

ENZYMES

INTRODUCTION

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- Enzymes are protein **catalysts** for chemical reactions in biological systems.
- They increase the rate of chemical reactions taking place within living cells **without changing themselves**.
- Catalyst is a substance, which accelerates the rate of a chemical reaction without being consumed in the reaction, or affects the end point.
- Enzymes are the active substances that are in the yeast (**en = in, zyme = yeast**).

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- **Enzymes are Biological Catalysts**
- Are (almost) always proteins
- Are extraordinarily **efficient** better than chemical catalysts –
- Are remarkably **specific**
- Need mild physiological conditions to function (temperature, salt, pH, etc.)
- Often require a cofactor (metal ion, prosthetic group, or coenzyme)
- May be modified for regulatory purposes

Why Study Enzymes?

- They catalyze stereo-specific reactions with virtually no undesirable “side-products”
- Many disease states are related to altered or absent enzyme activity
- Many vitamins, drugs, and toxins are enzyme modulators
- Measurement of activity is diagnostic for many diseases
- Enzymes are important practical tools in industry, medicine, and agriculture

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- Enzymes are **highly specific** in their action and act inside the cells (e.g., metabolic enzymes) and outside cells (e.g., the digestive enzymes and blood clotting factors).
- Most enzymes belong to **simple proteins**.
- Some enzymes require the help of certain non-protein factors that may be loosely attached to them(**a**

Classification of Enzymes

Enzymes are classified into 6 classes as follows:

1. Oxidoreductases:

- These are enzymes catalyzing oxidation-reduction reactions,
- Act on many chemical groupings to add or remove hydrogen atoms.

e.g., oxidases, oxygenases, reductases, dehydrogenases and peroxidases.

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2. Transferases: These are enzymes catalyzing the transfer of a chemical group from one compound to the other,

- They are subdivided into:
- (a) **Amino transferases**: These bring about the exchange of amino and keto group between an amino acid and a keto acid.

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- (b) **Kinases**: These bring about the transfer of a phosphate radical using ATP as the donor or ADP acceptor.
- © **Glycosyltransferases**: These bring about the transfer of a glycosyl group.
- (d) **C1-Transferases**: These bring about the transfer of an acyl group.

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3. Hydrolases: These are enzymes catalyzing the process of hydrolysis (breakdown of the compound by addition of water)



e.g. thiolases, amidases, ribonucleases, deoxyribonucleases, hydrolytic deaminases, phospholipases, phosphatases, glycosidases, esterase and peptidases.

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4. Lyases: these are enzymes, which catalyze breakdown of substrates by mechanisms other than hydrolysis and oxidation.

- They include **desulfhydrases** and **dehydratases** which reversibly remove or add H_2S or water from substrate.

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- Non-oxidative decarboxylases: which remove CO_2 , e.g. **pyruvic decarboxylase, aldolases, lyases**
- cleavage enzymes are lyases.
Phosphorylases cut the substrate by adding phosphate, e.g., **glycogen phosphorylase**, e.g., **$(\text{Glycogen})_n + \text{H}_3\text{PO}_4 \Rightarrow (\text{Glycogen})_{n-1} + \text{Glucose-1-phosphate}$**

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5. Isomerases: these are enzymes catalyzing isomerization, e.g.,

- **Epimerases**, e.g., **UDP-Glucose $\rightleftharpoons$ UDP-Galactose.**
- **Cis-trans-isomerase**, e.g., **trans-Retinol $\rightleftharpoons$ cis-Retinol.**
- **Mutase**, e.g., **glucose-1-phosphate $\rightleftharpoons$ glucose-6-phosphate.**
- **Aldo-keto-isomerase**, **Glucose-6-phosphate $\rightleftharpoons$ Fructose-6-phosphate.**

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6. Ligases or synthetases:

- These are enzymes catalyzing the process of ligation or binding of 2 molecules together in the presence of ATP.
- Glycogen synthase, DNA Ligase, Fatty acid synthase

e.g., **Fatty acid + CoASH + ATP $\Rightarrow$ Acyl-CoA + AMP + PPi.**

Systems of enzyme nomenclature

1. Substrate-dependent naming:

The name is derived from the substrate with **-ase** as a suffix, e.g., **urease** (for urea), **lipase** (for lipids) and **protease** (for protein).

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2. Chemical nature of the reaction-dependent naming:

- The name depends on nature of chemical reaction with **-ase** as a suffix,

E.g., **hydrolase** (for hydrolysis) and **dehydrogenase** (for removal of hydrogen).

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3. Systemic naming:

- The International Biochemical Union give every enzyme a code digital name.
- The digital name is of four digits, the first refers to the enzyme **class**, the second refers to the **subclass**, the third refers to the **sub-subclass** and the fourth refers to the name of the individual enzyme itself.

Functional sites in the enzymes system:

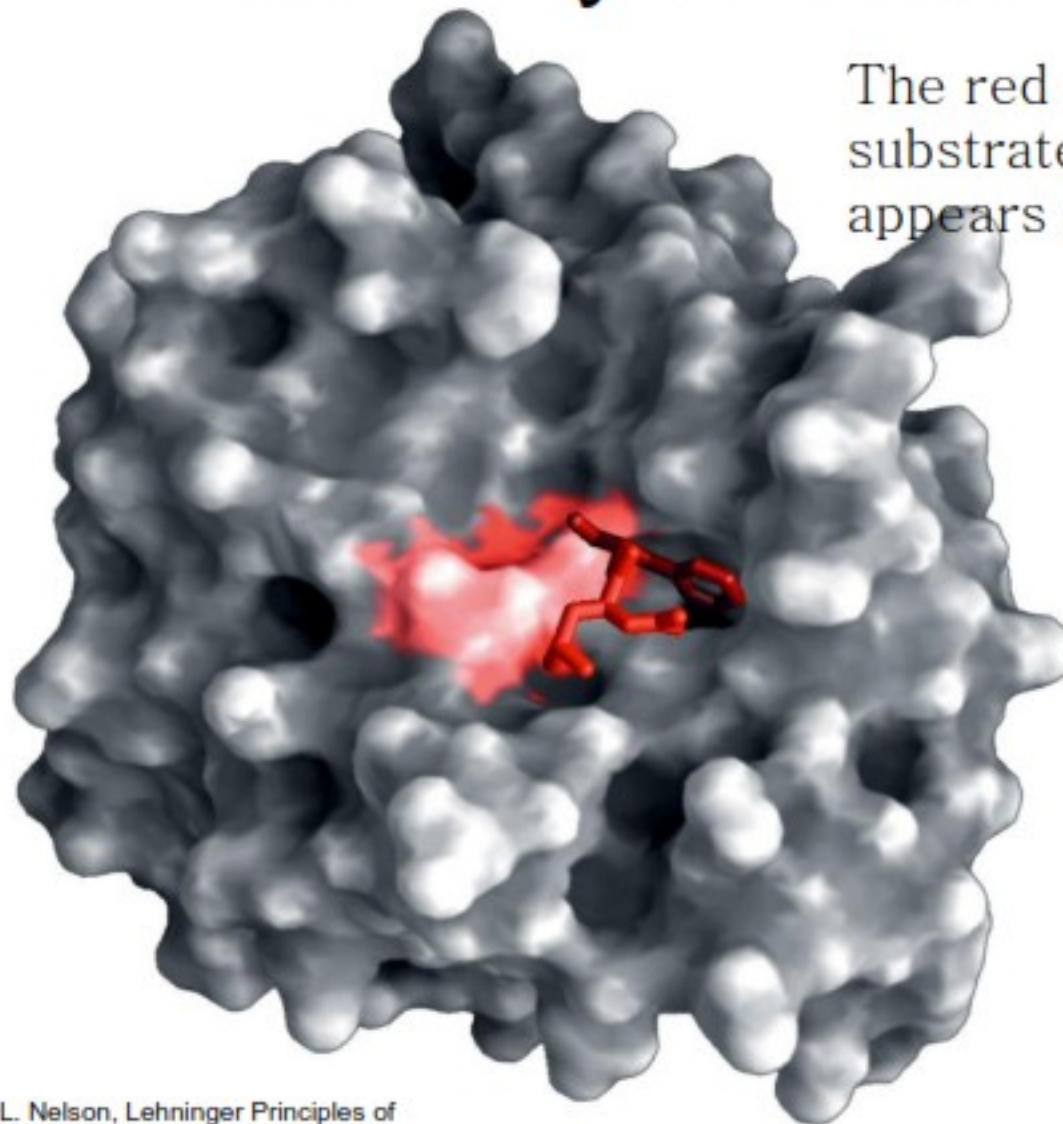
Active site:

Substrate-binding site at which substrate specifically binds and it is the site that carries out the chemical action.

Allosteric site:

The term allosteric site means “**the other site**” and allostery means “a change in shape”.

The enzyme active site



The red molecule is the substrate, the active site appears painted in red

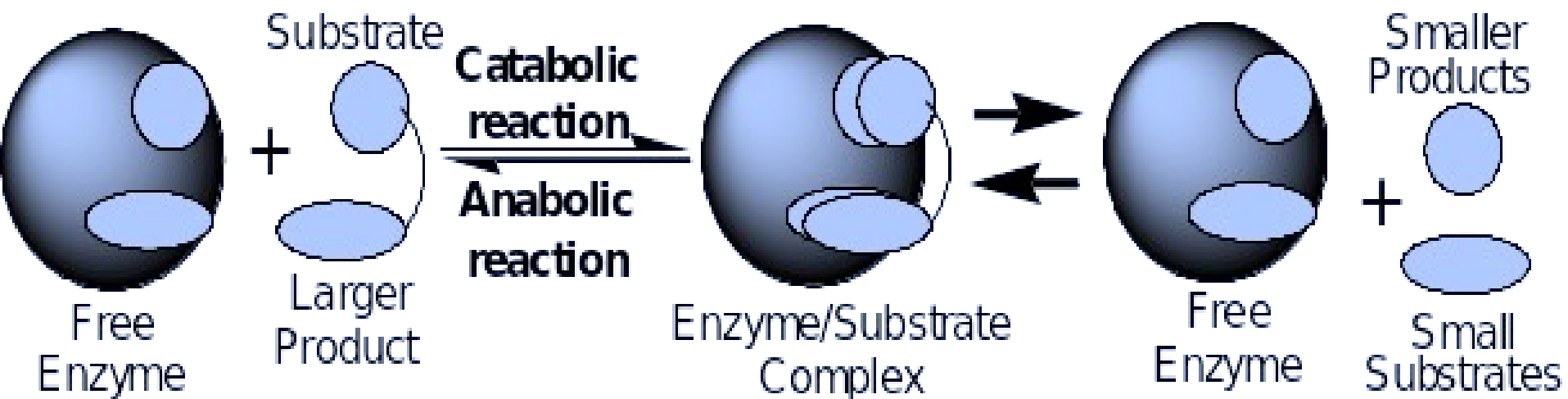
From: David L. Nelson, Lehninger Principles of Biochemistry, IV Edition, W. H. Freeman ed.



Enzyme-substrate combination:

- During the enzyme action, there is a temporary combination between the enzyme and its substrate.
- The enzyme combines with its substrate to give an **enzyme substrate complex**.
- The enzyme is liberated in a free state to combine with new substrate(s) and so on.
So the enzyme acts only as a catalyst for the proceeding of the reaction.

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- **LOCK-AND-KEY MODEL** proposed by Emil Fischer in 1894,
- the shape of the substrate and the active site of the enzyme are thought to fit together like a key into its lock.
- The two shapes are considered as rigid and fixed, and perfectly complement each other when brought together in the right alignment.

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- **Induced-Fit model:**
- The enzyme structure is flexible, not rigid.
- The active site adjusts to fit the shape of the substrate more closely. At the same time the substrate adjusts its shape to better adapt to the geometry of the active site. As a result, the reacting section of the substrate becomes aligned exactly with the groups in the active site that catalyze the reaction.

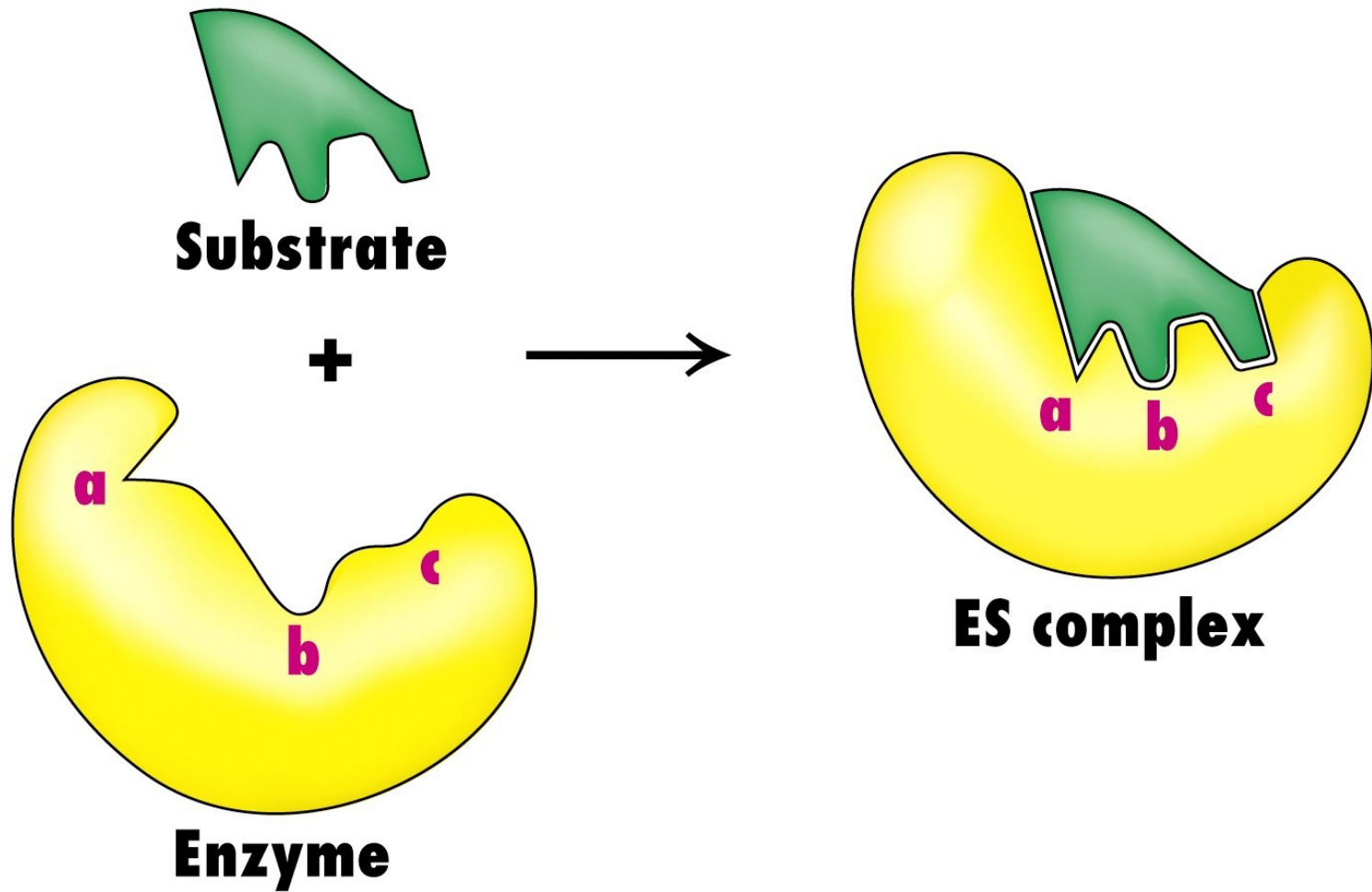


Figure 8-10
Biochemistry, Sixth Edition
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Structural components of the enzyme system (holoenzyme):

The apoenzyme

- the protein part of the enzyme that determines the substrate specificity and the major determinant of the nature of the chemical reaction.

The coenzyme

- is an organic mostly vitamin-derived compound or a free nucleotide, e.g., ATP, cAMP, UTP.
- Examples of vitamin coenzymes are **CoASH, TPP, NAD, and FAD.**
- The coenzyme is **loosely** (i.e., non-covalently) attached to the enzyme and is not consumed in the reaction.

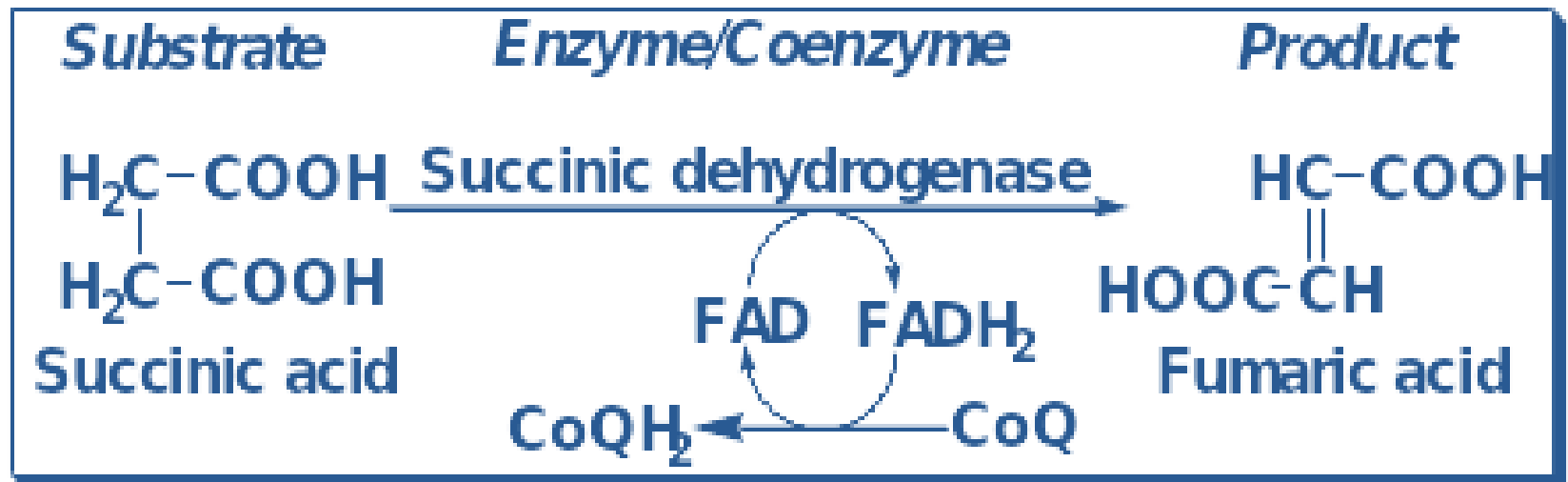
The activator

- It is an inorganic metal or non-metal ion, e.g., Ca^{2+} , Mg^{2+} , Fe^{2+} , Zn^{2+} or Cu^{2+} .
- Like coenzyme, the activator is **loosely** (i.e., non-covalently) attached to the enzyme and is not consumed in the reaction.
- Both the coenzymes and activators are known as cofactors.

The prosthetic group

- It is an inorganic metal or an organic compound or both, **firmly** (i.e., covalently) bound to the apoenzyme.
- e.g., a **heme** group in hemoglobin.

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Activation Energy

- It is the amount of energy required to raise all the molecules in one mole of a substance to the **transition state** at which it is ready to cleave or form a product.

How Enzymes Work

- For a molecule to be stable, it ***must*** have a high activation energy for conversion to product (that's what "stable" means!)
- Even though ***equilibrium*** (thermodynamic) may hugely favor product (remember coal and diamond?)
- So, to convert stable molecules into other stable molecules (even where equilibrium is unfavorable), requires ***selective reduction of the activation energy*** – the job of enzymes!

Mechanism of enzyme action

- Enzymes increase the rate of reactions by decreasing the activation energy required by substrates to reach a transition state.

Factors affecting the rate of enzyme catalyzed reaction

1. Temperature:

- Increase of temperature increases the rate of enzyme reaction until optimum temperature at which the enzyme acts maximally
- This optimum temperature is 37 - 40 °C for human enzymes

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- The velocity is almost doubled for every 10 °C increased.
- Since enzymes are protein in nature, temperature increase above the optimum temperature denatures and irreversibly inactivates the enzyme.

2. PH:

- It affects the state of **ionization** of amino acid residues at the active sites of the enzyme and ionization of the substrate.
- Extreme pH caused by strong acids or strong alkalis lead to **denaturation** and destroy the enzyme due to conformational and/or ionization change that also affects the substrate.
- Starting from a specific pH, increase in pH leads to increase of enzyme activity to certain pH at which the enzyme acts maximally. Such pH is called **optimum pH**.

3. Concentration of substrate:

- The rate of enzyme reaction is increased with the increase of substrate concentration till a certain point at which any increase in the substrate concentration will cause no further increase in the rate of enzyme reaction.
- This is due to **saturation** of all of the **active sites** of the enzyme by the substrate and so the excess substrate will find no free enzyme to interact with.

4. Concentration of enzyme:

- The rate of enzyme reaction **directly proportionate** with the concentration of enzyme provided that all other conditions are constant and product is withdrawn.
- This is true at the beginning of the reaction because the presence of some impurities and accumulation of the **product** at the end of the reaction **inhibit** the enzyme activity.

Enzyme kinetics

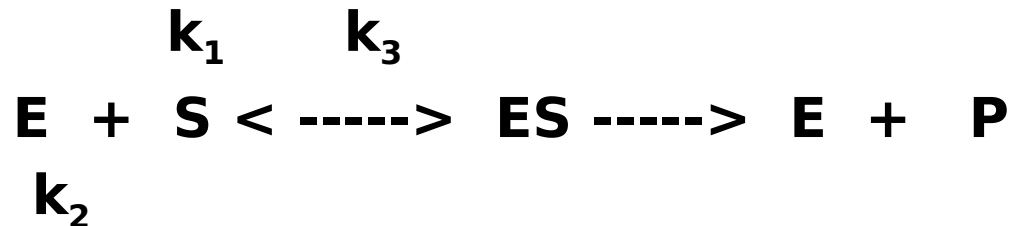
- A mathematical and graphical study of the rates of enzyme-catalyzed reactions.
- It studies reaction rates and how they change in response to changes in experimental parameters.

Michaelis-Menten equation

- For enzyme-catalyzed reactions:



- The rate or velocity is dependent upon both [enzyme] and [substrate].



- k_1 and k_2 govern the rates of association and dissociation of ES
- k_3 is the turnover number or catalytic constant

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- $$V_o = \frac{V_{\max}[S]}{K_m + [S]}$$
Michaelis-Menten equation

V_o = Initial reaction velocity(the rate of reaction as soon as ES is formed.

$V_{\max}$ = Maximum velocity (when all the active sites are occupied) it is the measure of the efficiency of enzymes.

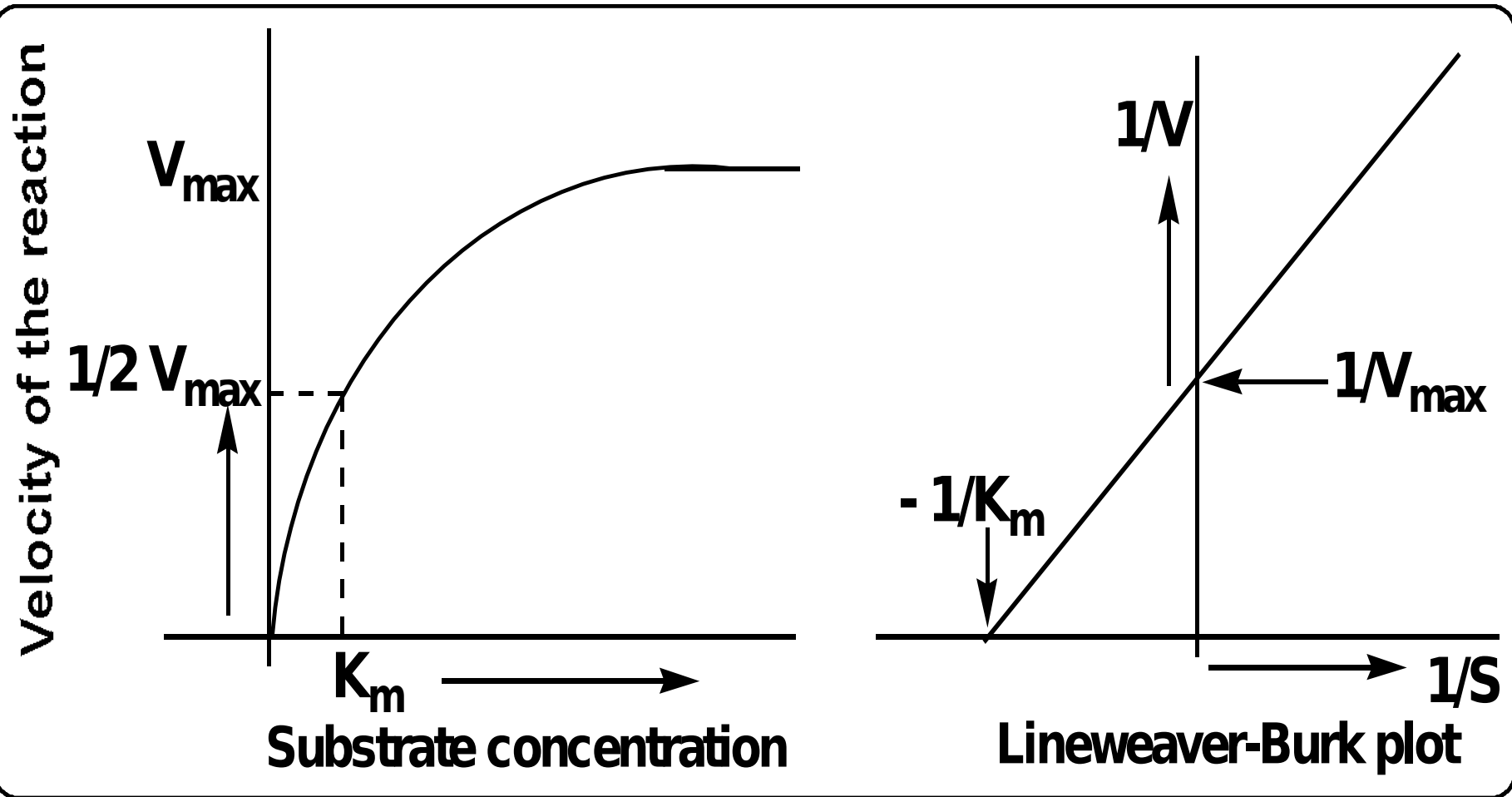
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- K_m = the substrate concentration that produces half-maximum velocity of the reaction ($\frac{1}{2}V_{max}$) that is termed **K_m** value or **Michaelis constant**, is constant and specific for every enzyme.
- Different enzymes reach V_{max} at different $[S]$ because enzymes differ in their affinity for the substrate or K_m .

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- 1) The greater the tendency for an enzyme and substrate to form an ES, the higher the enzyme's affinity for the substrate ---> lower K_m .
- 2) At a given [S], the more enzyme will be in ES for an enzyme with a higher affinity
- i.e. the greater the affinity, the lower the [S] needed to saturate the enzyme or to reach V_{max} .

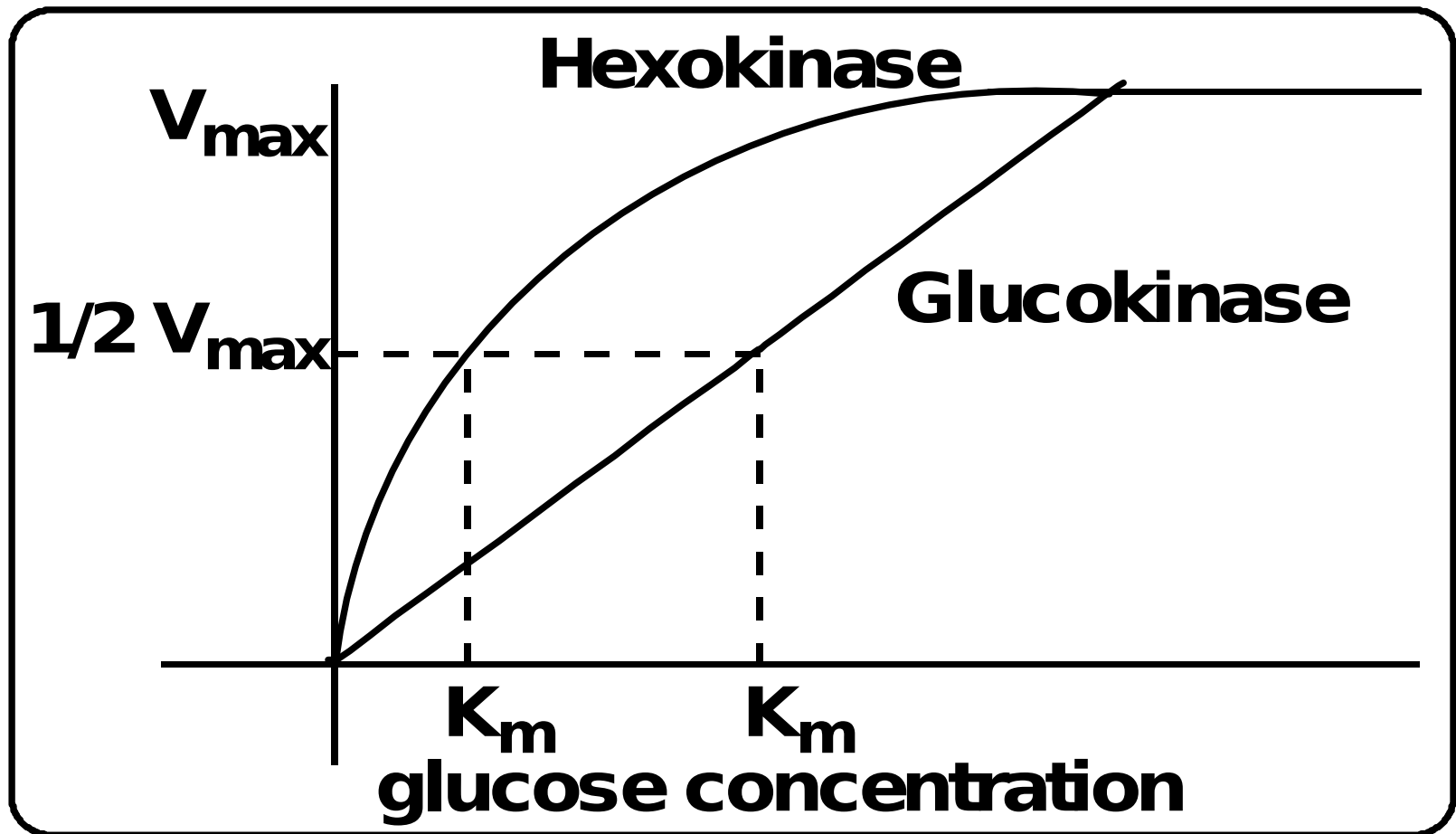
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- **Example:** Hexokinase and glucokinase are isoenzymes that catalize conversion of glucose into glucose-6-phosphate.
- Hexokinase is more active than glucokinase because the amount of glucose (substrate) needed to reach $\frac{1}{2} V_{\max}$ in case of hexokinase is less than in case of glucokinase, i.e., K_m of hexokinase is less than glucokinase.
- This difference is of utmost physiological importance to give each of them a distinct physiological role.

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Enzyme inhibitors

1. Non-specific Inhibitors

They are irreversible enzyme poisons that affect all enzymes, e.g.,

All denaturing factors, e.g., high temperature, strong acids, strong alkalis, high pressure, ultraviolet rays, Heavy metals

- All these non-specific inhibitors cause irreversible destruction of the enzyme.

2. Specific inhibitors

- **Coenzyme inhibitors:** e.g., cyanide, hydrazine and hydroxylamine inhibit pyridoxal phosphate (coenzyme form of vitamin B₆), anti-vitamins such as dicumarol against vitamin K and sulfanilamide against p-amino benzoic acid.

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2. Inhibitors of specific ion cofactor: e.g., **Fluoride** inhibits the Mg^{2+} of enolase enzyme.

3. Prosthetic group inhibitors: e.g., **cyanide** inhibits the heme prosthetic group of cytochrome oxidase.

- The coenzyme inhibitors, prosthetic group inhibitors and inhibitors of specific ion cofactor cause irreversible and non-competitive enzyme inhibition

Apoenzyme inhibitors

Competitive inhibitors:

- The inhibitor has a **structural similarity** to the substrate. So, it binds to the active site (substrate-binding site) of in competition with the substrate depending on their relative concentration.
- Increasing substrate concentration or lowering inhibitor concentration reverses this enzyme inhibition.

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- The reaction is summarized as follows,

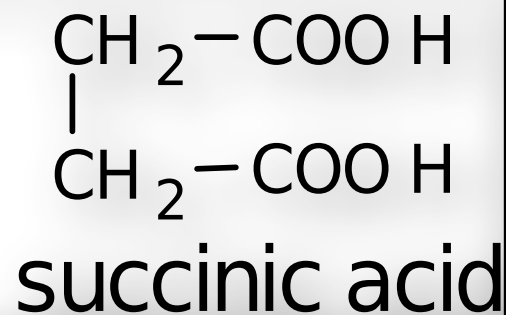
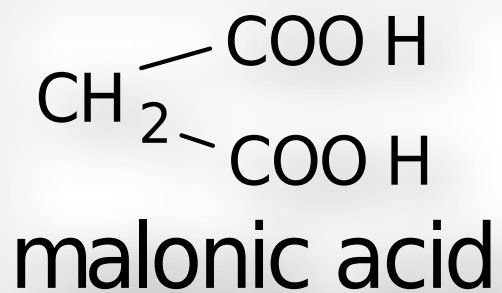
Without inhibitor, **$E + S \Rightarrow ES \Rightarrow E + P$** ,

With inhibitor, **$E + I \Rightarrow EI \Rightarrow \text{No product}$**

Examples:

- Classic example is inhibition of **succinic acid dehydrogenase** (that oxidizes succinate into malate) by

Conti.....



Conti...

- **Allopurinol** is a competitive inhibitor for xanthine oxidase that synthesizes uric acid from purines. Therefore, allopurinol is used for treating Gout.
- **Methotrexate** is competitive inhibitor of tetrahydrofolate reductase that produces tetrahydrofolate essential for cell division. Therefore, methotrexate is used as anticancer agent.

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- **Sulfonamides** are competitive inhibitors for conversion of p-aminobenzoic acid into folic acid in bacteria. Therefore, sulfonamides are used as bacteriostatic antibiotics.
- In competitive inhibition $V_{\max}$ is unaltered and K_m is increased.

Non competitive inhibitors:

- The inhibitor is **not structurally similar** to the substrate and does not bind its binding site.
- The inhibitor binds either to the **free enzyme** or the **enzyme-substrate complex** (i.e., inhibitor does not prevent substrate binding) but it decreases the enzyme catalytic rate.
- This inhibition **can not be reversed** by increasing substrate concentration, but by special measures such as dialysis.

Conti...

- Examples of noncompetitive inhibitors is the inhibition of an enzyme by **hydrogen ion** at acidic side and by the **hydroxyl ion** at the alkaline side of its optimum pH. The reaction is summarized as follows,
 - Without inhibitor, **$E + S \Rightarrow ES \Rightarrow E + P$** .
 - With inhibitor, **$E + S + I \Rightarrow EI$ or $ESI \Rightarrow$ no product**
- In noncompetitive inhibition V_{max} is decreased and K_m is unaltered.

Uncompetitive inhibitors

- This inhibitor binds at a site that only becomes available **after the substrate has bound to the enzyme.**
- This is encountered in multi-substrate enzymes, where the inhibitor compete with one substrate (e.g., S_2) of them and is uncompetitive for the another (e.g., S_1). The reaction is summarized as follows,

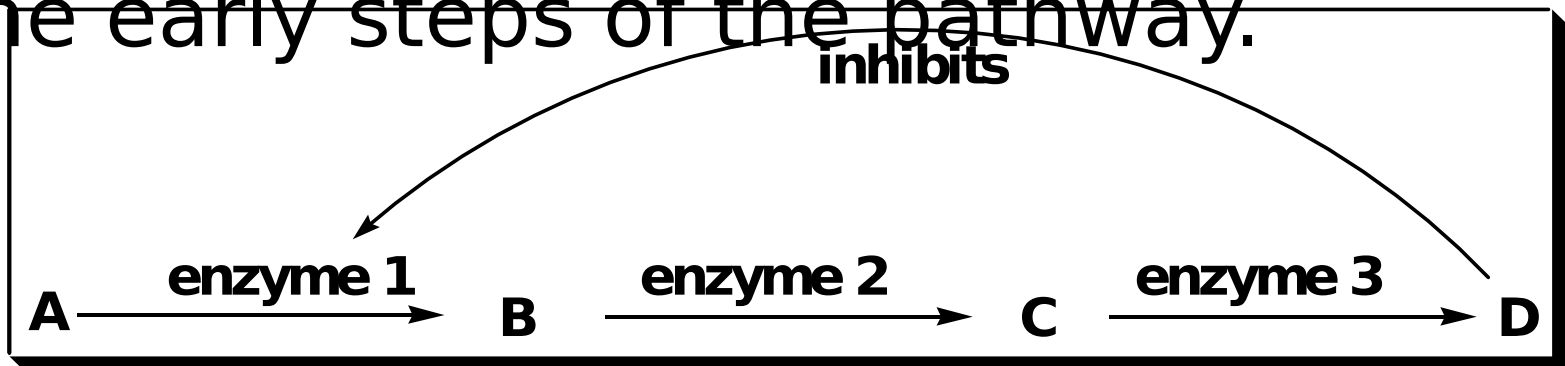
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- Without inhibitor, $\mathbf{E + S_1 \Rightarrow ES_1 + S_2}$
 $\Rightarrow \mathbf{ES_1S_2 \Rightarrow E + Ps.}$
- With inhibitor, $\mathbf{E + S_1 \Rightarrow ES_1 + I \Rightarrow}$
 $\mathbf{ES_1I \Rightarrow no\ product}$
- In uncompetitive inhibition both V_{max} and K_m decreases.

Regulation of enzyme activity

A. End products or feedback regulation

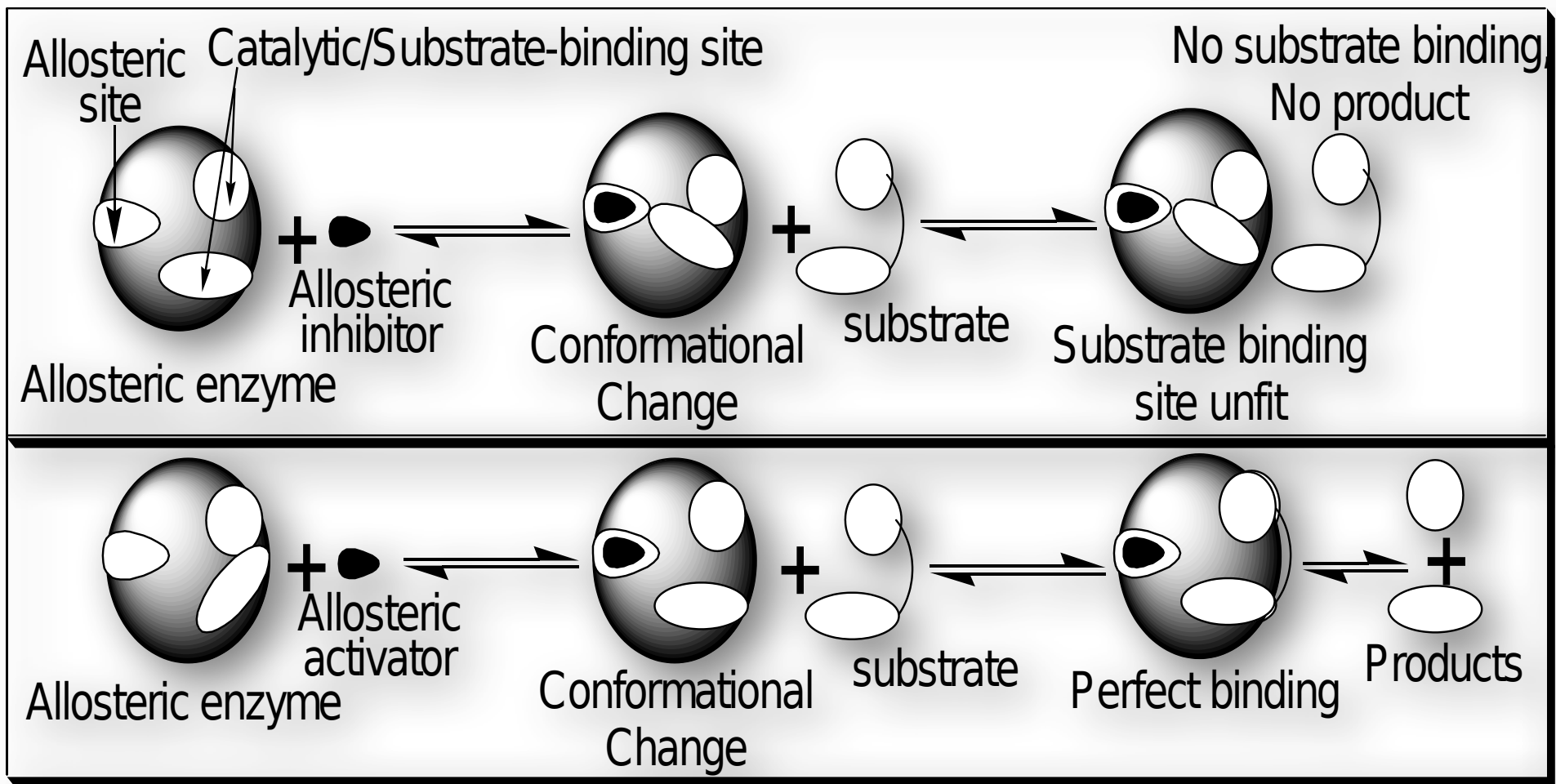
Feedback inhibition refers to the phenomenon whereby a late product in a cascade of catabolic or anabolic reactions inhibits an enzyme used in the early steps of the pathway.



B. Allosteric modulation

- Allosteric effectors are **positive** if they enhance the catalytic rate by increasing the enzyme-substrate affinity and **negative** if they decrease the catalytic rate

Conti...



C. Covalent modification

- It means the addition by a covalent bond of a foreign group, e.g., **phosphate group**, into the enzyme or removal of such group.
- Some enzymes such as **glycogen synthase** is inactivated upon phosphorylation, whereas, **glycogen phosphorylase** is activated by phosphorylation.

D. Proenzymes (Zymogens):

- some enzymes are manufactured by the body in an inactive form.
- To make them active, a small part of their polypeptide chain must be removed.
- These inactive forms of enzymes are called **proenzymes or zymogens.**

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- After excess polypeptide chain is removed, the enzyme becomes active. For example, **trypsin** (an important catalyst for the digestion of the proteins) is manufactured as the inactive molecule **trypsinogen** (a zymogen). When a fragment containing six amino acid residues is removed from the N-terminal end of trypsinogen, the molecule becomes a fully active

ISO-ENZYMES

- Iso-enzymes are physically distinct forms of the same enzyme activity.
- Higher organisms have several physically distinct versions of a given enzyme, each of which catalyzes the same reaction.
- Isozymes arise through gene duplication and exhibit differences in properties such as sensitivity to particular regulatory factors or substrate affinity that adapts them to specific tissues or circumstances.

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- Some isozymes enhance survival by providing a “backup” copy of an essential enzyme. Isozymes are expressed only in specific cell types.
- The expression of isozymes in specific cells occurs during certain periods of development, or in response to specific physiologic or pathophysiologic changes.
- Thus analysis of the presence and distribution of enzymes and isozymes are often useful in diagnosis.

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- Isoforms of Lactate dehydrogenase is useful in diagnosis of **myocardial infarction**.
- While study of alkaline phosphatase isoforms are helpful in diagnosis of various **bone disorder** and **obstructive liver diseases**.

CLINICAL SIGNIFICANCE OF ENZYMES

- The measurement of enzymes level in serum is applied in diagnostic application.
- Detection of certain enzymes in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into the blood.
- Hence, when a physician indicates that he/she is going to assay for liver enzymes, the purpose is to ascertain the potential for liver cell damage.

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- Commonly assayed enzymes are the amino transferases: **alanine transaminase**, ALT (sometimes still referred to as serum glutamate-pyruvate aminotransferase, **SGPT**) and **aspartate aminotransferase**, AST (also referred to as serum glutamate- oxaloacetate aminotransferase, **SGOT**); lactate dehydrogenase, **LDH**; creatine kinase, **CK** (also called creatine phosphokinase, **CPK**); gamma-glutamyl transpeptidase, **GGT**. Other enzymes are assayed under a variety of different clinical situations