1.3.76 is located at the 35 kDa N-terminal part which has a different α/β-fold, quarternary structure analysis, structure-mechanism relationship [27]; <4> the 25 kDa C-terminal part, with a hydrolase α/β-fold, harbors the epoxide hydrolase activity, the enzymes phosphatase activity of EC 3.1.3.76 is located at the 35 kDa N-terminal part which has a different α/β-fold, structure analysis [33]; <2, 4, 5, 6, 8, 20, 21, 23, 30, 34> the C-terminal domain catalyzes epoxy fatty acid hydrolysis, the N-terminal catalytic domain has also phosphatase activity with specificity for fatty acid diol phosphates [63]; <4> the C-terminal part harbors the epoxide hydrolase activity, the enzymes phosphatase activity of EC 3.1.3.76 is located at the N-terminal part, Arg287 may be involved in formation of the quaternary enzyme structure [44]; <4> the C-terminal part, with a

hydrolase α/β-fold, harbors the epoxide hydrolase activity, the enzymes phosphatase activity of EC3.1.3.76 is located at the N-terminal part which has a different α/β-fold [57]; <33> the enzyme contains an N-terminal transit peptide [51]; <32> the enzyme has no N-terminal membrane anchor in contrast to the microsomal epoxide hydrolase, EC 3.3.2.9 [45]) [3, 9, 27, 33, 41, 44, 45, 48, 51, 55, 57, 61, 63]

Posttranslational modification

glycoprotein <34> (<34> the enzyme sequence contains 1 putative O-glycosylation and 3 putative N-glycosylation sites [39]) [39]
phosphoprotein <34> (<34> the enzyme sequence contains 23 putative phosphorylation sites [39]) [39]

5 Isolation/Preparation/Mutation/Application

Source/tissue

NIH-3T3 cell <3> (<3> embryonic cell line [53]) [53]
adrenal <4> [63]
adrenal gland <2, 4> (<4> cytosolic localization [57]) [13, 57]
aerial part <13> [63]
alveolar wall <4> (<4> cytosolic localization [57]) [57]
anterior pituitary gland <4> (<4> cytosolic localization [57]) [57]
artery <4> (<4> renal, in the smooth muscle layer of the arterial wall [38]) [38]
blood <3> [62]
blood vessel <4> (<4> cytosolic localization [57]) [57]
brain <2, 5, 34> [13, 39, 52, 63]
brain stem <5> [52]
commercial preparation <9> [36]
cotyledon <31> (<31> the gene is expressed in cotyledon and hypocotyl [32]) [32]
culture condition:styrene oxide-grown cell <16> [28]
culture condition:styrene-grown cell <16> [28]
ejaculatory duct epithelium <4> (<4> cytosolic localization [57]) [57]
endometrium <4> (<4> cytosolic localization [57]) [57]
endosperm <19, 31> [32, 63]
endothelium <4> (<4> vascular [63]) [63]
epididymis <4, 5> (<5> caput, corpus, cauda [37]) [37, 63]
fruit <37> [63]
gonad <4> [63]
heart <5> [63]
hepatocyte <4> (<4> cytosolic and peroxisomal localization [57]) [57]
hippocampus <34> [39]
hypocotyl <31> (<31> the gene is expressed in cotyledon and hypocotyl [32]) [32]
hypothalamus <5> [52]

intestinal epithelium <4> (<4> cytosolic localization [57]) [57]
intestine <4> [63]
kidney <3, 4, 5, 8, 23, 34, 40> (<4,5,34> cortex [63]; <4,5,8,23,40> high activity [1]; <4> diseased and healthy, primarily vascular localization, low enzyme levels in the surrounding tubules [38]; <5> the activity for epoxyeicosatrienoic acids is elevated 5 to 54fold in renal cortical S9 fraction from the spontaneously hypertensive rats compared to normotensive Wistar-Kyoto rats [49]) [1, 37, 38, 41, 49, 63]
leaf <12, 13, 15, 36> (<12> meristem shows increased enzyme level compared to expanding and mature leaves [63]; <12> meristem, in expanding leaves, not in mature leaves [59]) [59, 63]
leukocyte <5> [63]
liver <2, 3, 4, 5, 8, 23, 34, 35, 40> (<4,5,8,23,40> high activity [1]) [1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 19, 20, 22, 24, 25, 37, 41, 42, 46, 49, 50, 53, 54, 56, 60, 61, 63, 65, 66]
lung <5> [63]
lymph node <4> [63]
lymphoid follicle <4> (<4> cytosolic localization [57]) [57]
mammary gland <34> [63]
muscle <34> (<34> striated [63]) [63]
neuron <5> (<5> culture, from hypothalamus [52]) [52]
ovary <4, 34> [63]
pancreas <4> [63]
pancreatic islet <4> (<4> cytosolic localization [57]) [57]
placenta <4> [63]
plasma <3> [62]
prostate <4> [63]
renal proximal tubule <4> (<4> cytosolic and peroxisomal localization [57]) [57]
seed <14, 31, 33> (<14> germinating [63]; <31> germination-specific isozyme [63]; <33> mature, dry seeds, high enzyme expression level at the very early stage of seed development 13 days after flowering, the expression decreases gradually duting seed development and is nearly ceased at the very late stage 58 days after flowering [51]) [32, 51, 63]
seedling <14> [16]
skin <5> [63]
small intestine <2> [13]
smooth muscle <4> [63]
spleen <5> [63]
stem <15, 33> [51, 63]
stomach <4> [63]
testis <4, 5, 8, 23, 40> (<4,5,8,23,40> high activity [1]) [1, 37, 63]
tonsil <4> [63]
urinary bladder <4> [63]
uterus <4> [63]
Additional information <1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 26, 28, 30, 31, 33, 34, 36, 37, 38, 39> (<1, 2, 4, 5, 6, 7, 8, 10,

11, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 26, 28, 30, 31, 34, 36, 37, 38, 39> tissue distribution [63]; <3,4,5> wide tissue distribution [41]; <5> enzyme expression is reduced in spontaneously hypertensive rats compared to normotensive rats [52]; <33> nearly no expression in leaves [51]) [41, 51, 52, 63]

Localization

Golgi apparatus <4, 5, 8, 23, 40> [1]
cytoplasm <8> [2]
cytosol <1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 26, 28, 30, 31, 34, 35, 36, 37, 38, 39> (<5> recombinant wild-type enzyme and some recombinant modified enzymes in V79 cells [53]; <3> recombinant wild-type enzyme, some recombinant modified enzymes [53]) [3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 16, 20, 21, 22, 24, 37, 41, 42, 46, 49, 50, 53, 54, 55, 56, 57, 59, 60, 61, 63, 65, 66]
endoplasmic reticulum <4, 5, 8, 23, 40> (<4,5,8,23,40> rough and smooth [1]) [1]
glyoxysome <19> [63]
membrane <4, 5, 8, 23, 40> [1]
microsome <4, 5> [12, 25]
nuclear membrane <4, 5, 8, 23, 40> [1]
peroxisome <3, 4, 5, 34> (<3> a recombinant modified enzyme in HeLa cells, recombinant wild-type enzyme and recombinant modified enzyme in plant BY-2 cells [53]; <5> a recombinant modified enzyme in V79 cells [53]; <34> in the light mitochondrial fraction [63]; <5> the enzyme contains an impaired peroxisomal targeting sequence leading to dual localization [63]) [19, 53, 57, 63]
plasma membrane <4, 5, 8, 23, 40> [1]
soluble <3, 4, 5, 12, 15, 17, 31, 32, 33, 34> (<32> the enzyme has no N-terminal membrane anchor in contrast to the microsomal epoxide hydrolase, 3.3.2.9 [45]) [23, 29, 32, 33, 38, 39, 40, 43, 44, 45, 48, 51, 52, 58, 62, 64]
Additional information <3, 4, 5, 8, 23, 40> (<4,5,8,23,40> highest level found in smooth endoplasmic reticulum, lower levels observed in rough endoplasmic reticulum, Golgi apparatus, nuclear membrane and plasma membrane. The enzyme is synthesized in membrane-bound polysomes and inserted cotranslationally into the endoplasmic reticulum [1]; <3,5> subcellular localization analysis [53]; <4> tissue-specific subcellular localization analysis [57]) [1, 53, 57]

Purification

<2> [13]
<2> (from liver) [63]
<3> [4, 5, 8, 19]
<3> (affinity purification) [10]
<3> (from liver by affinity chromatography) [50]
<3> (from liver by affinity chromatography on an epoxy activated resin derivatized with benzyl thiol) [54]
<3> (from livers, partially by subcellular fractionation) [46]

<3> (to homogeneity from liver by ultracentrifugation, ion exchange, hydrophobic interaction and hydroxyapatite chromatography) [56]
<4> [9, 12, 33]
<4> (2 cytosolic enzyme forms: 1. cEHTSO, epoxide hydrolase with diagnostic substrate specificity for trans-stilbene oxide and 2. cEHCSO, epoxide hydrolase with diagnostic substrate specificity for cis-stilbene oxide) [24]
<4> (425fold from liver cytosol to homogeneity by ammonium sulfate fractionation, cellulose resin, hydroxylapaptite, and ion exchange chromatography) [3]
<4> (from liver by affinity chromatography) [50]
<4> (recombinant His-tagged wild-type and mutant enzymes from Escherichia coli strain BL21-AI by nickel affinity chromatography, recombinant strep-tagged wild-type enzyme from Escherichia coli strain XL1-Blue by streptavidin affinity chromatography) [61]
<4> (recombinant His-tagged wild-type and mutant enzymes from Sf21 insect cells by nickel affinity chromatography) [44]
<4> (recombinant enzyme from insect cells in several steps including a final gel filtration step) [64]
<4> (recombinant wild-type and mutant enzymes from insect cells by affinity chromatography) [33]
<5> [11, 24]
<5> (from liver) [1]
<5> (native enzyme from liver to homogeneity by ultracentrifugation and affinity chromatography on a benzyl thio resin using 4-fluorochalcone oxide as eluent) [61]
<8> [2, 9]
<9> [17, 35]
<12> [23]
<14> [16]
<15> [23, 34]
<17> (recombinant enzyme) [29]
<18> [18]
<29> [23]
<32> (recombinant enzyme from Escherichia coli strain BL21(DE3) to homogeneity by ammonium sulfate fractionation, anion exchange and hydrophobic interaction chromatography, and gel filtration) [45]
<33> (native enzyme from dry mature seeds by ammonium sulfate fractionation, ion exchange chromatography and chromatofocusing, the latter step allows separation from the co-purifying seed-specific PR-8 chitinase Chib2, recombinant enzyme from Escherichia coli strain BL21(DE3) by heat treatment at 55°C for 10 min, chromatofocusing and gel filtration) [51]
<34> (from hippocampus for enzyme identification by 2D-electrophoresis and MALDI mass spectrometry) [39]
<34> (from liver) [63]
<35> (recombinant His-tagged enzyme from Sf21 or Tn5 insect cells by nickel affinity chromatography) [60]

Crystallization

<3> (analysis of several crystal structures of apoenzyme and enzyme bound to inhibitor urea for molecular dynamics simulations and determination of active site conformation replacing the inhibitor by substrate trans-methylstyrene oxide in the crystal structure model, minimized structure models) [55]

<3> (enzyme with bound inhibitor N-cyclohexyl-N'-decylurea) [41]

<4> [41]

<4> (purified recombinant enzyme in complex with N-cyclohexyl-N'-(iodophenyl)urea, sitting drop vapour diffusion method, 0.005 ml of 12-16 mg/ml protein in 3 mM DTT, 0.1 M sodium phosphate, pH 7.4, is mixed with 0.005 ml precipitation solution containing 0.1 M Tris, pH 9.0, 30% w/v PEG 4000, and 0.2 M Li_2SO_4, versus 1 ml reservoir of precipitation solution, 4°C, 10 days, soaking of crystals in inhibitor solution and n-hexadecyl-β-D-maltoside, X-ray diffraction structure determination and analysis at 2.35 A resolution, modeling) [27]

<4> (purified recombinant enzyme in complex with inhibitors 4-(3-cyclohexylureido)-ethanoic acid, N-cyclohexyl-N'-iodophenyl urea, 4-(3-cyclohexylureido)-butyric acid, 4-(3-cyclohexylureido)-hexanoic acid, and 4-(3-cyclohexylureido)-heptanoic acid, vapour diffusion method, 0.005 ml sitting drops of protein solution containing 12-16 mg/ml enzyme, 3 mM DTT, 0.1 M sodium phosphate, pH 7.4, mixed with 0.005 ml precipitant solution containing 0.1 M Tris, pH 8.4, and 34% w/v PEG3350, and 0.005 ml n-hexadecyl-β-D-maltoside solution, versus 1 ml reservoir solution, 4°C, 7 days, soaking of crystals in precipitation solution containing 30 mM inhibitor, 3 days, 4°C, cryoprotection by 20% sucrose, X-ray diffraction structure determination and analysis at 2.3-3.0 A resolution) [64]

<4> (sitting drop vapour diffusion method, X-ray crystal structure determined at 2.6 A resolution, structure of the complex with the inhibitor N-cyclohexyl-N'-(iodophenyl)urea determine at 2.35 A resolution) [27]

<27> (hanging-drop method) [30]

<32> (three-dimensional crystal structure is determined at 3.5 A resolution by the multiwavelength anomalous diffraction method using crystals of a seleno-methionine substituted form of enzyme and then refined at 1.8 A resolution) [35]

Cloning

<3> (baculovirus expression system) [15]

<3> (transient expression of wild-type and modified enzymes in HeLa cells, in NIH-3T3 cells, and in tobacco BY-2 cells, the latter via microprojectile bombardment) [53]

<4> (enzyme expression in insect cells using the baculovirus infection system) [64]

<4> (expression of His-tagged wild-type and mutant enzymes from cDNA in Escherichia coli strain BL21-AI, expression of strep-tagged enzyme from full-length gene in Escherichia coli strain XL1-Blue) [61]

<4> (expression of wild-type and mutant enzymes in insect cells using the baculovirus infection system) [33]
<4> (gene EPHX2, expression of His-tagged wild-type and mutant enzymes in Spodoptera frugiperda Sf21 cells using the baculovirus infection system) [44]
<5> [24]
<5> (expression in Escherichia coli) [14]
<5> (expression of the enzyme from full-length gene in Escherichia coli strain BL21-AI) [61]
<5> (stable expression of GFP-tagged wild-type and modified enzymes in V79 Chinese hamster cells) [53]
<12> (DNA and amino acid sequence determination and analysis, expression of the potato enzyme in Spodoptera frugiperda cells and in Trichoplusia ni cells using the baculovirus infection system) [59]
<12> (baculovirus expression system) [23]
<15> (overexpression in Pichia pastoris. A tag containing six His residues is added to the N-terminus to enable efficient one-step purification) [34]
<17> (expression in Pichia pastoris) [29]
<18> [18]
<29> (baculovirus expression system) [23]
<31> (expression in Escherichia coli) [32]
<32> (DNA and amino acid sequence determination and analysis, phylogenetic tree, sequence comparisons, functional expression of wild-type enzyme and expression of enzyme mutants in Escherichia coli strain BL21(DE3)) [45]
<33> (cDNA library construction, DNA and amino acid sequence determination of the gene encoding the enzyme, genetic organization in a small EH gene family in soybean, overexpression in Escherichia coli strain BL21(DE3)) [51]
<35> (DNA and amino acid sequence determination and analysis, expression of the His-tagged enzyme in Spodoptera frugiperda Sf21 cells or in Trichoplusia ni Tn5 cells using the baculovirus infection system) [60]

Engineering

C154Y <4> (<4> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 2.2, compared to the wild-type ratio of 1.0 [44]) [44]
D192A <32> (<32> site directed mutagenesis, inactive or nearly inactive mutant [45]) [45]
D192N <32> (<32> site directed mutagenesis, inactive or nearly inactive mutant [45]) [45]
D192S <32> (<32> site directed mutagenesis, inactive or nearly inactive mutant [45]) [45]
D333G <5> (<5> no activity [14]) [14]
D333N <3> (<3> 21.5% of the activity of the wild-type enzyme with trans-stilbene oxide [15]) [15]
D333S <3> (<3> no detectable activity with trans-stilbene oxide [15]) [15]
D348A <32> (<32> site directed mutagenesis, inactive or nearly inactive mutant [45]) [45]

D348E <32> (<32> site directed mutagenesis, the mutant shows 48% of wild-type enzyme activity [45]) [45]
E404D <5> (<5> site-directed mutagenesis, the mutant enzyme shows unaltered enantioselectivity with stearic acid oxide substrate compared to the wild-type enzyme [25]) [25]
E470G <4> (<4> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 0.9, compared to the wild-type ratio of 1.0 [44]) [44]
H237N <3> (<3> 117.2% of the activity of the wild-type enzyme with trans-stilbene oxide [15]) [15]
H263N <3> (<3> 52.3% of the activity of the wild-type enzyme with trans-stilbene oxide [15]) [15]
H332N <3> (<3> 3.3% of the activity of the wild-type enzyme with trans-stilbene oxide [15]) [15]
H332Q <5> (<5> strong decrease in enzymatic activity due to a change in substrate affinity, K_m deviates only slightly from that of the wild-type enzyme, V_{max} is reduced to 5% of the wild-type enzyme [14]) [14]
H374K <32> (<32> site directed mutagenesis, inactive or nearly inactive mutant [45]) [45]
H374N <32> (<32> site directed mutagenesis, inactive or nearly inactive mutant [45]) [45]
H374S <32> (<32> site directed mutagenesis, inactive or nearly inactive mutant, recombinantly expressed mutant enzyme is not soluble but remains in the particulate fraction of Escherichia coli cells [45]) [45]
H431S <3> (<3> mutation of His431 results in total loss of activity, the mutant can form the intermediate but cannot hydrolyze it [41]) [41]
H517Y/C521Y <5> (<5> no activity [14]) [14]
H523D <5> (<5> no activity [14]) [14]
H523Q <3> (<3> 0.1% of the activity of the wild-type enzyme with trans-stilbene oxide [15]) [15]
K55R <4> (<4> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 1.9, compared to the wild-type ratio of 1.0 [44]) [44]
Q134A <27> (<27> the K_i-value for phenylacetamide is 67fold higher than the K_i-value of the wild-type enzyme. The ratio of turnover number to K_M-value for (R)-styrene oxide is 10fold lower than that of the wild-type enzyme. The ratio of turnover number to K_M-value for (S)-styrene oxide is 25fold lower than that of the wild-type enzyme. The ratio of turnover number to K_M-value for (R)-*p*-nitrostyrene oxide is 4fold lower than that of the wild-type enzyme. The ratio of turnover number to K_M-value for (R)-*p*-nitrostyrene oxide is 13fold lower than that of the wild-type enzyme [26]) [26]
R103C <4> (<4> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 1.3, compared to the wild-type ratio of 1.0 [44]) [44]
R103C/R287Q <4> (<4> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 1.2, compared to the wild-type ratio of 1.0 [44]) [44]
R287E <4> (<4> naturally occuring mutation of gene EPXH2 leads to elevated plasma cholesterol and triglycerides [63]) [63]

R287Q <4> (<4> naturally occuring mutation of gene EPXH2 leads to elevated risk of coronary artery calcification found in African Americans [63]; <4> site-directed mutagenesis, the mutant shows the same hydrolase/phosphatase ratio of 1.0 as the wild-type, but reduced stability at 37°C [44]) [44, 63]
V422A <4> (<4> site-directed mutagenesis, the mutant shows a hydrolase/phosphatase ratio of 0.8, compared to the wild-type ratio of 1.0 [44]) [44]
W334F <5> (<5> 7fold increase in K_m-value, 2fold enhanced V_{max} [14]) [14]
W540L <5> (<5> no activity [14]; <5> strong decrease in enzymatic activity due to a change in substrate affinity, K_m deviates only slightly from that of the wild-type enzyme [14]) [14]
W540S <5> (<5> no activity [14]) [14]
Y152F <27> (<27> rate of irreversible inactivation for wild-type enzyme increases rapidly above pH 10, but the inactivation rates for Y215F and Y152F mutant remain low at increasing pH. The K_i-value for phenylacetamide is 67fold higher than the K_i-value of the wild-type enzyme [26]) [26]
Y152F/Y215F <27> (<27> mutant enzyme with low activity, more thermostable than wild-type enzyme [26]) [26]
Y215A <27> (<27> enzyme has lower enantioselectivity for styrene oxide and *p*-nitrostyrene oxide than the wild-type enzyme. The Ki-value for phenylacetamide is 9fold higher than the Ki-value of the wild-type enzyme. The ratio of turnover number to K_M-value for (R)-styrene oxide is 2667fold lower than that of the wild-type enzyme. The ratio of turnover number to K_M-value for (S)-styrene oxide is 2000fold lower than that of the wild-type enzyme. The ratio of turnover number to K_M-value for (R)-*p*-nitrostyrene oxide is 486fold lower than that of the wild-type enzyme. The ratio of turnover number to K_M-value for (R)-*p*-nitrostyrene oxide is 77fold lower than that of the wild-type enzyme [26]) [26]
Y215F <27> (<27> more thermostable than wild-type enzyme. Rate of irreversible inactivation for wild-type enzyme increases rapidly above pH 10, but the inactivation rates for Y215F and Y152F mutant remain low at increasing pH. The K_i-value for phenylacetamide is 25fold higher than the K_i-value of the wild-type enzyme [26]) [26]
Y381F <3> (<3> site-directed mutagenesis, mutant shows 90% reduced activity [41]) [41]
Y465F <3, 4> (<4> site-directed mutagenesis, mutant enzyme shows phosphatase, but no epoxide hydrolase activity [33]; <3> site-directed mutagenesis, mutant shows 90% reduced activity [41]) [33, 41]
Additional information <3, 4, 5, 9> (<9> the selectivity factor E reflecting the relative rate of the reaction of the two enantiomers is only 4.6 for the wild-type enzyme, in slight favor of the (S)-product, a value of 10.8 is observed with the natural EH variant IS002B1 with the three amino acid exchanges A217V, K332E and A390E. A value of 7.4 is observed with the natural variant IR003B1 with the amino acid exchange R219G, a value of 6.6 is observed with the natural variant IB001C_2 with the amino acid exchange F340Y, a value of 6.4 is observed with the natural variant IE001H6 with the amino acid exchange A327V, a value of 6.2 is observed with the natural variant IS001H8

with the amino acid exchange A327V and a value of 5.5 is observed with the natural variant IL001D4 with the amino acid exchange P222S [31]; <3> construction of mutants with point mutations in a wild-type SKI sequence motif, some mutant enzymes show altered subcellular localization, expression in tobacco BY-2 cells results in peroxisomal expression for recombinant wild-type and modified enzymes, overview [53]; <5> construction of mutants with point mutations in a wild-type SKI sequence motif, some mutant enzymes show altered subcellular localization, overview [53]; <4> enzyme polymorphisms, overview [63]) [31, 53, 63]

Application

agriculture <17> (<17> the isolation of BNSEH1 will facilitate metabolic studies of resistance and seed oil modification in the important oilcrop Brassica napus [29]) [29]

medicine <4, 5> (<4> enzyme inhibition is used in treatment of hypertension [64]; <4> the enzyme is a target for regulation of blood pressure, and inhibition of inflammation, atherosclerosis, kidney failure, and cancer progression [41]; <5> the enzyme is a therapeutic target for control of blood pressure [49]; <4> the enzyme is a therapeutic target in therapeutic intervention in renal hydrodynamic regulation and blood pressure control [38]) [38, 41, 49, 64]

pharmacology <3, 4, 5> (<3> the enzyme is a key target in treatment of acute systemic hypotension [62]; <3,4> the enzyme is a target for inhibition in therapy of disorders resulting from hypertension and vascular inflammation [40]; <3,4,5> the enzyme is a target for inhibitor design [41]) [40, 41, 62]

synthesis <9, 12, 15, 16, 32> (<9> the enzyme may be a good biocatalyst for the preparation of enantiopure epoxides or diols [17]; <12,15> potential as biocatalyst for the preparation of enantiopure epoxides [23]; <16> the enantioselective enzyme is useful in production of chiral substances, e.g. production of (2R,3S)-ethyl 3-phenylglycidate with 95% enantiomeric excess and 26% yield in 12 h from 0.2% (w/v) of the racemat by whole cells of Pseudomonas sp. strain BZS21, maximal activity with dimethyl formamide as cosolvent [28]; <32> the enzyme is useful for enantioselective bio-organic synthesis of chiral substances [45]) [17, 23, 28, 45]

6 Stability

pH-Stability

10 <27> (<27> rate of irreversible inactivation for wild-type enzyme increases rapidly above pH 10, but the inactivation rates for Y215F and Y152F mutant remain low at increasing pH [26]) [26]

Temperature stability

21 <15> (<15> 4 h, 50% loss of activity [34]) [34]

30 <3, 4, 5, 15> (<15> 3.5 h, 50% loss of activity [34]; <3> $t_{1/2}$ is 17.4 h [23]; <5> $t_{1/2}$: 20.3 h [23]; <4> $t_{1/2}$: 8.1 h [23]) [23, 34]

37 <3, 4, 5, 12, 15, 29> (<29> $t_{1/2}$: more than 24 h [23]; <5> $t_{1/2}$: 2.0 h [23]; <3> $t_{1/2}$: 18 h [23]; <4> $t_{1/2}$: 1.3 h [23]; <3> over 50% loss of activity within 10 min, more than 90% loss of activity within 25 min [9]; <5> $t_{1/2}$: 2 h [23]; <15> 2.8 h, 50% loss of activity [34]; <3> $t_{1/2}$ is 1.8 h [23]; <4> inactivation rate constants for recombinant wild-type and mutant enzymes [44]) [9, 23, 34, 44]
45 <15> (<15> 2.1 h, 50% loss of activity [34]; <15> $t_{1/2}$ is 10.2 h [23]) [23, 34]
51 <12, 15, 29> (<12> $t_{1/2}$: more than 24 h [23]; <29> $t_{1/2}$: 6.4 h [23]; <15> $t_{1/2}$ is 6.4 h [23]) [23]
54 <15> (<15> 1 h, 50% loss of activity [34]) [34]
55 <12, 15, 29> (<29> $t_{1/2}$: 0.93 h [23]; <12> $t_{1/2}$: 4.7 h [23]; <15> $t_{1/2}$ is 0.93 h [23]; <12> $t_{1/2}$ is 4.7 h [23]) [23]
59 <12, 15, 29> (<29> $t_{1/2}$: 0.38 h [23]; <12> $t_{1/2}$: 0.87 h [23]; <15> $t_{1/2}$ is 0.38 h [23]; <12> $t_{1/2}$ is 0.87 h [23]) [23]
62-67 <27> (<27> precipitation of wild-type and mutant enzymes Y215F and Y152F/Y215F [26]) [26]
80 <3> (<3> 10 min, cytosolic fraction, inactivation [56]) [56]

Oxidation stability

<3>, sensitive to oxygen [7]

Storage stability

<3>, -20°C, stable [5]
<3>, -20°C, stable for at least 6 months [7]
<15>, 4°C, 6 month, stable [34]
<35>, 25°C, purified recombinant enzyme, half-life is 9-24 h [60]
<35>, 37°C, purified recombinant enzyme, half-life is below 3 h [60]
<35>, 4°C, purified recombinant enzyme, half-life is more than 6 days [60]

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Cholesterol-5,6-oxide hydrolase 3.3.2.11

1 Nomenclature

EC number
3.3.2.11

Systematic name
5,6α-epoxy-5α-cholestan-3β-ol hydrolase

Recommended name
cholesterol-5,6-oxide hydrolase

Synonyms
CEHase <1> [3]
EH_CH <3, 4> [6]
cholEH <3> [5]
cholesterol 5α,6α-oxide hydrolase <3, 4> [6]
cholesterol epoxide hydrolase <1, 3> [3, 4, 5]
cholesterol oxide hydrolase <3> [7]
cholesterol-epoxide hydrolase <3> [8]

CAS registry number
55467-47-5

2 Source Organism

<1> *Mus musculus* (no sequence specified) [2, 3]
<2> *Homo sapiens* (no sequence specified) [2]
<3> *Rattus norvegicus* (no sequence specified) [1, 2, 4, 5, 6, 7, 8]
<4> *Oryctolagus cuniculus* (no sequence specified) [2, 6]
<5> *Mesocricetus auratus* (no sequence specified) [2]

3 Reaction and Specificity

Catalyzed reaction
5,6α-epoxy-5α-cholestan-3β-ol + H_2O = cholestane-3β,5α,6β-triol (<3> positively charged transition state [7])
5,6β-epoxy-5β-cholestan-3β-ol + H_2O = cholestane-3β,5α,6β-triol (<3> positively charged transition state [7])

Natural substrates and products

- **S** 5,6α-epoxy-5α-cholestan-3β-ol + H_2O <1, 2, 3, 4, 5> (<3> regulation, the enzyme is highly substrate specific [1]; <3,4> the enzyme is highly substrate-specific [6]; <1> the substrate is a product of lipid peroxidation in the liver [3]) (Reversibility: ?) [1, 2, 3, 5, 6, 7, 8]
- **P** cholestane-3β,5α,6β-triol
- **S** 5,6β-epoxy-5β-cholestan-3β-ol + H_2O <3> (Reversibility: ?) [2, 7, 8]
- **P** cholestane-3β,5α,6β-triol
- **S** Additional information <1, 2, 3, 4, 5> (<3> the enzyme is distinct from the microsomal epoxide hydrolase EC 3.3.2.9 [7,8]; <1,2,3,4,5> the enzyme is distinct from the microsomal epoxide hydrolase EC 3.3.2.9, which shows a wider substrate specificity [2,6]; <1,3> the enzyme is distinct from the microsomal epoxide hydrolase EC 3.3.2.9, which shows a wider xenobiotic substrate specificity [1,3]) (Reversibility: ?) [1, 2, 3, 6, 7, 8]
- **P** ?

Substrates and products

- **S** 5,6-epoxy-5α-cholestan-3β-ol + H_2O <3> (Reversibility: ?) [4]
- **P** cholestane-3β,5α,6β-triol
- **S** 5,6α-epoxy-5α-cholestan-3β-ol + H_2O <1, 2, 3, 4, 5> (<3> regulation, the enzyme is highly substrate specific [1]; <3,4> the enzyme is highly substrate-specific [1,6]; <1> the substrate is a product of lipid peroxidation in the liver [3]; <1,2,3,4,5> the enzyme is equally active with both stereoisomers [2,8]; <3> the enzyme is highly substrate specific [5]) (Reversibility: ?) [1, 2, 3, 5, 6, 7, 8]
- **P** cholestane-3β,5α,6β-triol (<3> i.e. 5,6-glycol [1])
- **S** 5,6β-epoxy-5β-cholestan-3β-ol + H_2O <1, 2, 3, 4, 5> (<1,2,3,4,5> the enzyme is equally active with both stereoisomers [2,8]) (Reversibility: ?) [2, 7, 8]
- **P** cholestane-3β,5α,6β-triol
- **S** Additional information <1, 2, 3, 4, 5> (<3> the enzyme is distinct from the microsomal epoxide hydrolase EC 3.3.2.9 [7,8]; <1,2,3,4,5> the enzyme is distinct from the microsomal epoxide hydrolase EC 3.3.2.9, which shows a wider substrate specificity [2,6]; <1,3> the enzyme is distinct from the microsomal epoxide hydrolase EC 3.3.2.9, which shows a wider xenobiotic substrate specificity [1,3]; <3> no activity with xenobiotic alkene oxides and arene oxides, e.g. octene 1,2-oxide or benz[a]anthracene 5,6-oxide [1]) (Reversibility: ?) [1, 2, 3, 6, 7, 8]
- **P** ?

Inhibitors

3-methylcholanthracene <3> [1]

5,6α-epoxy-5α-cholestan-3β-ol <3> (<3> competitive to 5,6β-epoxy-5α-cholestan-3β-ol [8]) [8]

5,6α-imino-5α-cholestan-3β-ol <3> (<3> strong inhibition [2]; <3> strong, specific, competitive inhibition [7]) [2, 7]

5,6α-imino-5α-cholestane-3β-ol <3, 4> (<3,4> specific inhibition of cholesterol-5,6-epoxide hydrolase [6]) [6]

5,6β-epoxy-5β-cholestan-3β-ol <3> (<3> competitive to 5,6α-epoxy-5α-cholestan-3β-ol [8]) [8]
6-oxocholestanol <3> [8]
7-oxocholesterol <3> [8]
7-dehydrocholesterol 5,6 β-oxide <3> (<3> specific, mechanism-based inhibitor [7]) [7]
7-oxocholestanol <3> [8]
9,10-iminophenanthrene <3> [2]
9-fluorenone <3> (<3> slight inhibition [4]) [4]
acetone <3> (<3> reduces the activity by 85% at 1.25% v/v, by 50% at 3.75% v/v, and by 56% at 6.25% v/v [1]) [1]
aroclor 1254 <3> [1]
chalcone <3> [4]
cholesterol <3> (<3> IC50 is above 0.125 mM [2]) [2]
clofibrate <3> [1]
dimethylsulfoxide <3> (<3> reduces the activity by 42% at 6.25% v/v [1]) [1]
ellipticine <3> [4]
Emulgen 108 <1> (<1> complete inactivation [3]) [3]
Emulgen 911 <1> (<1> complete inactivation [3]) [3]
iminosqualene <3> [2]
isoquinoline <3> (<3> slight inhibition [4]) [4]
Lubrol Px <1> (<1> complete inactivation [3]) [3]
methanol <3> (<3> reduces the activity by 86% at 3.75% v/v [1]) [1]
metyrapone <3> (<3> weak inhibition in hyperplastic nodules, not in normal tissue [4]) [4]
phenobarbital <3> [1]
sodium cholate <1> [3]
tetrahydrofuran <3> (<3> reduces the activity by 86% at 6.25% v/v [1]) [1]
trans-stilbene oxide <3> [1]
Triton WR-1339 <1> [3]
Tween 80 <1> [3]
benz[a]anthracene 5,6-oxide <3> (<3> non-competitive inhibition [1]) [1]
cholestane-3β,5α,6α-triol <3> (<3> IC50 is 0.110 mM [2]) [2]
cholestane-3β,5α,6β-triol <3> (<3> product inhibition [8]; <3> IC50 is 0.03 mM [2]) [2, 8]
cholestane-3β,5β,6α-triol <3> (<3> IC50 is 0.085 mM [2]) [2]
cholestane-3β,5β,6β-triol <3> (<3> IC50 is 0.118 mM [2]) [2]
cholestyramine <3> [1]
γ-chlordane <3> [1]
harman <3> [4]
isosafrole <3> (<3> slight inhibition [1]) [1]
norharman <3> [4]
octene 1,2-oxide <3> (<3> non-competitive inhibition [1]) [1]
oxidosqualene <3> (<3> IC50 is above 0.16 mM [2]) [2]
pregnenolone 16α-carbonitrile <3> [1]
Additional information <3, 4> (<3> comparison of inhibition level in microsomes of normal liver tissue and liver hyperplastic nodules, remaining activ-

ities, overview [4]; <3> no effect by 5,6α-epoxy-5α-cholestan-3β-ol in vivo, and by EDTA and 2,2-dipyridyl, preferred buffer is 300 mM potassium phosphate at pH 7.4 and 37°C, low activity in Tris and HEPES buffers [1]; <3,4> no inhibition by 1,1,1-trichloropropene oxide [6]; <3> no inhibition by isoquinoline, styrene oxide, cyclohexane oxide, benzil, cholesterol, and cholic acid [8]) [1, 4, 6, 8]

Activating compounds

acetonitrile <3> (<3> activates at 3% v/v [7]; <3> activates, maximal at 6.25% v/v [2]; <3> maximal enzyme activity at 6.25% v/v [4]; <3> maximal enzyme activity at 6.25% v/v, 19% of maximal activity at 1.25% v/v, 50% of maximal activity at 12.5% v/v, no activity at 18.8% v/v [1]) [1, 2, 4, 7]
Additional information <3> (<3> 2-acetylaminofluorene induces the enzyme [4]; <3> no effect by 5,6α-epoxy-5α-cholestan-3β-ol in vivo, and by EDTA and 2,2-dipyridyl, preferred buffer is 300 mM potassium phosphate at pH 7.4 and 37°C, low activity in Tris and HEPES buffers [1]) [1, 4]

Specific activity (U/mg)

0.00004 <4> (<4> liver microsomes, substrate 5,6β-epoxy-5α-cholestan-3β-ol [2]) [2]
0.00007 <3> (<3> microsomes from hyperplastic nodules [4]) [4]
0.00014 <4> (<4> liver microsomes, substrate 5,6α-epoxy-5α-cholestan-3β-ol [2]) [2]
0.00017 <3> (<3> microsomes from normal liver tissue [4]) [4]
0.00018 <1, 3> (<3> Golgi apparatus I [5]; <1> liver microsomes, substrate 5,6β-epoxy-5α-cholestan-3β-ol [2]) [2, 5]
0.0002 <5> (<5> liver microsomes, substrate 5,6α-epoxy-5α-cholestan-3β-ol [2]) [2]
0.00033 <1> (<1> liver microsomes [3]) [3]
0.00038 <2> (<2> liver microsomes, substrate 5,6β-epoxy-5α-cholestan-3β-ol [2]) [2]
0.00045 <3> (<3> liver microsomes, substrate 5,6β-epoxy-5α-cholestan-3β-ol [8]) [8]
0.00048 <3> (<3> liver microsomes, substrate 5,6α-epoxy-5α-cholestan-3β-ol [8]) [8]
0.00051 <1> (<1> liver microsomes, substrate 5,6α-epoxy-5α-cholestan-3β-ol [2]) [2]
0.00052 <3> (<3> liver microsomes, substrate 5,6β-epoxy-5α-cholestan-3β-ol [2]) [2]
0.00065 <3> (<3> smooth microsomes [5]) [5]
0.00068 <3> (<3> rough microsomes [5]) [5]
0.00108 <2, 3> (<2,3> liver microsomes, substrate 5,6α-epoxy-5α-cholestan-3β-ol [2]) [2]
0.00123 <3> (<3> liver microsomes [1]) [1]
Additional information <3> (<3> evaluation of substrate solubilization solvents for enzyme assay, preferred buffer is 300 mM potassium phosphate at pH 7.4 and 37°C [1]; <3> specific activity in different tissues and subcellular fractions [5]) [1, 5]

K_m-Value (mM)

0.00091 <3> (5,6-epoxy-5α-cholestan-3β-ol, <3> pH 7.4, 37°C, normal liver tissue microsomes [4]) [4]
0.0037 <3> (5,6α-epoxy-5α-cholestan-3β-ol, <3> pH 7.4, 37°C, versus 5,6β-epoxy-5α-cholestan-3β-ol [8]) [8]
0.0044 <3> (5,6β-epoxy-5β-cholestan-3β-ol, <3> pH 7.4, 37°C, versus 5,6α-epoxy-5α-cholestan-3β-ol [8]) [8]
Additional information <1, 2, 3, 4, 5> (<1,2,3,4,5> kinetics [2,8]) [2, 8]

K_i-Value (mM)

0.000085 <3> (5,6α-imino-5α-cholestan-3β-ol, <3> pH 7.4, 37°C, versus 5,6α-epoxy-5α-cholestan-3β-ol [2]) [2]
0.0037 <3> (5,6β-epoxy-5β-cholestan-3β-ol, <3> pH 7.4, 37°C, versus 5,6α-epoxy-5α-cholestan-3β-ol [8]) [8]
0.0044 <3> (5,6α-epoxy-5α-cholestan-3β-ol, <3> pH 7.4, 37°C, versus 5,6β-epoxy-5α-cholestan-3β-ol [8]) [8]
0.0366 <3> (iminosqualene, <3> pH 7.4, 37°C, versus 5,6α-epoxy-5α-cholestan-3β-ol [2]) [2]
0.126 <3> (9,10-iminophenanthrene, <3> pH 7.4, 37°C, versus 5,6α-epoxy-5α-cholestan-3β-ol [2]) [2]
Additional information <3> (<3> inhibition kinetics at pH 8.0, 37°C, 6.5% v/v acetonitrile [7]) [7]

pH-Optimum

7.4 <1, 2, 3, 4, 5> (<1,2,3,4,5> assay at [2,3,4,8]) [1, 2, 3, 4, 7, 8]

pH-Range

6.8-9 <3> (<3> 80-90% of maximal activity at pH 6.8 and pH 7.7, 33% at pH 9.0 [1]) [1]

Temperature optimum (°C)

37 <1, 2, 3, 4, 5> (<1,2,3,4,5> assay at [1,2,3,4,7,8]) [1, 2, 3, 4, 7, 8]

5 Isolation/Preparation/Mutation/Application

Source/tissue

brain <3> (<3> low activity [5]) [5]
intestine <3> (<3> epithelium, low activity [5]) [5]
kidney <3> (<3> low activity [5]) [5]
liver <1, 2, 3, 4, 5> (<3> highest enzyme activity [5]; <3> normal tissue and hyperplastic nodules [4]) [1, 2, 3, 4, 5, 6, 7, 8]
testis <3> (<3> low activity [5]) [5]
Additional information <3> (<3> tissue distribution analysis, no or poor activity in lung and spleen [5]) [5]

Localization

Golgi apparatus <3> [5]
microsome <1, 2, 3, 4, 5> (<3> similar enzyme activity in smooth and rough microsomes [5]) [1, 2, 3, 4, 5, 6, 7]

Additional information <3> (<3> subcellular distribution analysis, no or low activity in cytosol, plasma membrane, Golgi apparatus II, and nucleus, overview [5]) [5]

Purification

<1> (liver microsome preparation) [2]
<1> (partially from liver by micosome preparation, two steps of gel filtration, separation from the xenobiotic epoxide hydrolase, EC 3.3.2.9) [3]
<2> (liver microsome preparation) [2]
<3> (from microsomes, separation from EC 3.3.2.9 by immunoprecipitation) [6]
<3> (liver microsome preparation) [2]
<3> (partially by microsome preparation) [7, 8]
<3> (partially by subcellular fractionation) [5]
<3> (partially from liver by micosome preparation and immunoprecipitation, separation from the xenobiotic epoxide hydrolase, EC 3.3.2.9) [1]
<4> (from microsomes, separation from EC 3.3.2.9 by immunoprecipitation) [6]
<4> (liver microsome preparation) [2]
<5> (liver microsome preparation) [2]

6 Stability

Oxidation stability

<3>, acetone reduces the activity by 85% at 1.25% v/v, by 50% at 3.75% v/v, and by 56% at 6.25% v/v [1]
<3>, acetonitrile activates the enzyme at moderate concentration, maximal enzyme activity at 6.25% v/v, 19% of maximal activity at 1.25% v/v, 50% of maximal activity at 12.5% v/v, no activity at 18.8% v/v [1]
<3>, dimethylsulfoxide reduces the activity by 42% at 6.25% v/v [1]
<3>, methanol reduces the activity by 86% at 3.75% v/v [1]
<3>, tetrahydrofuran reduces the activity by 86% at 6.25% v/v [1]

Storage stability

<3>, -90°C, liver microsomes, enzyme is stable during repeated freezing and thawing [1]

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PepB Aminopeptidase 3.4.11.23

1 Nomenclature

EC number
3.4.11.23

Recommended name
PepB aminopeptidase

Synonyms
PepB <1> [1]
Salmonella enterica serovar Typhimurium peptidase B
aminopeptidase, tripeptide

CAS registry number
9056-26-2
928346-44-5

2 Source Organism

<1> *Salmonella enterica serovar Typhimurium* (no sequence specified) [1]

3 Reaction and Specificity

Catalyzed reaction
release of an N-terminal amino acid, Xaa, from a peptide or arylamide. Xaa is preferably Glu or Asp but may be other amino acids, including Leu, Met, His, Cys and Gln (<1> residue K310 is involved in substrate specificity determination [1])

Natural substrates and products
S Ala-Leu + H_2O <1> (Reversibility: ?) [1]
P Ala + Leu <1> [1]
S Asn-Leu + H_2O <1> (Reversibility: ?) [1]
P Asn + Leu <1> [1]
S Asp-Leu + H_2O <1> (<1> best substrate [1]) (Reversibility: ?) [1]
P Asp + Leu <1> [1]
S Cys-Leu + H_2O <1> (Reversibility: ?) [1]
P Cys + Leu <1> [1]
S Glu-Leu + H_2O <1> (Reversibility: ?) [1]
P Glu + Leu <1> [1]

S His-Leu + H_2O <1> (Reversibility: ?) [1]
P His + Leu <1> [1]
S Leu-Cys + H_2O <1> (Reversibility: ?) [1]
P Leu + Cys <1> [1]
S Leu-His + H_2O <1> (Reversibility: ?) [1]
P Leu + His <1> [1]
S Leu-Ile + H_2O <1> (Reversibility: ?) [1]
P Leu + Ile <1> [1]
S Leu-Leu + H_2O <1> (Reversibility: ?) [1]
P Leu + Leu <1> [1]
S Leu-Met + H_2O <1> (Reversibility: ?) [1]
P Leu + Met <1> [1]
S Leu-Phe + H_2O <1> (Reversibility: ?) [1]
P Leu + Phe <1> [1]
S Leu-Tyr + H_2O <1> (Reversibility: ?) [1]
P Leu + Tyr <1> [1]
S Leu-Xaa + H_2O <1> (<1> Xaa is an amino acid, substrate specificity, overview [1]; <1> lower activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Leu + Xaa <1> [1]
S Met-Leu + H_2O <1> (Reversibility: ?) [1]
P Met + Leu <1> [1]
S Ser-Leu + H_2O <1> (Reversibility: ?) [1]
P Ser + Leu <1> [1]
S Thr-Leu + H_2O <1> (Reversibility: ?) [1]
P Thr + Leu <1> [1]
S Xaa-peptide + H_2O <1> (<1> substrate specificity, overview [1]; <1> Xaa is an amino acid [1]) (Reversibility: ?) [1]
P Xaa + peptide <1> [1]

Substrates and products

S Ala-Leu + H_2O <1> (<1> 3% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Ala + Leu <1> [1]
S Asn-Leu + H_2O <1> (<1> 23% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Asn + Leu <1> [1]
S Asp-Ile + H_2O <1> (<1> best substrate, 120% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Asp + Ile <1> [1]
S Asp-Leu + H_2O <1> (<1> best substrate [1]) (Reversibility: ?) [1]
P Asp + Leu <1> [1]
S Asp-Leu-Gly + H_2O <1> (<1> higher activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Asp + Leu-Gly <1> [1]
S Asp-Phe + H_2O <1> (<1> 105% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]

P Asp + Phe <1> [1]
S Asp-Xaa + H_2O <1> (<1> Xaa is an amino acid, substrate specificity, overview [1]) (Reversibility: ?) [1]
P Asp + Xaa <1> [1]
S Cys-Leu + H_2O <1> (<1> 23% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Cys + Leu <1> [1]
S Gln-Leu + H_2O <1> (<1> 9% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Gln + Leu <1> [1]
S Glu-Leu + H_2O <1> (<1> 74% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Glu + Leu <1> [1]
S His-Leu + H_2O <1> (<1> 28% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P His + Leu <1> [1]
S Ile-Leu + H_2O <1> (<1> 2% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Ile + Leu <1> [1]
S Leu-Cys + H_2O <1> (<1> 24% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Leu + Cys <1> [1]
S Leu-His + H_2O <1> (<1> 9% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Leu + His <1> [1]
S Leu-Ile + H_2O <1> (<1> 59% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Leu + Ile <1> [1]
S Leu-Leu + H_2O <1> (<1> 40% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Leu + Leu <1> [1]
S Leu-Met + H_2O <1> (<1> 30% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Leu + Met <1> [1]
S Leu-Phe + H_2O <1> (<1> 12% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Leu + Phe <1> [1]
S Leu-Tyr + H_2O <1> (<1> 37% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Leu + Tyr <1> [1]
S Leu-Xaa + H_2O <1> (<1> Xaa is an amino acid, substrate specificity, overview [1]; <1> lower activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Leu + Xaa <1> [1]
S Met-Leu + H_2O <1> (<1> 30% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Met + Leu <1> [1]

S Phe-Leu + H_2O <1> (<1> 7% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Phe + Leu <1> [1]
S Ser-Leu + H_2O <1> (<1> 2% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Ser + Leu <1> [1]
S Thr-Leu + H_2O <1> (<1> 3% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Thr + Leu <1> [1]
S Trp-Leu + H_2O <1> (<1> 4% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Trp + Leu <1> [1]
S Tyr-Leu + H_2O <1> (<1> 6% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Tyr + Leu <1> [1]
S Val-Leu + H_2O <1> (<1> 2% activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Val + Leu <1> [1]
S Xaa-Gly-Gly + H_2O <1> (<1> Xaa is an amino acid, substrate specificity, overview [1]; <1> low activity compared to substrate Asp-Leu [1]) (Reversibility: ?) [1]
P Xaa + Gly-Gly <1> [1]
S Xaa-Leu + H_2O <1> (<1> Xaa is an amino acid, substrate specificity, overview [1]) (Reversibility: ?) [1]
P Xaa + Leu <1> [1]
S Xaa-peptide + H_2O <1> (<1> substrate specificity, overview [1]; <1> Xaa is an amino acid [1]) (Reversibility: ?) [1]
P Xaa + peptide <1> [1]

Inhibitors

bestatin <1> (<1> complete inhibition at 1 mM, 37°C, after 20 min [1]) [1]
EDTA <1> (<1> reversible by divalent cations [1]) [1]

Activating compounds

divalent cations <1> [1]
KCl <1> (<1> stimulation, optimal at 50 mM [1]) [1]

Turnover number (min^{-1})

866 <1> (Glu-Leu, <1> 37°C, pH 8.5 [1]) [1]
918 <1> (Leu-Leu, <1> 37°C, pH 8.5 [1]) [1]
950 <1> (Asp-Leu-Gly, <1> 37°C, pH 8.5 [1]) [1]
Additional information <1> (<1> substrate specificity, overview [1]) [1]

Specific activity (U/mg)

190 <1> (<1> purified recombinant enzyme [1]) [1]
Additional information <1> (<1> substrate specificity, overview [1]) [1]

K_m-Value (mM)

0.5 <1> (Asp-Leu-Gly, <1> 37°C, pH 8.5 [1]) [1]
0.9 <1> (Glu-Leu, <1> 37°C, pH 8.5 [1]) [1]

4 <1> (Leu-Leu, <1> 37°C, pH 8.5 [1]) [1]
Additional information <1> (<1> K_m-values for diverse substrates, kinetics [1]) [1]

pH-Optimum
8.5-9.5 <1> [1]

4 Enzyme Structure

Molecular weight
270000 <1> (<1> gel filtration [1]) [1]

Subunits
hexamer <1> (<1> 6 * 45000, SDS-PAGE [1]; <1> 6 * 46360-46362, mass spectrometry and amino acid sequence determination [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> (recombinant from strain TN5179) [1]

Cloning
<1> (DNA sequence determination, overexpression from plasmid, Salmonella typhimurium strain TN5179) [1]

Engineering
K310V <1> (<1> site-directed mutagenesis, more than 200fold reduction in the rate of Asp-Leu hydrolysis with no decrease in the rate of Leu-Leu hydrolysis [1]) [1]
R402W <1> (<1> site-directed mutagenesis, reduction in the rate of Asp-Leu hydrolysis with no decrease in the rate of Leu-Leu hydrolysis [1]) [1]

6 Stability

Temperature stability
70 <1> (<1> 20 min, stable [1]) [1]

References

[1] Mathew, Z.; Knox, T.M.; Miller, C.G.: Salmonella enterica serovar Typhimurium peptidase B is a leucyl aminopeptidase with specificity for acidic amino acids. J. Bacteriol., **182**, 3383-3393 (2000)

D-Ala-D-Ala dipeptidase 3.4.13.22

1 Nomenclature

EC number
3.4.13.22

Recommended name
D-Ala-D-Ala dipeptidase

Synonyms
D-, D-dipeptidase <3> [2]
D-Ala-D-Ala amino dipeptidase <5> [4]
D-Ala-D-Ala dipeptidase VanX <5> [3]
D-Ala-D-Ala-dipeptidase <3> [1, 2]
D-alanyl-D-alanine dipeptidase <1, 2> [5, 6]
M15.011 <1, 2, 3, 5> (<1,2,3,5> Merops-ID [1,2,3,4,5,6]) [1, 2, 3, 4, 5, 6]
VanX <1, 2, 3, 5> [1, 2, 3, 4, 5, 6]
VanXYc <6> [9]
vancomycin B-type resistance protein vanX <3> [2]

CAS registry number
213189-85-6

2 Source Organism

<1> *Enterococcus faecium* (no sequence specified) [1, 5, 7, 8, 10]
<2> *Streptomyces coelicolor A3(2)* (no sequence specified) [6]
<3> *Enterococcus faecium BM4147* (UNIPROT accession number: Q06241) [2]
<4> *Enterococcus faecium BM4147* (no sequence specified) [1]
<5> *Enterococcus sp.* (no sequence specified) [3,4,11]
<6> *Enterococcus gallinarium* (no sequence specified) [9]
<7> *Enterococcus faecium* (UNIPROT accession number: Q06241) [2]

3 Reaction and Specificity

Catalyzed reaction
D-Ala-D-Ala + H_2O = 2 D-Ala (<5> reaction mechanism [3]; <1> active site structure, substrate binding and mechanism [5])

Reaction type
hydrolysis of peptide bond

Natural substrates and products

- **S** D-Ala-D-Ala + H_2O <1, 2, 3, 5> (<3> enzyme is essential for vancomycin resistance in Enterococcus faecium [1,2]; <5> enzyme is required for vancomycin resistance in enterococci [4]) (Reversibility: ?) [1, 2, 3, 4, 5, 6]
- **P** D-Ala + D-Ala <1, 2, 3, 5> [1, 2, 3, 4, 5, 6]
- **S** Additional information <3> (<3> substrate specificty, no activity with D-Ala-D-lactate [2]) (Reversibility: ?) [2]
- **P** ? <3> [2]

Substrates and products

- **S** (2S)-N-D-alanyl-2-(phenylthio)-glycine + H_2O <5> (Reversibility: ?) [11]
- **P** D-alanine + 2-(phenylthio)-glycine
- **S** D-Ala-D-Ala + H_2O <5> (Reversibility: ?) [11]
- **P** D-Ala
- **S** D-Ala-D-Ala + H_2O <1, 2, 3, 5, 6> (<2> enzyme is required for vancomycin resistance [6]; <3> enzyme is essential for vancomycin resistance in Enterococcus faecium [1,2]; <5> enzyme is required for vancomycin resistance in enterococci [4]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10]
- **P** D-Ala + D-Ala <1, 2, 3, 5> [1, 2, 3, 4, 5, 6]
- **S** D-Ala-D-Gly + H_2O <3> (Reversibility: ?) [2]
- **P** D-Ala + D-Gly <3> [2]
- **S** D-Ala-D-Phe + H_2O <3> (Reversibility: ?) [2]
- **P** D-Ala + D-Phe <3> [2]
- **S** D-Ala-D-Ser + H_2O <6> (Reversibility: ?) [9]
- **P** D-Ala + D-Ser
- **S** D-Leu-4-nitroanilide + H_2O <1> (<1> specific but catalytically inefficient substrate [8]) (Reversibility: ?) [8]
- **P** D-Leu + 4-nitroaniline
- **S** D-Ser-D-Ala + H_2O <3> (Reversibility: ?) [2]
- **P** D-Ser + D-Ala <3> [2]
- **S** D/L-Ala-4-nitroanilide + H_2O <1> (<1> slight hydrolysis for both isomers [8]) (Reversibility: ?) [8]
- **P** ?
- **S** DL-Ala-DL-Asn + H_2O <3> (Reversibility: ?) [2]
- **P** DL-Ala + DL-Asn <3> [2]
- **S** DL-Ala-DL-Ser + H_2O <3> (Reversibility: ?) [2]
- **P** DL-Ala + DL-Ser <3> [2]
- **S** DL-Ala-DL-Val + H_2O <3> (Reversibility: ?) [2]
- **P** DL-Ala + DL-Val <3> [2]
- **S** Additional information <1, 3> (<3> substrate specificty, no activity with D-Ala-D-lactate [2]; <1> no substrate: L-Ala-4-nitroanilide [7]; <1> no substrate: L-Leu-4-nitroanilide [8]) (Reversibility: ?) [2, 7, 8]
- **P** ? <3> [2]

Inhibitors

Cu^{2+} <1, 3> (<3> 96% inhibition at 1 mM [1]) [1, 5]

D-3-[(1-aminoethyl)phosphinyl]-D-2-methylpropionic acid <1, 3> (<1> phosphinate analogue of the proposed tetrahedral intermediate of the hydro-

lysis reaction [5]; <3> phosphinate analogue of the proposed tetrahedral intermediate of the hydrolysis reaction, slow binding [1]) [1, 5]
D-3-[(1-aminoethyl)phosphonyl]-D-2-methylpropionic acid <1> (<1> phosphonate analogue of the proposed tetrahedral intermediate of the hydrolysis reaction [5]) [5]
D-Ala-D-(2-difluorothio)glycine-OH <5> (<5> dipeptide-like mechanism-based inhibitor, cleavage of the substance results in formation of a highly reactive 4-thioquinone fluoromethide which covalently reacts with the enzyme resulting in irreversible inhibition, inhibition mechanism [4]) [4]
D-Ala-D-Ala <5> (<5> competitive substrate [11]) [11]
D-Ala-D-lactate <3> (<3> very poor hydrolysis, blocking of the enzyme [2]) [2]
D-Ala-L-Ala <3> [1]
D-alanyl-2-[[4-(difluoromethyl)phenyl]thio]-glycine <5> (<5> K_{irr}-value 22 microM, kinact-value 9.3/min [11]) [11]
L-Ala-D-Ala <3> [1]
L-Ala-L-Ala <3> [1]
Ni^{2+} <6> (<6> 3.3 mM, no residual activity [9]) [9]
Zn^{2+} <3, 6> (<3> 72% inhibition at 1 mM [1]; <6> 1.3 mM, 50% inhibition [9]) [1, 9]

Metals, ions

Co^{2+} <1, 3> (<3> 1.2fold activation at 1 mM [1]; <3> activates, optimal at 2 mM [2]; <1> Co^{2+} is five-coordinate and has at least one solvent-derived ligand. About 0.7 equivalents per molecule [10]) [1, 2, 10]
Fe^{2+} <3> (<3> highly stimulating, optimal at 3 mM [2]) [2]
Ni^{2+} <3> (<3> 1.3fold activation at 1 mM [1]; <3> highly stimulating, optimal at 1 mM [2]) [1, 2]
Zn^{2+} <1, 3, 5> (<1> dependent on [5]; <3> activates, optimal at 0.5 mM [2]; <5> zinc binding residues H184, Asp123 and H116 in the active site, zinc content varies in mutant enzymes [3]; <1> 0.75 equivalents per protein molecule [7]; <1> may substitute for Co^{2+}. About 0.7 equivalents per molecule [10]) [2, 3, 5, 7, 10]
Additional information <1, 3> (<3> effect of diverse metal ions on enzyme activity, overview [2]; <1> no other metal ions than Co^{2+} or Zn^{2+} found [10]) [2, 10]

Turnover number (min^{-1})

0.0102 <1> (D-Leu-4-nitroanilide, <1> pH 7.2, 37°C [8]) [8]
0.35 <6> (D-Ala-D-Ser, <6> wild-type, pH 7.5, 37°C [9]) [9]
0.35 <3, 7> (D-Ser-D-Ala, <3,7> pH 7.0-9.0, 37°C [2]) [2]
0.64 <5> (D-Ala-D-Ala, <5> pH 8.0, 37°C, recombinant mutant C78S/C157S [3]) [3]
0.8 <6> (D-Ala-D-Ala, <6> mutant D59A, pH 7.5, 37°C [9]) [9]
1.4 <6> (D-Ala-D-Ala, <6> mutant D59S, pH 7.5, 37°C [9]) [9]
1.8 <3, 7> (D-Ala-D-Ser, <3,7> pH 7.0-9.0, 37°C [2]) [2]
2.5 <6> (D-Ala-D-Ala, <6> wild-type, pH 7.5, 37°C [9]) [9]
4.7 <3, 7> (D-Ala-D-Ala, <3,7> pH 7.0-9.0, 37°C [2]) [2]

9.1 <5> (D-Ala-D-Ala, <5> pH 8.0, 37°C, recombinant mutant H149A/H150A [3]) [3]
20.2 <5> (D-Ala-D-Ala, <5> pH 8.0, 37°C, recombinant mutant D145A/E146A [3]) [3]
30 <3, 7> (Zn^{2+}, <3,7> pH 7.0-9.0, 37°C [2]) [2]
41 <5> (D-Ala-D-Ala, <5> pH 7.0, 37°C [4]) [4]
44.3 <5> (D-Ala-D-Ala) [11]
54.1 <5> (D-Ala-D-Ala, <5> pH 8.0, 37°C, recombinant mutant C78S/C157S [3]) [3]
60 <1, 3> (D-Ala-D-Ala, <1,3> pH 8.0, 37°C [1]) [1]
69 <3, 7> (Co^{2+}, <3,7> pH 7.0-9.0, 37°C [2]) [2]
76 <5> ((2S)-N-D-alanyl-2-(phenylthio)-glycine) [11]
83 <5> (D-Ala-D-Ala, <5> pH 8.0, 37°C, recombinant mutant H13A [3]) [3]
94 <1> (D-Ala-D-Ala, <1> Co^{2+}-form, pH 8.0, 37°C [10]) [10]
110 <1> (D-Ala-D-Ala, <1> maltose-binding protein fusion enzyme, Co^{2+}-form, pH 8.0, 37°C [10]) [10]
125 <5> (D-Ala-D-Ala, <5> pH 8.0, 37°C, recombinant wild-type enzyme [3]) [3]
156 <1> (D-Ala-D-Ala, <1> pH 8.0, 37°C [7]; <1> Zn^{2+}-form, pH 8.0, 37°C [10]) [7, 10]
156 <3, 7> (Fe^{2+}, <3,7> pH 7.0-9.0, 37°C [2]) [2]
788 <3, 7> (Ni^{2+}, <3,7> pH 7.0-9.0, 37°C [2]) [2]

Specific activity (U/mg)

10.6 <2> (<2> recombinant enzyme [6]) [6]
Additional information <3, 5> (<5> direct continuous UV-Vis spectrometric assay based on substrate D-Ala-α-(R)-phenylthio-glycine [11]) [2, 11]

K_m-Value (mM)

0.1 <1> (D-Ala-D-Ala, <1> maltose-binding protein fusion enzyme, Co^{2+}-form, pH 8.0, 37°C [10]) [10]
0.109 <1> (D-Ala-D-Ala, <1> pH 8.0, 37°C [7]; <1> Zn^{2+}-form, pH 8.0, 37°C [10]) [7, 10]
0.11 <5> (D-Ala-D-Ala, <5> pH 7.0, 37°C [4]) [4, 11]
0.14 <1> (D-Ala-D-Ala, <1> Co^{2+}-form, pH 8.0, 37°C [10]) [10]
0.83 <5> ((2S)-N-D-alanyl-2-(phenylthio)-glycine) [11]
1 <3> (D-Ala-D-Ala, <3> pH 7.0-9.0, 37°C [2]) [2]
1.2 <3> (Co^{2+}, <3> pH 7.0-9.0, 37°C [2]) [2]
1.37 <3> (D-Ala-D-Ala, <3> pH 8.0, 37°C [1]) [1]
1.7 <3> (D-Ser-D-Ala, <3> pH 7.0-9.0, 37°C [2]) [2]
1.8 <5> (D-Ala-D-Ala, <5> pH 8.0, 37°C, recombinant mutant C78S/C157S [3]) [3]
2.1 <5> (D-Ala-D-Ala, <5> pH 8.0, 37°C, recombinant mutant H13A [3]) [3]
2.4 <5> (D-Ala-D-Ala, <5> pH 8.0, 37°C, recombinant mutant H149A/H150A [3]) [3]
2.5 <5, 6> (D-Ala-D-Ala, <5> pH 8.0, 37°C, recombinant wild-type enzyme [3]; <6> mutant D59S, pH 7.5, 37°C [9]) [3, 9]
2.8 <3> (D-Ala-D-Ser, <3> pH 7.0-9.0, 37°C [2]) [2]

3.6 <6> (D-Ala-D-Ala, <6> wild-type, pH 7.5, 37°C [9]) [9]
3.7 <5> (D-Ala-D-Ala, <5> pH 8.0, 37°C, recombinant mutant D145A/E146A [3]) [3]
8.9 <1> (D-Leu-4-nitroanilide, <1> pH 7.2, 37°C [8]) [8]
9 <6> (D-Ala-D-Ala, <6> mutant D59A, pH 7.5, 37°C [9]) [9]
15.5 <6> (D-Ala-D-Ser, <6> wild-type, pH 7.5, 37°C [9]) [9]
Additional information <3> (<3> kinetics [1]) [1]

K_i-Value (mM)

0.03 <5> (D-Ala-D-(2-difluorothio)glycine-OH, <5> pH 7.0, 37°C [4]) [4]
0.07 <5> (D-Ala-D-Ala) [11]
58 <3> (D-Ala-L-Ala, <3> pH 8.0, 37°C [1]) [1]
98 <3> (L-Ala-L-Ala, <3> pH 8.0, 37°C [1]) [1]
225 <3> (L-Ala-D-Ala, <3> pH 8.0, 37°C [1]) [1]
242 <3> (D-Ala-D-lactate, <3> pH 7.0-9.0, 37°C [2]) [2]
Additional information <3> (<3> inhibition kinetics, K_i is 0.0015 mM immediately after addition of the enzyme, but is then lowered to by a relatively slow isomerization step to a second complex to K_i = 0.00047 mM [1]) [1]

pH-Optimum

7 <5> (<5> assay at [4]) [4]
8 <3, 5> (<3,5> assay at [1,3]) [1, 3]

pH-Range

7-9 <3> [2]

Temperature optimum (°C)

37 <3, 5> (<3,5> assay at [1,3,4]) [1, 3, 4]

4 Enzyme Structure

Subunits

? <1, 2, 3> (<1> x * 23200, SDS-PAGE [7]; <2> x * 22700, recombinant enzyme, SDS-PAGE [6]; <3> x * 24200, recombinant ezyme, SDS-PAGE [2]) [2, 6, 7]
Additional information <1> (<1> monomer secondary structure [5]) [5]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [5]
<3> (recombinant from Escherichia coli, to homogeneity) [1]
<3> (soluble recombinant enzyme from Escherichia coli, to homogeneity) [2]

Crystallization

<1> (enzyme-D-Ala complex, enzyme-D-Ala-D-Ala complex, and enzyme in complex with phosphonate and phosphinate transition-state analogue inhibitors, complexsitting drops, 10 mg/ml protein, plus equal volume of precipi-

tant solution: 0.25 M ammonium sulfate, 25% w/v polyethylene glycol monomethyl ester 5000, 0.1 M MES, 1 mM $ZnCl_2$, X-ray diffraction at 2.1 A resolution structure determination and analysis) [5]

Cloning

<2> (gene vanX, genomic organization, functional expression of the soluble enzyme in Escherichia coli BL21(DE3)) [6]
<3> (gene vanX, overexpression of the soluble enzyme in Escherichia coli) [2]
<3> (overexpression in Escherichia coli) [1]
<5> (overexpression of wild-type and mutants as maltose-binding-protein fusion proteins) [3]

Engineering

C78S/C157S <5> (<5> site-directed mutagenesis, reduced activity [3]) [3]
D123A <5> (<5> site-directed mutagenesis, inactive, nearly no remaining Zn^{2+} [3]) [3]
D145A/E146A <5> (<5> site-directed mutagenesis, 6fold lowered k_{cat} compared to wild-type, 11% reduced zinc content compared to wild-type [3]) [3]
D59A <6> (<6> 73% decrease in ratio k_{cat}/K_M for D-Ala-D-Ala substrate [9]) [9]
D59S <6> (<6> 50% increase in ratio k_{cat}/K_M for D-Ala-D-Ala substrate [9]) [9]
E181A <5> (<5> site-directed mutagenesis, inactive, 21.8% reduced zinc content compared to wild-type [3]) [3]
H116A <5> (<5> site-directed mutagenesis, inactive, 92.1% reduced zinc content compared to wild-type [3]) [3]
H116A/H184A <5> (<5> site-directed mutagenesis, inactive, 97.5% reduced zinc content compared to wild-type [3]) [3]
H13A <5> (<5> site-directed mutagenesis, activity equivalent to wild-type, 8.3% reduced zinc content compared to wild-type [3]) [3]
H149A <5> (<5> site-directed mutagenesis, equivalent to wild-type [3]) [3]
H149A/H150A <5> (<5> site-directed mutagenesis, 11fold lowered k_{cat}, 14.9% reduced zinc content compared to wild-type [3]) [3]
H150A <5> (<5> site-directed mutagenesis, equivalent to wild-type [3]) [3]
H184A <5> (<5> site-directed mutagenesis, inactive, 99.08% reduced zinc content compared to wild-type [3]) [3]

Application

medicine <1, 3> (<3> enzyme may be a target for drug design to reverse clinical vancomycin resistance [2]; <1> key drug target in circumventing clinical vancomycin resistance [5]) [2, 5]

References

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Xaa-Xaa-Pro Tripeptidyl-peptidase 3.4.14.12

1 Nomenclature

EC number
3.4.14.12

Recommended name
Xaa-Xaa-Pro tripeptidyl-peptidase

Synonyms
prolyl tripeptidyl peptidase
prolyltripeptidyl aminopeptidase

CAS registry number
227604-68-4

2 Source Organism

<1> *Porphyromonas gingivalis* (no sequence specified) [2, 3]
<2> *Prevotella nigrescens* (no sequence specified) [1]
<3> *Streptomyces mobaraensis* (no sequence specified) [4]

3 Reaction and Specificity

Catalyzed reaction
hydrolysis of Xaa-Xaa-Pro-Yaa- releasing the N-terminal tripeptide of a peptide with Pro as the third residue (position P1) and where Yaa is not proline

Reaction type
hydrolysis of peptide bond

Natural substrates and products
S Additional information <1> (<1> the production of prolyl tripeptidyl peptidase may contribute to the pathogenesis of periodontal tissue destruction though the mutual interaction of this enzyme, host and bacterial collagenases, and dipeptidyl peptidases in the degradation of collagen during the course of infection [2]) (Reversibility: ?) [2]
P ?

Substrates and products
S Ala-Ala-Pro-*p*-nitroanilide + H_2O <2> (Reversibility: ?) [1]
P Ala-Ala-Pro + *p*-nitroaniline <2> [1]

S Ala-Arg-Pro-Ala-D-Lys-amide + H_2O <1> (Reversibility: ?) [2]
P Ala-Arg-Pro + Ala-D-Lys-amide
S Ala-Phe-Pro-*p*-nitroanilide + H_2O <1, 2> (<2> 66% of the activity with Ala-Ala-Pro-*p*-nitroanilide [1]) (Reversibility: ?) [1, 2]
P Ala-Phe-Pro + *p*-nitroaniline <1, 2> [1, 2]
S Ala-Pro-*p*-nitroanilide + H_2O <2> (<2> 13% of the activity with Ala-Ala-Pro-*p*-nitroanilide [1]) (Reversibility: ?) [1]
P Ala-Pro + *p*-nitroaniline
S Arg-Gly-Pro-Phe-Pro-Ile + H_2O <1> (Reversibility: ?) [2]
P Arg-Gly-Pro + Phe-Pro-Ile
S Arg-His-Pro-Lys-Tyr-Lys-Thr-Glu-Leu + H_2O <1> (Reversibility: ?) [2]
P Arg-His-Pro + Lys-Tyr-Lys-Thr-Glu-Leu
S Arg-Pro-Pro-Gly-Phe + H_2O <1> (Reversibility: ?) [2]
P Arg-Pro-Pro + Gly-Phe
S Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg + H_2O <1> (Reversibility: ?) [2]
P Arg-Pro-Pro + Gly-Phe-Ser-Pro-Phe-Arg
S Arg-Pro-*p*-nitroanilide + H_2O <2> (<2> 22% of the activity with Ala-Ala-Pro-*p*-nitroanilide [1]) (Reversibility: ?) [1]
P Arg-Pro + *p*-nitroaniline
S Gly-L-Ala-Gly-L-Pro-β-naphthylamide + H_2O <3> (Reversibility: ?) [4]
P Gly-L-Ala-Gly-L-Pro + β-naphthylamine
S Gly-L-Ala-L-Pro-4-nitroanilide + H_2O <3> (<3> 32% of the activity with L-Ala-L-Ala-L-Pro-4-nitroanilide [4]) (Reversibility: ?) [4]
P Gly-L-Ala-L-Pro + 4-nitroaniline
S Gly-L-Ala-L-Pro-β-naphthylamide + H_2O <3> (Reversibility: ?) [4]
P Gly-L-Ala-L-Pro + β-naphthylamine
S Gly-Pro-*p*-nitroanilide + H_2O <1, 2> (<1> no activity [2]; <2> 3% of the activity with Ala-Ala-Pro-*p*-nitroanilide [1]) (Reversibility: ?) [1, 2]
P Gly-Pro + *p*-nitroaniline
S Gly-Val-Pro-Lys-Thr-His-Leu-Glu-Leu + H_2O <1> (Reversibility: ?) [2]
P Gly-Val-Pro + Lys-Thr-His-Leu-Glu-Leu
S human cystatin C + H_2O <1> (<1> cleavage of a tripeptide, NH_2-Xaa-Xaa-Pro, from the amino terminus [2]) (Reversibility: ?) [2]
P ?
S L-Ala-L-Ala-L-Pro-4-nitroanilide + H_2O <3> (Reversibility: ?) [4]
P L-Ala-L-Ala-L-Pro + 4-nitroaniline
S L-Ala-L-Phe-L-Pro-4-nitroanilide + H_2O <3> (<3> 24% of the activity with L-Ala-L-Ala-L-Pro-4-nitroanilide [4]) (Reversibility: ?) [4]
P L-Ala-L-Phe-L-Pro + 4-nitroaniline
S L-Lys-L-Pro-L-Pro-4-nitroanilide + H_2O <3> (<3> 26% of the activity with L-Ala-L-Ala-L-Pro-4-nitroanilide [4]) (Reversibility: ?) [4]
P L-Pro-L-Pro-L-Pro + 4-nitroaniline
S Lys-Pro-*p*-nitroanilide + H_2O <2> (<2> 24% of the activity with Ala-Ala-Pro-*p*-nitroanilide [1]) (Reversibility: ?) [1]
P Lys-Pro + *p*-nitroaniline
S Phe-Pro-*p*-nitroanilide + H_2O <2> (<2> 13% of the activity with Ala-Ala-Pro-*p*-nitroanilide [1]) (Reversibility: ?) [1]

P Phe-Pro + *p*-nitroaniline
S Pro-Asn-Pro-Asn-Gln-Gly-Asn-Phe-Ile + H_2O <1> (Reversibility: ?) [2]
P Pro-Asn-Pro + Asn-Gln-Gly-Asn-Phe-Ile
S Ser-Pro-*p*-nitroanilide + H_2O <2> (<2> 13% of the activity with Ala-Ala-Pro-*p*-nitroanilide [1]) (Reversibility: ?) [1]
P Ser-Pro + *p*-nitroaniline
S Val-Glu-Pro-Ile-Pro-Tyr + H_2O <1> (Reversibility: ?) [2]
P Val-Glu-Pro + Ile-Pro-Tyr
S Val-Pro-Pro-Gly-Glu-Asp-Ser-Lys + H_2O <1> (Reversibility: ?) [2]
P Val-Pro-Pro + Gly-Glu-Asp-Ser-Lys
S Val-Pro-Pro-Gly-Glu-Asp-Ser-Lys-Glu-Val-Ala-Ala-Pro-His-Arg-Gln + H_2O <1> (Reversibility: ?) [2]
P Val-Pro-Pro + Gly-Glu-Asp-Ser-Lys-Glu-Val-Ala-Ala-Pro-His-Arg-Gln
S interleukin 6 + H_2O <1> (<1> cleavage of a tripeptide, NH_2-Xaa-Xaa-Pro, from the amino terminus [2]) (Reversibility: ?) [2]
P ?
S Additional information <1, 2, 3> (<1> the enzyme possesses the absolute requirement for the proline residue in the P1 position. A free α-amino group is absolutely required for cleavage after the third proline residue. No cleavage of: benzyloxycarbonyl-Gly-Pro-*p*-nitroanilide, benzyloxycarbonyl-Ala-Pro-*p*-nitroanilide, Pro-*p*-nitroanilide, Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, Tyr-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, Arg-Pro-Hyp-Gly-Phe-Ser-Pro-Phe-Arg, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-leu-Met-NH_2, acetyl-Val-Pro-Pro-Gly-Glu-Asp-Ser-Lys [2]; <2> no activity with Ala-Phe-*p*-nitroanilide, Ala-Ala-*p*-nitroanilide, Ala-*p*-nitroanilide, Pro-*p*-nitroanilide, benzoyl-Arg-*p*-nitroanilide, succinyl-Ala-Ala-Ala-*p*-nitroanilide, succinyl-Ala-Ala-Pro-*p*-nitroanilide, tosyl-Gly-Pro-Lys-4-nitroanilide [1]; <1> the production of prolyl tripeptidyl peptidase may contribute to the pathogenesis of periodontal tissue destruction though the mutual interaction of this enzyme, host and bacterial collagenases, and dipeptidyl peptidases in the degradation of collagen during the course of infection [2]; <3> enzyme exhibits tripeptidyl peptidase and tetrapeptidyl peptidase activity, but no other exo- or endo-activites [4]) (Reversibility: ?) [1, 2, 4]
P ?

Inhibitors

1,10-phenanthroline <3> (<3> 5 mM, 67% residual activity [4]) [4]
3,4-dichloroisocoumarin <1, 2> (<1> 1 mM [2]; <1> 44% inhibition [2]; <2> 5.0 mM, 36% inhibition [1]) [1, 2]
diisopropyl fluorophosphate <1, 2> (<1> 10 mM, complete inhibition [2]; <2> 5.0 mM, 69% inhibition [1]) [1, 2]
pefabloc SC <1, 2, 3> (<2> 1.0 mM, 33% inhibition [1]; <1> 80% inhibition at 1 mg/ml and complete inhibition at 10 mg/ml [2]; <3> 4 mM, 60% residual activity [4]) [1, 2, 4]
phenylmethylsulfonylfluoride <3> (<3> 1 mM, no residual activity [4]) [4]
tosyl-L-lysine chloromethyl ketone <2> (<2> 1.0 mM, 47% inhibition [1]) [1]

Additional information <2> (<2> no inhibition by [1]; <2> 1 mM NEM, 5 mM 1,10-phenanthroline, 1 mM EDTA, 0.2 mM E64, 0.4 mM antipain, 0.2 mM leupeptin, 1 mg/ml aprotinin, 0.2 mM bestatin or 1 mM mercaptoethanol [1]) [1]

Activating compounds

iodoacetamide <1> (<1> 5 mM, 2fold stimulation [2]) [2]

Turnover number (min^{-1})

43 <3> (Gly-L-Ala-Gly-L-Pro-β-naphthylamide, <3> pH 6.5, 30°C [4]) [4]
115 <3> (Gly-L-Ala-L-Pro-β-naphthylamide, <3> pH 6.5, 30°C [4]) [4]

Specific activity (U/mg)

Additional information <1> [2]

K_m-Value (mM)

0.069 <2> (Ala-Ala-Pro-*p*-nitroanilide, <2> pH 7.5, 37°C [1]) [1]
0.072 <3> (Gly-L-Ala-L-Pro-β-naphthylamide, <3> pH 6.5, 30°C [4]) [4]
0.084 <2> (Lys-Pro-*p*-nitroanilide, <2> pH 7.5, 37°C [1]) [1]
0.114 <2> (Arg-Pro-*p*-nitroanilide, <2> pH 7.5, 37°C [1]) [1]
0.129 <2> (Ala-Phe-Pro-*p*-nitroanilide, <2> pH 7.5, 37°C [1]) [1]
0.26 <3> (Gly-L-Ala-Gly-L-Pro-β-naphthylamide, <3> pH 6.5, 30°C [4]) [4]

pH-Optimum

6-6.5 <3> [4]
6-8 <1> [2]
7-7.5 <2> [1]

pH-Range

4.3-8.3 <3> [4]
6-8.5 <2> (<2> no significant activity is observed below pH 6.0 and above pH 8.5 [1]) [1]

Temperature optimum (°C)

25 <1> (<1> pH 7.6, 0.2 M HEPES, stable for at least 12 h [2]) [2]
37 <1> (<1> pH 7.6, 0.2 M HEPES, stable for at least 12 h [2]) [2]
45 <3> [4]
50 <3> (<3> significant decrease in activity above [4]) [4]

4 Enzyme Structure

Molecular weight

160000 <1> (<1> gel filtration [3]) [3]

Subunits

? <2, 3> (<3> x * 50000, SDS-PAGE [4]; <2> x * 56000, SDS-PAGE [1]) [1, 4]
dimer <1> (<1> 2 * 83000, SDS-PAGE, 2 * 82266, calculated [3]) [3]
Additional information <1> (<1> two protein bands which represent different forms of the enzyme are detected by SDS-PAGE: 75800 Da and 81800 Da [2]) [2]

Posttranslational modification
proteolytic modification <3> (<3> sequence contains an N-terminal signal peptide of 33 amino acids followed by mature protein of 444 amino acids [4]) [4]

5 Isolation/Preparation/Mutation/Application

Source/tissue
mycelium <3> [4]

Localization
cell surface <1> [2]
cell wall <3> (<3> connnected to wall through ionic interactions [4]) [4]

Purification
<1> [2]
<2> [1]
<3> [4]

Crystallization
<1> [3]

Cloning
<3> [4]

6 Stability

Temperature stability
30 <3> (<3> stable [4]) [4]
40 <3> (<3> significant loss of stability above [4]) [4]
60 <2> (<2> 5 min, complete loss of activity [1]) [1]

Storage stability
<2>, -40°C, stable for several weeks [1]
<2>, 4°C, stable for 24 h [1]

References

[1] Fujimura, S.; Ueda, O.; Shibata, Y.; Hirai, K.: Isolation and properties of a tripeptidyl peptidase from a periodontal pathogen Prevotella nigrescens. FEMS Microbiol. Lett., **219**, 305-309 (2003)
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Cyanophycinase 3.4.15.6

1 Nomenclature

EC number
3.4.15.6

Recommended name
cyanophycinase

Synonyms
CGPase <2> [2]
CphB <1> [1]
CphE <2> [2]
cyanophycin granule polypeptidase <2> [2]
cyanophycinase <1, 2> [1, 2]
extracellular CGPase <2> [2]

CAS registry number
131554-16-0

2 Source Organism

<1> *Synechocystis sp.* (UNIPROT accession number: P73832) [1]
<2> *Pseudomonas anguilliseptica* (UNIPROT accession number: Q8KQN8) [2]

3 Reaction and Specificity

Catalyzed reaction
$[\text{L-Asp(4-L-Arg)}]_n + H_2O = [\text{L-Asp(4-L-Arg)}]_{n-1} + \text{L-Asp(4-L-Arg)}$

Reaction type
hydrolysis of peptide bond (<2> the enzyme seems to belong to the familiy of serine proteases [2])

Natural substrates and products
S $[\text{L-Asp(4-Arg)}]_n$ + H20 <1, 2> (Reversibility: ?) [1, 2]
P $[\text{L-Asp(4-Arg)}]_{n-1}$ + [L-Asp(4-Arg)]

Substrates and products
S $[\text{L-Asp(4-Arg)}]_n$ + H20 <1, 2> (Reversibility: ?) [1, 2]
P $[\text{L-Asp(4-Arg)}]_{n-1}$ + [L-Asp(4-Arg)]

Inhibitors

3,4-dichloro-isocoumarin <1> (<1> 78% inhibition at 1 mM, 56% inhibition at 0.1 mM [1]) [1]
EDTA <2> (<2> 49% inhibition at 60 nM, 46% inhibition at 30 nM [2]) [2]
leupeptin <2> (<2> 13% inhibition within the range 0.001 nM to 0.01 nM [2]) [2]
N-bromo-succinimide <2> (<2> 100% inhibition at 5 nM, 99% inhibition at 1 nM [2]) [2]
pefabloc <1, 2> (<1> i.e. 4-[2-aminoethyl]benzenesulfonyl fluoride, 79% inhibition at 20 mM, 23% inhibition at 8 mM [1]; <2> i.e. 4-[2-aminoethyl]benzenesulfonyl fluoride, 92% inhibition at 80 nM, 13.6% inhibition at 8 nM [2]) [1, 2]
phenylmethanesulfonly fluoride <1> (<1> 45% inhibition at 10 mM, 8% inhibition at 1 mM [1]) [1]
phenylmethylsulfonyl fluoride <2> (<2> 63% inhibition at 10 nM, 28% inhibition at 1 nM [2]) [2]
Additional information <1> (<1> not inhibitory: pepstatin, diazoacetyl-DL-nor-leucine-methyl ester, aprotinin [1]) [1]

pH-Optimum

7.5 <1> (<1> broad pH maximum between pH 7 and pH 8, peaking at pH 7.5 [1]) [1]

pH-Range

6 <1> (<1> 30% of maximal activity [1]) [1]

Temperature optimum (°C)

35 <1> [1]

4 Enzyme Structure

Molecular weight

28000 <1> (<1> gel filtration [1]) [1]
43000 <2> (<2> monomer, SDS-PAGE [2]) [2]

Subunits

monomer <1> (<1> 1*27000, SDS-PAGE [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue

culture supernatant <2> (<2> cells grown in medium containing cyanophycin granules [2]) [2]

Localization

extracellular <2> [2]

Purification

<1> [1]

<2> (from culture supernatant of Pseudomonas anguilliseptica cells grown in medium containing cyanophycin granules, anion-exchange chromatography and L-arginine-agarose affinity chromatography. In case of heterologous expression in Escherichia coli the enzyme was not secreted and thus, isolation was done from the cell lysate) [2]

Renaturation

<2> (in-gel renaturation method) [2]

Cloning

<1> (expression in Escherichia coli DH5α) [1]

<2> (expression in Escherichia coli XL1-Blue) [2]

References

[1] Richter, R.; Hejazi, M.; Kraft, R.; Ziegler, K.; Lockau, W.: Cyanophycinase, a peptidase degrading the cyanobacterial reserve material multi-L-arginyl-poly-aspartic acid (cyanophycin). Molecular cloning of the gene of Synechocystis sp. PCC 6803, expression in Escherichia coli, and biochemical characterization of the purified enzyme. Eur. J. Biochem., **263**, 163-169 (1999)

[2] Obst, M.; Oppermann-Sanio, F.B.; Luftmann, H.; Steinbüchel, A.: Isolation of cyanophycin-degrading bacteria, cloning and characterization of an extracellular cyanophycinase gene (cphE) from Pseudomonas anguilliseptica strain BI. J. Biol. Chem., **277**, 25096-25105 (2002)

Physarolisin 3.4.21.103

1 Nomenclature

EC number
3.4.21.103

Recommended name
physarolisin

Synonyms
EC 3.4.23.27
EC 3.4.23.6
Physarum aspartic proteinase
physarolisin <4> [7]
physarolysin II <2> [6]
proteinase, Dictyostelium discoideum aspartic
proteinase, Dictyostelium discoideum aspartic, E
proteinase, Physarum flavicomum aspartic
proteinase, Physarum polycephalum acid

CAS registry number
94949-28-7

2 Source Organism

<1> *Dictyostelium discoideum* (no sequence specified) [2]
<2> *Physarum polycephalum* (no sequence specified) [1, 4, 5, 6]
<3> *Physarum flavicomum* (no sequence specified) [3]
<4> *Physarum polycephalum* (UNIPROT accession number: Q8MZS4) [7]

3 Reaction and Specificity

Catalyzed reaction
milk clotting activity. Preferential cleavage of Gly8-/-Ser in B chain of insulin most rapidly, followed by Leu11-/-Val, Cys(SO_3H)19-/-Gly and Phe24-/-Phe

Reaction type
hydrolysis of peptide bond

Natural substrates and products

S Additional information <1> (<1> involved in general protein degradation within the lysosomes of Dictyostelium discoideum [2]) (Reversibility: ?) [2]
P ?

Substrates and products

S azocasein + H_2O <3> (Reversibility: ?) [3]
P ?
S casein + H_2O <1> (Reversibility: ?) [2]
P ?
S cytochrome c + H_2O <1> (Reversibility: ?) [2]
P ?
S glucagon + H_2O <2> (Reversibility: ?) [6]
P ? <2> [6]
S hemoglobin + H_2O <1, 2, 3> (Reversibility: ?) [1, 2, 3]
P hydrolyzed hemoglobin
S hemoglobin + H_2O <2, 4> (Reversibility: ?) [4, 7]
P ? <2> [4]
S Hide powder azure + H_2O <1> (Reversibility: ?) [2]
P ?
S KPIEFF(NO_2)RL + H_2O <2> (Reversibility: ?) [6]
P ? <2> [6]
S Lys-Pro-Ile-Glu-Phe-(4-nitro)Phe-Arg-Leu + H_2O <2> (Reversibility: ?) [1]
P Lys-Pro-Ile-Glu-Phe + (4-nitro)Phe-Arg-Leu <2> [1]
S Lys-Pro-Ile-Glu-Phe-(4-nitrophenyl)Arg-Leu + H_2O <4> (Reversibility: ?) [7]
P Lys-Pro-Ile-Glu-Phe + (4-nitrophenyl)Arg-Leu
S oxidized insulin B-chain + H_2O <2> (<2> major cleavage sites: Gly8-Ser9 (most susceptible), Leu11-Val12, Cysteic acid19-Gly20 and Phe24-Phe25 [1]) (Reversibility: ?) [1]
P hydrolyzed insulin B-chain
S substance P + H_2O <2> (Reversibility: ?) [6]
P ? <2> [6]
S bovine albumin + H_2O <1> (Reversibility: ?) [2]
P ?
S histone + H_2O <1> (Reversibility: ?) [2]
P ?
S insulin B chain + H_2O <4> (Reversibility: ?) [7]
P ?
S ovalbumin + H_2O <1> (Reversibility: ?) [2]
P ?
S oxidized insulin B + H_2O <2> (Reversibility: ?) [6]
P ? <2> [6]
S Additional information <1, 2> (<1> activates trypsinogen [2]; <2> derivatives of basic amino acids and related peptides (proteinase E) [1]; <2>

not: N-acetyl-N-phenylalanyl-3,5-diiodo-L-tyrosine [1]; <2> no hydrolysis of hemoglobin, casein, azocoll and Ala-Ala-Phe-7-amido-4-methylcoumarin [6]; <1> involved in general protein degradation within the lysosomes of Dictyostelium discoideum [2]) (Reversibility: ?) [1, 2, 6]

P ? <2> [6]

Inhibitors

DAN <4> (<4> in the presence of cupric ions [7]) [7]
DFP <2, 4> (<2> strong [5]) [5, 7]
diazoacetyl-D,L-norleucine methyl ester <2> (<2> in presence of Cu^{2+} [1]) [1]
diazoacetyl-DL-norleucine methyl ester <2> (<2> 0.042 mg/ml, 95% inhibition [4]) [4, 5]
diisopropyl fluorophosphate <2> (<2> 23% inhibition by 0.5 mM, 76% inhibition by 10 mM [6]) [6]
PMSF <2> (<2> 10 mM, 21% inhibition [4]) [4]
pepstatin <1, 2, 3> (<1,2> not [1,2]) [1, 2, 3]
SH-reagents <3> [3]
tosyl-L-Leu-chloromethylketone <2> (<2> 10 mM, 18% inhibition [4]) [4]
Additional information <2> (<2> not: 1,2-epoxy-3-(4-nitrophenoxy)propane [1]; <2> no inhibition by 0.01 mM pepstatin, 10 mM Ala-Ala-Phe-CH_2Cl, 0.2 mM diazoacetyl-DL-norleucine methyl ester, 10 mM EDTA or 10 mM EGTA [6]) [1, 6]

Specific activity (U/mg)

Additional information <2> [1]

pH-Optimum

1.7 <2, 4> (<2> hemoglobin [1]; <2> hydrolysis of hemoglobin [4]) [1, 4, 7]
2-3 <3> (<3> hemoglobin, azocasein [3]) [3]
2.5 <1> (<1> hide powder azure [2]) [2]
4.2 <2> (<2> hydrolysis of oxidized insulin B chain [6]) [6]

pH-Range

1.3-3 <2> (<2> pH 1.3: about 25% of activity maximum, pH 3.0: about 35% of activity maximum [1]) [1]
3.7-5.3 <2> (<2> pH 3.7: about 40% of maximal actuvity, pH 5.3: about 35% of maximal activity, hydrolysis of oxidized insulin B chain [6]) [6]

Temperature optimum (°C)

16-22 <2> [6]
37 <2> (<2> assay at [1]) [1]
55 <3> [3]

Temperature range (°C)

3-30 <2> (<2> 3°C: about 50% of maximal activity, 30°C: about 60% of maximal activity, hydrolysis of oxidized insulin B chain [6]) [6]
50 <1> (<1> up to [2]) [2]

4 Enzyme Structure

Molecular weight
32000 <3> [3]
54000 <2> [4]
68000 <2> (<2> gel filtration [1]) [1]

Subunits
? <1> (<1> x * 58000 [2]) [2]
dimer <2, 4> (<2> 1 * 31000 + 1 * 23000, the two chains are cross-linked by disulfide bond(s), SDS-PAGE under reducing and nonreducing conditions [1]; <2> 1 * 31000 + 1 * 23000, the heavy and the light chain are cross-linked by disulfide bonds, SDS-PAGE [4]; <4> 1 * 31000 + 1 * 23000, SDS-PAGE, gel filtration [7]) [1, 4, 7]

Posttranslational modification
glycoprotein <1, 2> (<2> the heavy chain contains a carbohydrate moiety composed of 7 residues mannose, 5 glucosamine, 1 fucose and 1 glucose [1]; <2> the heavy chain, but not the light chain is glycosylated. One molecule of native enzyme contains about 7 residues of mannose, 5 residues of glucosamine, and 1 residue each of fucose and glucose [4]) [1, 2, 4]

5 Isolation/Preparation/Mutation/Application

Source/tissue
plasmodium <2> [4]

Localization
intracellular <2> [1, 4]
lysosome <3> [3]

Purification
<1> [2]
<2> [1, 4]
<3> [3]
<4> [7]

Cloning
<2> [5]
<2> (expression in Escherichia coli) [6]

6 Stability

pH-Stability
2-3 <3> (<3> maximal stability [3]) [3]
2-9 <1> (<1> 25°C, 24 h, stable [2]) [2]
3-6 <1, 2> (<1> 25°C, 48 h, stable [2]) [1, 2]

7 <3> (<3> unstable [3]) [3]
8 <2> (<2> 37°C, 15 min, 30% loss of activity [1]) [1]
9 <2> (<2> 37°C, 15 min, 45% loss of activity [1]) [1]

Temperature stability

18 <2> (<2> pH 4.2, $t_{1/2}$: 5 min [6]) [6]
25 <1, 2> (<2> pH 3.0-6.0, 48 h, stable [1]; <1> pH 2-9, 24 h stable [2]; <2> pH 3.0-6.0, stable [4]) [1, 2, 4]
37 <2> (<2> 15 min, 30% loss of activity at pH 8.0, 45% loss of activity at pH 9.0 [1]; <2> pH 4.2, $t_{1/2}$: 6 h [6]; <2> pH 8.0, 15 min, 30% loss of activity [4]) [1, 4, 6]
45 <3> (<3> good stability [3]) [3]

General stability information

<2>, the active enzyme is markedly unstable due to rapid autolysis, $t_{1/2}$: 5 min at 18°C, pH 4.2 [6]
<3>, thiol reagent required for stability [3]

References

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[3] Henney, H.R.; White, H.U.: Characterization of a lysosomal proteinase purified from haploid cells of Physarum flavicomum undergoing encystment. Can. J. Bot., **61**, 1357-1366 (1983)
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[7] Murakami-Murofushi, K.; Nishii, W.; Takahashi, K.: Physarolisin. Handbook of proteolytic enzymes (Barrett, A.J., Rawlings, N.D., Woessner, J.F., eds.) Academic Press, **2**, 1891-1893 (2004)

Mannan-binding lectin-associated serine protease-2 3.4.21.104

1 Nomenclature

EC number
3.4.21.104

Recommended name
mannan-binding lectin-associated serine protease-2

Synonyms
MASP <2> [12]
MASP-2 <1, 2, 3, 4, 5> [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21]
MBL-associated serine protease <1> [11]
MBL-associated serine protease-2 <2> [14]
MBP-associated serine protease <2> [12]
MBP-associated serine protease 2 <2> [13, 15]
MBP-associated serine protease-2 <3> [10]
S01.229 <2> (<2> Merops ID [8]) [8]
mannan-binding lectin-associated serine protease-2 <4, 5> [16, 17, 18, 19, 20, 21]
mannose-binding lectin-associated serine protease-2 <2> [14]

CAS registry number
214915-16-9

2 Source Organism

<1> *Mus musculus* (no sequence specified) [11]
<2> *Homo sapiens* (no sequence specified) [2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14, 15]
<3> *Rattus norvegicus* (no sequence specified) [1, 10]
<4> *Homo sapiens* (UNIPROT accession number: O00187) [16, 17, 18, 19, 20, 21]
<5> *Rattus norvegicus* (UNIPROT accession number: Q9JJS8) [20]

3 Reaction and Specificity

Catalyzed reaction
Selective cleavage after Arg223 in complement component C2 (-Ser-Leu-Gly-Arg-/-Lys-Ile-Gln-Ile) and after Arg76 in complement component C4 (-Gly-Leu-Gln-Arg-/-Ala-Leu-Glu-Ile) (<3> mechanism [1]; <2> catalytic serine re-

sidue is located on the light chain subunit [12]; <2> structure-function analysis of the catalytic unit, enzyme-substrate interaction is different from enzyme C1 which has the same substrate specificity, MASP-2 shows a subsite flexibility in substrate specificity, catalytic residues are Asp526 and His525, active site structure, overview [14]; <3> substrate recognition mechanism, mechanism of complement cascade activation [10])

Reaction type

cleavage of C-N-linkage
hydrolysis of peptide bond

Natural substrates and products

S Protein + H_2O <2, 3> (<3> enzyme circulates as a complex with mannose-binding protein, minimal functional unit for complement activation is a ASP homodimer bound to two mannose-binding protein trimeric subunits, complex is formed more readily in the presence of Ca^{2+} [1]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9]
P ? <2, 3> [1, 2, 3, 4, 5, 6, 7, 8, 9]
S complement C_2 + H_2O <2, 3> (<3> involved in activation of complement cascade [10]; <2> involved in lectin pathway being part of the innate immune system providing a first line of defense against infections by activation of the complement cascade [13]) (Reversibility: ?) [10, 12, 13, 14, 15]
P 2 fragments of complement C2 (<2> formation of the C3 convertase C4b2a [15])
S complement C_4 + H_2O <1, 2, 3> (<3> involved in activation of complement cascade [10]; <2> involved in lectin pathway being part of the innate immune system providing a first line of defense against infections by activation of the complement cascade [13]) (Reversibility: ?) [10, 11, 12, 13, 14, 15]
P 2 fragments of complement C4 (<2> formation of the C3 convertase C4b2a [15])
S Additional information <2> (<2> complement C3 is not a natural substrate [7]; <2> L-ficolin/P35 competes with mannose-binding protein for Ca^{2+}-dependent binding to MASP-2, complex activates complement [6]; <2> smallest functional unit for complement activation consists of mannose-binding protein dimers bound to MASP-2 homodimers [2]; <2> enzyme in serum is complexed with mannan-binding lectin MBL and ficolins [13]; <2> enzyme is complexed with the mannose-binding protein, a recognition molecule for the complement pathway [15]; <2> enzyme is Mg^{2+}-dependently associated with the mannose-binding protein, a C-type lectin binding to terminal mannose and N-acetylglucosamine moieties on surfaces of certain pathogens and activating the classical complement pathway, the enzyme is not identical with C1r or C1s [12]) (Reversibility: ?) [2, 6, 7, 12, 13, 15]
P ? <2> [2, 6, 7]

Substrates and products

- **S** 4,4'-dithiopyridine + H_2O <2> (Reversibility: ?) [14]
- **P** ?
- **S** Ile-Ala-Arg 4-nitroanilide + H_2O <2> (Reversibility: ?) [8]
- **P** ? <2> [8]
- **S** N-acetylglycine-L-lysine methyl ester + H_2O <2> (Reversibility: ?) [3]
- **P** ? <2> [3]
- **S** N-carboxybenzoylglycine-L-arginine thiobenzyl ester + H_2O <2> (Reversibility: ?) [3]
- **P** ? <2> [3]
- **S** N^{α}-benzoyl-L-arginine ethyl ester + H_2O <2> (Reversibility: ?) [3]
- **P** ? <2> [3]
- **S** N^{α}-carbobenzoxy-L-lysine-4-nitrophenyl ester + H_2O <2> (Reversibility: ?) [8]
- **P** ? <2> [8]
- **S** Phe-Gly-Arg-7-amido-4-methylcoumarin + H_2O <2> (<2> recombinant enzyme, low activity [15]) (Reversibility: ?) [15]
- **P** Phe-Gly-Arg + 7-amino-4-methylcoumarin
- **S** Phe-Ser-Arg-7-amido-4-methylcoumarin + H_2O <2> (<2> recombinant enzyme, low activity [15]) (Reversibility: ?) [15]
- **P** Phe-Ser-Arg + 7-amino-4-methylcoumarin
- **S** protein + H_2O <2, 3> (<2> cleaves oligopeptides that contain either Arg or Lys at their P1 positions [7]; <3> enzyme circulates as a complex with mannose-binding protein, minimal functional unit for complement activation is a ASP homodimer bound to two mannose-binding protein trimeric subunits, complex is formed more readily in the presence of Ca^{2+} [1]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9]
- **P** ? <2, 3> [1, 2, 3, 4, 5, 6, 7, 8, 9]
- **S** α-N-benzyloxycarbonyl-L-lysine thiobenzyl ester + H_2O <2> (Reversibility: ?) [14]
- **P** ?
- **S** benzyloxycarbonyl-Gly-Arg-S-benzyl + H_2O <4> (Reversibility: ?) [17]
- **P** ?
- **S** complement C2 + H_2O <2> (Reversibility: ?) [3]
- **P** complement component C2a + complement component C2b <2> [3]
- **S** complement C2 + H_2O <2, 4, 5> (<2> activates complement C2 [4]; <2> SP domain alone can autoactivate and cleave C2 [7]; <4> 37°C [18]) (Reversibility: ?) [4, 7, 18, 20]
- **P** ? <2> (<4,5> the complement C3 convertase C4bC2b is generated by cleavage of complement C4 and complement C2 [18,20]) [4, 7]
- **S** complement C2 + H_2O <2, 3> (<3> involved in activation of complement cascade [10]; <2> involved in lectin pathway being part of the innate immune system providing a first line of defense against infections by activation of the complement cascade [13]; <2> human substrate [15]; <3> only the activated enzyme binds to C2-component [10]) (Reversibility: ?) [10, 12, 13, 14, 15]

P 2 fragments of complement C2 (<2> formation of the C3 convertase C4b2a [15]; <3> C2a and C2b fragments, preferred substrate [10])
S complement C3 + H_2O <2> (<2> very weak substrate [3]) (Reversibility: ?) [3, 4]
P ? <2> [3, 4]
S complement C3i + H_2O <2> (Reversibility: ?) [7]
P ? <2> [7]
S complement C4 + H_2O <2> (Reversibility: ?) [3]
P complement component C4-α + complement component C4-α' <2> [3]
S complement C4 + H_2O <2, 4, 5> (<2> SP domain is able to cleave C4, but the presence of the CCP2 domain significantly increases the efficiency [7]; <4> 37°C [18]) (Reversibility: ?) [4, 7, 8, 17, 18, 20]
P ? <2> (<4,5> the complement C3 convertase C4bC2b is generated by cleavage of complement C4 and complement C2 [18,20]) [4, 7, 8]
S complement C4 + H_2O <1, 2, 3> (<3> involved in activation of complement cascade [10]; <2> involved in lectin pathway being part of the innate immune system providing a first line of defense against infections by activation of the complement cascade [13]; <1> activated MBL-MASP-2 complex [11]; <2> human substrate [15]; <3> no activity of the enzyme complexed with myelin basic protein before complex activation by binding to a suitable carbohydrate ligand [10]; <2> the complement control protein CCP module is essential for cleavage [14]) (Reversibility: ?) [10, 11, 12, 13, 14, 15]
P 2 fragments of complement C4 (<2> formation of the C3 convertase C4b2a [15]; <2> a fragment corresponding to C4b with appearance of the α-chain [12]; <3> C4a fragment, an N-terminal portion, and C4b fragment, the activated form [10])
S complement C4 + H_2O <4> (<4> 37°C [21]) (Reversibility: ?) [21]
P complement C4b
S *p*-tosyl-L-arginine methyl ester + H_2O <2> (Reversibility: ?) [3]
P ? <2> [3]
S Additional information <2> (<2> hardly any detectable activity on complement C3 [7]; <2> complement C3 is not a natural substrate [7]; <2> L-ficolin/P35 competes with mannose-binding protein for Ca^{2+}-dependent binding to MASP-2, complex activates complement [6]; <2> smallest functional unit for complement activation consists of mannose-binding protein dimers bound to MASP-2 homodimers [2]; <2> enzyme in serum is complexed with mannan-binding lectin MBL and ficolins [13]; <2> enzyme is complexed with the mannose-binding protein, a recognition molecule for the complement pathway [15]; <2> enzyme is Mg^{2+}-dependently associated with the mannose-binding protein, a C-type lectin binding to terminal mannose and N-acetylglucosamine moieties on surfaces of certain pathogens and activating the classical complement pathway, the enzyme is not identical with C1r or C1s [12]; <2> substrate specificity, fluorescent amide compounds are poor substrates, overview [15]) (Reversibility: ?) [2, 6, 7, 12, 13, 15]
P ? <2> [2, 6, 7]

Inhibitors

C1 inhibitor <2> (<2> abolishes activity at a 1:1 inhibitor:protease concentration [3]; <2> recombinant enzyme, at pH 7.4, inhibitor from human, the protease-serpin complex in highly unstable at alkaline pH [15]) [3, 15]
C1-inhibitor <2> (<2> forms a stable complex with the enzyme, 1-3fold excess completely inactivates [7]; <2> forms equimolar complexes with the enzyme and inhibits its proteolytic activity [4]; <2> linear relationship between inhibition and the molar ratio of inhibitor:enzyme up to about 50% inhibition [8]) [4, 7, 8]
Pefabloc SC <2> (<2> irreversible inhibition [8]) [8]
α-2-macroglobulin <2> (<2> forms a stable complex with the enzyme, inhibits at a large molar excess, 40fold [7]; <2> inhibition of recombinant, not native, enzyme at large molar excess [15]) [7, 15]
anti-thrombin III <2> (<2> recombinant enzyme, inhibition only in presence of heparin [15]) [15]
complement C1 inhibitor <4> [17, 20]
myelin basic protein <3> (<3> no activity with substrate C4-component of the enzyme complexed with myelin basic protein before activation of the complex by binding to a suitable carbohydrate ligand [10]) [10]
Additional information <2> (<2> no inhibitor: α-2-macroglobulin [3]; <2> no inhibition by Na^+ [14]; <2> no inhibition of recombinant enzyme by thrombin inhibitor Z-D-Phe-Pro-methoxypropylboroglycinepinanediol ester, i.e. boroMpg, and hirudin [15]) [3, 14, 15]

Activating compounds

Additional information <3> (<3> autoactivation activity is enhanced by complex formation with myelin basic protein, thus myelin basic protein has a regulating function [10]) [10]

Metals, ions

Ca^{2+} <1, 4, 5> (<4,5> enables dimerization [20]) [11, 20]
$CaCl_2$ <2> (<2> 5 mM [15]) [15]
Na^+ <1> [11]
NaCl <2> (<2> 140 mM [15]) [15]
Additional information <2> (<2> no induction by Na^+ [14]) [14]

Turnover number (min^{-1})

0.026 <4> (complement C4, <4> mutant MASP-2 CCP1-CCP2-SP R444Q [17]) [17]
0.9 <2, 4> (complement C4, <2> 37°C, pH 7.4 [7]; <4> MASP-2 CCP1-CCP2-SP [17]) [7, 17]
1.9 <2> (complement C2, <2> 37°C, pH 7.4 [7]) [7]
1.9 <4> (complement C4, <4> wild-type, 37°C [18]) [18]
2.2 <2> (complement C4, <2> pH 7.4, 30°C [3]) [3]
2.4 <2> (N^{α}-benzoyl-L-arginine ethyl ester, <2> pH 7.4, 30°C [3]) [3]
2.9 <4> (complement C4, <4> chimera C1s(MASP-2SP), 37°C [18]) [18]
3.2 <4> (complement C4, <4> chimera C1s(MASP-2CCP1/2), 37°C [18]) [18]
4.9 <2> (complement C2, <2> pH 7.4, 30°C [3]) [3]

5.1 <4> (complement C2, <4> chimera C1s(MASP-2CCP1/2), 37°C [18]) [18]
5.2 <4> (complement C2, <4> chimera C1s(MASP-2SP), 37°C [18]) [18]
5.6 <4> (complement C2, <4> wild-type, 37°C [18]) [18]
7.1 <2> (*p*-tosyl-L-arginine methyl ester, <2> pH 7.4, 30°C [3]) [3]
12.7 <2> (N-carboxybenzoylglycine-L-arginine thiobenzyl ester, <2> pH 7.4, 30°C [3]) [3]
133 <2> (N-acetylglycine-L-lysine methyl ester, <2> pH 7.4, 30°C [3]) [3]

Specific activity (U/mg)

Additional information <2> (<2> activity of recombinant enzyme with substrates Phe-Gly-Arg-7-amido-4-methylcoumarin and Phe-Ser-Arg-7-amido-4-methylcoumarin [15]) [15]

K_m-Value (mM)

0.000085 <4> (complement C4, <4> wild-type, 37°C [18]) [18]
0.000147 <4> (complement C4, <4> chimera C1s(MASP-2CCP1/2), 37°C [18]) [18]
0.000377 <4> (complement C4, <4> mutant MASP-2 CCP1-CCP2-SP R444Q [17]) [17]
0.0016 <4> (complement C4, <4> MASP-2 CCP1-CCP2-SP [17]) [17]
0.0028 <4> (complement C4, <4> chimera C1s(MASP-2SP), 37°C [18]) [18]
0.0052 <4> (complement C2, <4> wild-type, 37°C [18]) [18]
0.0073 <4> (complement C2, <4> chimera C1s(MASP-2SP), 37°C [18]) [18]
0.0092 <4> (complement C2, <4> chimera C1s(MASP-2CCP1/2), 37°C [18]) [18]
1.6 <2> (complement C4, <2> 37°C, pH 7.4 [7]) [7]
2.8 <2> (*p*-tosyl-L-arginine methyl ester, <2> pH 7.4, 30°C [3]) [3]
4 <2> (complement C2, <2> 37°C, pH 7.4 [7]) [7]
4.4 <2> (N^{α}-benzoyl-L-arginine ethyl ester, <2> pH 7.4, 30°C [3]) [3]
6.5 <2> (complement C2, <2> pH 7.4, 30°C [3]) [3]
6.7 <2> (N-acetylglycine-L-lysine methyl ester, <2> pH 7.4, 30°C [3]) [3]
40 <2> (N-carboxybenzoylglycine-L-arginine thiobenzyl ester, <2> pH 7.4, 30°C [3]) [3]
74 <2> (complement C4, <2> pH 7.4, 30°C [3]) [3]
Additional information <2, 3> (<2> kinetics [14]; <3> recombinant MASP-2K: kinetics of free enzyme and enzyme complexed with myelin basic protein [10]) [10, 14]

K_i-Value (mM)

8.8e-006 <2> (C1-inhibitor 2, <2> 37°C, pH 7.4 [7]) [7]

pH-Optimum

7.4 <1, 2> (<1,2> assay at [11,12,13,15]) [11, 12, 13, 15]
8.5 <2> (<2> assay at [14]) [14]

pH-Range

6-9 <2> [15]

Temperature optimum (°C)

37 <1, 2> (<1,2> assay at [11,12,14,15]) [11, 12, 14, 15]

4 Enzyme Structure

Molecular weight

29000 <2> (<2> calculated molecular mass of the MASP-2 CUB-EGF fragment in the presence of Ca^{2+} [5]) [5]
70000 <2> (<2> SDS-PAGE, proenzyme form [4]) [4]
74150 <2, 4, 5> (<2> calculated from nucleotide sequence [9]; <4,5> monomer, calculated from amino acid sequence [20]) [9, 20]
76000 <2> (<2> SDS-PAGE, non-reducing conditions [9]) [9]
80000 <2> (<2> SDS-PAGE, non-reducing conditions [6]) [6]

Subunits

? <1, 2, 4> (<4> x * 45000, SDS-PAGE [16]; <2> x * 75000, full-length proenzyme, SDS-PAGE, x * 50000, A-chain of the enzyme, SDS-PAGE [13]; <1> x * 87000, proenzyme form, SDS-PAGE, 1 * 52000, heavy enzyme chain, active enzyme form, SDS-PAGE [11]; <4> x * 44000, mutant MASP-2 CCP1-CCP2-SP R444Q, SDS-PAGE [17]; <4> x * 75100, SDS-PAGE [19]; <4> x * 78000, chimera C1s(MASP-2CCP1/2), SDS-PAGE [18]) [11, 13, 16, 17, 18, 19]
dimer <2, 3, 4, 5> (<4> 2 * 74000, SDS-PAGE [20]; <2> 1 * 45000 + 1 * 28000, SDS-PAGE, reducing conditions [6]; <2> 1 * 52000 + 1 * 31000, SDS-PAGE [9]; <3> crystallographic experiments [1]; <2> homodimer, sedimentation velocity experiments [5]; <2> 1 * 66000, heavy subunit, + 1 * 31000, light subunit, SDS-PAGE [12]; <5> 2 * 74000, SDS-PAGE, homodimers are stable in the presence of Ca^{2+} [20]; <4> 29000 + 60000, chimera C1s(MASP-2SP), SDS-PAGE [18]) [1, 5, 6, 9, 12, 18, 20]
Additional information <2> (<2> enzyme in serum is complexed with mannan-binding lectin MBL and ficolins [13]; <2> enzyme surface segment analysis, structure-function analysis [14]; <2> the subunits are linked by disulfide bonds [12]) [12, 13, 14]

Posttranslational modification

Glycoprotein <3> [10]
No glycoprotein <2> [14]
proteolytic modification <1, 2, 3> (<2> autoactivation [14]; <3> zymogen is cleaved into the catalytically active protease [1]; <1> autoactivation, autoactivation of the recombinant enzyme during purification [11]; <3> autoproteolytic activation of the zymogen, cleavage site contains a Lys residue near the C-terminus, cleavage into 2 fragements, a larger N-termnal and a smaller C-terminal one, the latter contains the protease active site, autoactivation activity is enhanced by complex formation with myelin basic protein [10]) [1, 10, 11, 14]
Additional information <2> (<2> no sites for N-linked glycosylation [9]) [9]

5 Isolation/Preparation/Mutation/Application

Source/tissue

Hep-G2 cell <4> [16]
blood <4> [21]
blood plasma <4, 5> [20]
esophagial squamous cell carcinoma cell line <4> (<4> increased MASP-2 expression in comparison with normal tissue [16]) [16]
liver <2, 3, 4, 5> [9, 10, 16, 20]
plasma <2> [13]
serum <2> [4, 12, 13, 15]
Additional information <2> (<2> enzyme content in plasma of blood donors during 1 year, overview [13]) [13]

Localization

extracellular <2, 3> [10, 12, 15]
Additional information <4, 5> (<4,5> MASP-2 is associated with mannan-binding lectin II [20]) [20]

Purification

<2> [2, 5, 6, 7, 9]
<2> (from serum by sequential affinity chromatography, separation from serum mannose-binding protein MBP in presence of EDTA) [12]
<2> (mature protein and truncated fragments) [3]
<2> (recombinant catalytic fragment encompassing the second complement control protein module and the serine protease domain, i.e. MASP-2-CCP2-SP, from Escherichia coli strain BL21(DE3)) [14]
<3> (MASP-2K mutant, inhibited in autoproteolytic activation, by affinity chromatography on an myelin basic protein-resin) [10]
<4> [17]
<4> (chimeric proteins C1s(MASP-2CCP1/2) and C1s(MASP-2SP)) [18]

Renaturation

<2> (combination of isolated MASP and MBP restores the complex and the complement activating activity) [12]

Crystallization

<2> (catalytic fragment encompassing the second complement control protein module and the serine protease domain, in presence of Na^+, in absence of Mg^{2+}, 0.8 mg/ml purified recombinant protein in 140 mM NaCl, 20 mM Tris-HCl, pH 7.4, and 0.05% w/v NaN_3, hanging drop vapour diffusion method at 20°C, mixing with equal volume of reservoir solution containing 30% w/v PEG 6000, 0.2 M NaCl, 10% v/v glycerol, 0.1 M Tris-HCl, pH 7.5, X-ray diffraction structure determination and analysis at 2.25 A resolution) [14]
<3> (hanging drop vapor diffusion method in the presence of Ca^{2+}) [1]
<4> [20]
<4> (zymogen and the activated form, 2.4 A resolution) [17]
<5> [20]

Cloning

<1> (expression using a Drosophila melanogaster expression system, C4-cleavage activity reconstitution of the enzyme-deficient mutant by addition of recombinant enzyme to deficient serum) [11]
<2> [5, 9]
<2> (MASP-2 and truncated fragments of the catalytic region) [7]
<2> (expressed in CHO cells) [2]
<2> (expression of the catalytic fragment encompassing the second complement control protein module and the serine protease domain, i.e. MASP-2-CCP2-SP, in Escherichia coli strain BL21(DE3), the recombinant construct contains an extra-tetrapeptide ASMT at the N-terminus) [14]
<2> (mature protein and truncated fragments) [3]
<4> [18, 19, 20]
<4> (expression in Escherichia coli) [17]
<5> [20]

Engineering

C1s(MASP-2CCP1/2) <4> (<4> hybrid C1s/MASP-2 molecule, swapped complement control protein, 21-27fold higher k_{cat}/K_m-ratio for complement C_4 than C1s(MASP-2SP) [18]) [18]
C1s(MASP-2SP) <4> (<4> hybrid C1s/MASP-2 molecule, swapped serine protease, 21-27fold lower k_{cat}/K_m-ratio for complement C_4 than C1s(MASP-2CCP1/2) [18]) [18]
D105G <4> (<4> inactive, MASP-2 deficiency, the index case suffers from recurrent severe infections and autoimmune reactions [20]; <4> incapable of forming a complex with mannan-binding lectin, found in 1.44% of studied Spaniards, 2% of studied North Africans, not found in Sub-Saharans [21]) [20, 21]
MASP-2 CCP1-CCP2-SP R444Q <4> (<4> lower K_M-value for complement C_4 [17]) [17]
P111L <4> (<4> found in 4% of studied North Africans, not found in Sub-Saharans and Spaniards [21]) [21]
R103C <4> (<4> found in 2% of studied North Africans, not found in Sub-Saharans and Spaniards [21]) [21]
R84Q <4> (<4> found in 13.33% of studied Sub-Saharans, 1% of studied North Africans, not found in Spaniards [21]) [21]
Additional information <1, 3> (<3> construction of an enzymatically inactive enzyme, also not exhibiting autocatalytic activity, by substitution of the active size catalytic Ser residue with alanine, i.e. MASP-2A, construction of a zymogen with reduced autoproteolytic activity by substitution of the Arg residue at the autocatalytic cleavage site with lysine, i.e. MASP-2K [10]; <1> construction of MASP-2-deficient mice [11]) [10, 11]

6 Stability

pH-Stability

5 <4> (<4> wild-type zymogen MASP-2 [17]) [17]

Temperature stability

48.2 <4> (<4> T_m for the zymogen of mutant MASP-2 CCP1-CCP2-SP R444Q [17]) [17]

50.8 <4> (<4> T_m for the active fragment of mutant MASP-2 CCP1-CCP2-SP R444Q [17]) [17]

General stability information

<2>, enzyme is stable during at least 10 freeze-thaw cycles [15]

Storage stability

<2>, -20°C, 4°C, and room temperature, 30 days, stable at [13]

<2>, 37°C, loss of about 95% activity within 30 days [13]

<2>, enzyme activity is decreased due to aging, loss of 90% activity after 48-120 h [15]

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Rhomboid protease 3.4.21.105

1 Nomenclature

EC number
3.4.21.105

Recommended name
rhomboid protease

Synonyms
AarA <8> [8, 16]
AqRho <9> [16]
GlpG <3> [2, 4, 8, 16]
PARL <4> [11]
PSARL <4, 17> [5]
Pcp1 <5, 13, 18> [10, 12, 13, 19]
RHBDL2 <4, 16> [1, 3, 8]
RHDBL-2 <4> [14]
Rbd1p <5> [15]
TgROM1 <10> [17]
TgROM2 <10> [18]
TgROM3 <10> [17]
TgROM4 <10> [17]
TgROM5 <10> [17]
Yqgp <1> [4, 8, 16]
presenilins-associated rhomboid-like protein <4> [11]
rhomboid pepditase Pcp1 <18> [19]
rhomboid protease PSARL <4, 17> [5]
rhomboid protease Pcp1 <5> [13]
rhomboid-1 <2> [3, 6, 8, 14]
rhomboid-2 <2> [7]
rhomboid-3 <2> [6]
rhomboid-4 <2> [6]
rhomboid-type protease Pcp1 <13> [10]

CAS registry number
713145-02-9

2 Source Organism

<1> *Bacillus subtilis* (no sequence specified) [4, 8, 16]
<2> *Drosophila sp.* (no sequence specified) (<2> gene R4CL, putative 4-coumarate coenzyme A ligase, i.e. Os02g0177600 protein [8]) [3, 6, 7, 8, 14]

<3> *Escherichia coli* (no sequence specified) [2, 4, 8, 16]
<4> *Homo sapiens* (no sequence specified) [1, 3, 5, 11, 14]
<5> *Saccharomyces cerevisiae* (no sequence specified) [12,13,15]
<6> *Pseudomonas aeruginosa* (no sequence specified) (<6> gene R4CL, putative 4-coumarate coenzyme A ligase, i.e. Os02g0177600 protein [8]) [4,8]
<7> *Thermotoga maritima* (no sequence specified) [4]
<8> *Providencia stuartii* (no sequence specified) (<8> gene R4CL, putative 4-coumarate coenzyme A ligase, i.e. Os02g0177600 protein [8]) [8,16]
<9> *Aquifex aeolicus* (no sequence specified) (<9> gene R4CL, putative 4-coumarate coenzyme A ligase, i.e. Os02g0177600 protein [8]) [8,16]
<10> *Toxoplasma gondii* (no sequence specified) [17, 18]
<11> no activity in *Methanothermobacter thermoautotrophicus* [9]
<12> no activity in *Thermoplasma volcanium* [9]
<13> *yeast* (no sequence specified) [10]
<14> no activity in *Encephalitozoon cuniculi* [9]
<15> no activity in *Xylella fastidiosum* [9]
<16> *Homo sapiens* (UNIPROT accession number: Q9NX52) (<16> gene R4CL, putative 4-coumarate coenzyme A ligase, i.e. Os02g0177600 protein [8]) [8]
<17> *Psammomys obesus* (no sequence specified) [5]
<18> *Saccharomyces cerevisiae* (UNIPROT accession number: P53259) [19]

3 Reaction and Specificity

Catalyzed reaction

cleaves type-1 transmembrane domains using a catalytic triad composed of serine, histidine and asparagine contributed by different transmembrane domains

Reaction type

hydrolysis of peptide bond

Natural substrates and products

S MIC adhesin + H_2O <10> (<10> only TgRMO5 is able to cleave MIC adhesins, it likely provides the key protease activity necessary for invasion [17]) (Reversibility: ?) [17]
P ?
S Spitz + H_2O <2, 4> (<2> rhomboids 1-4 are all dedicated to regulating EGF receptor signalling [6]; <2,4> the rhomboid active site in directly cleaves the membrane-anchored TGFα-like growth factor Spitz within its transmembarne domain [3]) (Reversibility: ?) [3, 6]
P ?

S ephrin B3 + H_2O <4> (<4> RHBDL-2 mediated proteolytic processing may regulate intercellular interactions between ephrinB3 and eph receptors [1]) (Reversibility: ?) [1]
P ?
S l-Mgm1 + H_2O <13> (<13> rhomboid-type protease Pcp1 is essential for wild type mitochondrial morphology. The processing of the large isoform l-Mgm1 by rhomboid-type protease Pcp1 to s-Mgm1, and the presence of both isoforms of Mgm1 appears to be crucial for wild-type mitochondrial morphology and maintenance of mitochondrial DNA [10]) (Reversibility: ?) [10]
P s-Mgm1 + N-terminal putative transmembrane segment
S large isoform of Mgm1 + H_2O <5> (<5> the enzyme is involved in the pathway of Mgm1 biogenesis. A strong shift in the ratio between both isoform of Mgm1 is sufficient to alter mitochondrial morphology [12]) (Reversibility: ?) [12]
P short isoform of Mgm1 + ?
S protein Gurken + H_2O <2> (Reversibility: ?) [6, 7]
P ?
S protein Keren + H_2O <2> (Reversibility: ?) [6, 7]
P ?
S protein Spitz + H_2O <2> (<2> rhomboids 1-4 are all dedicated to regulating EGF receptor signalling [6,7]; <2> when Spitz is cleaved by rhomboids in the endoplasmic reticulum it cannot be secreted. Star regulates Spitz cleavage by rhomboid-1 by transporting Spitz to the Golgi apparatus. Rhomboids 1-4 are all dedicated to regulating EGF receptor signalling [6]) (Reversibility: ?) [6, 7]
P ?
S Additional information <4, 5> (<5> intramolecular proteolysis by rhomboids controls cellular processes other than signalling [15]; <4> regulated intramembrane proteolysis in which the putative signaling moiety is part of the intramembrane-cleaving protease itself. Cytosolic N-terminal domain of PARL is cleaved at positions 52-53 (α-site) and 77-78 (β-site). Whereas α-cleavage is constitutive and removes the mitochondrial targeting sequence, β-cleavage appears to be developmentally controlled and dependent on PARL intramembrane-cleaving protease activity supplied in trans. The β-cleavage of PARL liberates Pβ, a nuclear targeted peptide whose sequence is conserved only in mammals. Thus, in addition to its evolutionarily conserved function in regulating mitochondrial dynamics, PARL might mediate a mammalian-specific, developmentally regulated mitochondria-to-nuclei signaling through regulated proteolysis of its N-terminus and release of the Pβ peptide [11]; <5> rhomboid protease Pcp1 catalyzes the second processing step of cytochrome c peroxidase, yielding the mature cytochrome c peroxidase protein [13]) (Reversibility: ?) [11, 13, 15]
P ?

Substrates and products

- **S** C100Spi-Flag + H_2O <1, 3, 8, 9> (<1,3,8,9> no cleavage of C_{10}0-Flag [16]) (Reversibility: ?) [16]
- **P** ?
- **S** Gurken + H_2O <1, 2, 3, 6> (<2> cleavage by rhomboid 4 [6]) (Reversibility: ?) [4, 6]
- **P** ?
- **S** Gurken-transmembrane domain + H_2O <2, 6, 8, 9, 16> (Reversibility: ?) [8]
- **P** ?
- **S** Keren + H_2O <1, 2, 3, 6, 7> (<2> cleavage by rhomboid 4 [6]; <7> inefficient cleavage [4]) (Reversibility: ?) [4, 6]
- **P** ?
- **S** MIC adhesin + H_2O <10> (<10> only TgRMO5 is able to cleave MIC adhesins, it likely provides the key protease activity necessary for invasion [17]) (Reversibility: ?) [17]
- **P** ?
- **S** Protein + H_2O <3> (<3> cleaves a model protein having an N-terminal and periplasmically localized β-lactamase domain, a LacY-derived transmembrane region, and a cytosolic maltose binding protein mature domain, cleavage occurs between Ser and Asp in a region of high local hydrophilicity, which might be located iin a juxtamembrane rather than an intramembrane position. The conserved Ser and His residue of GlpG are esential for proteolytic activity [2]) (Reversibility: ?) [2]
- **P** ?
- **S** Spitz + H_2O <1, 2, 3, 4, 6> (<2> rhomboids 1-4 are all dedicated to regulating EGF receptor signalling [6]; <2,4> the rhomboid active site in directly cleaves the membrane-anchored TGFα-like growth factor Spitz within its transmembarne domain [3]; <2> cleavage by rhomboid 4 [6]; <2> site-specific cleavage, the substrate Spitz is recognized by a small region of the Spitz transmembrane domain. This substrate motif is necessary and sufficient for cleavage [14]) (Reversibility: ?) [3, 4, 6, 14]
- **P** ?
- **S** Spitz-transmembrane domain + H_2O <2, 6, 8, 9, 16> (<2,16> little activity [8]) (Reversibility: ?) [8]
- **P** ?
- **S** adhesion protein from Toxoplasma gondii + H_2O <2, 4> (<2,4> MIC-2, MIC-6 and MIC-12 are efficient substrates [14]) (Reversibility: ?) [14]
- **P** ?
- **S** chimeric protein of the bacterial pelB leader peptide, GFP as the extracellularectodomain, the juxtamembrane-transmembrane-cytosolic residues 122-230 of Spitz and a C-terminal epitope + H_2O <1, 8> (Reversibility: ?) [16]
- **P** ?

S cytochrome c peroxidase precursor + H_2O <5, 18> (Reversibility: ?) [15, 19]
P cytochrome c peroxidase + ?
S dynamin-like GTPase + H_2O <5> (Reversibility: ?) [15]
P ?
S ephrin B3 + H_2O <4> (<4> RHBDL-2 mediated proteolytic processing may regulate intercellular interactions between ephrinB3 and eph receptors [1]; <4> cleaved efficiently, appears to be cleaved in its membrane domain [1]) (Reversibility: ?) [1]
P ?
S l-Mgm1 + H_2O <13> (<13> rhomboid-type protease Pcp1 is essential for wild type mitochondrial morphology. The processing of the large isoform l-Mgm1 by rhomboid-type protease Pcp1 to s-Mgm1, and the presence of both isoforms of Mgm1 appears to be crucial for wild-type mitochondrial morphology and maintenance of mitochondrial DNA [10]; <13> l-Mgm1 is the large isoform of Mgm1 [10]) (Reversibility: ?) [10]
P s-Mgm1 + N-terminal putative transmembrane segment (<13> s-Mgm1 is the small isoform of Mgm1 [10])
S large isoform of Mgm1 + H_2O <5> (<5> the enzyme is involved in the pathway of Mgm1 biogenesis. A strong shift in the ratio between both isoform of Mgm1 is sufficient to alter mitochondrial morphology [12]) (Reversibility: ?) [12]
P short isoform of Mgm1 + ?
S protein Gurken + H_2O <2> (<2> cleavage by rhomboid 1 [6]; <2> cleavage by rhomboid 2 [7]; <2> cleavage by rhomboid 3 [6]) (Reversibility: ?) [6, 7]
P ?
S protein Keren + H_2O <2> (<2> cleavage by rhomboid 1 [6]; <2> cleavage by rhomboid 2 [7]; <2> cleavage by rhomboid 3 [6]) (Reversibility: ?) [6, 7]
P ?
S protein Spitz + H_2O <2, 4> (<2> rhomboids 1-4 are all dedicated to regulating EGF receptor signalling [6,7]; <2> when Spitz is cleaved by rhomboids in the endoplasmic reticulum it cannot be secreted. Star regulates Spitz cleavage by rhomboid-1 by transporting Spitz to the Golgi apparatus. Rhomboids 1-4 are all dedicated to regulating EGF receptor signalling [6]; <2> cleavage by rhomboid 1 [6]; <2> cleavage by rhomboid 2 [7]; <2> cleavage by rhomboid 3 [6]; <4> UniProt Accession Code $QRHBDL_2$ cleaves the membrane domain of Drosophila protein Spitz, when the proteins are coexpressed in mammalian cells [1]) (Reversibility: ?) [1, 6, 7]
P ?
S Additional information <1, 2, 3, 4, 5, 7> (<5> intramolecular proteolysis by rhomboids controls cellular processes other than signalling [15]; <4> regulated intramembrane proteolysis in which the putative signaling moiety is part of the intramembrane-cleaving protease itself. Cytosolic N-terminal domain of PARL is cleaved at positions 52-53 (α-site) and 77-78 (β-site). Whereas α-cleavage is constitutive and removes the mitochon-

drial targeting sequence, β-cleavage appears to be developmentally controlled and dependent on PARL intramembrane-cleaving protease activity supplied in trans. The β-cleavage of PARL liberates Pβ, a nuclear targeted peptide whose sequence is conserved only in mammals. Thus, in addition to its evolutionarily conserved function in regulating mitochondrial dynamics, PARL might mediate a mammalian-specific, developmentally regulated mitochondria-to-nuclei signaling through regulated proteolysis of its N-terminus and release of the Pβ peptide [11]; <5> rhomboid protease Pcp1 catalyzes the second processing step of cytochrome c peroxidase, yielding the mature cytochrome c peroxidase protein [13]; <4> membrane domains of several mammalian EGF-faily proteins are not cleaved by RHBDL2, suggesting that the endogenous targets of the human protease are not EGF-related factors. Amino acid sequence at the luminal face of the membrane domain of a substrate protein determines whether it is cleaved by $RHBDL_2$ [1]; <2> no cleavage of EGFR, δ, TGN38 or TGFα [14]; <1,3> no cleavage of Gurken-transmembrane domain [8]; <7> no cleavage of Spitz anf Gurken [4]) (Reversibility: ?) [1, 4, 8, 11, 13, 14, 15]

P ?

Inhibitors

dichloroisocoumarin <1, 3, 8, 9> (<1,3,8,9> below 0.1 mM [16]) [16]
Additional information <1, 3, 8, 9> (<1,3,8,9> no inhibition by EDTA, *o*-phenanthroline, E64, PMSF, 4-(2-aminoethyl)benzenesulfonyl fluoride and pepstatin A [16]) [16]

Cofactors/prosthetic groups

Additional information <1, 3, 8, 9> (<1,3,8,9> enzyme does not require cofactors to catalyze intramembrane proteolysis [16]) [16]

Temperature optimum (°C)

25-37 <1> (<1> more active at 25°C than at 37°C [16]) [16]

Temperature range (°C)

25-37 <3> (<3> much more active az 37°C than at 25°C [16]) [16]

4 Enzyme Structure

Molecular weight

28000 <18> (<18> mature protein, monomer, SDS-PAGE [19]) [19]
33000 <18> (<18> precursor, monomer, SDS-PAGE [19]) [19]

Subunits

? <18> (<18> x * 28000, SDS-PAGE [19]) [19]
Additional information <1, 3> (<3> rhomboids do not function as a part of a multiprotein complex [8]; <1> rhomboids do not function as a partof a multiprotein complex [8]) [8]

5 Isolation/Preparation/Mutation/Application

Source/tissue

oocyte <10> (<10> TgROM2 [18]; <10> TgROM3 [17]) [17, 18]
skeletal muscle <4, 17> (<17> expression is reduced in diabetic Psammomys obesus, and restored after exercise training to successfully treat the diabetes [5]; <4> vastus lateralis. Expression of rhomboid protease PSARL is associated with mitochondrial oxidative capacity and insulin sensitivity [5]) [5]
tachyzoite <10> (<10> TgROM1 [17]; <10> TgROM4 is localized to the cell surface [17]; <10> TgROM5 is localized to the cell surface, primarily at the posterior of the parasite [17]) [17]

Localization

cell surface <10> (<10> TgROM4 is localized to the cell surface [17]; <10> TgROM5 is localized to the cell surface, primarily at the posterior of the parasite [17]) [17]
cytoplasmic membrane <3> (<3> membrane-embedded, traverses the membrane six times [2]) [2]
membrane <2, 4> (<2,4> intramembrane enzyme [3]) [3]
mitochondrial inner membrane <5> [15]
mitochondrion <4, 5, 13, 17, 18> (<5> inner membrane [15]; <4,17> intramembrane enzyme [5]) [5, 10, 15, 19]

Purification

<1> [8]
<3> [8]

Cloning

<4> (HEK 293 cells transfected with a FLAG-tagged PARL construct) [11]

Engineering

L262V <4> (<4> genetic variation is associated with insulin-resistance in an age-dependent manner [5]) [5]

Application

medicine <4, 10> (<10> TgROM5 is able to cleave MIC adhesins, it likely provides the key protease activity necessary for invasion. The enzyme TgROM5 offers a target for therapeutic intervention against invasion by the deadly pathogen Toxoplasma gondii [17]; <4> variation in rhomboid protease PSARL sequence and/or expression may be an important new risk factor for type 2 diabetes and other components of the metabolic syndrome [5]) [5, 17]

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Hepsin 3.4.21.106

1 Nomenclature

EC number
3.4.21.106

Recommended name
hepsin

Synonyms
S01.224 <4> (<4> Merops ID [2]) [2]
transmembrane serine protease 1 <6> [10]

CAS registry number
112398-23-9

2 Source Organism

<1> *Cricetulus griseus* (no sequence specified) [7]
<2> *Mus musculus* (no sequence specified) [8, 13]
<3> *Homo sapiens* (no sequence specified) [1, 3, 4, 5, 6, 7, 9, 11, 12, 15]
<4> *Rattus norvegicus* (no sequence specified) [2]
<5> *Papio ursinus* (no sequence specified) [3,5,7]
<6> *Homo sapiens* (UNIPROT accession number: P05981) [10,14,16]

3 Reaction and Specificity

Catalyzed reaction
cleavage after basic amino-acid residues, with Arg strongly preferred to Lys

Reaction type
cleavage of C-N-linkage
hydrolysis of peptide bond

Natural substrates and products
S protein + H_2O <1, 2, 3, 4, 5> (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9]
P ? <1, 2, 3, 4, 5> [1, 2, 3, 4, 5, 6, 7, 8, 9]
S hepatocyte growth factor precursor + H_2O <3> (<3> potential substrate for hepsin in vivo [11]) (Reversibility: ?) [11]
P ?
S Additional information <1, 3, 5> (<1,3,5> biological role in cell growth [7]; <3> hepsin reduces cell growth, cell invation and soft agar colony

formation after expression in PC-3, LNCaP and DU145 cells [4]) (Reversibility: ?) [4, 7]
P ? <1, 3, 5> [4, 7]

Substrates and products

S Glu-Pro-Arg-4-nitroanilide + H_2O <3> (Reversibility: ?) [9]
P ? <3> [9]
S N-benzoyl-Ile-Glu-Phe-Ser-Arg-4-nitroanilide + H_2O <1, 3, 5> (Reversibility: ?) [7]
P ? <1, 3, 5> [7]
S N-benzoyl-Leu-Ser-Arg-4-nitroanilide + H_2O <1, 2, 3, 5> (Reversibility: ?) [7, 8]
P ? <1, 2, 3, 5> [7, 8]
S N-benzoyl-Phe-Val-Arg-4-nitroanilide + H_2O <1, 3, 5> (Reversibility: ?) [7]
P ? <1, 3, 5> [7]
S N-benzyloxycarbonyl-Ala-Arg-Arg 4-methylcoumarin 7-amide + H_2O <4> (<4> weak substrate [2]) (Reversibility: ?) [2]
P N-benzyloxycarbonyl-Ala-Arg-Arg + 7-amino-4-methylcoumarin <4> [2]
S N-tert-butyloxycarbonyl-Gly-Lys-Arg 4-methylcoumarin 7-amide + H_2O <4> (<4> weak substrate [2]) (Reversibility: ?) [2]
P N-tert-butyloxycarbonyl-Gly-Lys-Arg + 7-amino-4-methylcoumarin <4> [2]
S protein + H_2O <1, 2, 3, 4, 5> (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9]
P ? <1, 2, 3, 4, 5> [1, 2, 3, 4, 5, 6, 7, 8, 9]
S S2366 + H_2O <6> (Reversibility: ?) [14]
P ?
S acetyl-DQLR-7-amido-4-methylcoumarin + H_2O <3> (Reversibility: ?) [11]
P ?
S acetyl-KKTR-7-amido-4-methylcoumarin + H_2O <3> (Reversibility: ?) [11]
P ?
S acetyl-KQLR-7-amido-4-methylcoumarin + H_2O <3> (<3> most active tetrapeptide substrate [11]) (Reversibility: ?) [11]
P ?
S acetyl-PVDR-7-amido-4-methylcoumarin + H_2O <3> (<3> least active tetrapeptide substrate [11]) (Reversibility: ?) [11]
P ?
S factor X + H_2O <3> (<3> activates factor X in the presence of factor VII [6]) (Reversibility: ?) [6]
P ? <3> [6]
S hepatocyte growth factor precursor + H_2O <3> (<3> potential substrate for hepsin in vivo [11]) (Reversibility: ?) [11]
P ?

S single-chain hepatocyte growth factor + H_2O <6> (<6> efficiently converted by soluble form of hepsin comprising the entire extracellular domain [14]) (Reversibility: ?) [14]
P two-chain hepatocyte growth factor
S tert-butyloxycarbonyl-Val-Pro-Arg 4-methylcoumarin 7-amide + H_2O <4> (<4> weak substrate [2]) (Reversibility: ?) [2]
P tert-butyloxycarbonyl-Val-Pro-Arg + 7-amino-4-methylcoumarin <4> [2]
S tert-butyloxycarbonyl-Gln-Ala-Arg 4-methylcoumarin 7-amide + H_2O <4> (<4> weak substrate [2]) (Reversibility: ?) [2]
P tert-butyloxycarbonyl-Gln-Ala-Arg + 7-amino-4-methylcoumarin <4> [2]
S tert-butyloxycarbonyl-Gln-Arg-Arg 4-methylcoumarin 7-amide + H_2O <4> (Reversibility: ?) [2]
P tert-butyloxycarbonyl-Gln-Arg-Arg + 7-amino-4-methylcoumarin <4> [2]
S zymogen factor VII + H_2O <3> (<3> cleaves between Arg152 and Ile153 [6]) (Reversibility: ?) [6]
P factor VIIa <3> [6]
S Additional information <1, 3, 4, 5> (<4> cleaves after basic amino acid residues, Arg being preferable to Lys [2]; <3> enzyme does not cleave factor VII R152E mutant [6]; <1,3,5> biological role in cell growth [7]; <3> hepsin reduces cell growth, cell invation and soft agar colony formation after expression in PC-3, LNCaP and DU145 cells [4]; <3> P1-P4 substrate specifity, hepsin exhibits strong preference at the P1 position for arginine over lysine, favours theonine, leucine or asparagine at the P2, glutamine or lysine at the P3, and proline or lysine at the P4 position [11]) (Reversibility: ?) [2, 4, 6, 7, 11]
P ? <1, 3, 4, 5> [2, 4, 6, 7]

Inhibitors

3,4-dichloroisocoumarin <4> (<4> 94% inhibition at 0.1 mM [2]) [2]
4-amidinophenylmethylsulfonyl fluoride <3> (<3> complete inhibition at 1 mM [6]) [6]
antithrombin III <3> (<3> 76% inhibition at 0.003 mM, 1U/ml heparin enhances inhibition [6]) [6]
aprotinin <3> (<3> 90% inhibition at 0.008 mM [6]) [6]
diisopropylfluorophosphate <4> (<4> complete inhibition at 5 mM [2]) [2]
leupeptin <4> (<4> 99% inhibition at 0.1 mM [2]) [2]
phenylmethanesulfonyl fluoride <4> (<4> 80% inhibition at 1 mM [2]) [2]
$ZnSO_4$ <4> (<4> 26% inhibition at 1 mM [2]) [2]
hepatocyte growth factor activator inhibitor-1 <3> (<3> potent inhibitor of hepsin activity [11]) [11]
hepatocyte growth factor activator inhibitor-1B <6> (<6> potent inhibitor of hepsin [14]) [14]
hepatocyte growth factor activator inhibitor-2 <3, 6> (<6> potent inhibitor of hepsin [14]; <3> potent inhibitor of hepsin activity [11]) [11, 14]
Additional information <3> (<3> no inhibition by soybean trypsin inhibitor and tissue factor pathway inhibitor [6]) [6]

Activating compounds
pepstatin A <4> (<4> 21% activation at 0.1 mM [2]) [2]

Metals, ions
Ca^{2+} <3> (<3> optimal concentration: 5 mM [6]) [6]

Specific activity (U/mg)
10.67 <4> [2]

K_i-Value (mM)
0.001 <6> (hepatocyte growth factor activator inhibitor-1B, <6> completely inhibits [14]) [14]
0.001 <6> (hepatocyte growth factor activator inhibitor-2, <6> completely inhibits [14]) [14]

pH-Optimum
7-8 <3> [6]

pH-Range
6-9 <4> (<4> activity with in the range pH 6-9 has no maximum, the activity increases continuously [2]) [2]

4 Enzyme Structure

Molecular weight
31000 <2> (<2> Western blot analysis, heavy chain of proteolytically processed hepsin [13]) [13]
35000 <3> (<3> amino sequence analysis in both non-reduction and reduction conditions of non-transmembrane isoform [12]) [12]
44000 <3> (<3> SDS-PAGE, enzyme after cell-free translation assay [5]) [5]
45000 <3> (<3> SDS-PAGE, enzyme derived from cell free translation assays [3]; <3> Western blot analysis, SDS-PAGE, transmembrane isoform expressed in HEK-293T cells, amino sequence analysis of transmembrane isoform [12]) [3, 12]
50000 <4> (<4> zymogen [2]) [2]
51000 <1, 3> (<3> SDS-PAGE [3]; <3> mature protein, SDS-PAGE [5]) [3, 5, 7]
75000 <3> (<3> Western blot analysis, SDS-PAGE, non-transmembrane isoform expressed in HEK-293T cells [12]) [12]

Subunits
? <3> (<3> x * 51000 + x * 28000 [7]) [7]
dimer <4> (<4> 1 * 31000 + 1 * 19000, SDS-PAGE under reducing conditions [2]) [2]

Posttranslational modification
glycoprotein <3> (<3> larger size of enzyme in cell extracts as compared to cell-free translation assay may be due to glycosylation, possible site for N-linked carbohydrate chain attachment is at amino acid 112 [3,5]) [3, 5]

proteolytic modification <3, 4> (<3> gene contains a cleavage site for protease activation [1]; <4> zymogen is converted to the active protease by cleavage between Arg161 and Ile162 [2]) [1, 2]

5 Isolation/Preparation/Mutation/Application

Source/tissue

BG-1 cell <3> (<3> strongest transformation on anchorage-independent cell growth [15]) [15]
BHK cell <1, 3> [3, 7]
CACO-2 cell <3> (<3> non-transmembrane isoform expressed at highest level among investigated cell lines, transmembrane isoform hardly detectable [12]) [12]
CAOV-3 cell <3> [15]
HEK-293T cell <3> (<3> two isoforms expressed with different molecular weight [12]) [12]
HeLa cell <3> [12]
Hep-G2 cell <3, 6> [1, 3, 7, 10]
LNCaP cell <3> (<3> used as positive control [15]) [4, 15]
LS174T cell <3> (<3> non-transmembrane and transmembrane isoform highly expressed [12]) [12]
OVCAR-3 cell <3> [15]
PLC/PRF/5 cell <2> [8]
SKOV-3 cell <3> [11]
SW-403 cell <3> (<3> non-transmembrane and transmembrane isoform highly expressed [12]) [12]
adrenal gland <5> (<5> low level [5]) [5]
brain <3> (<3> non-transmembrane isoform highly expressed, whereas transmembrane hepsin is expressed at a relatively lower level [12]) [12]
colon <2> [8]
colonic adenocarcinoma cell line <3> [12]
embryo <2> [8]
epithelial cell <3> [15]
epithelium <2> [8]
heart <3> (<3> transmembrane isoform only [12]) [12]
kidney <3, 5, 6> (<3> non-transmembrane and transmembrane isoform [12]) [3, 5, 12, 16]
liver <3, 4, 5> (<5> highest level [3,5]; <3> transmembrane isoform only [12]) [1, 2, 3, 5, 7, 12]
liver cancer cell <6> [10]
liver cell line <6> [10]
lung <3, 5> (<3> non-transmembrane and transmembrane isoform [12]) [3, 5, 12]
ovary cancer cell <3> [11]
ovary cancer cell line <3> [15]
pancreas <5> [3, 5]

pituitary gland <5> [3, 5]
prostate cancer cell line <3> (<3> used as positive control [15]) [15]
prostate epithelium <2> (<2> transgenic animals [13]) [13]
renal cell carcinoma cell <6> [16]
skeletal muscle <3> (<3> transmembrane isoform only [12]) [12]
small intestine <5> (<5> low level [5]) [5]
spleen <3, 5> (<5> low level [5]; <3> transmembrane isoform only [12]) [5, 12]
testis <5> [3, 5]
thymus <3, 5> (<5> low level [5]; <3> transmembrane isoform only [12]) [5, 12]
thyroid <5> [3, 5]
Additional information <2, 3, 5, 6> (<5> ubiquitous expression [3]; <3> enzyme is present in most tissues, highest level in liver [5]; <5> hepsin is present in most tissues, at a particularly high level in liver [7]; <2> hepsin presence increases significantly during embryo development in intensity and in the number of tissues [8]; <3> no activity in SV40 immortalized neonatal epithelial 267B1 cells, DU-145 cells, PC_3 cells and SV40 immortalized normal prostate cells [4]; <3> both isoforms hardly detectable in SW-1417 cells, Colo-201 cells, Colo-205 cells, SW-1222 cells, LS-180 cells, HT-29 cell and LoVo cells [12]; <6> Hep-G2.2.1.5 cell and L-02 cell [10]) [3, 4, 5, 7, 8, 10, 12]

Localization

cell membrane <1> [7]
cell surface <3> [3]
cytoplasm <3> (<3> non-transmembrane isoform [12]) [12]
membrane <3, 4> (<3> transmembrane isoform [12]) [2, 6, 12]
microsome <4> [2]
plasma membrane <3, 5> [3, 5, 7, 9]

Purification

<3> [9]
<3> (by gel filtration) [11]
<3> (gel filtration) [12]
<4> (homogeneity) [2]
<6> (by Ni-affinity chromatography) [14]

Crystallization

<3> (by sitting-drop method) [11]
<3> (extracellular portion of hepsin, starting at residue 46 and continuing to the end of the coding region, hanging drop vapor diffusion method) [9]

Cloning

<2> (cloned into the SpeI and EcoRI sites of the PB-IRES-hrGFP vector) [13]
<3> [1, 4, 7]
<3> (expressed in Pichia pastoris) [11]
<3> (fusion protein with maltose-binding protein) [6]
<3> (transmembrane isoform and non-transmembrane isoform cloned into vector pcDNA3/Myc-His-(-C), both isoforms expressed in BHK-21 cells, non-transmembrane isoform expressed in bacteria strain DE3, overexpression of myc-tagged non-transmembrane hepsin in HEK-293 cells) [12]

<6> (cDNA of full lenth hepsin inserted into vector pRK5E, His-tagged hepsin cDNA inserted into vector pCMV.PD5, expression in a chinese hamster ovary expression system) [14]
<6> (cloned into vector pCMV-HA and vector pCMV-flag) [10]

Application

medicine <2, 3, 6> (<3> expression of the non-transmembrane isoform in vivo does not exert any apparent inhibitory effect on cell growth [12]; <3> hepsin expression in tumours is dysregulated and may influence tumorigenesis through inappropiate activation and/or regulation of hepatocyte growth factor receptor functions [11]; <6> hepsin is a potent activator of pro-hepatocyte growth factor, thus contributing to tumor progression [14]; <6> hepsin is significantly decreased in pT2 or greater tumor samples, while expression does not differ between the normal and cancerous tissue of pT1 tumors, survival in patients bearing tumors with hepsin expression below the 10th percentile expression of hepsin in noncancerous tissue is poorer than that in patients with tumors with expression above that cutoff, the level of hepsin is found to be a significant predictor of death from renal cell carcinoma [16]; <3> hepsin significantly inhibits cell growth in the monolayer, anchorage-independent cell growth in soft agar in vitro, and tumorigenicity in vivo in ovarian cancer cell lines, through up-regulation of p53-dependent apoptosis and caspase-3, -6, and -7 activations [15]; <6> human hepsin interacts directly with X protein of human hepatitis B virus both in vitro and in vivo, their interaction appears to play a role in both promoting cell proliferation and blocking apoptosis in human liver tumor cell and normal liver cell lines, promotes expression of viral protein HBeAg in Hep-G2.2.1.5 cells, thus stimulating viral replication [10]; <2> overexpression of hepsin in a mouse model of nonmetastasizing prostate cancer has no impact on cell proliferation, but causes disorganization of the basement membrane and promotes primary prostate cancer progression and metastasis to liver, lung and bones [13]) [10, 11, 12, 13, 14, 15, 16]

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Peptidase Do 3.4.21.107

1 Nomenclature

EC number
3.4.21.107

Recommended name
peptidase Do

Synonyms
DegP <4, 6, 8, 9, 12> [1, 7, 9, 10, 16, 17, 18, 19, 21, 25, 28, 30]
DegP protease <4> (<4> classification of proteases [4]) [4]
Do protease <4> [18]
HtrA <4, 5, 9> (<4> classification of proteases [16]) [1, 3, 4, 8, 12, 14, 15, 16, 17, 18, 24, 27]
HtrA (DegP) protease <4> [27]
HtrA heat shock protease <4> [11]
HtrA protease <4, 7, 10, 13> [5, 23, 29]
HtrA/DegP <4> [29]
HtrA1 <3, 5> [26, 31]
HtrA2 <1, 2, 11> [18, 22]
S01.273 <4> (<4> Merops ID [9]) [9]
high temperature requirement factor A <3> [26]
protease do <4> [24]
serine protease <7> [29]
serine protease HtrA <8> [28]

CAS registry number
161108-11-8

2 Source Organism

<1> *Salmonella typhimurium* (no sequence specified) [18]
<2> *Mammalia* (no sequence specified) [22]
<3> *Mus musculus* (no sequence specified) [26]
<4> *Escherichia coli* (no sequence specified) (<4> α-subunit of Fdh3 [4]) [2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 27, 29, 30]
<5> *Homo sapiens* (no sequence specified) [16,31]
<6> *Arabidopsis thaliana* (no sequence specified) [16]
<7> *Streptococcus pneumoniae* (no sequence specified) [29]

<8> *Streptococcus pyogenes* (no sequence specified) [28]
<9> *Thermotoga maritima* (no sequence specified) [1,14]
<10> *Streptococcus mutans* (no sequence specified) [5]
<11> *Yersinia enterocolitica* (no sequence specified) [18]
<12> *Rickettsia sp.* (no sequence specified) [16]
<13> *Campylobacter jejuni* (no sequence specified) [23]
<14> *Lactococcus lactis* (UNIPROT accession number: Q9LA06) [20]

3 Reaction and Specificity

Catalyzed reaction

acts on substrates that are at least partially unfolded. The cleavage site P1 residue is normally between a pair of hydrophobic residues, such as Val-/-Val

Reaction type

cleavage of C-N-linkage
hydrolysis of peptide bond

Natural substrates and products

S protein + H_2O <1, 2, 4, 5, 6, 9, 10, 11, 12, 14> (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]

P ? <1, 2, 4, 5, 6, 9, 10, 11, 12, 14> [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]

S Additional information <1, 2, 4, 5, 6, 9, 10, 11, 14> (<11> deletion mutant is unable to grow at an elevated temperature and to survive within macrophages after phagocytosis [18]; <4> heat shock serine protease that degrades misfolded proteins at high temperatures [19]; <4, 6, 9, 10> involved in the degradation of damaged proteins [2, 3, 4, 5, 7, 8, 10, 13, 14, 16, 21, 22]; <2> involved in the degradation of damaged proteins, acts as protease, chaperone and regulator of apoptosis [22]; <9> involved in the degradation of damaged proteins, chaperone and proteolytic activity [1]; <4> involved in the degradation of damaged proteins, enzyme is indispensable for bacterial survival at elevated temperatures [6]; <4> involved in the degradation of damaged proteins, enzyme is indispensable for bacterial survival at temperatures above 42°C [11, 12]; <5> involved in the degradation of damaged proteins, involved in arthritis, cell growth, stress response, apoptosis and aging, possible tumor suppressor function [16]; <4> involved in the degradation of damaged proteins, participate in removal of aggregated proteins [17]; <4> involved in the degradation of damaged proteins, switches from chaperone to protease function in a temperature-dependent manner [16]; <4> involved in the degradation of denatured and unfolded proteins [9]; <4> involved in the degradation of misfolded proteins [18]; <4> involved in the degradation of unfolded proteins [15]; <1> mutants are deficient in their ability to survive in mice or macrophages [18]; <14> role in extracellular proteolysis, proteolysis occurs during or after export to the cell surface, involved in the degradation

of abnormal exported proteins [20]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]

P ? <1, 2, 4, 5, 6, 9, 10, 11, 14> [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]

Substrates and products

S Arc repressor + H_2O <4> (Reversibility: ?) [18]

P ? <4> [18]

S bovine serum albumin + H_2O <4> (<4> denatured [2]) (Reversibility: ?) [2]

P ? <4> [2]

S casein + H_2O <4> (<4> major pilin subunit of the Pap pilus [10]; <4> partially unfolded casein [15]) (Reversibility: ?) [2, 10, 15, 19]

P ? <4> [2, 10, 15, 19]

S D1 protein + H_2O <6> (<6> degrades photodamaged D1 protein of photosystem II [16]) (Reversibility: ?) [16]

P ? <6> [16]

S globin + H_2O <4> (Reversibility: ?) [2]

P ? <4> [2]

S HYTAVVKKSSAV + H_2O <4> (<4> model substrate [10]) (Reversibility: ?) [10]

P ? <4> [10]

S IciA protein + H_2O <4> (<4> inhibitor of DNA replication initiation [18]) (Reversibility: ?) [18]

P ? <4> [18]

S lysozyme + H_2O <4> (<4> can only be digested in the presence of reducing agents [3]) (Reversibility: ?) [3]

P ? <4> [3]

S PapA + H_2O <4> (<4> major pilin subunit of the Pap pilus [10]) (Reversibility: ?) [10]

P ? <4> [10]

S protein + H_2O <1, 2, 4, 5, 6, 9, 10, 11, 12, 14> (<4> cleaves between paired valine residues [10]; <4> cleaves model substrates at discrete Val/Xaa or Ile/Xaa sites [9]; <4> cleaves preferably at hydrophobic side chains at the P1 position [16]; <4> denatured proteins aggregate to form a distinct S fraction, one third of the isolated S fraction is converted to trichloroacetic acid-soluble products [17]; <4> denatured proteins aggregate to form a distinct S fraction, one third of the isolated S fraction is converted to trichloroacetic acid-soluble products, enzyme has a preference for valine or isoleucine as the residue preceding the cleavage site [18]; <4> enzyme recognizes an ssrA-encoded peptide tag which is tagged to misfolded proteins or protein fragments [13]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]

P ? <1, 2, 4, 5, 6, 9, 10, 11, 12, 14> [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]

S RseA + H_2O <4> (<4> physiological substrate of DegP [30]) (Reversibility: ?) [30]

P ?
S Staphylococcus aureus nuclease Nuc precursor + H_2O <14> (Reversibility: ?) [20]
P ? <14> [20]
S α-casein + H_2O <4> (<4> weaker substrate than β-casein [8]) (Reversibility: ?) [8]
P ? <4> [8]
S α-lactalbumin + H_2O <4, 9> (<4> acts on the fully unfolded protein but not on the native form [15]) (Reversibility: ?) [14, 15]
P ? <4, 9> [14, 15]
S amylase MalS + H_2O <12> (Reversibility: ?) [16]
P ? <12> [16]
S autolysin AcmA + H_2O <14> (Reversibility: ?) [20]
P ? <14> [20]
S β-casein + H20 <4> (<4> nonphysiological substrate of DegP [30]) (Reversibility: ?) [30]
P ?
S β-casein + H_2O <4, 7> (<4> better substrate than α-casein [8]; <4> cleaves β-casein yielding several polypeptide fragments [16]) (Reversibility: ?) [3, 6, 8, 11, 12, 16, 17, 18, 29]
P ? <4> [3, 6, 8, 11, 12, 16, 17, 18]
S citrate synthase + H_2O <4> (<4> acts on the thermally unfolded synthase but not on the native form [15]) (Reversibility: ?) [15]
P ? <4> [15]
S colicin A lysis protein + H_2O <4> (<4> i.e. pCal, hydrolyses the acylated precursor form, cleaves at two sites near the C-terminal end to give two truncated proteins which are matured into two truncated Cals [4]) (Reversibility: ?) [4]
P ? <4> [4]
S colicin A lysis protein precursor + H_2O <4> (Reversibility: ?) [18]
P ? <4> [18]
S insulin β-chain + H_2O <4> (<4> oxidized β-chain which is fully unfolded [15]) (Reversibility: ?) [15]
P ? <4> [15]
S λ repressor + H_2O <4> (<4> N-terminal domain [18]) (Reversibility: ?) [18]
P ? <4> [18]
S malate dehydrogenase + H_2O <4> (<4> acts on the thermally unfolded protein but not on the native form [15]) (Reversibility: ?) [15]
P ? <4> [15]
S Additional information <1, 2, 4, 5, 6, 9, 10, 11, 14> (<4> acts on substrates that are at least partially unfolded, does not cleave stably folded proteins, acts as a general chaperone forming stable complexes with several misfolded proteins [16]; <4> no substrates are: bovine serum albumin, ovalbumin, globin, insulin and other peptides that are routinely used as protease substrates [8]; <4> no substrates are: native bovine serum albumin, insulin, growth hormone or a variety of commonly used peptide

substrates [2]; <11> deletion mutant is unable to grow at an elevated temperature and to survive within macrophages after phagocytosis [18]; <4> heat shock serine protease that degrades misfolded proteins at high temperatures [19]; <4,6,9,10> involved in the degradation of damaged proteins [2,3,4,5,7,8,10,13,14,16,21,22]; <2> involved in the degradation of damaged proteins, acts as protease, chaperone and regulator of apoptosis [22]; <9> involved in the degradation of damaged proteins, chaperone and proteolytic activity [1]; <4> involved in the degradation of damaged proteins, enzyme is indispensable for bacterial survival at elevated temperatures [6]; <4> involved in the degradation of damaged proteins, enzyme is indispensable for bacterial survival at temperatures above 42°C [11,12]; <5> involved in the degradation of damaged proteins, involved in arthritis, cell growth, stress response, apoptosis and aging, possible tumor suppressor function [16]; <4> involved in the degradation of damaged proteins, participate in removal of aggregated proteins [17]; <4> involved in the degradation of damaged proteins, switches from chaperone to protease function in a temperature-dependent manner [16]; <4> involved in the degradation of denatured and unfolded proteins [9]; <4> involved in the degradation of misfolded proteins [18]; <4> involved in the degradation of unfolded proteins [15]; <1> mutants are deficient in their ability to survive in mice or macrophages [18]; <14> role in extracellular proteolysis, proteolysis occurs during or after export to the cell surface, involved in the degradation of abnormal exported proteins [20]; <4> unable to cleave inhibitor of apoptosis protein [30]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 30]

P ? <1, 2, 4, 5, 6, 9, 10, 11, 14> [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]

Inhibitors

Cardiolipin <4> [12]
diisopropyl fluorophosphate <4> (<4> binds covalently to the enzyme [2]) [2]
diisopropylfluorophosphate <4> (<4> efficient inhibitor [8]) [8, 16]
DnaJ <4> (<4> chaperone protein [17]) [17]
EDTA <4> (<4> inhibits at 10 mM [10]) [10]
phosphatidylglycerol <4> (<4> inhibits activity at 50-55°C [12]) [12]
diisopropyl fluorophosphates <4> [11]
Additional information <7, 13> (<7> rHtrA-mediated decline in transformation efficiency can not be corrected with excess competence-stimulating peptide [29]; <13> wild type and HtrA mutant are not sensitive to H_2O_2, cumene hydroperoxide and paraquat, moreover the wild type is not sensitive to oxygen while the mutant has a reduced oxygen tolerance [23]) [23, 29]

Cofactors/prosthetic groups

Additional information <4> (<4> largely indpependent of cofactors such as ATP [16]) [16]

Activating compounds

15 residue presenilin-1 peptide <4> (<4> activation [30]) [30]
IPTG <7> [29]
OMP C-terminal tripeptide YYF-COOH <4> (<4> activation less efficient as compared to 15 residue presenilin-1 peptide [30]) [30]
phosphatidylglycerol <4> (<4> activates at 37-45°C [12]) [12]
puromycin <13> (<13> increases level of misfolded proteins, HtrA is required for growth under conditions in which misfolded proteins accumulate [23]) [23]
Additional information <4> (<4> largely indpependent of activating compounds such as reducing agents [16]; <4> low osmolarity conditions result in HtrA repression together with the nucleoid associated proteins H-NS and HhA [27]; <4> no activation by 3 residue presenelin-1 peptide [30]) [16, 27, 30]

Metals, ions

$CaCl_2$ <4> (<4> stimulates at 5 mM [10]) [10]
Mg^{2+} <4> (<4> stimulates at 10 mM [17]) [17, 18]
$MgCl_2$ <4> (<4> stimulates at 5 mM [10]) [10]
$MnCl_2$ <4> (<4> stimulates at 5 mM [10]) [10]
Additional information <4> (<4> largely independent of divalent cations [16]) [16]

pH-Range

4.8-10 <4> (<4> proteolytic activity is largely independent of the pH [8]) [8]

Temperature optimum (°C)

55 <4> [11, 15, 18]
85 <9> [14]

Temperature range (°C)

20-30 <4> (<4> almost no activity below 20°C, activity rapidly increases above 30°C [16]) [16]
30-40 <8> [28]
30-42 <4> (<4> enzyme activity is constant within this temperature range [4]) [4]
37-44 <13> (<13> the HtrA mutant forms colonies with the same frequency as the wild type at 37°C and 42°C, the ability of the mutant to form colonies at 44°C was greatly reduced as compared to the wild type [23]) [23]
37-45 <4> (<4> enzyme is more efficient at 45°C than at 37°C [17]; <4> no change of cell density of wild type, cell density of the mutant rapidly falls, protease Do essential for the cell's survival at high temperatures [24]) [17, 24]
37-55 <4> (<4> activity rapidly increases with temperature [11]) [11]
44 <4> (<4> at 44°C function of DegP in mutant strain CLC198 can be complemented by HtrA2 [25]) [25]

4 Enzyme Structure

Molecular weight

26000 <9> (<9> SDS-PAGE, protease domain [1]) [1]
42000 <3> (<3> Western blot analysis, processed protein [26]) [26]
45000 <7> (<7> SDS-PAGE with coomassie staining [29]) [29]
48000 <4> (<4> SDS-PAGE [8]) [8]
50000 <3, 4> (<4> SDS-PAGE [7]; <3> Western blot analysis, native protein [26]) [7, 26]
51000 <4> (<4> precursor protein, SDS-PAGE [6]) [6]
200000 <4> (<4> above, gel filtration [3]) [3]
274000 <4> (<4> sedimentation analysis [19]) [19]
281000 <4> (<4> hexameric form, gel filtration [19]) [19]
300000 <4> (<4> gel filtration, 2 forms: 300000 Da and 500000 Da [2]; <4> gel filtration, hexameric form [9]) [2, 9]
328000 <4> (<4> heptameric form, gel filtration [19]) [19]
500000 <4> (<4> gel filtration, 2 forms: 300000 Da and 500000 Da [2]) [2]

Subunits

decamer <4> (<4> 10 * 54000, SDS-PAGE, enzyme exists in 3 different forms: pentamer, hexamer and decamer [2]) [2]
dodecamer <4> (<4> 12 * 50000, SDS-PAGE, enzyme can exist in two oligomeric forms which are interconvertible [9]; <4> consists of two stacks of hexameric rings, SDS-PAGE, cross-linking experiments [15]) [9, 15]
heptamer <4> (<4> 7 * 46000, mass spectroscopy [19]) [19]
hexamer <4, 9> (<4> 6 * 44000, gel filtration, SDS-PAGE [3]; <4> 6 * 46000, mass spectroscopy [19]; <4> 6 * 50000, SDS-PAGE, enzyme can exist in two oligomeric forms which are interconvertible [9]; <4> 6 * 54000, SDS-PAGE, enzyme exists in 3 different forms: pentamer, hexamer and decamer [2]; <4> formed by staggered association of trimeric rings [21]; <4> two trimeric rings for a functional DegP hexamer [16]; <4> two trimers for a hexameric structure by staggered association [22]; <9> two trimers form a hexamer, crystallization experiments [14]) [2, 3, 9, 14, 16, 19, 21, 22]
oligomer <4> (<4> largest complexes are dodecamers, probably formed by dimerization of trimers, gel filtration experiments [18]) [18]
pentamer <4> (<4> 5 * 54000, SDS-PAGE, enzyme exists in 3 different forms: pentamer, hexamer and decamer [2]) [2]
trimer <2> (<2> does not form hexamers like the Escherichia coli protein [22]) [22]

Posttranslational modification

proteolytic modification <4> (<4> enzyme is derived by cleavage of the first 26 amino acids of the pre-HtrA precursor polypeptide [8]) [8]

5 Isolation/Preparation/Mutation/Application

Source/tissue

colon <5> (<5> alternative splice form lacing exons 3 and 7 [16]) [16]
decidua <3> (<3> high level of enzyme expression in decidua capsularis specifically at the decidual-trophoblast interface where active involution occurs [26]) [26]
embryo <3> [26]
fibroblast <5> [16]
kidney <5> (<5> alternative splice form lacing exons 3 and 7 [16]) [16]
placenta <3, 5> (<5> first and third trimester (5-14 weeks and 28-40 weeks respectively) [31]) [26, 31]
thyroid <5> (<5> alternative splice form lacing exons 3 and 7 [16]) [16]
trophoblast <3> (<3> HtrA1 especially expressed in giant cells during the early stages of placental development [26]) [26]
uterus <3> [26]

Localization

cell surface <7> [29]
chloroplast <6> [16]
cytoplasm <4, 5> (<5> especially close to microvilli that characterizes the plasma membrane of syncytiotrophoblast cells, or in the extracytoplasmic space of the stroma of placental villi between the collagen fibers and on collagen fibers themselves [31]) [2, 31]
inner membrane <4> (<4> periplasmatic side of the membrane [11]; <4> periplasmic side of the inner membrane [18]) [11, 18]
mitochondrion <2> [22]
periplasm <4, 12, 13> [9, 15, 16, 18, 19, 23, 27]

Purification

<4> [3, 9, 11, 13, 15, 19, 21, 24]
<4> (90% purity) [8]
<4> (98% pure) [10]
<4> (near homogeneity) [2]
<4> (wild-type, S210A and H105R mutant) [6]
<4> (wild-type, htrA22 and htrA63 mutant) [17]
<7> (by Ni affinity chromatography) [29]
<8> [28]
<9> (95% pure) [1]

Crystallization

<4> (vapor diffusion method) [21]
<9> (hanging drop vapor diffusion method) [1]
<9> (protease domain) [14]

Cloning

<4> [6, 7, 8, 13]
<4> (cloned into a Bluescript plasmid and overexpressed in Escherichia coli DH1, disrupted gene transformed to Escherichia coli Jc7623) [24]

<4> (cloned into pET28a) [30]
<4> (truncated variant lacking both PDZ domains) [19]
<7> (expression in Escherichia coli as an IPTG-inducible, recombinant protein having both N-terminal and C-terminal His6-tags) [29]
<8> (expression in Escherichia coli strain DH5 α) [28]
<9> (protease domain) [1]
<10> [5]
<11> [18]
<13> (expression in Escherichia coli DH5α, BL20 and BL78) [23]
<14> [20]

Engineering

C57S/C69S <4> (<4> mutant enzyme is less stable, in contrast to wild-type enzyme the mutant protein is autocleaved even without reducing agents [3]) [3]
H105R <4> (<4> loss of protease activity, no change in secondary structure [6,11,18]) [6, 11, 18]
K455A <4> (<4> proteolytically inactive [13]) [13]
S210A <4> (<4> no proteolytic activity [19]; <4> loss of protease activity, no change in secondary structure [6,11,18]; <4> proteolytically inactive [3,21]) [3, 6, 11, 18, 19, 21]
S234A <7> (<7> mutant P1386, release of competence inhibition when mutant P1388 is present alone [29]) [29]
T230P <7> (<7> mutant P1388 with ciaH mutation [29]) [29]
Additional information <4, 7, 8, 13> (<4> degP null mutant strain CLC198 [25]; <4> disruption of the ptd gende to obtain lack of activity of protease Do, mutant with prolonged lag period, reduced ability to degrade cell proteins and unable to survive at high temperatures [24]; <4> Escherichia coli HtrA mutant [23]; <8> mutant HTR10 with in-frame deletion in htrA, mutant HTR10 is unable to efficiently process the protease proprotein, shows a dramatic increase in hemolytic activity and is virulent in a murine model of subcutaneous infection, mutant HSC11 with a deletion control strain that is a sibling of mutant HTR10 but contains wild-type htrA [28]; <7> mutant P1149 containing an insertion deletion mutation in the caiRH locus resulting in substantially lower HtrA expression [29]; <13> mutant strain NCTC11168δhtrA cat, main part of the HtrA gene replaced [23]) [23, 24, 25, 28, 29]

Application

biotechnology <4> (<4> htrA mutants show improved expression of envelope-associated proteins [18]) [18]
medicine <1, 3, 4, 5, 13> (<1> aroC aroD HtrA mutant shows promise as a typhoid vaccine in humans [18]; <4> htrA mutants can act as vaccines [18]; <13> HtrA is important both for adherence to and invasion of human INT407 epithelial cells [23]; <3> HtrA1 is developmentally regulated in the uterus and trophoblast during placental establishment [26]; <5> increased expression of HtrA1 in the human placenta in the third trimester of gestation,

especially in the outer layer forming the syncytiotrophoblast [31]) [18, 23, 26, 31]
Additional information <7> (<7> regulatory role for pneumococcal HtrA in modulating the activity of a two-component signaling system that controls the development of genetic competence [29]) [29]

6 Stability

Temperature stability

45 <4> (<4> stable for at least 4 h [11]) [11]
50 <4> (<4> stable for at least 1.5 h [11]) [11]
55 <4> (<4> stable for at least 1 h [11]) [11]
55-75 <4> (<4> denaturation of the enzyme starts at 55°C and ends at 75°C [11]) [11]
60 <4> (<4> drastic decrease of activity within 5 min [11]) [11]
Additional information <4> (<4> interaction with phosphatidylgycerol leads to a remarkable decrease in the thermal stability [12]) [12]

General stability information

<4>, enzyme cleaves itself under reducing conditions in the presence of 2-mercaptoethanol or dithiothreitol [3]
<4>, native enzyme undergoes slow self cleavage [21]

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HtrA2 Peptidase 3.4.21.108

1 Nomenclature

EC number
3.4.21.108

Recommended name
HtrA2 peptidase

Synonyms
HtrA2 <3, 8, 9> [2, 11, 16, 18, 22]
HtrA2 protease <3> [18]
HtrA2/Omi <1, 2, 3, 4, 5, 7> [1, 3, 6, 7, 8, 10, 12, 13, 14, 17, 25, 29, 30, 31, 32]
HtrA2/Omi serine protease <9> [32]
Nma11p <6> [28]
Omi <3, 9, 10> [4, 5, 6, 9]
Omi/HtrA2 <2, 3, 4> [19, 20, 21, 23, 24, 26, 27]
Omi/HtrA2 protease <2, 3> [15]
S01.278 <1> (<1> Merops ID [8]) [8]
high temperature requirement A serine protease <3> [17]
pro-apoptotic serine protease <6> [28]
serine protease <2, 3, 4, 5, 7> [15, 19, 20, 22, 23, 25, 26, 27, 30, 31]

CAS registry number
204655-80-1

2 Source Organism

<1> *Mammalia* (no sequence specified) [3, 7, 8, 12]
<2> *Mus musculus* (no sequence specified) [1, 13, 15, 21, 25, 30, 32]
<3> *Homo sapiens* (no sequence specified) [6, 9, 10, 11, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 29, 31]
<4> *Rattus norvegicus* (no sequence specified) [21, 25]
<5> *Sus scrofa* (no sequence specified) [25]
<6> *Saccharomyces cerevisiae* (no sequence specified) [28]
<7> *Bos taurus* (no sequence specified) [25]
<8> *Mus musculus* (UNIPROT accession number: Q9JIY5) [2]
<9> *Homo sapiens* (UNIPROT accession number: O43464) [2,4,32]
<10> *Homo sapiens* (UNIPROT accession number: AI979237) [5]

3 Reaction and Specificity

Catalyzed reaction

cleavage of non-polar aliphatic amino-acids at the P1 position, with a preference for Val, Ile and Met. At the P2 and P3 positions, Arg is selected most strongly with a secondary preference for other hydrophilic residues

Reaction type

cleavage of C-N-linkage
hydrolysis of peptide bond

Natural substrates and products

S Bir1p <6> (Reversibility: ?) [28]
P ?
S Additional information <1, 2, 3, 8, 9, 10> (<3> can promote caspase activation and cell death apoptosis [9]; <3> induces atypical cell death. Inhibits the caspase-inhibitory activity of XIAP by direct binding to it. Only mature form but not its precursor binds to IAPs [11]; <1> induces cell death in a caspase-dependent manner [8]; <2> loss of enzyme activity causes neuromuscular disorder [13]; <1> promotes apoptosis [3]; <1, 8, 9> role in apoptosis [2, 4, 12]; <3> role in apoptosis, amino-terminal IAP interaction motif displaces IAPs from caspases, leading to enhanced caspase activity [10]; <3> role in apoptosis, binds to XIAP-binding protein [6]; <3> role in apoptosis, degradation of aberrantly folded proteins during conditions of cellular stress [14]; <10> role in apoptosis, enzyme can induce apoptosis in a caspase-independent manner through its protease activity and in a caspase-dependent manner via its ability to disrupt caspase-IAP interaction [5]; <1, 2> role in apoptosis, mice with mutant HtrA2/Omi suffer from neurodegenerative disease due to progressive mitochondrial damage, mutant enzyme is proteolytically inactive but can bind to IAPs [1, 7]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14]
P ? <1, 2, 3, 8, 9, 10> [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14]

Substrates and products

S (^{35}S) Met-labeled proteolytically inactive S306 form of full length HtrA2 + H_2O <3> (Reversibility: ?) [22]
P ?
S (7-methoxycoumarin-4-yl)acetyl-IRRVSYSF(Dnp)KK + H_2O <3> (<3> best substrate [10]) (Reversibility: ?) [10]
P ? <3> [10]
S (7-methoxycoumarin-4-yl)acetyl-VTLCAVPS(Dnp)KK + H_2O <3> (<3> cleavage occurs between cysteine and alanine [10]) (Reversibility: ?) [10]
P ? <3> [10]
S 35S-β-casein + H_2O <3> (<3> C-terminus of presenilin-1 interacts the PDZ domain of with Omi/HtrA2, increasing its activity [26]) (Reversibility: ?) [26]
P ?

S A β 40 <3> (<3> HtrA2/Omi binds preferentially to the short form of A β peptides, the enzyme does not perform degradation by direct hydrolysis [29]) (Reversibility: ?) [29]
P ?
S A β 42 <3> (<3> HtrA2/Omi binds poorly to A β 42, the enzyme does not perform degradation by direct hydrolysis [29]) (Reversibility: ?) [29]
P ?
S Apollon + H_2O <3> (<3> $HtrA_2$ cleaves Apollon, a huge inhibitor of apoptosis protein, with its serine protease activity, conversely, Apollon ubiquitylates and facilitates proteasomal degradation of HtrA2, thus both downregulate each other [16]) (Reversibility: ?) [16]
P ?
S Bir1p <6> (Reversibility: ?) [28]
P ?
S Bir1p + H_2O <6> (<6> inhibitor of apoptosis proteins (IAP) Bir1p is a substrate for Nma11p, which is the yeast homologue of the human OMI/HtrA2, Bir1p physically interacts with the N-terminal HtrA-like repeat of Nma11p [28]) (Reversibility: ?) [28]
P ?
S Casein + H_2O <3> (<3> strong hydrolytic activity [29]) (Reversibility: ?) [16, 18, 29]
P ?
S Livin α + H_2O <1> (Reversibility: ?) [3]
P ? <1> [3]
S Livin β <1> (Reversibility: ?) [3]
P ? <1> [3]
S PDZ-interacting peptides + H_2O <3> (Reversibility: ?) [24]
P ?
S X-linked inhibitor of apoptosis protein <2> (<2> $HtrA_2$ promotes degradation of the X-linked inhibitor of apoptosis protein followed by subsequent caspase activation [21]) (Reversibility: ?) [21]
P ?
S X-linked inhibitor of apoptosis protein + H_2O <2, 3> (<3> autocatalytically processing of $HtrA_2$ to the 36-kDa protein fragment, which is required for the cytochrome c-dependent caspase activation, promoting apoptotic cell death [25]; <3> BIR2 [24]; <3> C-terminus of presenilin-1 interacts with the PDZ domain of Omi/HtrA2, increasing its activity [26]; <3> homotrimeric structure of enzyme is required for executing its activity [22]; <3> Omi/$HtrA_2$ cleaves as well as directly binds and inactivates X-linked inhibitor of apoptosis protein to promote caspase activation [19]) (Reversibility: ?) [15, 16, 19, 20, 22, 24, 25, 26, 27]
P ?
S α-casein + H_2O <1, 3, 9> (Reversibility: ?) [2, 3, 8, 14]
P ? <1, 3, 9> [2, 3, 8, 14]
S α-secretase peptide <3> (<3> HtrA2/Omi does not perform degradation by direct hydrolysis [29]) (Reversibility: ?) [29]
P ?

S β-casein <3> (<3> protease activity is activated by the binding of the PDZ domain of the mature form of Omi to the C-terminal region of the reduced form of Pag [23]) (Reversibility: ?) [23]
P ?
S β-casein + H_2O <1, 2, 3, 8, 9, 10> (<3> preferred substrate [14]; <3> main selectivity at position P1 is for non-polar aliphatic amino acids, in particular valine, isoleucine and methionine. At the P2 and P3 positions arginine is selected most strongly with a secondary selection for other hydrophilic residues [10]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 22, 25, 32]
P ? <1, 2, 3, 8, 9, 10> [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14]
S β-secretase peptide <3> (<3> HtrA2/Omi does not perform degradation by direct hydrolysis [29]) (Reversibility: ?) [29]
P ?
S c-inhibitor of apoptosis protein1 + H_2O <3> (<3> C-terminus of presenilin-1 interacts with the PDZ domain of Omi/HtrA2, increasing its activity [26]) (Reversibility: ?) [26]
P ?
S c-inhibitor of apoptosis protein2 + H_2O <3> (<3> C-terminus of presenilin-1 interacts with the PDZ domain of Omi/HtrA2, increasing its activity [26]) (Reversibility: ?) [26]
P ?
S dephosphorylated casein + H_2O <1> (Reversibility: ?) [8]
P ? <1> [8]
S γ-secretase peptide <3> (<3> HtrA2/Omi binds less strongly to γ-secretase substrate peptide as compared to α-secretase substrate peptide and β-secretase substrate peptide, the enzyme does not perform degradation by direct hydrolysis [29]) (Reversibility: ?) [29]
P ?
S glycprotein α_1 acid + H_2O <1> (Reversibility: ?) [3]
P ? <1> [3]
S hyaluronidase + H_2O <1> (Reversibility: ?) [3]
P ? <1> [3]
S inhibitor of apoptosis + H_2O <1> (<1> enzyme cleaves various IAPs [3]) (Reversibility: ?) [3]
P ? <1> [3]
S ped/pea-15 <3> (<3> $HtrA_2$ is a specific interactor of the ped/pea-15 death effector domain and leads to its degradation [27]) (Reversibility: ?) [27]
P ?
S protein DIAP1 + H_2O <1> (Reversibility: ?) [3]
P ? <1> [3]
S protein XIAP + H_2O <1> (Reversibility: ?) [3]
P ? <1> [3]
S protein cIAP1 + H_2O <1> (<1> best substrate, preferred cleavage sites are after Thr4, Asn133 and Leu161 [3]) (Reversibility: ?) [3]
P ? <1> [3]

S protein cIAP2 + H_2O <1> (Reversibility: ?) [3]
P ? <1> [3]
S reduced bovine serum albumin <3> (<3> HtrA2-Opt peptides stimulate the δ N-HtrA1 protease activity more than 3fold [17]) (Reversibility: ?) [17]
P ?
S unidentified substrate + H_2O <3> (<3> its cleavage by Omi/HtrA2 leads to permeabilization of the mitochondria outer membrane and release of chytochrome c followed by enhanced caspase activation [19]) (Reversibility: ?) [19]
P ?
S Additional information <1, 2, 3, 8, 9, 10> (<3> Omi can degrade inhibitor of apoptosis proteins [9]; <3> can promote caspase activation and cell death apoptosis [9]; <3> induces atypical cell death. Inhibits the caspase-inhibitory activity of XIAP by direct binding to it. Only mature form but not its precursor binds to IAPs [11]; <1> induces cell death in a caspase-dependent manner [8]; <2> loss of enzyme activity causes neuromuscular disorder [13]; <1> promotes apoptosis [3]; <1,8,9> role in apoptosis [2,4,12]; <3> role in apoptosis, amino-terminal IAP interaction motif displaces IAPs from caspases, leading to enhanced caspase activity [10]; <3> role in apoptosis, binds to XIAP-binding protein [6]; <3> role in apoptosis, degradation of aberrantly folded proteins during conditions of cellular stress [14]; <10> role in apoptosis, enzyme can induce apoptosis in a caspase-independent manner through its protease activity and in a caspase-dependent manner via its ability to disrupt caspase-IAP interaction [5]; <1,2> role in apoptosis, mice with mutant HtrA2/Omi suffer from neurodegenerative disease due to progressive mitochondrial damage, mutant enzyme is proteolytically inactive but can bind to IAPs [1,7]; <3> does not bind the reversed control peptide A β 41-1 and peptide H_2-Opt [29]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 29]
P ? <1, 2, 3, 8, 9, 10> [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14]

Inhibitors

diisopropylfluorophosphate <9> (<9> complete inhibition at 4 mM [2]) [2]
N-tosyllysine chloromethyl ketone <9> (<9> 51% inhibition at 5 mM [2]) [2]
phenylmethanesulfonyl fluoride <9> (<9> 51% inhibition at 5 mM [2]) [2]
ucf-101 <1, 2, 3> (<1> 78% inhibition at 0.02 mM, complete inhibition at 0.08 mM [8]; <3> 0.02 mM abrogates the death effect of the TNF-α + zVAD combination [20]; <2> blocks HtrA2 protease activity, inhibition of apoptosis [21]; <2> blocks protease activity of Omi, protects renal cells from cisplatin-induced cell death, mice treated with cisplatin and ucf-101 were more resistant to nephrotoxicy than animals treated with ciplatin alone [15]; <3> blocks protease activity of Omi, protects renal cells from cisplatin-induced cell death, together with 0.05 mM cisplatin, 0.07 mM ucf-101 can block X-linked inhibitor of apoptosis protein degradation [15]; <3> inhibits degradation of endogenous ped/pea-15 by Omi/HtrA2 [27]) [8, 15, 20, 21, 27]

ucf-102 <1> (<1> inhibits, but to a lesser extent than ucf-101 [8]) [8]
ucf-103 <1> (<1> inhibits, but to a lesser extent than ucf-101 [8]) [8]
ucf-104 <1> (<1> 58% inhibition at 0.02 mM [8]) [8]
Additional information <4> (<4> ucf-101 fails to exert any additional protective effect in transfected cells [21]) [21]

Activating compounds

cisplatin <2, 3> (<2,3> increase of Omi protein [15]) [15]
L-phenylalanine <3> (<3> essential for the formation of a homotrimer and for the HtrA2 serine protease activity [22]) [22]
staurosporine <3> (<3> apoptotic stimulus to release 36-kDa protein fragment of HtrA2 and cytochrome c from the mitochondria to the cytosol [25]) [25]
X-linked inhibitor of apoptosis protein <3> (<3> i.e. XIAP, binding of XIAP to the Reaper motif of the enzyme results in a marked increase in proteolytic activity [10]) [10]
heat shock <3> (<3> pre-incubation of Omi at 42°C for 30 min results in increased proteolytic activity [10]) [10]
Additional information <3> (<3> UV irradiation leads to an almost complete disappearance of mitochondrial Omi/HtrA2 and a parallel appearance of Omi/HtrA2 in the cytoplasm, induction of Omi/HtrA2 binding to ped/pea-15 [27]) [27]

Specific activity (U/mg)

Additional information <3> (<3> peptide derived from type III collagen α_1 C-propeptide does not activate nor significantly bind to HtrA2 as compared to HtrA1 [17]) [17]

pH-Optimum

7.4 <1> (<1> assay at [3]) [3]
8 <1> (<1> assay at [7]) [7]

Temperature optimum (°C)

37 <1> (<1> assay at [7]) [7]

Temperature range (°C)

25-55 <3> (<3> enzyme activity rapidly increases with temperature and it drastically decreases at and above 60°C, no significant change in secondary structure from 25-70°C or in the oligomeric size between 25-55°C, but significant change in tertiary level from 25-60°C [18]) [18]

4 Enzyme Structure

Molecular weight

28000-44000 <9> (<9> low molecular masses detected due to full-length HtrA2/Omi regions or amino acid residues that are very susceptible to a high degree of proteolytic events in Escherichia coli expression systems [32]) [32]

35000 <1, 3> (<1> calculated from nucleotide sequence [7]; <3> mass spectroscopy experiments [6]) [6, 7]
36000 <3> (<3> mature protein, SDS-PAGE [11]) [11]
38000 <10> (<10> SDS-PAGE [5]) [5]
42000 <3> (<3> SDS-PAGE [14]) [14]
50000 <9> (<9> calculated from nucleic acid sequence, SDS-PAGE [4]) [4]
63000 <3> (<3> most of the purified GST-HtrA$_2$ δ 133 proteins [22]) [22]
77000 <2> [32]
110000 <1> (<1> gel filtration [12]) [12]

Subunits

trimer <1, 3> (<1> 3 * 36000, gel filtration [12]; <3> homotrimer, SDS-PAGE [22]) [12, 22]

Posttranslational modification

proteolytic modification <1, 3> (<3> 133 N-terminal amino acids of the precursor protein are cleaved off [11]; <3> first 133 amino acids are removed by autoproteolysis [6]; <1> precursor protein is processed to yield an active protase [7]) [6, 7, 11]

5 Isolation/Preparation/Mutation/Application

Source/tissue

293 cell <3, 10> [5, 6, 9]
293T cell <3> [11]
HEK-293 cell <3> [15, 17, 24, 26, 27]
HL-60 cell <9> [4]
HeLa cell <3, 10> (<3> human cervical carcinoma [19]) [5, 19, 26, 27]
JURKAT cell <10> [5]
K-562 cell <9> [4]
MCF-7 cell <10> [5]
MEF cell <2> [30]
SH-SY5Y cell <3> [24]
SW-480 cell <9> [4]
brain <2, 3, 9> (<3> cerebral plaques in the brains of patients with Alzheimer's disease [31]; <9> from fetus [32]; <3> stem sections and cortical slices from patients with pathologically confirmed idiopathic Parkinsons disease [24]) [2, 24, 30, 31, 32]
embryonic stem cell <2> [30]
heart <2, 4, 9> (<4> neonatal ventricular cardiomyocytes [21]) [2, 21]
kidney <2, 3> (<3> embryonic kindey 293 cells [22]; <3> from embryos [27]; <3> from human embryos [15,19,24]; <2> from the cortices [15]) [15, 19, 22, 24, 27]
myocardium <2> [21]
neuroblastoma cell <3> [24]
neuron <2> (<2> primary neurons [30]) [30]
neutrophil <3> (<3> from the peripheral blood of healthy donors [20]) [20]

pancreas <9> [4]
placenta <9> [4]
proximal tubule <2> [15]
skeletal muscle <9> [2]
thalamus <2> [30]
thymocyte <2> [30]
Additional information <3> (<3> proximal cells, HK-2 cells [15]) [15]

Localization

cytoplasm <2, 3, 6> (<3> HtrA2 released from the mitochondrion to the cytoplasm during apoptosis in response to oxidative stress [23]; <3> HtrA2 relocates to the cytoplasm upon apoptosis [20]) [15, 20, 23, 24, 26, 27, 28, 30]
cytosol <2, 3> (<2> myocardial ischemia/reperfusion results in Omi/HtrA2 translocation from the mitochondria to the cytosol along with cytochrome c [21]) [21, 25]
endoplasmic reticulum <9> [4]
membrane <1> [7]
mitochondrion <1, 2, 3, 10> (<3> intermembrane space [19]; <3> extramitochondrially expressed enzyme induces atypical cell death [11]; <1> in response to UV irradiation the enzyme shifts into the cytosol [7]; <3> mature protein is released form the mitochondria into the cytosol during aopotosis [9]; <3> mitochondria also contain processed Omi which, following apoptotic insult, translocates to the cytosol [6]; <2> released into cytosol during apoptosis [1]; <2> HtrA2 in the intermembrane space of healthy cells, density of mitochondria decreased and showed abnormal forms in knockout mice [30]; <3> mainly localized [29]; <3> normally expression of HtrA2, enzyme does not leave the mitochondria upon the TNF-α + zVAD-induced cell death in neutrophils [20]; <3> predominant localisation, enlarged mitochondria with disorganized cristae in mutant cells [24]; <2> predominant presence of Omi [15]) [1, 5, 6, 7, 8, 9, 11, 13, 15, 16, 18, 19, 20, 21, 23, 24, 25, 26, 27, 29, 30]
nucleus <6, 9> [2, 28]
Additional information <3> (<3> halo of Lewy bodies from patients with pathologically confirmed idiopathic Parkinsons disease [24]) [24]

Purification

<1> [3]
<2> (rapid purification of GST-HtrA2/Omi fusion proteins under non-denaturing conditions by selective binding to glutathione-Sepharose 4B beads) [32]
<3> [2, 4, 5, 10, 11, 26]
<3> (90% pure) [14]
<3> (by glutathione-Sepharose 4B beads) [25]
<3> (by glutathione-Sepharose beads) [24]
<3> (by nickel affinity chromatography and HiTrap desalting) [19]
<3> (on Ni-NTA agarose column) [18]
<3> (rapid purification of GST-HtrA2 δ 133 proteins on glutathione-Sepharose 4B beads) [22]

<3> (recombinant enzyme by ProBondTM resin) [29]
<3> (sequential S-100 and S-200 gel filtration chromatography) [23]
<3> (with Ni-NTA-agarose column) [17]
<9> (rapid purification of full-length HtrA2/Omi as a GST fusion under non-denaturing conditions by selective binding to glutathione-Sepharose 4B beads) [32]

Crystallization

<1> (hanging drop vapor diffusion method) [12]

Cloning

<1> [3, 7, 8]
<2> [2, 13]
<2> (successful expression of full-length HtrA2/Omi as a GST fusion protein in Escherichia coli BL21 as compared to human HtrA2/Omi) [32]
<3> [2, 4, 5, 11]
<3> (PDZ domain) [10]
<3> (expression as GST fusions in Escherichia coli BL21) [25]
<3> (expression from pET-15b) [18]
<3> (expression in Escherichia coli BL21 (DE3)) [17]
<3> (expression in Escherichia coli BL21 (DE3) pLysS) [19]
<3> (expression in Escherichia coli BL21 Codon Plus (DE3)-RIL) [24]
<3> (expression in Escherichia coli and MEF cells from mnd2 mouse) [26]
<3> (expression of GST-HtrA2 δ 133 proteins in Escherichia coli BL21 from GST-HTRA2 δ 133 WT, S306 and F149D constructs) [22]
<3> (overexpression in Escherichia coli BL21 (DE3) and expressed in Saccharomyces cerevisiae EGY48/pLexA-hPag and their mutants) [23]
<3> (overexpression in HeLa cells and HEK-293 cells) [27]
<3> (subcloned into pET28a and propageted in Escherichia coli DH5α, expressed in Escherichia coli BL21 (DE3) pLys, overexpression in K269 cells) [29]
<3> (truncated version, expressed in Escherichia coli) [14]
<6> (overexpression of Nma11p in Saccharomyces cerevisiae strain BYa by insertion of a C-terminal GFP-tag to the BIR1ORF) [28]
<9> (barely expressed full-length HtrA2/Omi as a GST fusion protein in Escherichia coli BL21) [32]

Engineering

A144G <1> (<1> mutation reduces ability to promote cell death, weak interaction with XIAP [7]) [7]
A147I <1> (<1> increased affinity for the BIR3 domain of XIAP [7]) [7]
F149D <1> (<1> comparable level of binding to XIAP as the wild type-enzyme [12]) [12]
L286V <3> (<3> Alzheimer mutant presenilin-1 [26]) [26]
M146V <3> (<3> Alzheimer mutant presenilin-1 [26]) [26]
S276C <2, 3> (<2> loss of protease activity, maturation and IAP-binding activity are not affected [13]; <3> deletion of the PDZ domain of Omi/HtrA2 [26]) [13, 26]

S306A <1, 3, 9, 10> (<1> comparable level of binding to XIAP as the wild type-enzyme [12]; <3> localization is similar to wild-type [6]; <10> mutant is able to promote procaspase-3 activation [5]; <9> mutant with abolished autoproteolysis [2]; <1> mutation reduces ability to promote cell death [7]; <3> catalytically inactive Omi/HtrA2 [26]; <3> endoproteolytic activity abolished [25]) [2, 5, 6, 7, 12, 25, 26]
S306A/A144G <1> (<1> complete loss of proapoptotic activity [7]) [7]
Additional information <2, 3> (<3> truncated mutant lacking the putative mitochondrial localization motif in the first 33 amino acid residues shows distribution throughout the cell with no obvious concentration in the mitochondria [6]; <2> HtrA2 knockout mice with general decrease in organ size and neurological abnormalities, deletion of HtrA2 results in a mitochondrial dysfunction, death of these mice ca. 30 days after birth [30]) [6, 30]

Application

medicine <2, 3, 4> (<3> amyloid β, a pivotal factor implicated in the pathogenesis of Alzheimer's disease interacts with HtrA2/Omi [31]; <3> heterozygous G399S mutation in Parkinsons disease patients, which is absent in healthy controls, a A141S polymorphism is associated with Parkinsons disease, both mutations result in defective activation of Omi/HtrA2 and mitochondrial dysfunction associated with altered mitochondrial morphology [24]; <2> HtrA2 knockout mice display features characteristic of a Parkinson syndrome, deletion of HtrA2 results in increased susceptibility to cell death stimuli, Smac/DIABOLO can compensate for the removal of $HtrA_2$ in terms of neutralizing inhibitor of apoptosis proteins [30]; <3> involvement of HtrA2 in the etiology of Alzheimers disease [29]; <2> myocardial ischemia/reperfusion significantly increases cytosolic Omi/HtrA2 content and markedly increases apoptosis, ucf-101 can inhibit apoptosis [21]; <4> transfecting cells with siRNA molecules against Omi effectively eliminate Omi/HtrA2 protein expression [21]; <2,3> way to limit renal injury by specifically inhibiting its proteolytic activity [15]) [15, 21, 24, 29, 30, 31]

6 Stability

General stability information

<8, 9>, enzyme undergoes autoproteolysis [2]

Storage stability

<3>, -20°C [24]
<3>, -70°C, Tris-HCl buffer, 20% glycerol, several months [25]
<3>, -80°C, HEPES-KOH buffer, pH 7.5 [19]
<3>, -80°C, phosphate buffered saline, pH 7.4, 20% glycerol [29]
<9>, -70°C, Tris-HCl buffer, pH 7.6, 20% glycerol, several months [32]

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Matriptase 3.4.21.109

1 Nomenclature

EC number
3.4.21.109

Recommended name
matriptase

Synonyms
MT-SP-1 <2> [1, 5]
MT-SP1 <2> [15]
MT-SP1/matripase <2, 3> [8, 14]
matriptase-3 <1, 2> [12]
membrane-type serine protease 1 <2> [1, 5]
membrane-type serine protease 1/matripase <3> [14]
membrane-type serine proteinase matripase <2> [15]
serine protease SNC19/matripase <2> [21]

CAS registry number
241475-96-7

2 Source Organism

<1> *Mus musculus* (no sequence specified) [10, 12]
<2> *Homo sapiens* (no sequence specified) [1, 2, 4, 5, 6, 7, 8, 9, 12, 13, 15, 16, 17, 18, 19, 20, 21]
<3> *Rattus norvegicus* (no sequence specified) [14]
<4> *Homo sapiens* (UNIPROT accession number: Q9Y5Y6) [3, 11]

3 Reaction and Specificity

Catalyzed reaction
cleaves various synthetic substrates with Arg or Lys at the P1 position and prefers small side-chain amino acids, such as Ala and Gly, at the P2 position

Reaction type
cleavage of C-N-linkage
hydrolysis of peptide bond

Natural substrates and products

- **S** Additional information <1, 2, 4> (<1> component of the profilaggrin-processing pathway and a key regulator of terminal epidermal differentiation, involved in lipid matrix formation, cornified envelope morphogenesis and stratum corneum desquamation [10]; <2, 4> degrades extracellular matrix [1, 2, 7, 8, 11]; <4> degrades extracellular matrix, matriptase-binding protein is a Kunitz-type serine protease inhibitor [3]; <2> involved in multiple aspects of tumor progression and cancer invasion [4]) (Reversibility: ?) [1, 2, 3, 4, 7, 8, 10, 11]
- **P** ? <1, 2, 4> [1, 2, 3, 4, 7, 8, 10, 11]
- **S** Additional information <2> (<2> initiator of matrix-degrading protein cascade, activates hepatocyte growth factor scattering factor [6]) (Reversibility: ?) [6]

<2> [6]

Substrates and products

- **S** Arg-Xaa-Ser-Arg-Ala + H_2O <2> (<2> X: non-basic amino acid, good substrate [5]) (Reversibility: ?) [5]
- **P** Arg-Xaa-Ser + Arg-Ala <2> [5]
- **S** Boc-Glu-Ala-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [20]
- **P** ?
- **S** Boc-Glu-Ala-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [16]
- **P** ?
- **S** bovine serum albumin + H_2O <1> (Reversibility: ?) [12]
- **P** ?
- **S** casein + H_2O <1> (Reversibility: ?) [12]
- **P** ?
- **S** gelatin + H_2O <2> (Reversibility: ?) [15, 17, 20]
- **P** ?
- **S** H-Glu-Gly-Arg-*p*-nitroanilide + H_2O <1> (<1> low activity [12]) (Reversibility: ?) [12]
- **P** H-Glu-Gly + Arg-*p*-nitroanilide
- **S** Lys-Xaa-Ser-Arg-Ala + H_2O <2> (<2> X: non-basic amino acid, good substrate [5]) (Reversibility: ?) [5]
- **P** Lys-Xaa-Ser + Arg-Ala <2> [5]
- **S** N-Ala-Ala-Ala-Tyr-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [17]
- **P** ?
- **S** N-succinyl-Ala-Phe-Lys-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [17]
- **P** ?
- **S** N-succinyl-Ala-Phe-Lys-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [6]
- **P** N-succinyl-Ala-Phe-Lys + 7-amino-4-methylcoumarin <2> [6]

S N-succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [17]
P ?
S N-tert-butoxy-carbonyl-Gln-Ala-Arg-7-amido-4-methylcoumarin <2> (Reversibility: ?) [2]
P N-tert-butoxy-carbonyl-Gln-Ala-Arg + 7-amino-4-methylcoumarin <2> [2]
S N-tert-butoxycarbonyl-Gln-Ala-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [17]
P ?
S N-tert-butoxycarbonyl-Gln-Ala-Arg-7-amido-4-methylcoumarin + H_2O <4> (Reversibility: ?) [3, 11]
P N-tert-butoxycarbonyl-Gln-Ala-Arg + 7-amino-4-methylcoumarin <4> [3, 11]
S N-tert-butoxycarbonyl-Gly-Lys-Arg-7-amido-4-methylcoumarin + H_2O <4> (Reversibility: ?) [3]
P N-tert-butoxycarbonyl-Gly-Lys-Arg + 7-amino-4-methylcoumarin <4> [3]
S N-tert-butoxycarbonyl-Leu-Arg-Arg-7-amido-4-methylcoumarin + H_2O <4> (Reversibility: ?) [3]
P N-tert-butoxycarbonyl-Leu-Arg-Arg + 7-amino-4-methylcoumarin <4> [3]
S N-tert-butoxycarbonyl-Leu-Gly-Arg-7-amido-4-methylcoumarin + H_2O <4> (Reversibility: ?) [3]
P N-tert-butoxycarbonyl-Leu-Gly-Arg + 7-amino-4-methylcoumarin <4> [3]
S N-tert-butoxycarbonyl-Leu-Ser-Thr-Arg-7-amido-4-methylcoumarin + H_2O <4> (Reversibility: ?) [3]
P N-tert-butoxycarbonyl-Leu-Ser-Thr-Arg + 7-amino-4-methylcoumarin <4> [3]
S N-tert-butoxycarbonyl-Phe-Ser-Arg-7-amido-4-methylcoumarin + H_2O <4> (Reversibility: ?) [3]
P N-tert-butoxycarbonyl-Phe-Ser-Arg + 7-amino-4-methylcoumarin <4> [3]
S N-tert-butoxycarbonyl-Val-Pro-Arg-7-amido-4-methylcoumarin + H_2O <4> (Reversibility: ?) [3]
P N-tert-butoxycarbonyl-Val-Pro-Arg + 7-amino-4-methylcoumarin <4> [3]
S N-tert-butoxycarbonyl-benzyl-Asp-Pro-Arg-7-amido-4-methylcoumarin + H_2O <4> (Reversibility: ?) [3]
P N-tert-butoxycarbonyl-benzyl-Asp-Pro-Arg + 7-amino-4-methylcoumarin <4> [3]
S N-tert-butoxycarbonyl-benzyl-Glu-Gly-Arg-7-amido-4-methylcoumarin + H_2O <4> (Reversibility: ?) [3]
P N-tert-butoxycarbonyl-benzyl-Glu-Gly-Arg + 7-amino-4-methylcoumarin <4> [3]

S N-tert-butoxycarbonyl-γ-benzyl-Glu-Ala-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [17]
P ?
S N-tert-butoxycarbonyl-γ-benzyl-Gln-Ala-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [6]
P N-tert-butoxycarbonyl-γ-benzyl-Gln-Ala-Arg + 7-amino-4-methylcoumarin <2> [6]
S N-tert-butoxycarbonyl-γ-benzyl-Glu-Ala-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [6]
P N-tert-butoxycarbonyl-γ-benzyl-Glu-Ala-Arg + 7-amino-4-methylcoumarin <2> [6]
S N-tert-butoxycarbonyl-γ-benzyl-Glu-Gly-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [6]
P N-tert-butoxycarbonyl-γ-benzyl-Glu-Gly-Arg + 7-amino-4-methylcoumarin <2> [6]
S N-tert-butoxycarbonyl-γ-benzyl-Leu-Gly-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [6]
P N-tert-butoxycarbonyl-γ-benzyl-Leu-Gly-Arg + 7-amino-4-methylcoumarin <2> [6]
S Suc-Ala-Ala-Pro-Arg-*p*-nitroanilide + H_2O <1> (<1> low activity [12]) (Reversibility: ?) [12]
P Suc-Ala-Ala-Pro + Arg-*p*-nitroanilide
S Suc-Ala-Ala-Pro-Lys-*p*-nitroanilide + H_2O <1> (Reversibility: ?) [12]
P Suc-Ala-Ala-Pro-Lys + *p*-nitroaniline
S Xaa-Arg-Ser-Arg-Ala + H_2O <2> (<2> X: non-basic amino acid, good substrate [5]) (Reversibility: ?) [5]
P Xaa-Arg-Ser + Arg-Ala <2> [5]
S Xaa-Lys-Ser-Arg-Ala + H_2O <2> (<2> X: non-basic amino acid, good substrate [5]) (Reversibility: ?) [5]
P Xaa-Lys-Ser + Arg-Ala <2> [5]
S Z-Phe-Val-Arg-*p*-nitroanilide + H_2O <1> (Reversibility: ?) [12]
P Z-Phe-Val + Arg-*p*-nitroaniline
S denatured collagen + H_2O <1> (Reversibility: ?) [12]
P ?
S hepatocyte growth factor + H_2O <2> (Reversibility: ?) [6]
P ? <2> [6]
S insulin growth factor binding protein-related protein-1 + H_2O <2> (<2> cleaved by the soluble form of active matripase [15]) (Reversibility: ?) [15]
P ?
S methyl-sulfonyl-D-cyclo-hexyltyrosyl-glycyl-L-arginine-4-nitroanilide + H_2O <2> (Reversibility: ?) [1]
P ? <2> [1]
S plasminogen + H_2O <2> (Reversibility: ?) [6]
P ? <2> [6]
S pro-urokinase plasminogen activator + H_2O <2> (Reversibility: ?) [6]
P ? <2> [6]

S pro-urokinase-type plasminogen activator + H_2O <2> (Reversibility: ?) [19]
P ?
S single-chain urokinase-type plaminogen activator + H_2O <2> (<2> is activated by matriptase [5]) (Reversibility: ?) [5]
P ? <2> [5]
S succinyl-Ala-Phe-Lys-7-amido-4-methylcoumarin + H_2O <4> (Reversibility: ?) [3]
P succinyl-Ala-Phe-Lys + 7-amino-4-methylcoumarin <4> [3]
S Additional information <1, 2, 4> (<2> cleaves synthetic substrates with arginine or lysine as their P1 sites and prefers small side chain amino acids, such as Ala and Gly, at P2 sites [6]; <2> preferred amino acid residues: at P4 position: Lys and Arg, at P3 position: basic residues and Glu, at P2 position: Gly, Ser and Phe [5]; <1> component of the profilaggrin-processing pathway and a key regulator of terminal epidermal differentiation, involved in lipid matrix formation, cornified envelope morphogenesis and stratum corneum desquamation [10]; <2,4> degrades extracellular matrix [1,2,7,8,11]; <4> degrades extracellular matrix, matriptase-binding protein is a Kunitz-type serine protease inhibitor [3]; <2> involved in multiple aspects of tumor progression and cancer invasion [4]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 8, 10, 11]
P ? <1, 2, 4> [1, 2, 3, 4, 5, 6, 7, 8, 10, 11]
S Additional information <2> (<2> initiator of matrix-degrading protein cascade, activates hepatocyte growth factor scattering factor [6]) (Reversibility: ?) [6]
P <2> [6]

Inhibitors

4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride <2> [15]
antithrombin III <1> (<1> formation of a stable inhibitor complex [12]) [12]
aprotinin <2> [15]
protein C inhibitor <1> (<1> formation of a stable inhibitor complex [12]) [12]
R1K4-eglin <2> (<2> wild type eglin c with Pro45 at P1 position and Tyr49 residues at P4 position replaced with Arg and Lys respectively, most potent, selective, high affinity and proteolytically stable inhibitor [16]) [16]
R4R1-eglin <2> (<2> with substituted P42 and L45 for arginine residues, variants containing an Arg or Lys at position 49 instead of the original Tyr residue show enhanced inhibition [16]) [16]
α_1 proteinase <1> (<1> formation of a stable inhibitor complex [12]) [12]
α_2-antiplasmin <1> (<1> formation of a stable inhibitor complex [12]) [12]
antisense-matripase <2> (<2> significantly reduces matripase protein expression by 70-80%, anti-tumoral activity on HRA cells intraperitoneal injected into nude mice [19]) [19]
hepatocyte growth factor activator inhibitor I <1> [12]
hepatocyte growth factor activator inhibitor-1 <2> (<2> i.e. HAI^-1, binding is reversible and acid labile [2]) [2, 13, 21]

hexamidine <4> (<4> and its structural analogs [11]) [11]
plasminogen activator inhibitor-1 <1> (<1> formation of a stable inhibitor complex [12]) [12]
single chain variable fragment of antibodies <2> (<2> different variants [1]) [1]
Additional information <2> (<2> not inhibited by leupeptin, pepstatin and N-(R)-(2-(hydroxyaminocarbonyl)methyl)-4-methylpentanoyl-L-3-(2-naphthyl)alaninyl-L-alanine 2-aminoethyl amide [15]; <2> wild type eglin c has no effect [16]) [15, 16]

Activating compounds

C_2 ceramide 1-phosphate <2> (<2> weaker activator than sphingosine 1-phosphate, activation at 0.000237 mM [7]) [7]
C_8 ceramide 1-phosphate <2> (<2> weaker activator than sphingosine 1-phosphate, activation at 0.001 mM [7]) [7]
Epidermal growth factor <2> (<2> stimulates the release of matripase/hepatocyte growth factor activator inhibitor-1 complexes [20]) [20]
Lysophosphatidic acid <2> (<2> weaker activator than sphingosine 1-phosphate [7]) [7]
Serum <2> (<2> from human, horse, mouse, rat, rabbit, duck, chicken, goat, calf, turtle and foetal bovine serum, leads to increase of the level of the active two-chain form [2]) [2]
Sphingosine 1-phosphate <2> (<2> activation at 0.00001 mg/ml, activation is dependent on Ca^{2+} [7]; <2> mediates translocation of the enzyme to cell-cell junctions, sphingosine 1-phosphate-induced matripase activation is enhancend by reduced hepatocyte growth factor activator inhibitor-1 expression [13]) [7, 13]
Trypsin <2> (<2> activates membrane-bound, latent matripase on the cell surface [20]) [20]
dihydrosphingosine 1-phosphate <2> (<2> weaker activator than sphingosine 1-phosphate [7]) [7]
hepatocyte growth factor activator inhibitor-1 <2> [13]
sphingosine phosphocholine <2> (<2> weaker activator than sphingosine 1-phosphate [7]) [7]

K_m-Value (mM)

0.00381 <2> (N-tert-butoxycarbonyl-γ-benzyl-Glu-Ala-Arg-7-amido-4-methylcoumarin, <2> pH 8.5 [6]) [6]
0.00489 <2> (N-tert-butoxycarbonyl-γ-benzyl-Gln-Ala-Arg-7-amido-4-methylcoumarin, <2> pH 8.5 [6]) [6]
0.0136 <2> (N-tert-butoxycarbonyl-γ-benzyl-Leu-Gly-Arg-7-amido-4-methylcoumarin, <2> pH 8.5 [6]) [6]
0.0335 <2> (N-tert-butoxycarbonyl-γ-benzyl-Gln-Ala-Arg-7-amido-4-methylcoumarin, <2> pH 8.5 [6]) [6]
0.0475 <2> (N-tert-butoxycarbonyl-γ-benzyl-Glu-Gly-Arg-7-amido-4-methylcoumarin, <2> pH 8.5 [6]) [6]
0.0699 <2> (N-succinyl-Ala-Phe-Lys-7-amido-4-methylcoumarin, <2> pH 8.5 [6]) [6]

240 <1> (Suc-Ala-Ala-Pro-Arg-*p*-nitroanilide) [12]
534 <1> (Suc-Ala-Ala-Pro-Lys-*p*-nitroanilide) [12]

K_i-Value (mM)

4e-006 <2> (R1K4-eglin) [16]
2.6e-005 <2> (R4R1-eglin) [16]
0.000924 <2> (hexamidine, <2> pH 8.5 [11]) [11]

pH-Optimum

8.5 <4> (<4> assay at [3]) [3]
9-11 <2> (<2> purified enzyme [16]) [16]

Temperature optimum (°C)

25 <4> (<4> assay at [3]) [3]

4 Enzyme Structure

Molecular weight

29000 <2> (<2> refolded zymogen [16]) [16]
70000 <2> (<2> SDS-PAGE [6]; <2> non-complexed form, SDS-PAGE [7]; <2> immunoblotting, latent NH_2 terminal processed form matripase [13]) [6, 7, 13]
75000 <2, 4> (<4> calculated from nucleic acid sequence [3]; <2> by immunoblotting or zymography [20]; <2> gelatin zymography, soluble active enzyme [15]) [3, 15, 20]
80000 <2> (<2> noncomplexed form purified from T47D cells, SDS-PAGE [2]; <2> SDS-PAGE, recombinant protease domain [5]; <2> immunoblotting, membrane-bound enzyme [15]) [2, 5, 15]
85000 <2> (<2> and 95000 Da, SDS-PAGE, complex derived from milk [4]; <2> SDS-PAGE, full length enzyme [5]; <2> immunoblotting, full-length hepatocyte growth factor activator inhibitor-1 complexed with the serine protease domain of matripase [13]) [4, 5, 13]
90000 <3> (<3> Western blot analysis, post-translational processed enzyme, cleavage between Gly149 and Ser150 within the SEA domain [14]) [14]
93000 <2> (<2> immunoblotting, latent full-length matripase [13]) [13]
95000 <2, 4> (<2> and 85000 Da, SDS-PAGE, complex derived from milk [4]; <4> complex can be converted by boiling to matriptase plus a 40000 Da protein doublet [3]; <2> complexed with HAI-1, purified from milk, SDS-PAGE [2]; <2> gelatin zymography or immunoblotting, matripase/hepatocyte growth factor activator inhibitor complex [15]) [2, 3, 4, 15]
100000 <2> (<2> immunoblotting, matripase-hepatocyte growth factor activator inhibitor-1 complex [19]) [19]
110000 <2, 3> (<2> gelatin zymography or immunoblotting, matripase/hepatocyte growth factor activator inhibitor complex [15]; <3> Western blot analysis, unprocessed enzyme [14]) [14, 15]

120000 <2> (<2> complexed form, SDS-PAGE [7]; <2> immunoblotting, 70000 active matripase plus full-length hepatocyte growth factor activator inhibitor-1 [13]) [7, 13]

Subunits

? <1> (<1> x * 90000, SDS-PAGE [12]) [12]
dimer <2> (<2> 1 * 70000 + 1 * 240000, complex purified from milk, SDS-PAGE [2]) [2]
monomer <2> (<2> 1 * 80000 noncomplexed enzyme from T47D cells, SDS-PAGE [2]) [2]

Posttranslational modification

glycoprotein <1, 4> (<1> N-glycosylated [12]; <4> 3 potential N-glycosylation sites [3]) [3, 12]
proteolytic modification <2> (<2> one-chain zymogen is converted to an active two-chain protease, enzyme with mutation in its catalytic triad is unable to undergo this activational cleavage [9]; <2> zymogen is activated by proteolytic cleavage [2]) [2, 9]

5 Isolation/Preparation/Mutation/Application

Source/tissue

184A1N4 cell <2> (<2> nontumorigenic mammary epithelial cells [2]; <2> proportional increases or decreases in matripase and hepatocyte growth factor activator inhibitor-1 [13]) [2, 13]
BT-20 cell <2> (<2> low level [20]) [20]
Colo-201 cell <2> (<2> high level [20]) [20]
DLD-1 cell <2> (<2> high level [20]; <2> major amount of matripase [15]) [15, 20]
HEK-293 cell <2> [15]
HSC-4 cell <2> [15]
HeLa cell <2> [1, 5]
KATO-III cell <2> (<2> low level [20]) [20]
MCF-10A cell <2> [2]
MCF-7 cell <2> (<2> low level [20]) [20]
MKN-1 cell <2> (<2> low level [20]) [20]
MKN-28 cell <2> (<2> low level [20]) [20]
MKN-45 cell <2> (<2> high level [20]; <2> major amount of matripase [15]) [15, 17, 20]
MKN-74 cell <2> (<2> high level [20]) [20]
NUGC-3 cell <2> (<2> low level [20]) [20]
PC-3 cell <2> [1, 5, 8]
SW-480 cell <2> (<2> high level [20]) [20]
T47D cell <2> [2]
WiDr cell <2> (<2> high level [20]) [20]
brain <1, 2> [12]
breast carcinoma cell <2> [20]

colonic cancer cell line <2> [20]
enterocyte <3> [14]
epidermis <1> [10]
epididymis <1> [12]
epithelial cell <2, 3> (<3> basolateral sides [14]) [2, 14]
epithelium <2> [4, 7]
eye <1> [12]
gastrointestinal tract <2> (<2> in cancer tissue the expression of serine protease SNC19/matripase and hepatocyte growth factor activator inhibitor-1 is significantly lower than in the corresponding adjacent normal tissue, the ratio, however, shows no difference between normal and malingant tissue [21]) [21]
heart <1> [12]
jejunum <3> [14]
lung <2> [12]
mamma <2> [2]
mammary gland <2> [4]
milk <2, 4> [2, 3, 4, 6]
ovary <1, 2> [12]
ovary adenocarcinoma cell line <2> (<2> major amount of matripase [15]) [15]
ovary cancer cell line <2> (<2> HRA cell [19]) [19]
prostate <1, 2> [1, 12]
salivary gland <1, 2> [12]
skeletal muscle <1> [12]
skin <1> [10, 12]
testis <1, 2> [12]
thymus <1> [12]
thyroid cancer cell <2> (<2> 44.1% of papillary carcinomas, 15.4% of follicular adenomas, none follicular or anaplastic carcinomas [18]) [18]
trachea <2> [12]
uterus <1> [12]
Additional information <2> (<2> COS cell [17]; <2> no activity in foreskin fibroblasts or HT-1080 fibrosacroma cells [4]; <2> not in normal thyroid tissue [18]; <2> stomach carcinoma cell line, low level in AZ521 cell, SCH cell, STKM-2 cell, MDA-MB cell, MMK-29 cell, YLC cell, KKI-cell, LU-65 cell and A549 cell,high level in STKM-1, RCM-1 cell, LU-99 cell and VMRC-LCP cell [20]) [4, 17, 18, 20]

Localization

cell surface <1, 2> [12, 15, 20]
membrane <1, 2> (<1> transmembrane protein, composed of a short N-terminal cytoplasmic region followed by a transmembrane domain [12]) [5, 12, 15, 20]
plasma membrane <2> [4]

Purification

<1> (activated matriptase-3 serine protease domain expressed in Spodoptera frugiperda Sf9 cells) [12]
<2> [2, 3, 8, 11]
<2> (by immobilized metal-chelate affinity chromatography and ion exchange chromatography) [16]
<2> (by molecular-sieve chromatography, reverse-phase HPLC) [15]
<2> (from N-acetylglucosminyltransferase V transfectant by immunoaffinity chromatography) [17]
<2> (partial) [4]
<3> (vector pcMT-SP1 MycHis by Ni^{2+} charged resin) [14]

Crystallization

<2> (complexed with benzamidine or bovine panreatic trypsin inhibitor, hanging drop vapor diffusion method) [8]

Cloning

<1> (activated matriptase-3 serine protease domain expressed in insect cells, expression in COS7 cells) [12]
<2> [8, 9]
<2> (expression in MDA-MB-435 breast caner cells) [7]
<2> (into pQE-vecto and expressed in Escherichia coli M15 strain) [16]
<2> (into vector pcDNA3.1, expressed in BT549 breast cancer cells) [13]
<3> (into vector pcMT-SP1 MycHis and MT-SP1wt, expressed in COS-1 monkey kidney cells) [14]

Engineering

D482Y <2> (<2> inhibited activation of matriptase [9]) [9]
D519Y <2> (<2> inhibited activation of matriptase [9]) [9]
D555Y <2> (<2> inhibited activation of matriptase [9]) [9]
D598Y <2> (<2> inhibited activation of matriptase [9]) [9]
D799A <2> (<2> matripase mutant altered in the substrate binding pocket, is able to traffic in the absence of hepatocyte growth factor activator inhibitor-1 [13]) [13]
G149N <2> (<2> results in production of only nonprocesse, full-lenth matriptase [9]) [9]
N109Q <2> (<2> no reduced formation of matriptase-HAI-1-complex [9]) [9]
N302Q <2> (<2> reduced formation of matriptase-HAI-1-complex [9]; <2> significant resistance to degradation in the N-acetylglucosminyltransferase V transfectants [17]) [9, 17]
N485Q <2> (<2> no reduced formation of matriptase-HAI-1-complex [9]; <2> significant resistance to degradation in the N-acetylglucosminyltransferase V transfectants [17]) [9, 17]
N772Q <2> (<2> reduced formation of matriptase-HAI-1-complex [9]; <2> completely degraded in the N-acetylglucosminyltransferase V transfectants [17]) [9, 17]

S805A <2> (<2> matripase mutant altered in the catalytic triad, is able to traffic in the absence of hepatocyte growth factor activator inhibitor-1 [13]) [13]

Application

medicine <2, 3> (<2> expression of serine protease SNC19/matripase and hepatocyte growth factor is decreased in gastrointestinal cancer tissues compared to their normal counterparts, decreased expression may correlate with invasion and lymph node metastasis [21]; <2> matripase mediates cell invasion through activation of receptor-bound pro-urokinase-type plasminogen activator [19]; <2> multiple roles for hepatocyte growth factor activator inhibitor-1 to regulate matripase, including its proper expression, intracellular trafficking, activation and inhibition [13]; <3> possibly controlls epithelial-cell turnover by regulating cell-cell and/or cell-substratum adhesions [14]; <2> prolonged stabilization of matripase is stabilized by UDP-N-acetylglucosamine: α-mannoside β-1,6-N-acetylglucosminyltransferase-mediated glycosylation in vivo, thus extending its halftime and permitting it to play role in the early phases of papillary carcinoma, but not in its later progression [18]) [13, 14, 18, 19, 21]

Additional information <2> (<2> resistance to degradation and stabilization of β1-6GlcNAc matripase are specifically due to the presence of β1-6GlcNAc branching on the sugar chains, binds strongly to leukoagglutinating phytohemagglutinin, which preferentially recognizes β1-6 GlcNAc branches of tri- or tetraantennary sugar chains, β1-6 GlcNAc matriptase is resistant to autodegradation, as well as trypsin digestion, compared with matriptase purified from mock-transfected cells, N-glycosidase-F treatment of β1-6 GlcNAc matriptase greatly reduces its resistance to degradation [17]) [17]

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C5a Peptidase 3.4.21.110

1 Nomenclature

EC number
3.4.21.110

Recommended name
C5a peptidase

Synonyms
GBS <3> [7]
GBS C5a peptidase <1> [4]
S08.020 <1> (<1> Merops ID [5]) [5]
SCFI <3> [13]
SCPA <1, 3> [2, 5, 8, 9, 10, 11, 14]
ScpB <1, 2> [3, 4, 12, 14]
streptococcal chemotactic factor inactivator <3> [13]
surface-bound C5a peptidase <3> [10]

CAS registry number
100179-39-3

2 Source Organism

<1> *Streptococcus pyogenes* (no sequence specified) [1, 2, 3, 4, 5, 6, 11, 14]
<2> *Streptococcus agalactiae* (no sequence specified) [12]
<3> *Streptococcus sp.* (no sequence specified) [7, 8, 9, 10, 13]

3 Reaction and Specificity

Catalyzed reaction
the primary cleavage site is at His67-/-Lys68 in human C5a with a minor secondary cleavage site at Ala58-/-Ser59

Reaction type
cleavage of C-N-linkage
hydrolysis of peptide bond

Natural substrates and products
S chemotaxin C5a <1> (Reversibility: ?) [1, 3, 4, 5, 6]
P ? <1> [1, 3, 4, 5, 6]
S chemotaxin C5a + H_2O <1> (Reversibility: ?) [2]

P ? <1> [2]
S Additional information <1> (<1> alters the normal host inflammatory response by delaying the accumulation of PMNLs at the foci of streptococcal infection [6]; <1> binds directly to HEp2 and A549 cells, binds to the extracellular matrix protein fibronectin, role in cell invasion [3]; <1> mediates adherence to fibronectin, role in immune evasion by attenuating recruitment of polymorphonuclear leukocytes to the site of infection [4]) (Reversibility: ?) [3, 4, 6]
P ? <1> [3, 4, 6]

Substrates and products

S Ac-SQLRANISH-4-nitroanilide + H_2O <1> (<1> weak chromogenic substrate [1]) (Reversibility: ?) [1]
P ? <1> [1]
S Ac-SQLRANISH-*p*-nitroamilide + H_2O <1> (Reversibility: ?) [14]
P ?
S Cys-Val-Val-Ala-Ser-Gln-Leu-Arg-Ala-Asn-Ile-His-Gln-Asp-Met-Gln-Leu-Gly-Arg <1> (<1> substrate is cleaved into 2 fragments [2]) (Reversibility: ?) [2]
P ? <1> [2]
S Cys-Val-Val-Ala-Ser-Gln-Leu-Arg-Ala-Asn-Ile-Ser-His-Lys-Asp-Met-Gln-Leu-Gly-Arg <1> (Reversibility: ?) [2]
P ? <1> [2]
S SQLRANISHKDMQLGR + H_2O <1> (<1> good substrate [1]) (Reversibility: ?) [1]
P ? <1> [1]
S VVASQLRANISH + H_2O <1> (Reversibility: ?) [1]
P VVA + SQLRANISH <1> [1]
S VVASQLRANISHKDMQLGR + H_2O <1> (Reversibility: ?) [1]
P VVASQLRANISH + KDMQLGR <1> [1]
S chemotaxin C5a <1> (<1> cleavage site is His-Lys rather than Lys-Asp, native protein is resistant to cleavage, suggesting that His-Lys bond is inaccessible prior to proteolytic cleavage by C5 convertase [2]) (Reversibility: ?) [1, 2, 3, 4, 5, 6]
P ? <1> [1, 2, 3, 4, 5, 6]
S chemotaxin C5a + H_2O <1, 3> (<1> cleaves between His67 and Lys68 or Ala58 and Ser59 [1]; <1> removes a six-amino-acid fragment and thereby inactivates this chemotaxin [6]; <3> cleavage between histidine-67 and lysine-68 near the C-terminus [7]) (Reversibility: ?) [1, 2, 3, 4, 5, 6, 7, 11, 13]
P ? <1> [1, 2, 3, 4, 5, 6]
S glutathione S-transferase-chemotaxin C5a-GFP + H_2O <1> (<1> fusion protein [5]) (Reversibility: ?) [5]
P ? <1> [5]
S rGST-C5a-enhanced green fluorescent protein + H_2O <1> (Reversibility: ?) [11]
P ?

S Additional information <1, 3> (<1> no substrate: Cys-Val-Val-Ala-Ser-Gln-Leu-Arg-Ala-Asn-Ile-His-Pro-Lys-Asp-Met-Gln-Leu-Gly-Arg [2]; <1> alters the normal host inflammatory response by delaying the accumulation of PMNLs at the foci of streptococcal infection [6]; <1> binds directly to HEp2 and A549 cells, binds to the extracellular matrix protein fibronectin, role in cell invasion [3]; <1> mediates adherence to fibronectin, role in immune evasion by attenuating recruitment of polymorphonuclear leukocytes to the site of infection [4]; <3> native bovine serum albumin and other native proteins are highly resistant to proteolysis [13]; <1> no cleavage of rGST-enhanced green fluorescent protein [11]) (Reversibility: ?) [2, 3, 4, 6, 11, 13]

P ? <1> [2, 3, 4, 6]

Inhibitors

Ca^{2+} <1> (<1> inhibition at 10 mM [5]) [5]
EDTA <1> (<1> inhibition at 5 mM [1]) [1]
Mn^{2+} <1> (<1> inhibition at 10 mM [5]) [5]
NaCl <1> [5]
Zn^{2+} <1> (<1> significant inhibition at 0.1-10 mM [5]) [5]
anti-SCPB antibody <3> (<3> also provides protection against group A streptococcal infection [10]) [10]
anti-ScpA antibody <1, 3> (<3> blocks internalization of streptococci and neutralizes the C5a peptidase acitvity, enabling recruitment of polymorphoneutrophil leukocytes to sites of infection [8]; <3> reduces group A streptococci colonization of nasal mucosa-associated lymphoid tissue administered alone or with cholera toxin [10]; <3> rise in convalescent anti-SCPA antibody in all children aged 2-12 years, mean convalescent anti-SCPA antibody level is twice the level of mean actue anti-SCPA antibody, which is statistically significant [9]) [8, 9, 10, 11]
Additional information <1, 3> (<1> no inhibitor: EDTA [5]; <3> a high degree of group A streptococci colonization of nasal mucosa-associated lymphoid tissue in control mice immunized with phosphate-buffered saline only [10]) [5, 10]

Turnover number (min^{-1})

0.2 <1> (VVASQLRANISH, <1> pH 7.4, 25°C [1]) [1]
1.1 <1> (SQLRANISHKDMQLGR, <1> pH 7.4, 25°C [1]) [1]

Specific activity (U/mg)

Additional information <1> [5]

K_m-Value (mM)

0.36 <1> (SQLRANISHKDMQLGR, <1> pH 7.4, 25°C [1]) [1]
0.936 <1> (VVASQLRANISH, <1> pH 7.4, 25°C [1]) [1]
2 <1> (Ac-SQLRANISH-4-nitroanilide, <1> pH 7.4, 25°C, K_m above 2 mM [1]) [1]

pH-Optimum
7.4 <1> (<1> SQLRANISHKDMQLGR as substrate [1]) [1]
8 <1> [5]
8.6 <1> (<1> activity against chromogenic substrate Ac-SQLRANISH-4-nitroanilide is 60% higher than at pH 7.4 [1]) [1]

pH-Range
7 <1> (<1> activity sharply declines below pH 7 [5]) [5]
8.6 <1> (<1> activity against chromogenic substrate Ac-SQLRANISH-4-nitroanilide is 60% higher than at pH 7.4 [1]) [1]

Temperature optimum (°C)
40-43 <1> [1]

Temperature range (°C)
5-43 <1> (<1> increase of temperature from 5°C to 43°C results in 10fold increase in enzyme activity [1]) [1]

4 Enzyme Structure

Posttranslational modification
proteolytic modification <1> (<1> propeptide is cleaved either via an autocatalytic intramolecular cleavage or by exogenous protease streotopain [1]) [1]

5 Isolation/Preparation/Mutation/Application

Localization
cell surface <1, 3> [3, 8]

Purification
<1> [1, 11]
<2> (by ion-exchange chromatography) [12]
<3> (standard method) [13]

Crystallization
<2> (SCP mutant, by hanging drop method, to 2 A resolution, SCPB composed of five distinct domains, N-terminal subtilisin-like protease domain with a 134-residue protease-associated domain inserted into a loop between two β-strands, and with one of two Arg-Gly-Asp sequences found in SCPB, at the C-terminus are three fibronectin type III domains, second Arg-Gly-Asp sequence is located between fibronectin type III (1) and fibronectin type III (2)) [12]

Cloning
<1> [6]
<1> (expressed in Escherichia coli) [1]
<1> (full length clone and ScpB-PDF fragment) [4]

<2> (in Escherichia coli B834(DE3)) [12]
<3> (strain 90 226 SCPA-mutant expressed in Escherichia coli) [10]

Engineering

D130A <1, 3> (<1> mutant with reduced proteolytic activity [5]; <3> strain 90 226 SCPA-mutant, impaired capacity to infect nasal mucosa-associated lymphoid tissue [10]) [5, 10]
D130A/S512A <1, 2> (<1> no hydrolysis of substrate [14]; <2> SCP mutant with 949-residue fragment [12]) [12, 14]
H193A <1> (<1> mutant with reduced proteolytic activity [5]) [5]
L512A <1> (<1> mutant with reduced proteolytic activity [5]) [5]
N295A <1> (<1> mutant with reduced proteolytic activity [5]) [5]
S512A <1> (<1> no autocatalytic cleavage [1]) [1]
Additional information <1, 3> (<3> isogenic M1-mutant, expressses SCPA on cell surface [8]; <1> isogenic scpA-deficient mutant strain TR-9 [11]) [8, 11]

Application

medicine <1, 3> (<3> acts as a potential invasin of group A streptococcus and promotes invasion independent of fibronectin [8]; <1> immunization with C5a peptidase protein from either group A or group B streptococci provides protection against group A streptococcus infections in mice, moreover, mice immunized with antibodies directed against protein from group B streptococci also cleared streptococci from their lungs more efficiently than those immunized with tetanus toxoid [14]; <3> inactivates recombinant chemotaxin C5a by destroying its capacity to interact with the C5a receptor on human polymorphonuclear leukocytes [7]; <3> intranasal immunization with SCPA prevents colonization and infection of human tonsils, thereby eliminating potential reservoirs that maintain endemic disease [10]; <3> SCPA is highly immunogenic in children infected with group A streptococcal pharyngitis [9]) [7, 8, 9, 10, 14]
Additional information <1, 2, 3> (<3> greatly reduces ability to bind to receptors of polymorphonuclear leukocytes as compared with native C5a, inactivation is temperature dependent, mediates a small decrease in the molecular weight of C5adesArg and a six-residue peptide in inactivated C5a is lost [13]; <1> ScpA and the multifunctional protein streptococcal plasmin receptor/surface dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase are both necessary for the cleavage of C5a on the bacterial surface [11]; <2> streptococcal C5a peptidase binding to integrins by the Arg-Gly-Asp motifs may stabilize conformational changes required for substrate binding [12]) [11, 12, 13]

6 Stability

Temperature stability

60 <1> (<1> complete inactivation [1]) [1]
65 <1> (<1> 85% activity after 30 min, 75% activity after 60 min [5]) [5]
100 <1> (<1> 30% activity after boiling for 10 min [5]) [5]

Storage stability
<2>, -20°C [12]

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Aqualysin 1 3.4.21.111

1 Nomenclature

EC number
3.4.21.111

Recommended name
aqualysin 1

Synonyms
S08.051 <1> (<1> Merops ID [16]) [16]

CAS registry number
88747-68-6

2 Source Organism

<1> *Thermus aquaticus* (no sequence specified) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18]
<2> *Thermus thermophilus* (no sequence specified) [13]

3 Reaction and Specificity

Catalyzed reaction
exhibits low specificity towards esters of amino acids with small hydrophobic or aromatic residues at the P1 position

Reaction type
cleavage of C-N-linkage
hydrolysis of peptide bond

Natural substrates and products
S Additional information <1> (<1> requires its propeptide ProA to function as an intramolecular chaperone, N-terminal IMC can inhibit aqualysin activity and form a stable complex with subtilisin BPN [16]) (Reversibility: ?) [16]
P ? <1> [16]

Substrates and products
S casein + H_2O <1, 2> (Reversibility: ?) [1, 7, 13, 17]
P ? <1, 2> [1, 7, 13, 17]

S Hammarsten casein + H_2O <1> (<1> at 70°C, pH 7.5-10.4 [18]) (Reversibility: ?) [18]
P ?
S N-benzoyloxycarbonyl-L-leucyl-4-nitrophenyl ester + H_2O <1> (Reversibility: ?) [1]
P ? <1> [1]
S N-succinyl-Ala-Ala-Ala-4-nitroanilide + H_2O <1> (<1> synthetic substrate for elastase [12]) (Reversibility: ?) [4, 9, 10, 12, 15]
P N-succinyl-Ala-Ala-Ala + 4-nitroaniline <1> [4, 9, 10, 12, 15]
S N-succinyl-Ala-Ala-Phe-4-nitroanilide + H_2O <1> (Reversibility: ?) [4]
P N-succinyl-Ala-Ala-Phe + 4-nitroaniline <1> [4]
S N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide + H_2O <1> (<1> synthetic substrate for subtilisin [16]) (Reversibility: ?) [2, 4, 5, 6, 8, 9, 11, 15, 16]
P N-succinyl-Ala-Ala-Pro-Phe + 4-nitroaniline <1> [2, 4, 5, 6, 8, 9, 11, 15, 16]
S N-succinyl-Ala-Ala-Val-Ala-4-nitroanilide + H_2O <1> (Reversibility: ?) [6]
P N-succinyl-Ala-Ala-Val-Ala + 4-nitroaniline <1> [6]
S N-succinyl-Ala-Pro-Ala-4-nitroanilide + H_2O <1> (Reversibility: ?) [9]
P N-succinyl-Ala-Pro-Ala + 4-nitroaniline <1> [9]
S N-succinyl-Gly-Gly-Phe-4-nitroanilide + H_2O <1> (Reversibility: ?) [4, 9]
P N-succinyl-Gly-Gly-Phe + 4-nitroaniline <1> [4, 9]
S N-succinyl-Gly-βAla-Ala-4-nitroanilide + H_2O <1> (Reversibility: ?) [9]
P N-succinyl-Gly-βAla-Ala + 4-nitroaniline <1> [9]
S N-succinyl-Ile-Val-Ala-4-nitroanilide + H_2O <1> (Reversibility: ?) [9]
P N-succinyl-Ile-Val-Ala + 4-nitroaniline <1> [9]
S N-succinyl-Ile-βAla-Ala-4-nitroanilide + H_2O <1> (Reversibility: ?) [9]
P N-succinyl-Ile-βAla-Ala + 4-nitroaniline <1> [9]
S N-succinyl-Leu-βAla-Ala-4-nitroanilide + H_2O <1> (Reversibility: ?) [9]
P N-succinyl-Leu-βAla-Ala + 4-nitroaniline <1> [9]
S N-succinyl-Phe-Ala-Ala-4-nitroanilide + H_2O <1> (Reversibility: ?) [4, 9, 10, 15]
P N-succinyl-Phe-Ala-Ala + 4-nitroaniline <1> [4, 9, 10, 15]
S N-succinyl-Phe-Leu-Ala-4-nitroanilide + H_2O <1> (Reversibility: ?) [4, 9]
P N-succinyl-Phe-Leu-Ala + 4-nitroaniline <1> [4, 9]
S N-succinyl-Phe-Nle-Ala-4-nitroanilide + H_2O <1> (Reversibility: ?) [4, 8, 9, 17]
P N-succinyl-Phe-Nle-Ala + 4-nitroaniline <1> [4, 8, 9, 17]
S N-succinyl-Phe-Val-Ala-4-nitroanilide + H_2O <1> (Reversibility: ?) [4, 9]
P N-succinyl-Phe-Val-Ala + 4-nitroaniline <1> [4, 9]
S N-succinyl-Phe-Val-Leu-4-nitroanilide + H_2O <1> (Reversibility: ?) [9]
P N-succinyl-Phe-Val-Leu + 4-nitroaniline <1> [9]
S N-succinyl-Phe-Val-Phe-4-nitroanilide + H_2O <1> (Reversibility: ?) [9]
P N-succinyl-Phe-Val-Phe + 4-nitroaniline <1> [9]
S N-succinyl-Phe-βAla-Ala-4-nitroanilide + H_2O <1> (Reversibility: ?) [9]
P N-succinyl-Phe-βAla-Ala + 4-nitroaniline <1> [9]

S N-tert-butyloxycarbonyl-Val-Leu-Gly-Arg-4-nitroanilide + H_2O <1> (Reversibility: ?) [6]
P ? <1> [6]
S Suc-Ala-Ala-Pro-Phe-*p*-nitroamilide + H_2O <1> (<1> at 40°C, pH 7.5 [18]) (Reversibility: ?) [18]
P Suc-Ala-Ala-Pro-Phe + *p*-nitroamilide
S chromogenic succinyl-tripeptide-4-nitroanilide + H_2O <1> (Reversibility: ?) [10]
P ? <1> [10]
S elastin-orcein + H_2O <1> (<1> at 40°C, pH 7.5 [18]) (Reversibility: ?) [18]
P ?
S oxidized insulin chain B + H_2O <1> (<1> cleavage sites are not specific [12]; <1> at 40°C, pH 7.5 [18]) (Reversibility: ?) [12, 18]
P ? <1> [12]
S Additional information <1> (<1> enzyme has 3 subsites S1, S2 and S3 in its substrate binding site. S_1 site prefers alanine and phenylalanine, S2 site prefers alanine and norleucine, S3 site prefers phenylalanine and isoleucine [9]; <1> preferentially hydrolyzes the ester bond of an alanine ester, but also shows relatively strong activity toward Gly, Trp, Phe and Tyr esters [12]; <1> requires its propeptide ProA to function as an intramolecular chaperone, N-terminal IMC can inhibit aqualysin activity and form a stable complex with subtilisin BPN [16]; <1> exhibits specificity toward ester of amino acids with small haydrophobic or aromatic residues in P1 [18]) (Reversibility: ?) [9, 12, 16, 18]
P ? <1> [9, 12, 16]

Inhibitors

Cu^{2+} <1> (<1> inhibits S102H/G131H mutant at 0.76 mM, inhibition can be restored by addition of EDTA [11]) [11]
DFP <1> (<1> strongly inhibits [18]) [18]
N-terminal propeptide of aqualisin I <1> (<1> potent inhibitor [18]) [18]
Ni^{2+} <1> (<1> inhibits S102H/G131H mutant at 0.76 mM, inhibition can be restored by addition of EDTA [11]) [11]
Streptomyces subtilisin inhibitor <1> (<1> 93% inhibition at 0.001 mg/ml [12]; <1> strongly inhibits [18]) [12, 18]
Z-Ala-Gly-Phe-CH_2Cl <1> (<1> strongly inhibits [18]) [18]
Z-Ala-Gly-Phe-CH_2Cl <1> (<1> almost complete inhibition at 0.5 mM [12]) [12]
Zn^{2+} <1> (<1> inhibits S102H/G131H mutant at 0.76 mM, inhibition can be restored by addition of EDTA [11]) [11]
microbial alkaline protease inhibitor <1> (<1> 98% inhibition at 0.001 mg/ml [12]) [12]

Metals, ions

Ca^{2+} <1> (<1> 1 mol per mol enzyme [5]; <1> binds to low-affinity Ca^{2+} binding site, binding is essential for thermostabilization [10]; <1> stabilizes, at least two Ca^{2+}-binding sites, i. e. stronger and weaker binding sites, weaker

binding site essential for heat stability of the enzyme, Ca^{2+} bound to the stronger binding site is hardly removed with EDTA [18]) [5, 10, 18]
La^{3+} <1> (<1> stabilizes [18]; <1> binds to low-affinity Ca^{2+} binding site as well as to an additional metal ion-binding site that can also be involved in thermostabilization [10]) [10, 18]
Na^{+} <1> (<1> binds to low-affinity Ca^{2+} binding site, binding is essential for thermostabilization [10]) [10]
Nd^{3+} <1> (<1> stabilizes [18]) [18]
Sr^{2+} <1> (<1> stabilizes [18]) [18]
Tb^{3+} <1> (<1> stabilizes [18]) [18]

Turnover number (min^{-1})

0.009 <1> (N-succinyl-Ala-Ala-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101L mutant [4]) [4]
0.014 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101L mutant [4]) [4]
0.024 <1> (N-succinyl-Gly-Gly-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101L mutant [4]) [4]
0.047 <1> (N-succinyl-Ala-Ala-Val-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
0.069 <1> (N-succinyl-Phe-Val-Leu-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
0.091 <1> (N-succinyl-Ala-Ala-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101V mutant [4]) [4]
0.1 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]; <1> pH 7.5, 40°C, absence of DMSO [10]) [9, 10]
0.11 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, S202E mutant [15]; <1> pH 7.5, 40°C, S202H mutant [15]) [15]
0.15 <1> (N-succinyl-Gly-Gly-Phe-4-nitroanilide, <1> pH 7.4, 40°C [9]; <1> pH 7.5, 40°C, wild-type [4]) [4, 9]
0.17 <1> (N-succinyl-Gly-Gly-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101A mutant [4]) [4]
0.18 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, presence of DMSO [10]) [10]
0.18 <1> (N-succinyl-Phe-Nle-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G101A mutant [4]) [4]
0.18 <1> (N-succinyl-Phe-Val-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G101A mutant [4]) [4]
0.22 <1> (N-succinyl-Ala-Ala-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101A mutant [4]) [4]
0.25 <1> (N-succinyl-Gly-Gly-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101V mutant [4]) [4]
0.48 <1> (N-succinyl-Ala-Pro-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
0.5 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, S202E mutant [15]) [15]
0.88 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101A mutant [4]) [4]
0.9 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101V mutant [4]) [4]

1.1 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, presence of DMSO [10]) [10]
1.1 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G131H mutant [15]) [15]
1.2 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, wild-type [15]) [15]
1.3 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, absence of DMSO [10]) [10]
1.4 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
1.5 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G131K mutant [15]) [15]
1.6 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, S202H mutant [15]) [15]
2.4 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G131D mutant [15]) [15]
2.5 <1> (N-succinyl-Ala-Ala-Phe-4-nitroanilide, <1> pH 7.4, 40°C [9]; <1> pH 7.5, 40°C, wild-type [4]) [4, 9]
2.6 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7, 50°C [12]) [12]
2.9 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, S202K mutant [15]) [15]
3.3 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.4, 40°C [9]; <1> pH 7.5, 40°C [8]) [8, 9]
3.3 <1> (N-succinyl-Phe-Leu-Ala-4-nitroanilide, <1> pH 7.5, 40°C, wild-type [4]) [4]
3.6 <1> (N-succinyl-Phe-Leu-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
4.7 <1> (N-succinyl-Phe-Val-Ala-4-nitroanilide, <1> pH 7.5, 40°C, wild-type [4]) [4]
5 <1> (N-succinyl-Ile-Val-Ala-4-nitroanilide, <1> pH 7.5, 40°C, absence of DMSO [10]) [10]
5 <1> (N-succinyl-Phe-Val-Phe-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
5.4 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G131K mutant [15]) [15]
6.08 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101A mutant [4]) [4]
7.3 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G131H mutant [15]) [15]
7.9 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G131D mutant [15]) [15]
8.1 <1> (N-succinyl-Ile-Val-Ala-4-nitroanilide, <1> pH 7.5, 40°C, presence of DMSO [10]) [10]
8.5 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, S202K mutant [15]) [15]
8.8 <1> (N-succinyl-Phe-Nle-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]; <1> pH 7.5, 40°C [8]; <1> pH 7.5, 40°C, wild-type [4]) [4, 8, 9]
8.9 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G131H mutant [15]) [15]

9 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G131K mutant [15]) [15]
9 <1> (N-succinyl-Ala-Ala-Val-Ala-4-nitroanilide, <1> 40°C pH 7.5, N219T mutant [6]) [6]
11 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, wild-type [4,15]) [4, 15]
17 <1> (N-tert-butyloxycarbonyl-Val-Leu-Gly-Arg-4-nitroanilide, <1> 40°C pH 7.5, N219T mutant [6]) [6]
18 <1> (N-succinyl-Ala-Ala-Val-Ala-4-nitroanilide, <1> 40°C pH 7.5, wild-type [6]) [6]
24 <1> (N-succinyl-Ala-Ala-Val-Ala-4-nitroanilide, <1> 40°C pH 7.5, N219S mutant [6]) [6]
25 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, S202E mutant [15]) [15]
26 <1> (N-tert-butyloxycarbonyl-Val-Leu-Gly-Arg-4-nitroanilide, <1> 40°C pH 7.5, wild-type [6]) [6]
33 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, wild-type [15]) [15]
42 <1> (N-tert-butyloxycarbonyl-Val-Leu-Gly-Arg-4-nitroanilide, <1> 40°C pH 7.5, N219S mutant [6]) [6]
54 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, S202H mutant [15]) [15]
66 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> 40°C pH 7.5, N219T mutant [6]) [6]
84 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> 40°C pH 7.5, wild-type [6]) [6]
144 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, S202K mutant [15]) [15]
296 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> 40°C pH 7.5, N219S mutant [6]) [6]
20400 <1> (N-succinyl-Phe-Nle-Ala-4-nitroanilide, <1> 40°C, pH 7.5, precursor protein [17]) [17]
47400 <1> (N-succinyl-Phe-Nle-Ala-4-nitroanilide, <1> 40°C, pH 7.5, mature protein [17]) [17]

Specific activity (U/mg)

Additional information <1> [1, 12, 17]

K_m-Value (mM)

0.036 <1> (N-succinyl-Phe-Nle-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]; <1> pH 7.5, 40°C [8]) [8, 9]
0.037 <1> (N-succinyl-Phe-Nle-Ala-4-nitroanilide, <1> 40°C, pH 7.5, precursor protein [17]) [17]
0.039 <1> (N-succinyl-Phe-Val-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
0.04 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]; <1> pH 7.5, 40°C, absence of DMSO [10]) [9, 10]
0.044 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, wild-type [15]) [15]

0.05 <1> (N-succinyl-Phe-Nle-Ala-4-nitroanilide, <1> 40°C, pH 7.5, mature protein [17]) [17]
0.064 <1> (N-succinyl-Ile-Val-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]; <1> pH 7.5, 40°C, absence of DMSO [10]) [9, 10]
0.087 <1> (N-succinyl-Phe-Leu-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
0.15 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, S202K mutant [15]) [15]
0.24 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, S202H mutant [15]) [15]
0.34 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, S202E mutant [15]) [15]
0.4 <1> (N-succinyl-Ala-Ala-Val-Ala-4-nitroanilide, <1> 40°C pH 7.5, N219T mutant [6]) [6]
0.41 <1> (N-succinyl-Phe-Val-Leu-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
0.49 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G131D mutant [15]) [15]
0.5 <1> (N-succinyl-Ala-Ala-Val-Ala-4-nitroanilide, <1> 40°C pH 7.5, N219S mutant [6]) [6]
0.51 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G131K mutant [15]) [15]
0.52 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G131H mutant [15]) [15]
0.7 <1> (N-succinyl-Ala-Ala-Val-Ala-4-nitroanilide, <1> 40°C pH 7.5, wild-type [6]) [6]
0.73 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101V mutant [4]) [4]
0.76 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101L mutant [4]) [4]
0.79 <1> (N-succinyl-Ala-Pro-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
0.91 <1> (N-succinyl-Ala-Ala-Phe-4-nitroanilide, <1> pH 7.4, 40°C [9]; <1> pH 7.5, 40°C, wild-type [4]) [4, 9]
1 <1> (N-succinyl-Ala-Ala-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101V mutant [4]) [4]
1 <1> (N-succinyl-Ile-Val-Ala-4-nitroanilide, <1> pH 7.5, 40°C, presence of DMSO [10]) [10]
1 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G131D mutant [15]) [15]
1.1 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, absence of DMSO [10]; <1> pH 7.5, 40°C, wild-type [15]) [10, 15]
1.1 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> 40°C pH 7.5, N219T mutant [6]; <1> pH 7.5, 40°C, S202E mutant [15]) [6, 15]
1.2 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.4, 40°C [9]; <1> pH 7.5, 40°C [8]; <1> pH 7.5, 40°C, wild-type [4,15]) [4, 8, 9, 15]
1.3 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
1.3 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> 40°C pH 7.5, wild-type [6]; <1> pH 7.5, 40°C, G101A mutant [4]) [4, 6]

1.5 <1> (N-succinyl-Ala-Ala-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101L mutant [4]) [4]
1.6 <1> (N-succinyl-Ala-Ala-Val-4-nitroanilide, <1> pH 7.4, 40°C [9]) [9]
1.6 <1> (N-succinyl-Gly-Gly-Phe-4-nitroanilide, <1> pH 7.4, 40°C [9]; <1> pH 7.5, 40°C, wild-type [4]) [4, 9]
1.7 <1> (N-succinyl-Ala-Ala-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101A mutant [4]) [4]
1.7 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, S202K mutant [15]) [15]
1.8 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, S202H mutant [15]) [15]
1.9 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G131H mutant [15]) [15]
2 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G131K mutant [15]) [15]
2.1 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G131D mutant [15]; <1> pH 7.5, 40°C, S202H mutant [15]) [15]
2.2 <1> (N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, <1> 40°C pH 7.5, N219S mutant [6]) [6]
2.2 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, presence of DMSO [10]) [10]
2.3 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G131K mutant [15]) [15]
2.6 <1> (N-tert-butyloxycarbonyl-Val-Leu-Gly-Arg-4-nitroanilide, <1> 40°C pH 7.5, N219T mutant [6]) [6]
2.7 <1> (N-tert-butyloxycarbonyl-Val-Leu-Gly-Arg-4-nitroanilide, <1> 40°C pH 7.5, N219S mutant [6]) [6]
2.9 <1> (N-tert-butyloxycarbonyl-Val-Leu-Gly-Arg-4-nitroanilide, <1> 40°C pH 7.5, wild-type [6]) [6]
3.4 <1> (N-succinyl-Gly-Gly-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101V mutant [4]) [4]
3.8 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, S202E mutant [15]) [15]
4.2 <1> (N-succinyl-Gly-Gly-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101A mutant [4]) [4]
5.3 <1> (N-succinyl-Gly-Gly-Phe-4-nitroanilide, <1> pH 7.5, 40°C, G101L mutant [4]) [4]
7.1 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G131H mutant [15]) [15]
9.3 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, presence of DMSO [10]) [10]
10 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, S202K mutant [15]) [15]
20 <1> (N-succinyl-Ala-Ala-Ala-4-nitroanilide, <1> pH 7, 50°C [12]) [12]
36 <1> (N-succinyl-Phe-Nle-Ala-4-nitroanilide, <1> pH 7.5, 40°C, wild-type [4]) [4]

39 <1> (N-succinyl-Phe-Val-Ala-4-nitroanilide, <1> pH 7.5, 40°C, wild-type [4]) [4]
44 <1> (N-succinyl-Phe-Ala-Ala-4-nitroanilide, <1> pH 7.5, 40°C, wild-type [4]) [4]
71 <1> (N-succinyl-Phe-Leu-Ala-4-nitroanilide, <1> pH 7.5, 40°C, wild-type [4]) [4]
730 <1> (N-succinyl-Phe-Nle-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G101A mutant [4]) [4]
1200 <1> (N-succinyl-Phe-Val-Ala-4-nitroanilide, <1> pH 7.5, 40°C, G101A mutant [4]) [4]

pH-Optimum
10 <1> [12, 18]
10-11.5 <1> [1]

pH-Range
7.5-10.4 <1> [18]

Temperature optimum (°C)
70 <1, 2> (<2> assay at [13]; <1> in the absence of Ca^{2+} [12]) [12, 13]
70-80 <1> [1]
75-95 <1> [7]
80 <1> (<1> in the presence of Ca^{2+} [12]; <1> in the presence of 1 mM $CaCl_2$, which stabilizes the enzyme [18]) [5, 12, 17, 18]

Temperature range (°C)
40-80 <1> [18]

4 Enzyme Structure

Molecular weight
12090 <1> (<1> ProA peptide, calculated from nucleic acid sequence [16]) [16]
14000 <1> (<1> ProA peptide, gel filtration [16]) [16]
28000 <1, 2> (<1,2> SDS-PAGE [5,13]; <1> mature protein, SDS-PAGE [10]; <1> mature protein after heat treatment, SDS-PAGE [2,3,17]) [2, 3, 5, 10, 13, 17]
28350 <1> (<1> calculated [18]) [18]
28500 <1> (<1> SDS-PAGE [12]) [12]
38000 <1> (<1> precursor protein, SDS-PAGE [2,3,10,17]; <1> precursor with a C-terminal pro sequence from the membrane fraction of Escherichia coli cells [18]) [2, 3, 10, 17, 18]
53910 <1> (<1> synthesized as a large precursor, calculated [18]) [18]

Posttranslational modification
proteolytic modification <1> (<1> intermolecular autocatalytic activation upon heat treatment at 70°C [10]; <1> intramolecular self-processing mechanism [17]) [10, 17]

5 Isolation/Preparation/Mutation/Application

Localization

cytoplasm <1> (<1> 60-70% of the deletion mutant enzyme can be found in cytoplasm, whereas only 30% of the wild-type enzyme is cytoplasmic [3]) [3]
membrane <1> (<1> precursor protein [17]) [3, 17]
periplasm <1> (<1> most activity [2]) [2]
soluble <1> (<1> mature protein [17]) [17]
spheroplast <1> (<1> little activity [2]) [2]

Purification

<1> [5, 10]
<1> (38000 Da precursor protein) [17]
<1> (ProA peptide) [16]
<1> (disulfide-containing chymotryptic peptides) [14]
<1> (homogeneity) [1]
<1> (recombinant enzyme from Escherichia coli cells harboring an expression plasmid using the tac promoter by cation-exchange chromatography, larger scale production by using bacteriophage T7 RNA polymerase/promoter) [18]

Crystallization

<1> (hanging and sitting drop vapor diffusion method) [1]

Cloning

<1> [4, 6, 11, 15]
<1> (C-terminal deletion mutant, expressed in Escherichia coli) [3]
<1> (ProA peptide) [16]
<1> (expression in Escherichia coli) [17]
<1> (expression in Escherichia coli and Thermus thermophilus) [18]
<1> (expression in Pichia pastoris) [10]
<1> (mutant with deletion of the C-terminal pro-sequence, expressed in Saccharomyces cerevisiae) [2]
<2> (wild-type and C-terminal deletion mutants) [13]

Engineering

C5A/C8A <1> (<1> intracellular distribution similar to wild-type enzyme [3]) [3]
C5S/C8S <1> (<1> intracellular distribution similar to wild-type enzyme [3]) [3]
G101A <1> (<1> catalytic efficiencies for bulky amino acid residues in P2 site such as valine and lucine drastically decreased [4]) [4]
G101L <1> (<1> reduced catalytic efficiencies for any substrate [4]) [4]
G101V <1> (<1> catalytic efficiency toward glycine was retained [4]) [4]
G131D <1> (<1> reduced efficiency for N-succinyl-Phe-Ala-Ala-4-nitroanilide, raised k_{cat} for N-succinyl-Ala-Ala-Ala-4-nitroanilide [15]) [15]
G131H <1> (<1> reduced efficiency for N-succinyl-Phe-Ala-Ala-4-nitroanilide, raised k_{cat} for N-succinyl-Ala-Ala-Ala-4-nitroanilide [15]) [15]

G131K <1> (<1> reduced efficiency for N-succinyl-Phe-Ala-Ala-4-nitroanilide, raised k_{cat} for N-succinyl-Ala-Ala-Ala-4-nitroanilide [15]) [15]
N219S <1> (<1> increased catalytic activity [6]) [6]
N219T <1> (<1> slightly decreased catalytic activity [6]) [6]
S102E <1> (<1> reduced efficiency for N-succinyl-Phe-Ala-Ala-4-nitroanilide, raised k_{cat} for N-succinyl-Ala-Ala-Ala-4-nitroanilide [15]) [15]
S102H <1> (<1> reduced efficiency for N-succinyl-Phe-Ala-Ala-4-nitroanilide, raised k_{cat} for N-succinyl-Ala-Ala-Ala-4-nitroanilide [15]) [15]
S102H/G131H <1> (<1> enzyme can be inhibited by metal ions [11]) [11]
S102K <1> (<1> reduced efficiency for N-succinyl-Phe-Ala-Ala-4-nitroanilide, raised k_{cat} for N-succinyl-Ala-Ala-Ala-4-nitroanilide [15]) [15]

Application

Additional information <1> (<1> C-terminal pro sequence of the enzyme functions as an intramolecular chaperone that stabilizes the partially unfolded structure of the protease domain, and thereby facilitates its secretion [18]) [18]

6 Stability

Temperature stability

70 <1> (<1> 80% activity after 30 min [7]) [7]
80 <1> (<1> rapid inactivation in absence of Ca^{2+} [6]; <1> Sr^{2+} and Ca^{2+} stabilize the enzyme to heat treatment [12]; <1> stable in presence of 1 mM Ca^{2+}, after gel filtration to remove free Ca^{2+} the enzyme still binds Ca^{2+} but is no longer stable, La^{3+}, Sr^{2+}, Nd^{3+} and Tb^{3+} stabilize the enzyme [5]) [5, 6, 12]
90 <1> (<1> half-life of wild-type and N219S mutant: 120 min, half-life of N219T mutant: 60 min in presence of Ca^{2+} [6]; <1> presence of Ca^{2+}, 40% activity after 30 min [12]) [6, 12]
95 <1> (<1> presence of Ca^{2+}, 25% activity after 30 min [12]) [12]

Organic solvent stability

DMSO <1> (<1> 10% DMSO alters the substrate specificity of the enzyme, solvent may take part in the substrate recognition of the enzyme [10]) [10]

General stability information

<1>, stable against 2 M urea and 2 M guanidine hydrochloride [14]
<1>, stable to denaturing reagents like 7 M urea, 6 M guanidine/HCl and 1% SDS [12]
<1>, stable towards denaturing reagents such as 2.5% Tween-20, 4 M guanidine HCl or 4 M urea [7]

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Site-1 protease 3.4.21.112

1 Nomenclature

EC number
3.4.21.112

Recommended name
site-1 protease

Synonyms
S08.063 <5> (<5> Merops ID [8]) [8]
S1P <1, 2, 4, 5> [1, 2, 3, 4, 5, 7, 8, 10, 13, 14]
SK-1 <4> [9]
SKI-1 <4, 5> [1, 12]
SKI-1/S1P <1, 3, 4, 5, 6, 7> [6, 11, 15]
hSKI-1 <4> [6]
kexin <4> [9]
subtilisin kexin isozyme-1 <4> [9]
subtilisin kexin isozyme-1(SK-1)/site 1 protease (S1P) <4> [3]
subtilisin kexin-isozyme-1 <4> [11]
subtilisin-kexin-isozyme <5> [1]

CAS registry number
167140-48-9

2 Source Organism

<1> *Cricetulus griseus* (no sequence specified) [2, 4, 11, 14]
<2> *Mammalia* (no sequence specified) [5]
<3> *Mus musculus* (no sequence specified) [11]
<4> *Homo sapiens* (no sequence specified) [3, 6, 7, 8, 9, 10, 11, 12, 13, 15]
<5> *Rattus norvegicus* (no sequence specified) [1,8,11]
<6> *Arabidopsis thaliana* (no sequence specified) [11]
<7> *Oryza sativa* (no sequence specified) [11]

3 Reaction and Specificity

Catalyzed reaction
processes precursors containing basic and hydrophobic/aliphatic residues at P4 and P2, respectively, with a relatively relaxed acceptance of amino acids at P1 and P3

Reaction type

cleavage of C-N-linkage
hydrolysis of peptide bond

Natural substrates and products

S Additional information <1, 2> (<2> functions to control lipid biosynthesis and uptake in animal cells [5]; <1> initiates a process by which the active fragments of the SREBPs translocate to the nucleus and activate genes controlling the synthesis and uptake of cholersterol and unsaturated fatty acids in animal cells [2]) (Reversibility: ?) [2, 5]
P ? <1, 2> [2, 5]

Substrates and products

S 2-aminobenzoic acid-Asp-Ile-Tyr-Ile-Ser-Arg-Arg-Leu-Leu-Gly-Thr-Phe-Thr-(3-nitro)Tyr-Ala + H_2O <4> (<4> cleaves between Leu and Gly [3]) (Reversibility: ?) [3]
P 2-aminobenzoic acid-Asp-Ile-Tyr-Ile-Ser-Arg-Arg-Leu-Leu + Gly-Thr-Phe-Thr-(3-nitro)Tyr-Ala <4> [3]
S 2-aminobenzoyl-ALVLRKPLFLDSY(NO_2)-Ala + H_2O <4> (<4> cleaves between Leu and Phe [7]) (Reversibility: ?) [7]
P ? <4> [7]
S 2-aminobenzoyl-Arg-Asn-Thr-Pro-Arg-Arg-Glu-Arg-Arg-Arg-Lys-Lys-Arg-Gly-Leu-(3-nitro)Tyr-Ala + H_2O <4> (<4> cleaves between Leu and 3-nitrotyrosine [3]) (Reversibility: ?) [3]
P 2-aminobenzoyl-Arg-Asn-Thr-Pro-Arg-Arg-Glu-Arg-Arg-Arg-Lys-Lys-Arg-Gly-Leu + (3-nitro)Tyr-Ala <4> [3]
S 2-aminobenzoyl-Arg-His-Ser-Ser-Arg-Arg-Leu-Leu-Arg-Ala-Ile-(3-nitro)-Tyr-Ala + H_2O <4> (<4> cleaves between Leu and Arg [3]) (Reversibility: ?) [3]
P 2-aminobenzoyl-Arg-His-Ser-Ser-Arg-Arg-Leu-Leu + Arg-Ala-Ile-(3-nitro)Tyr-Ala <4> [3]
S 2-aminobenzoyl-Arg-Ser-Leu-Lys-Tyr-Ala-Glu-Ser-Asp-(3-nitro)-Tyr-Ala + H_2O <4> (<4> cleaves between Lys and Tyr [3]) (Reversibility: ?) [3]
P 2-aminobenzoyl-Arg-Ser-Leu-Lys + Tyr-Ala-Glu-Ser-Asp-(3-nitro)-Tyr-Ala <4> [3]
S 2-aminobenzoyl-SSGSRRLLSEESY(NO_2)-Ala + H_2O <4> (<4> cleaves between Leu and Ser [7]) (Reversibility: ?) [7]
P ? <4> [7]
S 2-aminobenzoyl-Ser-Arg-Arg-Leu-Leu-Arg-Ala-Leu-Glu-(3-nitro)Tyr-Ala + H_2O <4> (<4> cleaves between Leu and Arg [3]) (Reversibility: ?) [3]
P 2-aminobenzoyl-Ser-Arg-Arg-Leu-Leu + Arg-Ala-Leu-Glu-(3-nitro)Tyr-Ala <4> [3]
S 2-aminobenzoyl-Val-Phe-Arg-Ser-Leu-Lys-Tyr-Ala-Glu-Ser-Asp-(3-nitro)-Tyr-Ala + H_2O <4> (<4> cleaves between Lys and Tyr [3]) (Reversibility: ?) [3]
P 2-aminobenzoyl-Val-Phe-Arg-Ser-Leu-Lys + Tyr-Ala-Glu-Ser-Asp-(3-nitro)Tyr-Ala <4> [3]

S ATF6 + H_2O <1> (<1> i.e. activating transcription factor 6 [11,14]) (Reversibility: ?) [11, 14]
P
S ATF6 + H_2O <4> (<4> i.e. activating transcription factor 6 [10]) (Reversibility: ?) [10]
P ?
S Abz-Asp-Ile-Tyr-Ile-Ser-Arg-Arg-Leu-Leu-Gly-Thr-Phe-Thr-(3-nitro)Tyr-Ala-$CONH_2$ + H_2O <4> (<4> favored quenched fluorogenic substrate [11]) (Reversibility: ?) [11]
P ?
S Abz-DIYISRRLL-GTFT-Tyx-A + H_2O <4> (Reversibility: ?) [9]
P Abz-DIYISRRLL + GTFT-Tyx-A
S Ac-VFRSLK-4-methylcoumarin 7-amide <2> (Reversibility: ?) [5]
P ? <2> [5]
S CREB4 + H_2O <4> (<4> i.e. androgen-induced leucine zipper protein, C-terminal domain of CREB4 somehow confers resistance to cleavage by S1P, which can be released either by removal of the region or physiologically by some regulatory signal [13]) (Reversibility: ?) [13]
P ?
S Crimean Congo hemorrhagic fever virus glycoprotein + H_2O <4> (<4> cleavage at RRLL-sites [11]) (Reversibility: ?) [11]
P ?
S Lassa virus glycoprotein + H_2O <4> (<4> cleavage at RRLL-sites [11]) (Reversibility: ?) [11]
P ?
S Lassa virus glycoprotein precursor GP-C + H_2O <4> (<4> cleavage at the C-terminal end of the recognition motif R-R-L-L [15]) (Reversibility: ?) [15]
P GP-2
S brain-derived neurotrophic factor precursor + H_2O <4> (<4> cleaves at an RGLTLS site between Thr and Ser [8]) (Reversibility: ?) [8]
P ? <4> [8]
S glycoprotein precursor Gc + H_2O <4> (Reversibility: ?) [12]
P ?
S glycoprotein precursor Gn + H_2O <4> (Reversibility: ?) [12]
P ?
S sterol regulatory element-binding protein + H_2O <1, 2> (<1,2> SREBP [4,5]; <1> SREBP, cleaves the luminal loop following the tetrapeptide sequence Arg-Xaa-Xaa-Leu [2]) (Reversibility: ?) [2, 4, 5]
P ? <1, 2> [2, 4, 5]
S Additional information <1, 2, 4, 5> (<5> can cleave postsingle and possibly pairs of Thr residues in certain precursors [1]; <2> cleaves after lysine or leucine rather than arginine [5]; <1> initiates a process by which the transcriptionally active N-terminal fragments of SREBPs are released from membranes [4]; <4> peptides containing RSLK and RRLL are cleaved best [6]; <4> RRLL peptide representing the Gn processing site is efficiently cleaves but an RKPL peptide representing the GC processing

site is cleaved at negligible levels [7]; <2> functions to control lipid biosynthesis and uptake in animal cells [5]; <1> initiates a process by which the active fragments of the SREBPs translocate to the nucleus and activate genes controlling the synthesis and uptake of cholersterol and unsaturated fatty acids in animal cells [2]) (Reversibility: ?) [1, 2, 4, 5, 6, 7]

P ? <1, 2, 4, 5> [1, 2, 4, 5, 6, 7]

Inhibitors

1,10-Phenanthroline <2, 4> (<4> complete inactivation at 5 mM [6]; <2> partial inhibition at very high concentrations [5]) [5, 6]
4-(2-aminoethyl benzene)sulfonyl fluoride <4> (<4> i.e. AEBSF, competitive [9]) [9]
4-(2-aminoethyl)-benzenesulfonyl fluoride <4> [10]
Ac-VFRSLK-4-(2-aminoethyl benzene)sulfonyl fluoride <4> [9]
Ca^{2+} <2> (<2> inhibition above 3 mM [5]) [5]
$CuSO_4$ <4> (<4> complete inactivation at 1 mM [6]) [6]
Dithiothreitol <2> (<2> partial inhibition at very high concentrations [5]) [5]
EDTA <2, 4> (<4> complete inactivation at 10 mM [6]; <2> partial inhibition at very high concentrations [5]) [5, 6]
EGTA <2, 4> (<4> 85% inactivation at 10 mM [6]; <2> partial inhibition at very high concentrations [5]) [5, 6]
Pefabloc SC <2> (<2> at high concentrations [5]) [5]
$ZnSO_4$ <4> (<4> complete inactivation at 1 mM [6]) [6]
prosegment of SKI-1 <4> [6]
Additional information <4> (<4> decanoyl-RVKR-chloromethylketone does not affect SKI-1-mediated processing [12]) [12]

Metals, ions

Ca^{2+} <4> (<4> dependent [6,8]) [6, 8]

K_m-Value (mM)

0.00096 <4> (2-aminobenzoyl-Arg-Ser-Leu-Lys-Tyr-Ala-Glu-Ser-Asp-(3-nitro)Tyr-Ala, <4> pH 7.4, 37°C [3]) [3]
0.00428 <4> (2-aminobenzoyl-Val-Phe-Arg-Ser-Leu-Lys-Tyr-Ala-Glu-Ser-Asp-(3-nitro)Tyr-Ala, <4> pH 7.4, 37°C [3]) [3]
0.0044 <4> (2-aminobenzoyl-Asp-Ile-Tyr-Ile-Ser-Arg-Arg-Leu-Leu-Gly-Thr-Phe-Thr-(3-nitro)Tyr-Ala, <4> pH 7.4, 37°C [3]) [3]
0.0051 <4> (2-aminobenzoic acid-SSGSRRLLSEESY(NO_2)-Ala-NH_2, <4> pH 7.4 [7]) [7]
0.02 <4> (2-aminobenzoyl-Arg-His-Ser-Ser-Arg-Arg-Leu-Leu-Arg-Ala-Ile-(3-nitro)Tyr-Ala, <4> pH 7.4, 37°C [3]) [3]
0.023 <4> (2-aminobenzoyl-Ser-Arg-Arg-Leu-Leu-Arg-Ala-Leu-Glu-(3-nitro)Tyr-Ala, <4> pH 7.4, 37°C [3]) [3]
0.028 <4> (2-aminobenzoyl-Arg-Asn-Thr-Pro-Arg-Arg-Glu-Arg-Arg-Arg-Lys-Lys-Arg-Gly-Leu-(3-nitro)Tyr-Ala, <4> pH 7.4, 37°C [3]) [3]
0.1 <2> (Ac-VFRSLK-4-methyl-coumaryl-7-amide, <2> pH 8, 37°C [5]) [5]

K_i-Value (mM)

57.7 <4> (Ac-VFRSLK-4-(2-aminoethyl benzene)sulfonyl fluoride, <4> substrate Abz-DIYISRRLL-GTFT-Tyx-A, 37°C, pH 7.4 [9]) [9]
231 <4> (4-(2-aminoethyl benzene)sulfonyl fluoride, <4> substrate Abz-DIYISRRLL-GTFT-Tyx-A, 37°C, pH 7.4 [9]) [9]

pH-Optimum

6.5 <4> [6]
7-8 <2> [5]

4 Enzyme Structure

Molecular weight

98000 <4> (<4> SDS-PAGE [6]) [6, 11]
148000 <4> (<4> zymogen, SDS-PAGE [8]) [8]

Posttranslational modification

glycoprotein <1, 4> (<4> putative N-glycosylation at Asn148 [6]; <1> six potential N-glycosylation sites [4]) [4, 6, 8]
proteolytic modification <1, 2, 4> (<2> enzyme undergoes autocatalytic processing, generating an active form S1P-B, which is further cleaved after residue 186 to generate S1P-C [5]; <4> proSKI-1 is converted to active SKI-1 [6]; <4> proSKI-1 is processed into two membrane-bound forms of SKI-1: 120000 Da and 106000 Da [8]; <1> protein is inactive and undergoes autocatalytic processing, generating an active form S1P-B, which is further cleaved at an internal RRLL sequence to generate an active S1P-C [4]; <1> SP1-A is inactive and undergoes autocatalytic processing, generating an active form S1P-B, which is further cleaved after residue 186 to generate S1P-C [2]) [2, 4, 5, 6, 8]

5 Isolation/Preparation/Mutation/Application

Source/tissue

HeLa cell <4> [10]
adrenal gland <5> [1, 8]
bone <5> [8]
brain <5> [8]
cerebellum <5> [8]
hepatocyte <3> [11]
intestine <5> [8]
kidney <5> [8]
liver <3, 5> [8, 11]
melanocyte <5> [1]
molar <5> [8]
muscle <5> [8]
pituitary gland <5> [1, 8]
retina <5> [8]

skin <5> [1, 8]
submaxillary gland <5> [1, 8]
thymus <5> [1, 8]
thyroid gland <5> [8]
tooth <5> [8]
Additional information <4> (<4> SW-13 cell, infected with crimean-Congo hemorrhagic fever virus [12]) [12]

Localization

Golgi apparatus <1, 4> (<1> cis-and medial-Golgi, but not in the trans-Golgi [2]; <4> SP1 localizes to the same compartment as SP2, the cis/medial Golgi [10]) [2, 8, 10, 13]
endoplasmic reticulum <1, 4> (<4> relocalization of SP1 and SP2 with brefeldin A, reconstitution of the sequential cleavage of ATF6 [10]) [10, 11, 14, 15]
membrane <1, 4, 5> [1, 2, 11]

Purification

<2> [5]
<4> [6]

Cloning

<1> [4]
<1> (into pcMV-S1P-Myc) [14]
<1> (truncated from lacking the COOH-terminal membrane anchor, expressed in CHO cells) [2]
<2> (truncated from lacking the COOH-terminal membrane anchor, expressed in CHO cells) [5]
<4> [6, 8]
<4> (pCDNA3.1-SKI/S1P expressed in CHO-K1, BHK, Vero, or SRD-12B cells) [15]

Engineering

S414A <1> (<1> inactive mutant, no autocatalytic processing to generate S1P-C [4]) [4]
Additional information <1, 3> (<3> complete silencing of SKI-1/SP1 results in an embryonic lethal phenotype, elimination of about 70-90% of SKI-1/SP1 protein expression in liver results in a drop of ca. 50% in the level of circulating cholesterol and triglycerides, cholesterol and fatty acid biosynthesis in hepatocytes declines by 75%, low density lipoprotein receptor mRNA declines by 50% [11]; <1> S1P-deficient cells, ATF6 processing partially blocked [14]) [11, 14]

Application

degradation <4> (<4> S1P reduces the size of the luminal domain to prepare ATF6 to be an optimal S2P substrate [10]) [10]
Additional information <1, 4> (<4> cleaves glycoprotein, the only cleaved glycoprotein is incorporated into virions and this is necessary for the formation of infectious virus [15]; <1> S1P is required for the endoplasmic reticulum stress response as well as for lipid synthesis [14]) [14, 15]

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Pestivirus NS3 polyprotein peptidase 3.4.21.113

1 Nomenclature

EC number
3.4.21.113

Recommended name
pestivirus NS3 polyprotein peptidase

Synonyms
BVD NS3 endopeptidase <1> [5]
BVDV NS3 endopeptidase <1> [5]
CSFV NS3 endopeptidase <1> [5]
NS3 <1> [1, 5]
NS3 proteinase <1> [1, 5]
NS3 serine protease <1> [4, 5]
P80pestivirus P80 protein <1> [5]
S31.001 <2> (<2> Merops ID [3]) [3]
border disease virus NS3 endopeptidase <1> [5]
bovine <1> [5]
classical swine fever virus NS3 endopeptidase <1> [5]
nonstructural protein NS3 <1, 2> [3, 5]
viral diarrhea virus endopeptidase <1> [5]

CAS registry number
37259-58-8

2 Source Organism

<1> *Bovine viral diarrhea virus* (no sequence specified) [1, 2, 4, 5]
<2> *Bovine viral diarrhea virus* (UNIPROT accession number: Q01499) [3]

3 Reaction and Specificity

Catalyzed reaction
Leu is conserved at position P1 for all four cleavage sites. Alanine is found at position P1' of the NS4A-NS4B cleavage site, whereas serine is found at position P1' of the NS3-NS4A, NS4B-NS5A and NS5A-NS5B cleavage sites

Reaction type
hydrolysis of peptide bond

Natural substrates and products

S bovine viral diarrhea virus polyprotein + H_2O <1> (<1> the enzyme is required for pestivirus replication. The enzyme is required for processing at nonstructural protein sites 3/4A, 4A/4B, 4B/5A and 5A/5B but not for cleavage at the junction between NS2 and NS3. A conserved Leu is found at the P1 position of all four cleavage sites, followed by either Ser (3/4A, 4B5A, and 5A/5B sites) or Ala (4A/4B site) at the P1 position [1]; <1> pestivirus P80 protein is a viral proteinase responsible for nonstructural polyprotein cleavage events [5]) (Reversibility: ?) [1, 5]

P ? <1> [1]

Substrates and products

S bovine viral diarrhea virus polyprotein + H_2O <1> (<1> cleavages leading to the release of NS4A, NS4B, NS5A, and NS5B [4]; <1> Leu at P1 is the only position conserved for all cleavage sites. At P1 alanine is found at the NS4A-NS4B site, whereas serine residues at this position at the NS3/NS4A, NS4B-NS5A, and NS5A/NS5B cleavage sites. For all cleavage sites the amino acids found at P1 and P1 are conserved for different genotypes of pestiviruses, despite the high degree of sequence variation found between these viruses [2]; <1> the enzyme is required for processing at nonstructural protein sites 3/4A, 4A/4B, 4B/5A and 5A/5B but not for cleavage at the junction between NS2 and NS3. A conserved Leu is found at the P1 position of all four cleavage sites, followed by either Ser (3/4A, 4B5A, and 5A/5B sites) or Ala (4A/4B site) at the P1 position [1]; <1> the enzyme is required for pestivirus replication. The enzyme is required for processing at nonstructural protein sites 3/4A, 4A/4B, 4B/5A and 5A/5B but not for cleavage at the junction between NS2 and NS3. A conserved Leu is found at the P1 position of all four cleavage sites, followed by either Ser (3/4A, 4B5A, and 5A/5B sites) or Ala (4A/4B site) at the P1 position [1]; <1> pestivirus P80 protein is a viral proteinase responsible for nonstructural polyprotein cleavage events [5]) (Reversibility: ?) [1, 2, 4, 5]

P ? <1> [1, 2, 4]

S Additional information <1> (<1> His1678, Asp1686 and Ser1752 constitute the catalytic triad [4]) (Reversibility: ?) [4]

P ? <1> [4]

Activating compounds

Additional information <1> (<1> cofactor required for cleavage at the 4B/5A and 5A/5B sites [1]; <1> the central region of NS4A represents the cofactor domain [4]) [1, 4]

5 Isolation/Preparation/Mutation/Application

Cloning

<1> (complete genomic nucleotide sequence) [3]

Engineering

D1686G <1> (<1> mutation does not induce obvious changes in processing [4]) [4]
D1695A <1> (<1> mutation does not induce obvious changes in processing [4]) [4]
S1752C <1> (<1> mutant enzyme exhibits only cleavage activity at the NS3/4A site [4]) [4]
S1752T <1> (<1> mutant enzyme exhibits only cleavage activity at the NS3/4A site [4]; <1> mutant enzyme still shows residual activity [4]) [4]
Additional information <1> (<1> the NS3 protease is sensitive to N-terminal truncation because a deletion of 6 amino acids significantly reduces the cleavage efficiency at the NS4A/4B site [4]) [4]

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Equine arterivirus serine peptidase 3.4.21.114

1 Nomenclature

EC number
3.4.21.114

Recommended name
equine arterivirus serine peptidase

Synonyms
S32.001 <3> (<3> Merops ID [6]) [6]
equine arteritis virus serine endopeptidase
non-structural protein 4 <3> [9]
nonstructural protein 4 <2, 3> [1, 6, 8]
nsp4 <1, 2, 3> [1, 2, 3, 5, 6, 7, 8, 9]
nsp4 serine protease <3> [5, 7]
nsp4SP <3> [7]

CAS registry number
97162-88-4

2 Source Organism

<1> *equine arteritis virus* (UNIPROT accession number: P19811) [3, 4]
<2> *equine arteritis virus* (UNIPROT accession number: X53549) [1, 2]
<3> *equine arteritis virus* (no sequence specified) [5, 6, 7, 8, 9]

3 Reaction and Specificity

Catalyzed reaction
cleavage of (Glu/Gln)-/-(Gly/Ser/Ala) in arterivirus replicase translation products ORF1a and ORF1ab

Reaction type
cleavage of C-N-linkage
hydrolysis of peptide bond

Natural substrates and products
S arterivirus replicase ORF1a + H_2O <3> (<3> ORF1a can be cleaved following two alternative pathways. In the majority nsp3-8 precursors are cleaved at the nsp4/5 site yielding nsp3-4 and nsp5-8. The latter product is then cleaved at nsp7/8 only. In the alternative proteolytic cascade the

nsp4/5 site remains uncleaved while the nsp5/6 and nsp6/7 sites are processed. nsp3-8 has to interact with cleaved nsp2 to allow processing of the nsp4/5 junction, when nsp2 cofactor is absent the alternative pathway is used [6]) (Reversibility: ?) [6]

P ? <3> [6]

S Additional information <3> (<3> enzyme is the main viral protease [7]; <3> the enzyme controls the maturation of the replicase complex. The C-terminal domain has a modulating role in nsp4-mediated autoprocessing of the arterivirus replicase polyproteins [8]) (Reversibility: ?) [7, 8]

P ? <3> [7]

Substrates and products

S arterivirus replicase ORF1a + H_2O <3> (<3> cleavage sites are Glu1430/Gly and Glu1452/Ser [6]; <3> ORF1a can be cleaved following two alternative pathways. In the majority nsp3-8 precursors are cleaved at the nsp4/5 site yielding nsp3-4 and nsp5-8. The latter product is then cleaved at nsp7/8 only. In the alternative proteolytic cascade the nsp4/5 site remains uncleaved while the nsp5/6 and nsp6/7 sites are processed. nsp3-8 has to interact with cleaved nsp2 to allow processing of the nsp4/5 junction, when nsp2 cofactor is absent the alternative pathway is used [6]) (Reversibility: ?) [6]

P ? <3> [6]

S arterivirus replicase ORF1a protein + H_2O <2> (<2> nsp4 and an unidentified protease is responsible for the cleavage sites nsp2/3, nsp3/4, nsp4/5 and nsp5/6 in the ORF1a protein [1]) (Reversibility: ?) [1, 2]

P ? <2> [1, 2]

S arterivirus replicase ORF1ab + H_2O <3> (<3> cleavage sites are the junctions at nsp3/4, Nsp4/5, 2 internal nsp5 sites, nsp5/6, p80/p50, p50/p26, p26/p12, enzyme cleaves between Glu and Gly, Glu and Ser or Glu and Lys [5]) (Reversibility: ?) [5]

P ? <3> [5]

S arterivirus replicase ORF1b + H_2O <3> (<3> protein is processed by nsp4 serine protease [5]; <3> protein is processed by nsp4 serine protease at the following sites: nsp9/10 i.e. p80/p50, cleavage occurs between Glu2370 and Ser, nsp10/11 i.e. p50/p26, cleavage occurs between Gln2837 and Ser, nsp11/12 i.e. nsp26/p12, cleavage between Glu3056 and Gly [7]) (Reversibility: ?) [5, 7]

P ? <3> [5, 7]

S Additional information <2, 3> (<2> nsp4 cleaves multiple GluX(Gly/Ser/Ala) sites, X: cleavage site [2]; <3> protease has a specificity for Glu/Gly or Glu/Ser substrates [6]; <3> enzyme is the main viral protease [7]; <3> the enzyme controls the maturation of the replicase complex. The C-terminal domain has a modulating role in nsp4-mediated autoprocessing of the arterivirus replicase polyproteins [8]; <3> the enzyme cleaves the cognate nsp6/7- and nsp7/8 site in in vitro synthesized substrates [9]) (Reversibility: ?) [2, 6, 7, 8, 9]

P ? <2, 3> [2, 6, 7]

4 Enzyme Structure

Molecular weight

21000 <2> (<2> calculated from amino acid sequence [2]) [2]
31000 <2> (<2> SDS-PAGE [2]) [2]

Posttranslational modification

proteolytic modification <2> (<2> ORF1a is subject to at least 5 proteolytic cleavages, resulting in five non-structural protein 1-6 [1]; <2> replicase of EAV processed by at least 3 viral proteases, resulting in the generation of non-structural proteins Nsp1-6 [2]) [1, 2]

5 Isolation/Preparation/Mutation/Application

Purification

<3> [9]

Crystallization

<1> (hanging drop vapor diffusion method) [3]

Cloning

<1> [2, 4, 7]
<1> (ORF1a, fusion protein with glutathione S-transferase) [1]
<1> (ORF1b) [5]
<1> (fusion protein with maltose-binding protein) [3]
<3> (large scale production of recombinant nsp4 in Escherichia coli. The nsp4 proteinase is expressed either fused to maltose binding protein or carrying a C-terminal hexahistidine tag) [9]

Engineering

D1129E <2> (<2> mutant is able to cleave the Nsp4/5 site with wild-type efficiently but does not process the Nsp3/4 and Nsp5/6 junctions [2]) [2]
D1129K <2> (<2> no Nsp3456 cleavage [2]) [2]
D1129V <2> (<2> no Nsp3456 cleavage [2]) [2]
H1103G <2> (<2> complete inhibition of Nsp3456 processing [2]) [2]
H1103R <2> (<2> complete inhibition of Nsp3456 processing [2]) [2]
H1198L <2> (<2> completely abolished processing of Nsp4/5 and Nsp5/6 [2]) [2]
H1198R <2> (<2> completely abolished processing of Nsp4/5 and Nsp5/6 [2]) [2]
H1198Y <2> (<2> completely abolished processing of Nsp4/5 and Nsp5/6 [2]) [2]
S1184C <2> (<2> mutation has no effect on Nsp3456 processing [2]) [2]
S1184F <2> (<2> mutation has no effect on Nsp3456 processing [2]) [2]
S1184I <2, 3> (<2> mutation has no effect on Nsp3456 processing [2]; <3> no cleavage between nsp9 and nsp10 [7]) [2, 7]
S1184Y <2> (<2> mutation has no effect on Nsp3456 processing [2]) [2]

T1179D <2> (<2> completely abolished processing of Nsp4/5 and Nsp5/6 [2]) [2]
T1179G <2> (<2> reduced cleavage of Nsp4/5, no cleavage of Nsp5/6 site [2]) [2]
T1179N <2> (<2> retains wild-type activity towards Nsp4/5, cleaves Nsp5/6 with increased efficiency [2]) [2]
T1179S <2> (<2> reduced cleavage of Nsp4/5, no cleavage of Nsp5/6 site [2]) [2]

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Infectious pancreatic necrosis birnavirus Vp4 peptidase 3.4.21.115

1 Nomenclature

EC number
3.4.21.115

Recommended name
infectious pancreatic necrosis birnavirus Vp4 peptidase

Synonyms
Drosophila X virus Vp4 protease <6> [3]
NS <1> [1]
NS protease <2> [5]
S50.001 <1> (<1> Merops ID [1]) [1]
VP4 <1, 3> [1, 2, 3, 4]
avian infectious busrsal disease birnavirus Vp4 endopeptidase <5> [3]
blotched snakehead birnavirus endopeptidase <3> [3]
equine arterivirus serine peptidase
infectious pancreatic necrosis birnavirus Vp4 protease <4> [3]

CAS registry number
330588-95-9

2 Source Organism

<1> *infectious pancreatic necrosis virus* (UNIPROT accession number: P90205) [1]
<2> *infectious pancreatic necrosis virus* (no sequence specified) [5]
<3> *Blotched snakehead virus* (no sequence specified) [2, 3, 4]
<4> *infectious pancreatic necrosis birnavirus* (no sequence specified) [3]
<5> *avian infectious busrsal disease birnavirus* (no sequence specified) [3]
<6> *Drosophila X virus* (no sequence specified) [3]

3 Reaction and Specificity

Catalyzed reaction
cleaves the (Ser/Thr)-Xaa-Ala-/-(Ser/Ala)-Gly motif in the polyprotein NH_2-pVP2-VP4-VP3-COOH of infectious pancreatic necrosis virus at the pVP2-VP4 and VP4-VP3 junctions

Reaction type
cleavage of C-N-linkage
hydrolysis of peptide bond

Natural substrates and products
S polyprotein of BSNV + H_2O <3, 4, 5, 6> (Reversibility: ?) [2, 3, 4]
P pVP2 + VP3 + VP4
S polyprotein of IPNV + H_2O <2> (<2> the viral NS protease is active and cleaves the polyprotein when it is expressed in Escherichia coli [5]) (Reversibility: ?) [5]
P pVP2 + VP3 + VP4

Substrates and products
S polyprotein of BSNV + H_2O <3, 4, 5, 6> (Reversibility: ?) [2, 3, 4]
P pVP2 + VP3 + VP4
S polyprotein of IPNV + H_2O <1, 2> (<1> cleaves at the (Ser/Thr)-X-Ala-/-(Ser/Ala)-Gly motif, -/- = cleavage site [1]; <2> the viral NS protease is active and cleaves the polyprotein when it is expressed in Escherichia coli [5]) (Reversibility: ?) [1, 5]
P pVP2 + VP3 + VP4 <1> [1]

4 Enzyme Structure

Molecular weight
28500 <2, 3> (<2,3> SDS-PAGE [2,5]) [2, 5]

Posttranslational modification
proteolytic modification <1> (<1> self-cleavage of IPNV VP4 at the pVP2-VP4 and VP4-VP3 junctions [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue
cell culture <2, 3> [2, 4, 5]

Purification
<1> [1]
<2> (eluted from SDS-PAGE gel) [5]
<3> (ammonium sulfate fractionation and gel filtration) [4]
<3> (two chromatography steps to homogeneity) [2]

Crystallization
<3> (a truncated variant with 18 residues removed from the C-terminal) [2, 4]

Cloning

<1> (wild-type IPNA and truncated forms, expressed in Escherichia coli) [1]
<2> (expression in Escherichia coli JM107) [5]
<3> (expression in Escherichia coli) [2, 4]

Engineering

D537Q <1> (<1> mutant with wild-type activity [1]) [1]
D585I <1> (<1> mutant with wild-type activity [1]) [1]
D595L <1> (<1> mutant with wild-type activity [1]) [1]
D601S <1> (<1> mutant with wild-type activity [1]) [1]
D644I <1> (<1> mutant with wild-type activity [1]) [1]
D660G/D661S <1> (<1> mutant with wild-type activity [1]) [1]
D672N <1> (<1> mutant with wild-type activity [1]) [1]
D693L <1> (<1> reduced activity for the VP4-VP3 junction [1]) [1]
H547S <1> (<1> reduced activity for the VP4-VP3 junction [1]) [1]
H697L <1> (<1> reduced activity for the VP4-VP3 junction [1]) [1]
H704S <1> (<1> mutant with wild-type activity [1]) [1]
P774Z <3> (<3> 18 residues removed from the C-terminus [4]) [4]
S633A <1> (<1> no enzyme activity [1]) [1]
S633C <1> (<1> 40% reduction of VP2-VP4 cleavage, 80% reduction of VP4-VP3 cleavage [1]) [1]
S633Q <1> (<1> no enzyme activity [1]) [1]
S633T <1> (<1> no enzyme activity [1]) [1]

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SpoIVB peptidase 3.4.21.116

1 Nomenclature

EC number
3.4.21.116

Recommended name
SpoIVB peptidase

Synonyms
M50.002 <1, 3> (<1,3> Merops-ID [2,9]) [2, 9]
S55.001 <1> (<1> Merops ID [6]) [6]
SPOIVFB <1> [4]
SpoIVB <1> [10]
SpoIVB serine peptidase <1> [8]
SpolVFB <1, 3> [2, 9]
sporulation protein SpolVFB
stage IV sporulation protein FB <1, 3> [2, 4, 9]

CAS registry number
258282-12-1
296241-18-4

2 Source Organism

<1> *Bacillus subtilis* (no sequence specified) [3, 4, 5, 6, 7, 8, 9, 10, 11, 12]
<2> *Bacillus subtilis* (UNIPROT accession number: P17896) [1]
<3> *Bacillus subtilis* (UNIPROT accession number: P26937) [2]

3 Reaction and Specificity

Catalyzed reaction
enzyme cleaves its own precursor protein between Val52 and Asn53, Ala62 and Phe63, Val74 and Thr75, plays a signalling role in the sigma-K checkpoint of sporulation
self-cleaves Val52-/-Asn53, Ala62-/-Phe63 and Val74-/-Thr75 at the N-terminus of SpoIVB (<1> D137 is essential for catalytic activity, mechanism model, enzyme contains conserved metalloprotease sequence motifs HEXXH and NPDG, which are expected to form the catalytic center and are required for catalytic activity [9]; <3> enzyme contains conserved metalloprotease sequence motif HEXXH [2])

Reaction type

cleavage of C-N-linkage
hydrolysis of peptide bond

Natural substrates and products

S SpoIIQ + H_2O <1> (<1> forespore-expressed, intrinsic protein [11]) (Reversibility: ?) [11]
P ?
S SpoIVFA + H_2O <1> (<1> cleavage of SpoIVFA triggers regulated intramembrane proteolysis of pro-σK, releasing finally active σK into the mother cell. Model of signaling events [12]; <1> intrinsic protein substrate [10]) (Reversibility: ?) [10, 12]
P ?
S pro-σK <1> (<1> enzyme is essential for intercompartmental signalling in the σK-checkpoint, activates poteolytic processing of pro-σK to its mature and active form σK, function in formation of heat-resistant spores [6]) (Reversibility: ?) [6]
P σK + ? <1> [6]
S pro-σK + H_2O <1, 3> (<1,3> processing of proσK, which is a protein involved in sporulation [2,4,9]) (Reversibility: ?) [2, 4, 9]
P σK + 20 amino acid peptide <1, 3> [2, 4, 9]
S Additional information <1> (<1> critical component of the intercompartmental signal-transduction pathway that activates the sigma factor, simgaK, in the mother cell of the sporulating cell, possible non-signalling function in germ cell wall biosynthesis and the formation of heat-resistant spores [5]; <1> enzyme is a critical component of the σK regulatory checkpoint during spore formation, PDZ domain can interact with BofC [3]; <1> initiates proteolytic processing of pro-σK to its mature and active form in the opposed mother cell chamber of the developing cell, interacts with BofC [7]; <1> key role in signaling in the σK checkpoint [4]; <1> autoproteolytical activation. PDZ domain of enzyme binds to enzyme N-terminus to maintain its zymogen form. Following secretion across a spore membrane, domain binds in trans to the C-terminus of another enzyme molecule thus facilitating first cleavage event of enzyme near the N-terminus which releases the enzyme from the forespore membrane [10]; <1> enzyme activity or its access to substrates is regulated by engulfment [11]) (Reversibility: ?) [3, 4, 5, 7, 10, 11]
P ? <1> [3, 4, 5, 7]

Substrates and products

S SpoIIQ + H_2O <1> (<1> forespore-expressed, intrinsic protein [11]) (Reversibility: ?) [11]
P ?
S SpoIVFA + H_2O <1> (<1> cleavage of SpoIVFA triggers regulated intramembrane proteolysis of pro-σK, releasing finally active σK into the mother cell. Model of signaling events [12]; <1> intrinsic protein substrate [10]) (Reversibility: ?) [10, 12]
P ?

S SpoIVFA + H_2O <1> (<1> component of the pro-σK processing complex, cleaves between residues 145 and 175 [8]) (Reversibility: ?) [8]
P 17 KDa fragment + 14 KDa fragment <1> [8]
S pro-σK <1> (<1> enzyme is essential for intercompartmental signalling in the σK-checkpoint, activates poteolytic processing of pro-σK to its mature and active form σK, function in formation of heat-resistant spores [6]) (Reversibility: ?) [6]
P σK + ? <1> [6]
S pro-σK + H_2O <1, 3> (<1,3> processing of proσK, which is a protein involved in sporulation [2,4,9]) (Reversibility: ?) [2, 4, 9]
P σK + 20 amino acid peptide <1, 3> [2, 4, 9]
S Additional information <1> (<1> self-cleavage occurs at three different sites: Val52 and Asn53, Ala62 and Phe63, Val74 and Thr75 [6]; <1> critical component of the intercompartmental signal-transduction pathway that activates the sigma factor, simgaK, in the mother cell of the sporulating cell, possible non-signalling function in germ cell wall biosynthesis and the formation of heat-resistant spores [5]; <1> enzyme is a critical component of the σK regulatory checkpoint during spore formation, PDZ domain can interact with BofC [3]; <1> initiates proteolytic processing of pro-σK to its mature and active form in the opposed mother cell chamber of the developing cell, interacts with BofC [7]; <1> key role in signaling in the σK checkpoint [4]; <1> autoproteolytical activation. PDZ domain of enzyme binds to enzyme N-terminus to maintain its zymogen form. Following secretion across a spore membrane, domain binds in trans to the C-terminus of another enzyme molecule thus facilitating first cleavage event of enzyme near the N-terminus which releases the enzyme from the forespore membrane [10]; <1> enzyme activity or its access to substrates is regulated by engulfment [11]) (Reversibility: ?) [3, 4, 5, 6, 7, 10, 11]
P ? <1> [3, 4, 5, 6, 7]

Inhibitors

BofA <1> (<1> intrinsic inhibitor protein [4]) [4]
SpoIVFA <1> (<1> intrinsic inhibitor protein [4]) [4]

Metals, ions

Zn^{2+} <1> (<1> enzyme is a zinc metalloprotease [4]) [4]

Temperature optimum (°C)

37 <3> (<3> in vivo assay at [2]) [2]

4 Enzyme Structure

Molecular weight

31000 <1> (<1> SDS-PAGE [8]) [8]
42000 <1> (<1> three predominant species of approximately 45000 Da, 43000 Da and 42000 Da after processing [6]) [6]

43000 <1> (<1> three predominant species of approximately 45000 Da, 43000 Da and 42000 Da after processing [6]) [6]
45000 <1> (<1> three predominant species of approximately 45000 Da, 43000 Da and 42000 Da after processing [6]) [6]
45980 <2> (<2> calculated from amino acid sequence [1]) [1]
50000 <1> (<1> preprotein, SDS-PAGE [4]; <1> proenzyme, SDS-PAGE [3]) [3, 4]
52000 <1> (<1> S378A mutant, unprocessed proprotein [6]) [6]

Subunits

? <1> (<1> x * 46000, full-length protein, x * 40000, x * 39000, x * 38000, autoactivation products, SDS-PAGE [10]) [10]
Additional information <1> (<1> Enzyme binds specifically to intrinsic protein BofA [10]) [10]

Posttranslational modification

proteolytic modification <1> (<1> enzyme becomes activated by SpoIVB, a membrane-integrated serine peptidase [4]; <1> enzyme is able to self-cleave in trans into at least three discrete products, zymogen is subject to two levels of proteolysis: autoproteolysis generating intermediate products of 42000 Da, 43000 Da and 45000 Da, at least one of which is proposed to be the active form, followed by processing by one or more enzymes to smaller species of 42000 Da and 40000 Da [6]; <1> enzyme is synthesized as a 50 kDa polypeptide, after 3 h it is cleaved into a number of products, one or more of which is the active form, almost simultaneously these forms are subject to secondary proteolysis which presumably inactivates the active form [4]; <1> self-cleavage releases its active form [3]; <1> autoproteolytical activation. PDZ domain of enzyme binds to enzyme N-terminus to maintain its zymogen form. Following secretion across a spore membrane, domain binds in trans to the C-terminus of another enzyme molecule thus facilitating first cleavage event of enzyme near the N-terminus which releases the enzyme from the forespore membrane [10]) [3, 4, 6, 10]

5 Isolation/Preparation/Mutation/Application

Localization

extracellular <1> (<1> sectretion of enzyme from the forespore [12]) [11, 12]
membrane <1, 3> (<3> belongs to the family of putative membrane metalloproteases [2]; <1> catalytic center is located adjacent tot or within the membrane [9]) [2, 4, 9]

Cloning

<1> [3, 4, 5, 6]
<1> (expressed in Escherichia coli) [8]
<1> (expression of wild-type and enzyme mutants in mutants strain BSL51 and R13) [9]

<1> (functional expression of wild-type, and expression of amino acid exchange mutants in the mutant strain BSL51, lacking spoIVFA and spoIVFB genes, and in the mutant OR745, lacking gene spoIVFB) [2]
<1> (fusion protein with β-galactosidase) [1]

Engineering

D137A <1, 3> (<3> mutant shows no remaining sporulation activity [2]; <1> site-directed mutagenesis, mutant is completely impaired in pro-σK processing [9]) [2, 9]
D137E <1> (<1> site-directed mutagenesis, mutant is completely impaired in pro-σK processing [9]) [9]
D137H <3> (<3> mutant shows no remaining sporulation activity [2]) [2]
D137N <1, 3> (<3> mutant shows highly reduced sporulation activity [2]; <1> site-directed mutagenesis, mutant is completely impaired in pro-σK processing [9]) [2, 9]
D149N <1> (<1> impaired self-cleavage, no pro-σK processing [3]) [3]
D213L <1> (<1> signaling similar to wild-type enzyme [4]) [4]
D242L <1> (<1> no signaling, mutant protein is unstable [4]) [4]
D242N <1> (<1> signaling similar to wild-type enzyme [4]) [4]
D364N <1> (<1> signaling similar to wild-type enzyme [4]) [4]
E44A <1, 3> (<1> site-directed mutagenesis, no activity [9]; <3> mutant shows nearly no remaining sporulation activity [2]) [2, 9]
E44D <1, 3> (<3> mutant shows increased sporulation activity [2]; <1> site-directed mutagenesis, unaltered activity, similar to wild-type [9]) [2, 9]
E44Q <1, 3> (<1> site-directed mutagenesis, no activity [9]; <3> mutant shows highly reduced sporulation activity [2]) [2, 9]
G114A <1> (<1> self-cleavage and pro-σK processing similar to wild-type enzyme [3]) [3]
G114Q <1> (<1> impaired self-cleavage, delayed pro-σK processing [3]) [3]
G126A <1> (<1> self-cleavage and pro-σK processing similar to wild-type enzyme [3]) [3]
G126Q <1> (<1> self-cleavage and pro-σK processing similar to wild-type enzyme [3]) [3]
G138A <1> (<1> site-directed mutagenesis, slightly reduced activity [9]) [9]
G139A <1> (<1> site-directed mutagenesis, slightly reduced activity [9]) [9]
G144A <1> (<1> self-cleavage and pro-σK processing similar to wild-type enzyme [3]) [3]
G144A/N155D <1> (<1> self-cleavage and pro-σK processing similar to wild-type enzyme [3]) [3]
G144Q <1> (<1> self-cleavage and pro-σK processing similar to wild-type enzyme [3]) [3]
H236F <1> (<1> no signaling, slow cleavage of the preprotein [4]) [4]
H236N <1> (<1> no signaling, slow cleavage of the preprotein [4]) [4]
H394D <1> (<1> delayed signaling [4]) [4]
H43A <1> (<1> site-directed mutagenesis, no activity [9]) [9]

H43F <1, 3> (<3> mutant shows no remaining sporulation activity, no immunologically detectable enzyme protein [2]; <1> site-directed mutagenesis, extremely low enzyme expression, no activity [9]) [2, 9]
H47F <1, 3> (<1> site-directed mutagenesis, no activity [9]; <3> mutant shows no remaining sporulation activity [2]) [2, 9]
I42P <3> (<3> mutant shows increased sporulation activity [2]) [2]
K240L <1> (<1> signaling similar to wild-type enzyme [4]) [4]
K321A <1> (<1> signaling similar to wild-type enzyme [4]) [4]
K363L <1> (<1> no signaling [4]) [4]
K387A <1> (<1> signaling similar to wild-type enzyme [4]) [4]
N122A <1> (<1> site-directed mutagenesis, extremely low enzyme expression [9]) [9]
N122D <1> (<1> site-directed mutagenesis, extremely low enzyme expression [9]) [9]
N122Q <1> (<1> site-directed mutagenesis, unaltered activity, similar to wild-type [9]) [9]
N129A <1> (<1> site-directed mutagenesis, slightly reduced activity [9]) [9]
N155D <1> (<1> self-cleavage and pro-σK processing similar to wild-type enzyme [3]) [3]
N155Y <1> (<1> self-cleavage and pro-σK processing similar to wild-type enzyme [3]) [3]
N290I <1> (<1> no signaling [4]) [4]
P132A <1> (<1> site-directed mutagenesis, highly reduced enzyme activity [9]) [9]
R185H <1> (<1> self-cleavage and pro-σK processing similar to wild-type enzyme [3]) [3]
R185K <1> (<1> self-cleavage and pro-σK processing similar to wild-type enzyme [3]) [3]
S378A <1> (<1> mutant with blocked self-cleavage [6]; <1> no signaling, slow cleavage of the preprotein [4]) [4, 6]
S378K <1> (<1> no signaling, slow cleavage of the preprotein [4]) [4]
T228A <1> (<1> can signal processing of pro-σK but is unable to complete its non-signalling function [5]) [5]
Additional information <1, 3> (<3> comparison of effects of spoIVFB mutation with spoIVFB mutation on accumulation level of the enzymes in the recombinant strains, overview [2]; <1> enzyme deletion mutant, strong inhibition of extracellular proteolysis of native protein SpoIIQ and of intracellular SpoIIQ-GFP fusion protein [11]; <1> N-terminal deletion of residues 1-35, 1-52, 1-62 or 1-74 and additional C-terminal deletion of up to 33 residues. Study on autoactivation process, which involves one trans cleavage by another enzyme protein and two cis cleavages [10]) [2, 10, 11]

References

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Stratum corneum chymotryptic enzyme 3.4.21.117

1 Nomenclature

EC number
3.4.21.117

Recommended name
stratum corneum chymotryptic enzyme

Synonyms
K7 <3> [3]
KLK7 <1> [13, 14, 15, 20, 23]
S01.300
SCCE <1, 3> [1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 20, 21, 22]
hK7 <1> [20, 22]
kallikrein 7 <1> [16, 17, 22, 23]
tissue kallikrein <2> [2]
tissue kallikrein 7 <1> [18]

CAS registry number
155215-90-0
9001-01-8

2 Source Organism

<1> *Homo sapiens* (no sequence specified) [4, 5, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23]
<2> *Rattus norvegicus* (no sequence specified) [2]
<3> *Homo sapiens* (UNIPROT accession number: P49862) [1, 3, 6, 11]

3 Reaction and Specificity

Catalyzed reaction
cleavage of proteins with aromatic side chains in the P1 position (<1> specific for amino acid residues with aromatic side chains in the P1 position [7]; <2> enzyme exhibits a marked preference for cleavage downstream of Arg residues [2]; <3> substrate binding pocket contains an Asn residue [3]; <1,3> enzyme is a chymotrypsin-like serine protease [4,5,6,7,8,9,10])

Reaction type
hydrolysis of peptide bond

Natural substrates and products

- **S** polypeptide + H_2O <1, 2, 3> (<1> mediates shedding and desquamation of skin cells [9]; <1> involved in desquamation in plantar stratum corneum [4,5,7]; <1> catalyzes the degradation of intercellular cohesive structures in the cornified layer of the skin in the continous sheeding of the cells from the skin surface [7,9]; <3> involved in pathological keratinization, psoriasis, and ovarian cancer [3]) (Reversibility: ?) [2, 3, 4, 5, 6, 7, 8, 9, 10, 11]
- **P** peptides <1, 2, 3> [2, 3, 4, 5, 6, 7, 8, 9, 10, 11]
- **S** Additional information <1, 3> (<3> enzyme is regulated by steroid hormones [11]; <1> enzyme may contribute to tumor cell growth, tumor spread, and the metastatic potential of ovarian tumor cells [9]; <1> AACC insertion in the stratum corneum chymotryptic enzyme gene may result in a change to activity of stratum corneum chymotryptic enzyme within the skin barrier. Stratum corneum chymotryptic enzyme could have an important role in the development of atopic dermatitis [21]; <1> epidermal hyperproliferation and decreased skin barrier function in mice overexpressing stratum corneum chymotryptic enzyme [12]; <1> gene expression might be involved in lung tumorigenesis [13]; <1> increased expression of kallikrein 7 and decreased expression of its inhibitor antileukoprotease might play an important role in cervical adenocarcinoma development [22]; <1> kallikrein 7 is involved in desquamation [20]) (Reversibility: ?) [9, 11, 12, 13, 20, 21, 22]
- **P** ?

Substrates and products

- **S** casein + H_2O <1> (Reversibility: ?) [4]
- **P** ?
- **S** D-Ile-Pro-Arg-4-nitroanilide + H_2O <1> (<1> chromogenic substrate [5,8]; <1> low activity [8]; <1> i.e. S2288 [5,8]) (Reversibility: ?) [5, 8]
- **P** D-Ile-Pro-Arg + 4-nitroaniline
- **S** N-carbobenzoxy-Gly-Pro-Arg-4-nitroanilide + H_2O <1> (<1> chromogenic substrate [8]; <1> low activity [8]) (Reversibility: ?) [8]
- **P** N-carbobenzoxy-Gly-Pro-Arg + 4-nitroaniline
- **S** N-carbobenzoxy-Val-Gly-Arg-4-nitroanilide + H_2O <1> (<1> chromogenic substrate [8]; <1> low activity [8]) (Reversibility: ?) [8]
- **P** N-carbobenzoxy-Val-Gly-Arg + 4-nitroaniline
- **S** PFR-7-amino-4-methylcoumarin + H_2O <2> (<2> best substrate [2]) (Reversibility: ?) [2]
- **P** ?
- **S** acetyl-Phe-Val-Arg-4-nitroanilide + H_2O <1> (<1> chromogenic substrate [8]) (Reversibility: ?) [8]
- **P** acetyl-Phe-Val-Arg + 4-nitroaniline
- **S** benzoyl-Phe-Val-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [2]
- **P** benzoyl-Phe-Val-Arg + 7-amino-4-methylcoumarin

S benzyloxycarbonyl-Phe-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [2]
P benzyloxycarbonyl-Phe-Arg + 7-amino-4-methylcoumarin
S corneodesmosin + H_2O <1> (Reversibility: ?) [20]
P ?
S desmocollin 1 + H_2O <1> (Reversibility: ?) [20]
P ?
S human pro-interleukin 1β + H_2O <1> (<1> several claevage sites [10]; <1> recombinant enzyme and substrate [10]; <1> substrate is in the inactive form [10]) (Reversibility: ?) [10]
P human interleukin β_1 + ? <1> (<1> product can be further degraded by the enzyme leading to its inactivation [10]; <1> product is in the active form [10]) [10]
S methoxy-succinyl-Ala-Ala-Pro-Met-4-nitroanilide + H_2O <1> (<1> chromogenic substrate [8]; <1> low activity [8]) (Reversibility: ?) [8]
P methoxy-succinyl-Ala-Ala-Pro-Met + 4-nitroaniline
S methoxy-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide + H_2O <1> (<1> chromogenic substrate [8]; <1> low activity [8]) (Reversibility: ?) [8]
P methoxy-succinyl-Ala-Ala-Pro-Phe + 4-nitroaniline
S methoxy-succinyl-Arg-Pro-Tyr-4-nitroanilide + H_2O <1> (<1> best substrate [5,8]; <1> chromogenic substrate [5,8]; <1> i.e. S2586 [5,8]) (Reversibility: ?) [5, 8]
P methoxy-succinyl-Arg-Pro-Tyr + 4-nitroaniline
S *o*-aminobenzoyl-FRAPR-(2,4-dinitrophenyl)ethylenediamine + H_2O <2> (<2> low activity [2]) (Reversibility: ?) [2]
P ?
S *o*-aminobenzoyl-FRLVR-(2,4-dinitrophenyl)ethylenediamine + H_2O <2> (<2> low activity [2]) (Reversibility: ?) [2]
P *o*-aminobenzoyl-FR + LVR-(2,4-dinitrophenyl)ethylenediamine <2> [2]
S *o*-aminobenzoyl-SVIRRVQ-(2,4-dinitrophenyl)ethylenediamine + H_2O <2> (Reversibility: ?) [2]
P ?
S *o*-aminobenzoyl-TSVIRRPQ-(2,4-dinitrophenyl)ethylenediamine + H_2O <2> (<2> low activity [2]) (Reversibility: ?) [2]
P ?
S oxidized bovine insulin B chain + H_2O <1> (<1> recombinant enzyme [7]) (Reversibility: ?) [7]
P peptides + (Tyr)26 <1> (<1> product identification, cleavage sites are: Leu6-cysteic acid7, Tyr16-Leu17, Phe25-Tyr26, Tyr26-Tr27 [7]) [7]
S polypeptide + H_2O <1, 2, 3> (<1> mediates shedding and desquamation of skin cells [9]; <1> involved in desquamation in plantar stratum corneum [4,5,7]; <1> catalyzes the degradation of intercellular cohesive structures in the cornified layer of the skin in the continous sheeding of the cells from the skin surface [7,9]; <3> involved in pathological keratinization, psoriasis, and ovarian cancer [3]) (Reversibility: ?) [2, 3, 4, 5, 6, 7, 8, 9, 10, 11]
P peptides <1, 2, 3> [2, 3, 4, 5, 6, 7, 8, 9, 10, 11]

S succinyl-Ala-Ala-Pro-Phe-4-nitroanilide + H_2O <1> (<1> chromogenic substrate [5]) (Reversibility: ?) [5]
P succinyl-Ala-Ala-Pro-Phe + 4-nitroaniline
S succinyl-Phe-Leu-Phe-4-nitroanilide + H_2O <1> (<1> chromogenic substrate [8]; <1> low activity [8]) (Reversibility: ?) [8]
P succinyl-Phe-Leu-Phe + 4-nitroaniline
S succinyl-Val-Pro-Phe-4-nitroanilide + H_2O <1> (<1> chromogenic substrate [8]; <1> low activity [8]) (Reversibility: ?) [8]
P succinyl-Val-Pro-Phe + 4-nitroaniline
S tert-butyloxycarbonyl-Leu-Gly-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [2]
P tert-butyloxycarbonyl-Leu-Gly-Arg + 7-amino-4-methylcoumarin
S tert-butyloxycarbonyl-Phe-Ser-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [2]
P tert-butyloxycarbonyl-Phe-Ser-Arg + 7-amino-4-methylcoumarin
S Additional information <1, 2, 3> (<1> substrate specificity [7]; <2> no kininogenase activity [2]; <2> enzyme exhibits a marked preference for cleavage downstream of Arg residues [2]; <3> recombinant enzyme proform is activated by digestion with trypsin [6]; <3> enzyme is regulated by steroid hormones [11]; <1> enzyme may contribute to tumor cell growth, tumor spread, and the metastatic potential of ovarian tumor cells [9]; <1> AACC insertion in the stratum corneum chymotryptic enzyme gene may result in a change to activity of stratum corneum chymotryptic enzyme within the skin barrier. Stratum corneum chymotryptic enzyme could have an important role in the development of atopic dermatitis [21]; <1> epidermal hyperproliferation and decreased skin barrier function in mice overexpressing stratum corneum chymotryptic enzyme [12]; <1> gene expression might be involved in lung tumorigenesis [13]; <1> increased expression of kallikrein 7 and decreased expression of its inhibitor antileukoprotease might play an important role in cervical adenocarcinoma development [22]; <1> kallikrein 7 is involved in desquamation [20]; <1> no cleavage of desmoglein 1 [20]) (Reversibility: ?) [2, 6, 7, 9, 11, 12, 13, 20, 21, 22]
P ?

Inhibitors

α_1-proteinase inhibitor <1> (<1> 89.6% inhibition at 0.00015 mM [8]) [8]
aprotinin <1> (<1> 90.3% inhibition at 0.0027 mM [8]) [4, 8]
chymostatin <1> [4]
eglin c <1> (<1> 99.8% inhibition at 0.00015 mM [8]) [8]
elafin <1> (<1> 33.4% inhibition at 0.0015 mM [8]) [8]
pepstatin A <1> (<1> weak inhibition, 23.6% inhibition at 0.1 mM [8]) [8]
soybean trypsin inhibitor <1, 2> (<2> strong inhibition [2]; <1> 99.6% inhibition at 0.00015 mM [8]) [2, 8]
Zn^{2+} <1> (<1> 92.6% inhibition at 0.1 mM [8]) [4, 8]
α_1-antichymotrypsin <1> (<1> 99.6% inhibition at 0.00015 mM [8]) [8]
α_1-macroglobulin <1> (<1> 44.2% inhibition at 0.00015 mM [8]) [8]

antileukoprotease <1> (<1> 88.8% inhibition at 0.0022 mM [8]) [8]
Additional information <1> (<1> no inhibition by leupeptin [4]; <1> inhibition assay: 15 min preincubation of the enzyme with inhibitor at 21°C [8]; <1> no inhibition by Mg^{2+}, E-64, 1,10-phenanthrolin, EDTA [8]) [4, 8]

Activating compounds

kallikrein 5 <1> (<1> activates proform of kallikrein 7 [20]) [20]
Additional information <3> (<3> estrogen and glucocorticoids cause up-regulation of gene expression [3,11]) [3, 11]

Turnover number (min^{-1})

0.04 <1> (acetyl-Phe-Val-Arg-4-nitroanilide, <1> pH 8.0, 37°C [8]) [8]
0.05 <1> (succinyl-Phe-Leu-Phe-4-nitroanilide, <1> pH 8.0, 37°C [8]) [8]
1.2 <1> (methoxy-Suc-Arg-Pro-Tyr-4-nitroanilide, <1> pH 8.0, 37°C [8]) [8]
Additional information <1> [8]

Specific activity (U/mg)

Additional information <1, 2> [2, 5]

K_m-Value (mM)

0.08 <1> (acetyl-Phe-Val-Arg-4-nitroanilide, <1> pH 8.0, 37°C [8]) [8]
0.22 <1> (succinyl-Phe-Leu-Phe-4-nitroanilide, <1> pH 8.0, 37°C [8]) [8]
0.65 <1> (methoxy-Suc-Arg-Pro-Tyr-4-nitroanilide, <1> pH 8.0, 37°C [8]) [8]
Additional information <1> [8]

K_i-Value (mM)

3.3e-007 <1> (eglin c, <1> pH 8.0, 37°C [8]) [8]
2.7e-005 <1> (aprotinin, <1> pH 8.0, 37°C [8]) [8]
6.3e-005 <1> (antileukoprotease, <1> pH 8.0, 37°C [8]) [8]
0.0016 <1> (elafin, <1> pH 8.0, 37°C [8]) [8]

pH-Optimum

7 <1> (<1> assay at [10]) [10]
7.2 <1> (<1> assay at [7]) [7]
8 <1> (<1> assay at [5,8]) [4, 5, 8]
9 <2> (<2> assay at [2]) [2]

pH-Range

5.5-8 <1> (<1> no activity at pH 4.0, highest activity at 8.0 [4]) [4]

Temperature optimum (°C)

37 <1, 2> (<1,2> assay at [2,5,7,8,10]) [2, 5, 7, 8, 10]

4 Enzyme Structure

Subunits

? <1, 2> (<1> x * 25000, SDS-PAGE [4,5]; <2> x * 9000 + x * 19000, SDS-PAGE [2]) [2, 4, 5]

Posttranslational modification

Glycoprotein <1, 3> (<3> recombinant and native enzyme [6]; <3> enzyme contains 1 potential N-glycosylation site [6]) [5, 6]
proteolytic modification <3> (<3> recombinant enzyme proform is activated by digestion with trypsin [6]) [6]

5 Isolation/Preparation/Mutation/Application

Source/tissue

BT-474 cell <3> (<3> breast carcinoma cell line, enzyme is upregulated by estrogens and slightly by glucocorticoids [3]) [3]
amniotic fluid <1> [17]
ascites <1> (<1> from ovarian cancer patients [17]) [17]
brain <3> [11]
breast <3> [3]
breast carcinoma cell <1> (<1> breast cancer patients with KLK7 positive tumors have relatively shorter disease-free survival and overall survival than patients with KLK7 negative tumors [23]) [23]
central nervous system <3> [3]
cerebellum <3> [11]
cerebrospinal fluid <1> (<1> decrease in level of kallikrein 7 in patients with Alzheimers disease and patients with frontotemporal dementia [16]) [16, 17]
cervical adenocarcinoma cell <1> (<1> increased expression of kallikrein 7 and decreased expression of its inhibitor antileukoprotease might play an important role in cervical adenocarcinoma development [22]) [22]
cervical cancer cell line <1> (<1> 50% of the primary and 50% of the established cervical cancer cell lines express kallikrein 7 [19]) [19]
cervical squamous cell carcinoma cell line <1> (<1> high expression of the enzyme [19]) [19]
corneocyte <1> (<1> plantar [5]) [5]
epidermis <1, 3> (<1,3> enzyme expression [6,7]) [6, 7]
epithelium <1> (<1> keratinizing squamous [10]) [10]
esophagus <1> [17]
keratinocyte <1, 3> (<1,3> enzyme expression [6,7]) [6, 7, 10]
kidney <1, 3> [3, 11, 17]
lung <1> (<1> highest concentrations of KLK7 mRNA are found in the non-cancerous tissue of patients with (well or moderately) differentiated tumors and the lowest in those with poorly differentiated tumors [13]) [13, 17]
lung adenocarcinoma cell <1> (<1> expression of KLK7 is decreased [13]) [13]
lung cancer cell <1> (<1> KLK7 is strongly expressed in the tissue of patients with squamous cell carcinoma and differentiated tumors [13]) [13]
mammary gland <3> [11]
milk <1> [17]
ovarian carcinoma cell <1, 3> (<1> different carcinoma cell lines and types, overview [9]; <1,3> overexpression of the enzyme [3,9]) [3, 9]

ovary <1, 3> (<1> a short mRNA variant for KLK7 (alternative AUG, predicted protein N-terminally truncated and lacking catalytic histidine residue) expressed in normal ovary and in ovarian cancer [14]) [3, 14]
ovary cancer cell <1> (<1> overexpression of the human tissue kallikrein genes KLK4, 5, 6, and 7 increases the malignant phenotype of ovarian cancer cells [15]; <1> short mRNA variant for KLK7 (alternative AUG, predicted protein N-terminally truncated and lacking catalytic histidine residue) expressed in normal ovary and in ovarian cancer [14]) [14, 15]
placenta <3> (<3> low content [3,11]) [3, 11]
saliva <1> [17]
salivary gland <3> (<3> low content [3,11]) [3, 11]
seminal plasma <1> [17]
serum <1> [17]
skin <1, 3> (<1> of the forearm [4]) [3, 4, 5, 6, 7, 9, 11, 17]
spinal cord <3> [11]
stratum corneum <1, 3> (<1> hyperplastic [5]; <1> plantar [4,5,8]; <1> from heels [4,8]) [4, 5, 6, 7, 8, 9, 10, 11]
submandibular gland <2> [2]
sweat <1> [17]
synovial fluid <1> [17]
testis <3> (<3> low content [3,11]) [3, 11]
thymus <3> (<3> low content [3,11]) [3, 11]
thyroid <3> (<3> low content [3,11]) [3, 11]
trachea <3> (<3> low content [3,11]) [3, 11]
urine <1> [17]
uterus <3> (<3> low content [3,11]) [3, 11]
Additional information <1, 3> (<3> no expression in liver, pancreas, small intestine, bone marrow, ovary, skeletal muscle, spleen, colon, adrenal gland, prostate, heart, lung, and stomach [11]; <1> no activity in healthy ovary [9]; <3> enzyme expression in restricted to skin [6]; <1> enzyme is specifically expressed in keratinizing squamous epithelia [10]; <1> no expression of kallikrein 7 by normal cervical keratinocytes [19]) [6, 9, 10, 11, 19]

Purification

<1> [5, 8]
<1> (partially) [4]
<1> (recombinant) [17]
<2> [2]
<3> (recombinant and native enzyme) [6]

Cloning

<1> (recombinant kallikrein 7 is produced in HEK 293T cells) [17]
<1> (transgenic mice overexpressing human scce-gene. Epidermal hyperproliferation and decreased skin barrier function in mice overexpressing stratum corneum chymotryptic enzyme) [12]
<3> (DNA sequence determination and analysis, gene KLK7 mapps to chromosomal locus 19q13.3-13.4, human kallikrein gene family locus) [11]

<3> (cloning from keratinocyte library, DNA and RNA sequence determination and analysis, expression of the enzyme in its proform in murine C127 cells, enzyme activation by trypsin) [6]
<3> (gene KLK7, genetic organization and structure of the kallikrein gene family, clustered on chromosome 19q13.3-q13.4) [3]

Engineering

analysis <1> (<1> ELISA-type immunoassay for human kallikrein quantification [17]) [17]

Application

medicine <1> (<1> because the enzyme appears only in abundance in tumor tissue and contains a secretion signal sequence, suggesting that the enzyme is secreted, it may prove to be a useful diagnostic/prognostic tool for the detection of metastatic or recurrent disease or as a novel molecular target for cervical cancer therapy [19]; <1> breast cancer patients with KLK7 positive tumors have relatively shorter disease-free survival and overall survival than patients with KLK7 negative tumors. KLK7 gene expression may be used as a marker of unfavorable prognosis for breast cancer patients [23]; <1> expression of full-length KLK7 mRNA may represent a new prognostic marker in breast cancer disease [18]; <1> kallikrein 7 may be useful in clinics as biomarkers for the detection and monitoring of non-small cell lung cancer [13]) [13, 18, 19, 23]

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Kallikrein 8 3.4.21.118

1 Nomenclature

EC number
3.4.21.118

Recommended name
kallikrein 8

Synonyms
GK-8 <8> [1]
NP <4, 5, 6> [1, 8]
P1 kallikrein <7> [1]
RGK-8 <7> [1]
S01.244
TADG-14 <2> [20]
TADG14 <5> (<5> i.e. tumor-associated differentially expressed gene-14 [5]) [1, 5, 12]
brain serine protease 1 <6> [1]
hK8 <2> [20]
kallikrein-8
neuropsin <1> [17]
ovasin <5> [1, 12]
tissue kallikrein <7> [1]

CAS registry number
171715-15-4
9001-01-8

2 Source Organism

<1> *Mus musculus* (no sequence specified) [17, 18]
<2> *Homo sapiens* (no sequence specified) [15, 16, 20]
<3> *Rattus norvegicus* (no sequence specified) [11]
<4> *Mus musculus* (UNIPROT accession number: Q61955) [1, 2, 3, 4, 6, 7, 8, 9, 10, 13]
<5> *Homo sapiens* (UNIPROT accession number: O60259) [1,3,5,12,14]
<6> *Rattus norvegicus* (UNIPROT accession number: O88780) [1]
<7> *Rattus norvegicus* (UNIPROT accession number: P36374) [1]
<8> *Mus musculus* (UNIPROT accession number: P07628) [1]
<9> *Homo sapiens* (UNIPROT accession number: Q5V9X2) [19]

3 Reaction and Specificity

Catalyzed reaction

cleavage of amide substrates following the basic amino acids Arg or Lys at the P1 position, with a preference for Arg over Lys (<5> substrate binding pocket contains an Asp residue [12]; <4,5> enzyme is a trypsin-type serine protease [2,4,5,6,7,10]; <4> substrate binding site structure defining the specificity for Arg and Lys in the S_1 pocket [6]; <4> the ordered kallikrein loop projects proline towards the active site to restrict smaller residues or proline at the P2 position of substrates [6]; <3> kallikrein k8 exhibit a marked preference for cleavage downstream of Arg residues [11])

Reaction type

hydrolysis of peptide bond

Natural substrates and products

- **S** fibronectin + H_2O <4> (<4> affects cell adhesion or cell migration by modulating the content and/or chemical characteristics of fibronectin in the extracellular matrix [7]) (Reversibility: ?) [7]
- **P** ?
- **S** polypeptide + H_2O <3, 4, 5> (<4> enzyme has significant limbic effects by changing the extracellular matrix environment [4]; <4> involvement in neural plasticity [4,8]; <4> enzyme is implicated in various neurological processes including formation of memory [2]) (Reversibility: ?) [2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]
- **P** peptides <3, 4, 5> [2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]
- **S** Additional information <1, 2, 5> (<5> enzyme might be involved in ovarian cancer, brain damage and kindling epilepsy [12]; <1> enzyme is involved in the synaptogenesis/maturation of orphan and small synaptic boutons in the Schaffer-collateral pathway [17]; <1> enzyme is necessary for establishment of long-term potentiation and has a significant role in memory acquisition [18]; <2> enzyme may be impicated in extracellular matrix protein degradation in the area surrounding enzyme producing cells [16]) (Reversibility: ?) [12, 16, 17, 18]
- **P** ?

Substrates and products

- **S** casein + H_2O <2> (Reversibility: ?) [16]
- **P** ?
- **S** D-Val-Leu-Arg-4-nitroanilide + H_2O <4> (<4> native and recombinant enzyme [4]) (Reversibility: ?) [4]
- **P** D-Val-Leu-Arg + 4-nitroaniline <4> [4]
- **S** D-Val-Leu-Lys-4-nitroanilide + H_2O <4> (<4> native and recombinant enzyme [4]) (Reversibility: ?) [4]
- **P** D-Val-Leu-Lys + 4-nitroaniline <4> [4]
- **S** fibrinogen + H_2O <2> (Reversibility: ?) [16]
- **P** ?

S fibronectin + H_2O <2, 4> (<4> recombinant enzyme [4,7]; <4> affects cell adhesion or cell migration by modulating the content and/or chemical characteristics of fibronectin in the extracellular matrix [7]) (Reversibility: ?) [4, 7, 16]
P ?
S gelatin + H_2O <2> (Reversibility: ?) [16]
P ?
S L-Pro-L-Phe-L-Arg-7-amido-4-methylcoumarin + H_2O <2> (<2> best substrate [16]) (Reversibility: ?) [16]
P L-Pro-L-Phe-L-Arg + 7-amino-4-methylcoumarin
S Pro-Phe-Arg-4-methylcoumaryl-7-amide <3, 4> (<4> recombinant wild-type and mutants [9]) (Reversibility: ?) [9, 11]
P Pro-Phe-Arg + 7-amino-4-methylcoumarin <3, 4> [9, 11]
S acetyl-Pro-Phe-Arg-4-nitroanilide + H_2O <4> (<4> low activity [4]; <4> recombinant, not native enzyme [4]) (Reversibility: ?) [4]
P acetyl-Pro-Phe-Arg + 4-nitroaniline <4> [4]
S benzoyl-Phe-Val-Arg-4-methylcoumarin 7-amide + H_2O <3> (Reversibility: ?) [11]
P benzoyl-Phe-Val-Arg + 7-amino-4-methylcoumarin
S benzyloxycarbonyl-Gly-Gly-L-Arg-7-amido-4-methylcoumarin + H_2O <5> (<5> ratio k_{cat}/K_M-value is 1000 M/s [14]) (Reversibility: ?) [14]
P benzyloxycarbonyl-Gly-Gly-L-Arg + 7-amino-4-methylcoumarin
S benzyloxycarbonyl-L-Phe-L-Arg-7-amido-4-methylcoumarin + H_2O <5> (<5> ratio k_{cat}/K_M-value is 1000 M/s [14]) (Reversibility: ?) [14]
P benzyloxycarbonyl-L-Phe-L-Arg + 7-amino-4-methylcoumarin
S benzyloxycarbonyl-L-Val-L-Val-L-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [16]
P benzyloxycarbonyl-L-Val-L-Val-L-Arg + 7-amino-4-methylcoumarin
S benzyloxycarbonyl-Phe-Arg-4-methylcoumarin 7-amide + H_2O <3> (Reversibility: ?) [11]
P benzyloxycarbonyl-Phe-Arg + 7-amino-4-methylcoumarin
S collagen type IV + H_2O <2> (Reversibility: ?) [16]
P ?
S high-molecular weight kininogen + H_2O <2> (Reversibility: ?) [16]
P ?
S polypeptide + H_2O <3, 4, 5> (<4> enzyme has significant limbic effects by changing the extracellular matrix environment [4]; <4> involvement in neural plasticity [4,8]; <4> enzyme is implicated in various neurological processes including formation of memory [2]) (Reversibility: ?) [2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]
P peptides <3, 4, 5> [2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]
S single-chain tissue-type plasminogen activator + H_2O <2> (<2> specific cleavage of Arg275-Ile276 bond [16]) (Reversibility: ?) [16]
P two-chain tissue-type plasminogen activator
S t-butyloxycarbonyl-L-Leu-L-Lys-L-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [16]
P t-butyloxycarbonyl-L-Leu-L-Lys-L-Arg + 7-amino-4-methylcoumarin

S t-butyloxycarbonyl-L-Phe-L-Ser-L-Arg-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [16]
P t-butyloxycarbonyl-L-Phe-L-Ser-L-Arg + 7-amino-4-methylcoumarin
S t-butyloxycarbonyl-L-Val-L-Leu-L-Lys-7-amido-4-methylcoumarin + H_2O <2> (Reversibility: ?) [16]
P t-butyloxycarbonyl-L-Val-L-Leu-L-Lys + 7-amino-4-methylcoumarin
S t-butyloxycarbonyl-L-Val-L-Pro-L-Arg-7-amido-4-methylcoumarin + H_2O <2, 5> (<5> ratio k_{cat}/K_M-value is 21000 M/s [14]) (Reversibility: ?) [14, 16]
P t-butyloxycarbonyl-L-Val-L-Pro-L-Arg + 7-amino-4-methylcoumarin
S tert-butyloxycarbonyl-Ala-Gly-Pro-Arg-4-methylcoumaryl-7-amide + H_2O <4> (<4> recombinant, not native, enzyme [4]) (Reversibility: ?) [4]
P tert-butyloxycarbonyl-Ala-Gly-Pro-Arg + 7-amino-4-methylcoumarin <4> [4]
S tert-butyloxycarbonyl-Asp(benzyloxy)-Pro-Arg-4-methylcoumaryl-7-amide + H_2O <4> (<4> wild-type and mutants [9]) (Reversibility: ?) [9]
P tert-butyloxycarbonyl-Asp(benzyloxy)-Pro-Arg + 7-amino-4-methylcoumarin <4> [9]
S tert-butyloxycarbonyl-Asp-Pro-Arg-4-methylcoumaryl-7-amide + H_2O <4> (<4> recombinant, not native, enzyme [4]) (Reversibility: ?) [4]
P tert-butyloxycarbonyl-Asp-Pro-Arg + 7-amino-4-methylcoumarin <4> [4]
S tert-butyloxycarbonyl-Glu-Gly-Arg-4-methylcoumaryl-7-amide + H_2O <4> (<4> native and recombinant enzyme [4]; <4> recombinant, not native, enzyme [4]) (Reversibility: ?) [4]
P tert-butyloxycarbonyl-Glu-Gly-Arg + 7-amino-4-methylcoumarin <4> [4]
S tert-butyloxycarbonyl-Glu-Lys-Lys-4-methylcoumaryl-7-amide + H_2O <4> (<4> low activity [4]; <4> recombinant and native enzyme [4]) (Reversibility: ?) [4]
P tert-butyloxycarbonyl-Glu-Lys-Lys + 7-amino-4-methylcoumarin <4> [4]
S tert-butyloxycarbonyl-Leu-Arg-Arg-4-methylcoumaryl-7-amide + H_2O <4> (<4> low activity [4]; <4> recombinant, not native, enzyme [4]) (Reversibility: ?) [4]
P tert-butyloxycarbonyl-Leu-Arg-Arg + 7-amino-4-methylcoumarin <4> [4]
S tert-butyloxycarbonyl-Leu-Thr-Arg-4-methylcoumaryl-7-amide + H_2O <4> (<4> low activity [4]; <4> recombinant, not native, enzyme [4]) (Reversibility: ?) [4]
P tert-butyloxycarbonyl-Leu-Thr-Arg + 7-amino-4-methylcoumarin <4> [4]
S tert-butyloxycarbonyl-Phe-Ser-Arg-4-methylcoumaryl-7-amide + H_2O <3, 4> (<3> no activity [11]; <4> native and recombinant enzyme [4]; <4> recombinant wild-type and mutants [9]) (Reversibility: ?) [4, 9, 11]
P tert-butyloxycarbonyl-Phe-Ser-Arg + 7-amino-4-methylcoumarin <4> [4, 9]
S tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide + H_2O <3, 4> (<4> best substrate [4,9]; <4> native and recombinant enzyme [4];

<4> recombinant wild-type and mutants [9]) (Reversibility: ?) [4, 8, 9, 10, 11]

P tert-butyloxycarbonyl-Val-Pro-Arg + 7-amino-4-methylcoumarin <3, 4> [4, 8, 9, 10, 11]

S tert-butyloxycarbonyl-pyroglutamyl-Gly-Arg-4-methylcoumaryl-7-amide + H_2O <4> (<4> recombinant and native enzyme [4]) (Reversibility: ?) [4]

P tert-butyloxycarbonyl-pyroglutamyl-Gly-Arg + 7-amino-4-methylcoumarin <4> [4]

S tosyl-Gly-L-Pro-L-Lys-7-amido-4-methylcoumarin + H_2O <5> (<5> ratio k_{cat}/K_M-value is 670 M/s [14]) (Reversibility: ?) [14]

P tosyl-Gly-L-Pro-L-Lys + 7-amido-4-methylcoumarin

S Additional information <1, 2, 3, 4, 5> (<4> recombinant enzyme is produced as inactive proform and needs to be processed by an endoprotease, e.g. protease-1, EC 3.4.21.50, or trypsin, EC 3.4.21.4, for activation by specific cleavage of the Lys32-Ile33 bond near the N-terminus [4,9]; <3> kallikrein k8 exhibits a marked preference for cleavage downstream of Arg residues [11]; <4> loop C and the N-linked oligosaccharide chain on the kallikrein loop affect the catalytic efficiency and P2 specificity, respectively [9]; <3> no activity with tert-butyloxycarbonyl-Leu-Gly-Arg-4-methylcoumarin 7-amide [11]; <4> substrate specificty of native and recombinant enzyme [4]; <4> disulfide bonds SS_1 in loop E, Gly142-Leu155, and SS6 in loop G, Ser185-Gly197, are essential for catalytic activity [9]; <5> enzyme might be involved in ovarian cancer, brain damage and kindling epilepsy [12]; <1> enzyme is involved in the synaptogenesis/maturation of orphan and small synaptic boutons in the Schaffer-collateral pathway [17]; <1> enzyme is necessary for establishment of long-term potentiation and has a significant role in memory acquisition [18]; <2> enzyme may be impicated in extracellular matrix protein degradation in the area surrounding enzyme producing cells [16]; <5> enzyme shows trypsin-like specificity. Synthetic substrates containing either Arg or Lys at P1 positions are cleaved [14]; <2> trypsin-like specificity with strong preference for Arg over Lys in P1-position. No substrate: plasminogen [16]) (Reversibility: ?) [4, 9, 11, 12, 14, 16, 17, 18]

P ?

Inhibitors

(4-amidinohenyl)methanesulfonyl 1-fluoride <4> (<4> 61% inhibition at 1 mM [4]) [4]

aprotinin <2, 4> (<4> 65% inhibition at 0.1 mM [4]; <2> 0.01 mg/ml, 98% inhibition [16]) [4, 16]

benzamidine <2, 4> (<4> 56% inhibition at 1 mM [4]; <2> 0.2 mM, 18% inhibition [16]) [4, 16]

chymostatin <4, 5> (<4> 80% inhibition at 0.1 mM [4]; <5> 50% inhibition at 0.008 mM [14]) [4, 14]

diisopropyl fluorophosphate <2> (<2> 2 mM, 100% inhibition [16]) [16]

diisopropylfluorophosphate <4> (<4> 16% inhibition at 0.1 mM [4]) [4]

leupeptin <2, 4, 5> (<4> complete inhibition at 0.1 mM [4]; <5> 50% inhibition at 0.066 mM [14]; <2> 0.2 mM, 95% inhibition [16]) [4, 14, 16]
Ni^{2+} <5> (<5> 50% inhibition at 0.011 mM [14]) [14]
Zn^{2+} <5> (<5> 50% inhibition at 0.0033 mM [14]) [14]
antipain <2, 4, 5> (<4> 95% inhibition at 0.1 mM [4]; <2> 0.2 mM, 100% inhibition [16]; <5> 50% inhibition at 0.00046 mM [14]) [4, 14, 16]
human α_1-antichymotrypsin <4> (<4> weak, 16% inhibition at 2 mM [4]) [4]
human α_1-antitrypsin <4> (<4> weak, 20% inhibition at 10 mM [4]) [4]
murinoglobulin I <4> (<4> 24% inhibition at enzyme-inhibitor ratio of 1:2 [8]; <4> formation of a SDS-stable complex [8]; <4> i.e. MUG I [8]) [8]
serine protease inhibitor-3 <4> (<4> from pyramidal neurons [8]; <4> formation of a SDS-stable complex, bimolecular kinetics [8]; <4> slowly and tightly binding inhibitor [8]) [8]
trans-epoxysuccinyl-L-leucylamido(4-guanidinobutane) <4> (<4> i.e. E-64, 15% inhibition at 0.1 mM [4]) [4]
Additional information <2, 3, 4> (<3> no inhibition by soybean trypsin inhibitor [11]; <4> no or very slight inhibition by pepstatin A, trypsin inhibitor, Ca^{2+}, Mg^{2+}, and metal chelators [4]; <4> enzyme is strongly inhibited by low molecular weight protease inhibitors that bind to His and Ser residues in the active center of serine proteases [4]; <2> not inhibitory: N^{α}-*p*-tosyl-L-phenylalanine chloromethyl ketone, pepstatin, *o*-phenynthroline, E-64 [16]) [4, 11, 16]

Activating compounds

Additional information <4> (<4> enzyme is induced and activated by uterine decidualization [10]) [10]

Metals, ions

Ca^{2+} <5> (<5> 4fold stimulation [14]) [14]
K^{+} <5> (<5> 1.7fold stimulation [14]) [14]
Mg^{2+} <5> (<5> 3foldstimulation [14]) [14]

Turnover number (min^{-1})

31.3 <4> (tert-butyloxycarbonyl-Asp(benzyloxy)-Pro-Arg-4-methylcoumaryl-7-amide, <4> recombinant wild-type enzyme, pH 8.0, 25°C [9]) [9]
34.8 <4> (tert-butyloxycarbonyl-Phe-Ser-Arg-4-methylcoumaryl-7-amide, <4> recombinant wild-type enzyme, pH 8.0, 25°C [9]) [9]
40 <2> (t-butyloxycarbonyl-L-Phe-L-Ser-L-Arg-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]
48 <2> (t-butyloxycarbonyl-L-Val-L-Leu-L-Lys-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]
65 <2> (t-butyloxycarbonyl-L-Leu-L-Lys-L-Arg-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]
98 <2> (t-butyloxycarbonyl-L-Val-L-Pro-L-Arg-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]
100 <4> (tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide, <4> recombinant wild-type enzyme, pH 8.0, 25°C [9]) [9]
140 <2> (benzyloxycarbonyl-L-Val-L-Val-L-Arg-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]

180 <2> (L-Pro-L-Phe-L-Arg-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]
193 <4> (Pro-Phe-Arg-4-methylcoumaryl-7-amide, <4> recombinant wild-type enzyme, pH 8.0, 25°C [9]) [9]

Specific activity (U/mg)

1.23 <4> (<4> purified native enzyme, substrate tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide [4]) [4]
6.26 <4> (<4> purified recombinant enzyme, substrate tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide [4]) [4]
Additional information <3> [11]

K_m-Value (mM)

0.07 <2> (L-Pro-L-Phe-L-Arg-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]
0.07 <2> (benzyloxycarbonyl-L-Val-L-Val-L-Arg-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]
0.07 <2> (t-butyloxycarbonyl-L-Phe-L-Ser-L-Arg-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]
0.07 <2> (t-butyloxycarbonyl-L-Val-L-Pro-L-Arg-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]
0.1 <2> (t-butyloxycarbonyl-L-Leu-L-Lys-L-Arg-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]
0.1 <2> (t-butyloxycarbonyl-L-Val-L-Leu-L-Lys-7-amido-4-methylcoumarin, <2> 37°C, pH 8.0 [16]) [16]
0.22 <4> (tert-butyloxycarbonyl-Phe-Ser-Arg-4-methylcoumaryl-7-amide, <4> recombinant wild-type enzyme, pH 8.0, 25°C [9]) [9]
0.23 <4> (D-Val-Leu-Arg-4-nitroanilide, <4> recombinant enzyme, pH 8.0, 37°C [4]) [4]
0.27 <4> (tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide, <4> recombinant enzyme, pH 8.0, 37°C [4]) [4]
0.28 <4> (D-Val-Leu-Arg-4-nitroanilide, <4> native enzyme, pH 8.0, 37°C [4]) [4]
0.28 <4> (tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide, <4> recombinant wild-type enzyme, pH 8.0, 25°C [9]) [9]
0.3 <4> (tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide, <4> native enzyme, pH 8.0, 37°C [4]) [4]
0.32 <4> (tert-butyloxycarbonyl-Asp(benzyloxy)-Pro-Arg-4-methylcoumaryl-7-amide, <4> recombinant wild-type enzyme, pH 8.0, 25°C [9]) [9]
0.34 <4> (tert-butyloxycarbonyl-Asp-Pro-Arg-4-methylcoumaryl-7-amide, <4> recombinant enzyme, pH 8.0, 37°C [4]) [4]
0.5 <4> (tert-butyloxycarbonyl-Phe-Ser-Arg-4-methylcoumaryl-7-amide, <4> recombinant enzyme, pH 8.0, 37°C [4]) [4]
0.54 <4> (tert-butyloxycarbonyl-Phe-Ser-Arg-4-methylcoumaryl-7-amide, <4> native enzyme, pH 8.0, 37°C [4]) [4]
8.36 <4> (Pro-Phe-Arg-4-methylcoumaryl-7-amide, <4> recombinant wild-type enzyme, pH 8.0, 25°C [9]) [9]

K_i-Value (mM)

0.0008 <4> (serine protease inhibitor-3, <4> complex formation, pH 7.4, 37°C [8]) [8]

pH-Optimum

7.4 <4> (<4> assay at [8,10]) [8, 10]
8 <4> (<4> recombinant enzyme [4]; <4> assay at [9]) [4, 9]
8.5 <2, 5> [14, 16]

pH-Range

5.5 <5> (<5> no detectable activity below [14]) [14]
7.4-10 <5> (<5> more than 50% of maximum activity within this range [14]) [14]

Temperature optimum (°C)

25 <4> (<4> assay at [9]) [9]
37 <4> (<4> assay at [4,8,10]) [4, 8, 10]

4 Enzyme Structure

Molecular weight

26600 <4> (<4> recombinant enzyme, MALDI-TOF MS [4]) [4]
28520 <4> (<4> precursor, calculated from sequence of cDNA [13]) [13]
29000 <4> (<4> recombinant enzyme, gel filtration [4]) [4]

Subunits

? <2, 3, 5> (<5> x * 30000, SDS-PAGE [5]; <3> x * 10000 + x * 18000, SDS-PAGE [11]; <2> x * 31500, SDS-PAGE of active recombinant enzyme [16]) [5, 11, 16]
monomer <4> (<4> 1 * 26000, non-reducing SDS-PAGE [4]; <4> 1 * 32000, reducing SDS-PAGE [4]) [4]
Additional information <4> (<4> determination of loop structure and organization [9]) [9]

Posttranslational modification

glycoprotein <4> (<4> enzyme has a putative N-glycoslyation site at Asn95 of the kallikrein loop [6]; <4> N-glycosylated at the kallikrein loop, residues His91-Ile103, oligosacchride chain is essential for activity [9]; <4> enzyme contains N-glycosylation sites [8]) [6, 8, 9]
proteolytic modification <4, 5> (<4> recombinant enzyme is produced as inactive proform and needs to be processed by an endoprotease, e.g. protease-1, EC 3.4.21.50, or trypsin, EC 3.4.21.4, for activation by specific cleavage of the Lys32-Ile33 bond near the N-terminus [4,9]; <5> pro-enzyme may be activiated by lysyl-endopeptidase by cleavage of a 9 amino acid propeptide [14]) [4, 9, 14]

5 Isolation/Preparation/Mutation/Application

Source/tissue

brain <4, 9> (<4> expressed in the forebrain limbic area of adult brain [8]; <9> frontal lobe of adult brain [19]) [2, 4, 6, 8, 9, 19]
central nervous system <5> (<5> high expression level [12]) [12]
epidermis <4> [3]
epithelium <2> (<2> epithelial ovarian tumor [15,20]) [15, 20]
gastrointestinal tract <4> (<4> epidermis, keratinocytes [3]) [3]
hippocampus <1, 4> [2, 4, 6, 7, 18]
keratinocyte <4, 5> (<5> high expression level at active, growing stage [3]) [3]
limbic system <4> [2]
neuron <4> (<4> in the hippocampal CA1-CA3 subfields [8]) [8]
ovarian carcinoma cell <5> (<5> overexpression [12]; <5> enzyme is overexpressed by 40-100%, overview [5]; <5> serous, mucinous, endometrioid, and clear cell [5]) [5, 12]
ovary <5> (<5> high expression level [12]) [5, 12]
ovary cancer cell <2> (<2> epithelial ovarian tumor [15,20]) [15, 20]
skin <4, 5> (<4> epidermis [3]; <5> high expression level [12]) [3, 12]
submaxillary gland <3> [11]
uterus <4> (<4> low activity in nonpregnant uteri, increased activity in pregnant uteri, highest at midgestational period [10]) [10]

Localization

extracellular <4> (<4> secreted [7,9,10]; <4> disruption of loop C and the kallikrein loop enhances the regulated secretion, no loop disruption does lead to inhibition of secretion [9]) [7, 9, 10]

Purification

<3> [11]
<4> (1100fold from brain) [4]
<4> (recombinant from SF21 insect cells) [4, 8]
<4> (recombinant from SF9 insect cells) [2, 6]

Crystallization

<4> (hanging drop vapor diffusion method, recombinant enzyme, protein solution: 24.5 mg/ml, 5 mM HEPES, pH 7.4, 100 mM NaCl, 4°C, precipitant with PEG, X-ray diffraction structure determination and analysis) [2, 6]

Cloning

<4> (expression and secretion of wild-type and mutants from Neuro2a cells) [9]
<4> (overexpression of proneuropsin in Spodoptera frugiperda insect cells via baculovirus infection) [2, 4, 6, 8]
<5> (DNA sequence determination and analysis) [3, 5]
<5> (gene KLK6, genetic organization and structure of the kallikrein gene family, clustered on chromosome 19q13.3-q13.4) [12]
<5> (gene KLK8, genetic organization and structure of the kallikrein gene family, clustered on chromosome 19q13.3-q13.4) [12]

Engineering

C108S <4> (<4> oligonucleotide-directed mutagenesis, reduced K_m, increased k_{cat}, increased activity with substrate tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide [9]) [9]
C145S <4> (<4> oligonucleotide-directed mutagenesis of disulfide bond SS3, reduced K_m and k_{cat}, increased activity with substrate tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide [9]) [9]
C208S <4> (<4> oligonucleotide-directed mutagenesis of disulfide bond SS6, highly reduced K_m and reduced k_{cat}, increased activity with substrate tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide [9]) [9]
C233S <4> (<4> oligonucleotide-directed mutagenesis, highly reduced K_m and slightly reduced k_{cat}, reduced activity with substrate tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide [9]) [9]
C246S <4> (<4> oligonucleotide-directed mutagenesis, reduced K_m and k_{cat}, decreased activity with substrate tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide [9]) [9]
C39S <4> (<4> oligonucleotide-directed mutagenesis of disulfide bond SS1, increased K_m, reduced k_{cat}, reduced activity with substrate tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide [9]) [9]
C7S <4> (<4> oligonucleotide-directed mutagenesis, reduced K_m and k_{cat}, slightly decreased activity with substrate tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide [9]) [9]
D206V <4> (<4> oligonucleotide-directed mutagenesis, reduced K_m and highly reduced k_{cat}, decreased activity with substrate tert-butyloxycarbonyl-Val-Pro-Arg-7-amino-4-methylcoumarin [9]) [9]
N110A <4> (<4> oligonucleotide-directed mutagenesis, reduced K_m and k_{cat}, slightly increased activity with substrate tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide [9]) [9]
medicine <2> (<2> enzyme expression in epithelial ovarian tumors correlates significantly with favorable patient survival. Use of enzyme as a prognostic marker in patients with ovarian cancer [20]) [20]
Additional information <1, 4> (<4> additional deletion N113-E115 in mutant N110S: decreased k_{cat} and K_m, increased activity with substrate tert-butyloxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide [9]; <1> enzyme knockout mice are significantly impaired in the Morris water maze and Y-mazes and fail to exhibit early phase long-term potentiation by a single tetanus. A dose of recombinant enzyme alone, without tetanic stimulation, elicits either long-lasting potentiation or depression, depending on the dose, by increase of phosphorylation at different sites on the GluR1 subunit of the AMPA receptor [18]; <1> enzyme-deficient mouse, number of L1-immunoreactive boutons is markedly higher than in wild-type, their number reverts to wild-type level upon microinjection of enzyme. L1-immunoreactive boutons are hypertrophied in the mutant [17]) [9, 17, 18]

Application

biotechnology <4> (<4> enzyme might contribute to the remodeling of extracellular components after decidualization [10]) [10]

medicine <2, 5> (<5> TAGD14 may be a useful molecular target for therapy or a diagnostic tool as tumor marker due to its overexpression in ovarian carcinoma [5]; <2> patients with ovarian cancer, women with enzyme-positive tumors most often have lower-grade tumors, no residual tumor after surgery, optimal debulking success, and significantly longer progression-free survival and overall survival. Use of enzyme as independent marker of favorable prognsis in ovarian cancer [15]) [5, 15]
pharmacology <4> (<4> enzyme is a drug target in the treatment of epilepsy [2]) [2]

6 Stability

Storage stability
<2>, -20°C, active recombinant enzyme, stable for at least two months [16]

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Kallikrein 13 3.4.21.119

1 Nomenclature

EC number
3.4.21.119

Recommended name
kallikrein 13

Synonyms
GK-13 <1> [5]
K13 <2> [6]
MGK-13 <4> [1]
PRECE <1> [4]
S01.306 (Merops-ID)
glandular kallikrein 13 <1> [5]
glandular kallikrein K13 <4> [1]
mK13 <1> [12]
prorenin converting enzyme <1> [4, 5, 11]
prorenin-converting enzyme <1> [2, 3, 5]
tissue kallikrein <4> [1]
Additional information <1> (<1> enzyme is neither trypsin-like nor kallikrein [3]) [3]

CAS registry number
342900-44-1
9001-01-8

2 Source Organism

<1> *Mus musculus* (no sequence specified) [2, 3, 4, 5, 11, 12]
<2> *Homo sapiens* (no sequence specified) [6, 8, 9, 10, 13, 14]
<3> *Homo sapiens* (UNIPROT accession number: Q9UKR3) [7]
<4> *Mus musculus* (UNIPROT accession number: P36368) [1]

3 Reaction and Specificity

Catalyzed reaction
hydrolyses mouse Ren2 protein (a species of prorenin present in the submandibular gland) on the carboxy side of the arginine residue at the Lys-Arg pair in the N-terminus, to yield mature renin (<1> substrate binding [5]; <1,2,3>

enzyme is a serine protease [5,6,7]; <1> preference for Xaa-Arg-Arg- motif [2]; <1> active site is located in the cleft between two β-barrel domains, with an ordered kallikrein loop conformation close to it [5])

Reaction type

hydrolysis of peptide bond

Natural substrates and products

- **S** prorenin + H_2O <1> (<1> inactive precursor [3,4,5]) (Reversibility: ?) [3, 4, 5]
- **P** Renin + ? <1> (<1> product is an active enzyme [3,4,5]) [3, 4, 5]
- **S** polypeptide + H_2O <1, 2, 3> (Reversibility: ?) [3, 4, 5, 6, 7]
- **P** peptides <1, 2, 3> [3, 4, 5, 6, 7]
- **S** Additional information <2> (<2> enzyme is involved in tissue remodeling and/or tumor invasion and metastasis [8]) (Reversibility: ?) [8]
- **P** ?

Substrates and products

- **S** 35 kDa pro-interleukin 1β + H_2O <1> (<1> cleavage of Leu113-Leu114 bond [12]) (Reversibility: ?) [12]
- **P** 17.5 interleukin 1β + 20 kDa interleukin 1β
- **S** FSR-amido-4-methylcoumarin + H_2O <2> (<2> fluorogenic trypsin-like peptide substrate [6]) (Reversibility: ?) [6]
- **P** FSR + 7-amino-4-methylcoumarin
- **S** prorenin + H_2O <1> (<1> cleavage site at paired basic amino acid residues [2,3,4,5]; <1> no cleavage of the peptide bond between Arg and Pro [4]; <1> specifically cleaves the peptide bond on the COOH-side of the Arg residue at the Lys-Arg pair of mouse Ren 2 prorenin, but does not cleave mouse Ren 1 and human prorenins [4]; <1> mouse prorenin from submandibular gland [5]; <1> recombinant murine prorenin 2 [2]; <1> murine or human origin substrate from Xenopus oocytes expression system [3]; <1> inactive precursor [3,4,5]) (Reversibility: ?) [2, 3, 4, 5]
- **P** renin + ? <1> (<1> product is an active enzyme [3,4,5]) [2, 3, 4, 5]
- **S** VPR-amido-4-methylcoumarin + H_2O <2> (<2> fluorogenic trypsin-like peptide substrate [6]) (Reversibility: ?) [6]
- **P** VPR + 7-amino-4-methylcoumarin
- **S** benzyloxycarbonyl-Arg-Val-Arg-Arg-4-methyl-coumaryl-7-amide + H_2O <1> (<1> low activity [2]) (Reversibility: ?) [2]
- **P** benzyloxycarbonyl-Arg-Val-Arg-Arg + 7-amino-4-methylcoumarin
- **S** benzyloxycarbonyl-Gln-Arg-Arg-4-methyl-coumaryl-7-amide + H_2O <1> (<1> best substrate [2]) (Reversibility: ?) [2]
- **P** benzyloxycarbonyl-Gln-Arg-Arg + 7-amino-4-methylcoumarin
- **S** benzyloxycarbonyl-Glu-Lys-Lys-4-methyl-coumaryl-7-amide + H_2O <1> (<1> low activity [2]) (Reversibility: ?) [2]
- **P** benzyloxycarbonyl-Glu-Lys-Lys + 7-amino-4-methylcoumarin
- **S** benzyloxycarbonyl-Leu-Lys-Arg-4-methyl-coumaryl-7-amide + H_2O <1> (Reversibility: ?) [2]
- **P** benzyloxycarbonyl-Leu-Lys-Arg + 7-amino-4-methylcoumarin

S mouse prorenin Ren2 + H_2O <1> (<1> specific cleavage of Lys-Arg bond of mouse prorenin Ren2 [11]) (Reversibility: ?) [11]
P renin + ?
S polypeptide + H_2O <1, 2, 3> (<1> cleavage site at paired basic amino acid residues [3,4]) (Reversibility: ?) [3, 4, 5, 6, 7]
P peptides <1, 2, 3> [3, 4, 5, 6, 7]
S pyroglutamyl-Arg-Tyr-Lys-Arg-4-methyl-coumaryl-7-amide + H_2O <1> (Reversibility: ?) [2]
P pyroglutamyl-Arg-Tyr-Lys-Arg + 7-amino-4-methylcoumarin
S Additional information <1, 2> (<1> glycosylation near the processing site might prevent hydrolysis [4]; <1> substrate specificty [4]; <2> enzyme is involved in tissue remodeling and/or tumor invasion and metastasis [8]; <1> no substrate: mouse prorenin Ren1, human prorenin [11]) (Reversibility: ?) [4, 8, 11]
P ?

Inhibitors

benzamidine <1> (<1> 2 mM, 100% inhibition [11]) [3, 11]
diisopropyl fluorophosphate <1> (<1> 2 mM, 35% inhibition [11]) [11]
leupeptin <1> (<1> 2 mM, 100% inhibition [11]) [3, 11]
phenylmethylsulfonylfluoride <1> [12]
antipain <1> (<1> 2 mM, 100% inhibition [11]) [3, 11]
Additional information <1> (<1> no inhibition by EDTA and pepstatin A [3]; <1> little or no effect by the following protease inhibitors: PMSF, DFP, aprotinin, iodoacetamide, NEM, PCMBS, and E-64 [3]; <1> not inhibitory: EDTA, iodoacetamide, aprotinin [11]) [3, 11]

Specific activity (U/mg)

0.0011 <1> (<1> purified enzyme, substrate benzyloxycarbonyl-Gln-Arg-Arg-4-methyl-coumaryl-7-amide [2]) [2]

pH-Optimum

7.5 <1, 2> (<1,2> assay at [2,6]) [2, 6]
7.5-8.5 <1> [11]
8-8.5 <1> [3]

Temperature optimum (°C)

37 <1, 2> (<1,2> assay at [2,3,6]) [2, 3, 6]

4 Enzyme Structure

Molecular weight

27000 <1> (<1> gel filtration, SDS-PAGE under nonreducing conditions [11]) [11]
30000 <2> (<2> secreted enzyme, gel filtration [6]) [6]
54000 <1> (<1> about, gel filtration and crystal structure [5]) [5]

Subunits

? <2> (<2> x* 50000, recombinant glycosylated enzyme, SDS-PAGE [6]; <2> x *25000-28000, recombinant deglycosylated enzyme, SDS-PAGE [6]) [6]
dimer <1> (<1> 2 * 27000, SDS-PAGE [5]; <1> 1 * 17000 + 1 * 10000, SDS-PAGE [11]) [5, 11]
Additional information <1, 2> (<1> enzyme contains 2 disulfide bridges at cystines 22-157 and 136-201 [5]; <2> enzyme forms SDS-stable complexes with serum protease inhibitors such as α_2-antiplasmin, α_2-macroglobulin and α_1-antichymotrypsin [9]) [5, 9]

Posttranslational modification

Glycoprotein <2> (<2> enzyme is highly glycosylated [6]) [6]

5 Isolation/Preparation/Mutation/Application

Source/tissue

amniotic fluid <2> [6]
ascites fluid <2> (<2> from women with advanced ovarian carcinoma [6]) [6]
breast <2, 3> [6, 7]
breast carcinoma cell <3> (<3> down-regulated by steroid hormones [7]) [7]
cerebrospinal fluid <2> (<2> traces [6]) [6]
esophagus <2> (<2> highest activity in esophagus and tonsil [6]) [6]
kidney <1, 2> (<1> low activity [3]) [3, 6]
lung <2> [6]
milk <2> [6]
ovarian follicle <2> [6]
ovary cancer cell <2> [8, 13]
prostate <2, 3> [6, 7]
salivary gland <2, 3> (<2> tumor cell of salivary gland [10]) [6, 7, 10]
seminal plasma <2> [6]
skin <2> [6]
stratum corneum <2> (<2> quantification of eight different tissue kallikreins. Enzyme is present at significantly lower concentrations in females than in males [14]) [14]
submandibular gland <1> (<1> highest activity [3]; <1> enzyme regulation is parallel and similar to that of renin [3]; <1> larger amounts in male than female mice [3]) [2, 3, 4, 5, 11, 12]
sweat <2> (<2> quantification of eight different tissue kallikreins. Enzyme is detected only in the eccrine sweat glands regardless of gender [14]) [14]
testis <2, 3> [6, 7]
thyroid gland <2> [6]
tonsil <2> (<2> highest activity in esophagus and tonsil [6]) [6]
trachea <2> [6]
ureter <2> [6]

Additional information <1, 2, 3> (<3> gene is expressed in various tissues [7]; <1> no activity in liver, brain, heart, and pancreas [3]; <2> fetal tissue pattern is similar to the adult one [6]) [3, 4, 6, 7]

Localization

cytosol <1, 2> [3, 6]
extracellular <2> (<2> enzyme is also secreted into biological fluids [6]) [6, 8]
secretory granule <1> (<1> of granular convoluted tubular cell, co-localization of enzyme and pro-interleukin 1β [12]) [12]

Purification

<1> [2, 4, 5]
<2> (recombinant enzyme from Pichia pastoris) [6]

Crystallization

<1> (both hanging and sitting drop vapour diffusion method, protein solution: 57 mg/ml, precipitant solution: 10-15% PEG 8000, 200 mM Li_2SO_4, 100 mM sodium cacodylate, pH 6.5, room temperature, X-ray diffraction structure determination and analysis) [5]

Cloning

<2> (expression in Pichia pastoris) [6]
<3> (gene KLK-L4, DNA sequence determination and analysis, gene mapps to chromosome 19q13.3-q13.4, tissue expression analysis) [7]

Engineering

Additional information <2> (<2> treatment of ovarian cancer cell lines secreting enzyme with an enzyme neutralizing antibody results in reduced migration of cells [8]) [8]

Application

medicine <2, 3> (<3> gene may be involved in the pathogenesis and/or progression of breast cancer and may find applicability as cancer biomarker [7]; <2> most salivary gland tumors show high levels of enzyme expression. Pleomorphic adenoma shows less staining than normal tissue, adenoid cystic carcinomas, polymorphous low grade adenocarcinomas and adenocarcinomas not otherwise specified stain significantly more than normal salivary gland tissue [10]; <2> patients with ovarian carcinoma, women with enzyme-positive tumors most often have early stage disease, no residual tumor after surgery and optimal debulking success. Patients with enzyme-positive tumors have a significantly longer progression-free survival and overall survival. Use of enzyme as biomarker for ovarian carcinoma [13]; <2> treatment of ovarian cancer cell lines secreting enzyme with an enzyme neutralizing antibody results in less migration of cells [8]) [7, 8, 10, 13]

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Oviductin 3.4.21.120

1 Nomenclature

EC number
3.4.21.120

Recommended name
oviductin

Synonyms
Gp43 processing protease <7> [2]
α-fetoprotein <2> (<2> human oviductin (hOV-I) and human α-fetoprotein (hAFP) are chemically similar and immunologically identical molecules [10]) [10]
hAFP <2> (<2> human oviductin (hOV-I) and human α-fetoprotein (hAFP) are chemically similar and immunologically identical molecules [10]) [10]
hOV-1 <2> (<2> human oviductin (hOV-I) and human α-fetoprotein (hAFP) are chemically similar and immunologically identical molecules [10]) [10]
oviductal protease <7> [2, 6]
oviductin <11> [8]
oviductin-1 <8> [3]
oviductin-I <2, 8> (<2> human oviductin (hOV-I) and human α-fetoprotein (hAFP) are chemically similar and immunologically identical molecules [10]) [10, 12]

2 Source Organism

<1> *Mus musculus* (no sequence specified) [5, 16]
<2> *Homo sapiens* (no sequence specified) [10, 11, 16]
<3> *Sus scrofa* (no sequence specified) [16]
<4> *Bos taurus* (no sequence specified) [16]
<5> *Oryctolagus cuniculus* (no sequence specified) [9,16]
<6> *Ovis aries* (no sequence specified) [16]
<7> *Xenopus laevis* (no sequence specified) [2,6]
<8> *Mesocricetus auratus* (UNIPROT accession number P19080) (<8> There are four published structures of the Bacillus subtilis wild-type chroismate mutase (CM) with Protein Data Bank (PDB) codes 1COM, 2CHS, 2CHT, and 1DBF [7,14,17]) [1,3,4,7,12,14,15,16,17]
<9> *Papio hamadryas* (no sequence specified) [16]
<10> *Bufo arenarum* (no sequence specified) [13]
<11> *Bufo japonicus* (no sequence specified) [8]

3 Reaction and Specificity

Catalyzed reaction

preferential cleavage at Gly-Ser-Arg373-/-glycoprotein gp43 in Xenopus laevis coelemic egg envelope to yield gp41

Natural substrates and products

S gp43 + H_2O <7> (<7> oviductin alone is the oviductal factor responsible for converting the egg envelope to a sperm-penetrable form, via an increase in sperm binding. gp43 processing is the only requirement for envelope conversion [6]; <7> preferential cleavage at Gly-Ser-Arg373-/-glycoprotein gp43 in Xenopus laevis coelemic egg envelope to yield gp41. Processing of gp43 causes physical changes in the egg envelope which occurs during egg transit through the oxiduct [2]) (Reversibility: ?) [2, 6]
P gp41 + ?
S Additional information <8> (<8> it is proposed that hamster oviductin is a mucin-type glycoprotein which might act as a protective secretion influencing the first steps of the reproductive process necessary for the normal triggering of fertilization and early embryonic development [1]) (Reversibility: ?) [1]
P ?

Substrates and products

S 55 kDa glycoprotein + H_2O <10> (<10> the oviductal protease selectively hydrolyzes in vitro the 84 kDa and the 55 kDa glycoproteins of the coelomic envelope [13]) (Reversibility: ?) [13]
P ?
S 84 kDa glycoprotein + H_2O <10> (<10> the oviductal protease selectively hydrolyzes in vitro the 84 kDa and the 55 kDa glycoproteins of the coelomic envelope [13]) (Reversibility: ?) [13]
P ?
S N^{α}-tert-butoxycarbonyl-Leu-Ser-Thr-Arg-4-methylcoumarin-7-amide + H_2O <7> (<7> 14% of the activity with N^{α}-tert-butoxycarbonyl-Phe-Ser-Arg-4-methylcoumarin-7-amide [2]) (Reversibility: ?) [2]
P N^{α}-tert-butoxycarbonyl-Leu-Ser-Thr-Arg + 7-amino-4-methylcoumarin
S N^{α}-tert-butoxycarbonyl-Leu-Thr-Arg-4-methylcoumarin-7-amide + H_2O <7> (<7> 12% of the activity with N^{α}-tert-butoxycarbonyl-Phe-Ser-Arg-4-methylcoumarin-7-amide [2]) (Reversibility: ?) [2]
P N^{α}-tert-butoxycarbonyl-Leu-Thr-Arg + 7-amino-4-methylcoumarin
S N^{α}-tert-butoxycarbonyl-Phe-Ser-Arg-4-methylcoumarin-7-amide + H_2O <7> (Reversibility: ?) [2]
P N^{α}-tert-butoxycarbonyl-Phe-Ser-Arg + 7-amino-4-methylcoumarin
S Pro-Phe-Arg-4-methylcoumarin-7-amide + H_2O <7> (<7> 9% of the activity with N^{α}-tert-butoxycarbonyl-Phe-Ser-Arg-4-methylcoumarin-7-amide [2]) (Reversibility: ?) [2]
P Pro-Phe-Arg + 7-amino-4-methylcoumarin

S Pyr-Gly-Arg-4-methylcoumarin-7-amide + H_2O <7> (<7> 9% of the activity with N^{α}-tert-butoxycarbonyl-Phe-Ser-Arg-4-methylcoumarin-7-amide [2]) (Reversibility: ?) [2]
P Pyr-Gly-Arg + 7-amino-4-methylcoumarin
S gp43 + H_2O <7> (<7> oviductin alone is the oviductal factor responsible for converting the egg envelope to a sperm-penetrable form, via an increase in sperm binding. gp43 processing is the only requirement for envelope conversion [6]; <7> preferential cleavage at Gly-Ser-Arg373-/-glycoprotein gp43 in Xenopus laevis coelemic egg envelope to yield gp41. Processing of gp43 causes physical changes in the egg envelope which occurs during egg transit through the oxiduct [2]; <7> preferential cleavage at Gly-Ser-Arg373-/-glycoprotein gp43 in Xenopus laevis coelemic egg envelope to yield gp41 [2]) (Reversibility: ?) [2, 6]
P gp41 + ?
S Additional information <8> (<8> it is proposed that hamster oviductin is a mucin-type glycoprotein which might act as a protective secretion influencing the first steps of the reproductive process necessary for the normal triggering of fertilization and early embryonic development [1]) (Reversibility: ?) [1]
P ?

Inhibitors

aprotinin <7> (<7> 0.05 mM, 97% inhibition [2]) [2]
diisopropyl fluorophosphate <7> (<7> 0.05 mM, 37% inhibition. Rapid and irreverible [2]) [2]
EDTA <7> (<7> 0.05 mM, 90% inhibition [2]) [2]
EGTA <7> (<7> 0.05 mM, 94% inhibition [2]) [2]
guanidine hydrochloride <7> (<7> competitive [2]) [2]
leupeptin <7> (<7> 0.05 mM, 55% inhibition [2]) [2]
soybean trypsin inhibitor <7> (<7> 0.0025 mM, 84% inhibition [2]) [2]
antipain <7> (<7> 0.05 mM, 35% inhibition [2]) [2]
p-aminobenzamidine <7> (<7> competitive [2]) [2]
Additional information <7> (<7> no inhibition by iodoacetamide, E-64, pepstatin or 1,10-phenanthroline [2]) [2]

Metals, ions

Ca^{2+} <7> (<7> 38% stimulation at 10 mM. 11% stimulation at 100 mM [2]) [2]
KCl <7> (<7> maximal activity in 200-400 mM solution of monovalent salts is 200% of that in absence of added salt [2]) [2]
LiCl <7> (<7> maximal activity in 200-400 mM solution of monovalent salts is 200% of that in absence of added salt [2]) [2]
NaCl <7> (<7> maximal activity in 200-400 mM solution of monovalent salts is 200% of that in absence of added salt [2]) [2]
NaF <7> (<7> maximal activity in 200-400 mM solution of monovalent salts is 200% of that in absence of added salt [2]) [2]
$NaNO_3$ <7> (<7> maximal activity in 200-400 mM solution of monovalent salts is 200% of that in absence of added salt [2]) [2]

Turnover number (min^{-1})
3.8 <7> (N^{α}-tert-butoxycarbonylphenylalanylserylarginyl-4-methylcoumarin-7-amide) [2]

Specific activity (U/mg)
2.53 <7> [2]

K_m-Value (mM)
0.058 <7> (N^{α}-tert-butoxycarbonylphenylalanylserylarginyl-4-methylcoumarin-7-amide) [2]

K_i-Value (mM)
0.0041 <7> (*p*-aminobenzamidine) [2]
0.0075 <7> (guanidine hydrochloride) [2]

pH-Optimum
8 <7> (<7> N^{α}-tert-butoxycarbonylphenylalanylserylarginyl-4-methylcoumarin-7-amide [2]) [2]

pH-Range
7.5-8.5 <7> (<7> more than 90% of maximal activity at pH 7.5 and at pH 8.5, less than 30% of maximal activity below pH 6.5 [2]) [2]

4 Enzyme Structure

Subunits
? <7> (<7> x * 66000, SDS-PAGE under both nonreducing and reducing conditions [2]) [2]

Posttranslational modification
Glycoprotein <1, 2, 3, 4, 5, 6, 8, 9> (<8> contains terminal α-D-GalNAc and either terminal α-D-NeuAc or non-terminal β-D-$(GlcNAc)_2$ residues. Both the α- and β-forms are sulphated on O-linked oligosaccharide side chains but are not phosphorylated. The hamster oviductin polymorphism is a consequence of different glycosylation patterns and not the polypeptide chain itself. Hamster oviductin is mostly O-glycosylated and contains a few N-linked oligosaccharide side chains (approx. 10 kDa). It is proposed that hamster oviductin is a mucin-type glycoprotein which might act as a protective secretion influencing the first steps of the reproductive process necessary for the normal triggering of fertilization and early embryonic development [1]; <8> glycosylation of hamster oviductin appears to be differentially regulated during the estrous cycle [7]; <2,8> mucin-type motifs [16]; <8> polymorphism in the heavily O-glycosylated region of hamster oviductin [4]; <1> the mature MOGP contained three potential N-linked glycosylation sites and 24 possible O-linked glycosylation sites [5]) [1, 4, 5, 7, 9, 14, 16, 17]
proteolytic modification <7, 11> (<7> protease is translated as the N terminus of an unusual mosaic protein. It is proposed that during post-translational proteolytic processing of the mosaic oviductin glycoprotein, the processed N-terminal protease domain is released coupled to two C-terminal

CUB domains and constitutes the enzymatically active protease molecule [6]; <11> translational products stored in the pars recta granules need to be processed to become a proteolytically active 66000 Da form. Extensive posttranslational processing for activation of proteolytic activity. Oviductin translated as 107.6-kDa precursors are processed both N- and C-terminally to give rise to a 66-kDa active form comprising a serine protease and two CUB domains [8]) [6, 8]

5 Isolation/Preparation/Mutation/Application

Source/tissue

OE-E6/E7 cell <2> (<2> immortalized human oviductal epithelial cell line [11]) [11]
blastomere <8> (<8> endocytic compartments in the blastomeres of developing embryos [3]) [3]
embryo <8> (<8> endocytic compartments in the blastomeres of developing embryos [3]) [3]
endocervix <5> (<5> negligible expression during the first three months of postnatal cervical differentiation. Transcripts are minimally detectable in the cervives of 4-month-old juveniles. Strong expression in the endocervices of adults is eliminated by ovariectomy and restored by estrogen treatment [9]) [9]
epithelial cell <8> (<8> luminal epithelial cells of uterus [12]) [12]
non-ciliated oviduct cell <8> [14]
oocyte <8> (<8> after its release into the lumen of the oviduct, oviductin becomes associated with the zona pellucida of post-ovulatory oocytes. In unfertilized oocytes, oviductin is also detected in membrane invaginations along the oolemma and in some vesicles within multivesicular bodies [14]) [14]
oviduct <1, 7, 8> (<8> expression of the oviductin gene is confined strictly to nonciliated secretory cells [17]; <8> hamster oviduction is inhibitory to in vitro fertilization, but oocytes with cumulus intact maintain their fertilizing capacity in the presence of oviductin [15]; <8> in Golgi saccules and secretory granules of the non-ciliated oviduct cells. After its release into the lumen, oviductin becomes associated with the zona pellucida of post-ovulatory oocytes [14]; <1> major secretory glycoprotein of the oviduct [5]; <8> oviductin mRNA expression remains constant throughout the estrous cycle. Production of oviductin is not regulated differentially during the estrous cycle. The oviduct contains several forms of oviductin at each stage of the estrous cycle, the native glycosylated form(s) of 160-350 kDa, and several precursor forms of 70-100 kDa [7]; <7> secretory granules [2]) [2, 5, 7, 14, 15, 17]
pars recta <7, 10, 11> [6, 8, 13]
uterine endometrium <8> (<8> it is speculated that receptors for hamster oviductin-1 are present at the apical cell surface of endometrial cells and that implantation of the developing blastocyst into the uterine wall is possible only following downregulation of these receptors [12]) [12]

uterine epithelial cell <8> (<8> oviductin is detected over the microvilli and within multivesicular bodies [14]) [14]
uterus <8> (<8> luminal epithelial cells [12]) [12]
zona pellucida <2, 8> (<2,8> oviductins are targeted to the oocyte via the interaction of their chitinase-like domains with specific oligosaccharide moieties of the zona pellucida. Once localized to this structure, oviductin molecules would act as a protective shield around the oocyte and early embryo by virtue of their densely glycosylated mucin-type domains [16]; <8> some of the oviductin associated with the zona pellucida appears to be internalized by blastomeres of the embryo and further processed through the endosomal/lysosomal pathway [3]) [3, 4, 16]

Localization

Golgi apparatus <8> (<8> of the non-ciliated oviduct cells [14]) [14]
granule <11> (<11> translational products stored in the pars recta granules need to be processed to become a proteolytically active 66000 Da form [8]) [8]
membrane <8> (<8> in unfertilized oocytes, oviductin is detected in membrane invaginations along the oolemma and in some vesicles within multivesicular bodies [14]) [14]
microvillus <8> (<8> of uterine epithelial cells [14]) [14]
multivesicular body <8> (<8> of uterine epithelial cells [14]) [14]
secretory granule <7, 8> (<8> of the non-ciliated oviduct cells [14]; <7> oviductal [2]) [2, 14]

Purification

<7> [2]
<8> [1, 15]

Cloning

<1> [5]
<7> [6]
<8> [7, 16]
<11> [8]

6 Stability

General stability information

<7>, relatively stable to rapid freezing in the presence of 20% glycerol. 90% of the activity remains after each freeze-thaw cycle [2]

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