
UNIT 3
ENZYMES

3.1 Nature of enzymes

What are enzyme molecules like?

- All enzymes are globular proteins.
- Globular proteins all have a unique tertiary structure, which gives them a unique shape.
- within that very complex structure is a region called the ***active site***.
- This is the part of the enzyme molecule that binds with its *substrate* so that the enzyme can catalyse the chemical reaction.
- The active site of an enzyme is shaped to allow:

a. binding with a particular substrate and that substrate only, and

b. binding in such a way that the reaction can take place requiring less energy than if the enzyme was not present.

- We can use the example of the enzyme sucrase catalysing the hydrolysis of *sucrose* into *glucose* and *fructose* to show enzyme substrate complex
- The substrate for the enzyme is the molecule of sucrose.
- This binds with the active site to form an enzyme-substrate complex.

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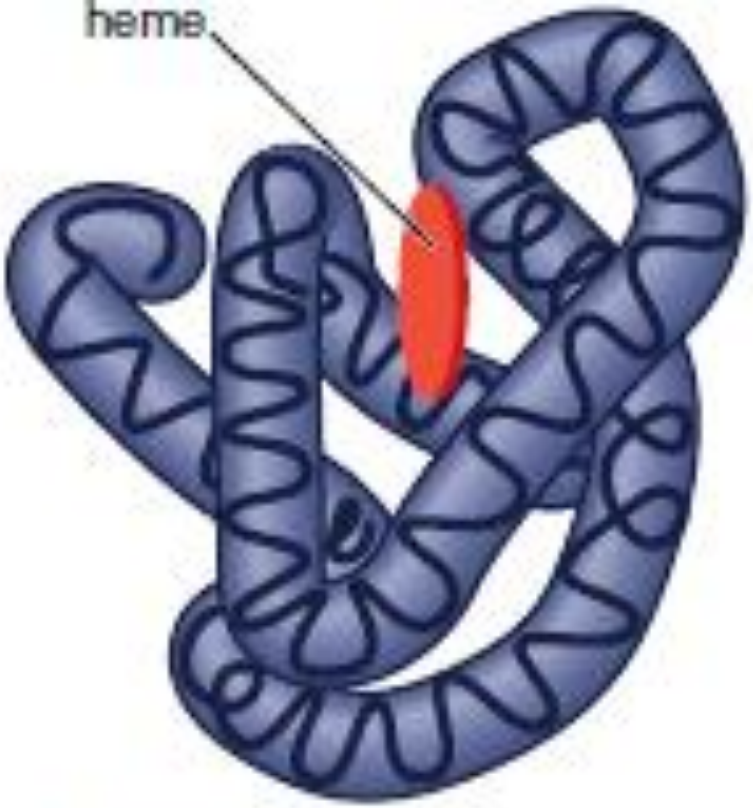
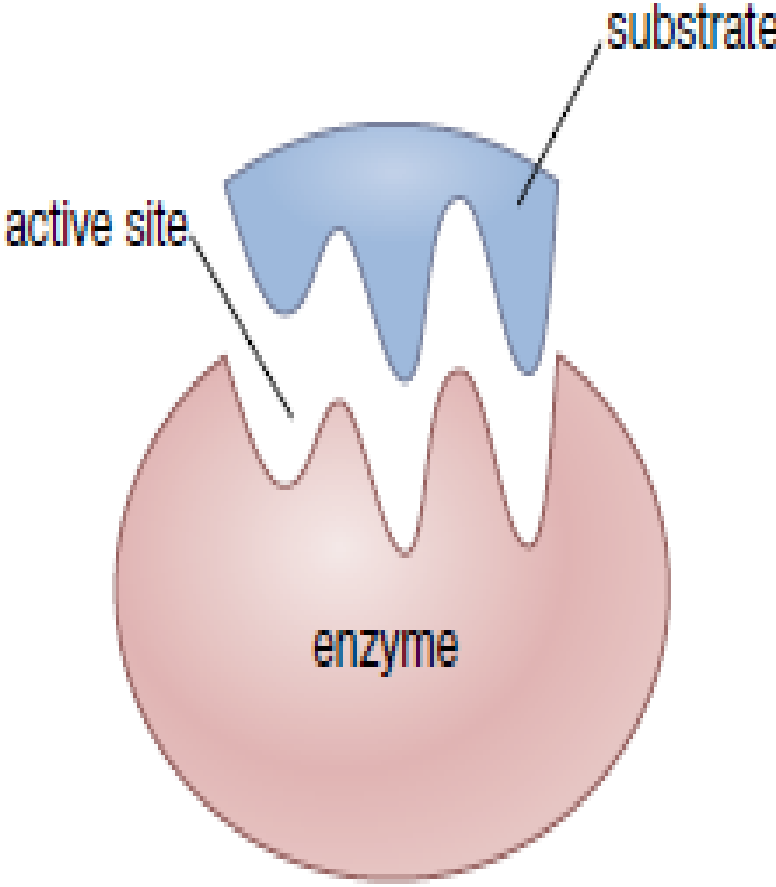
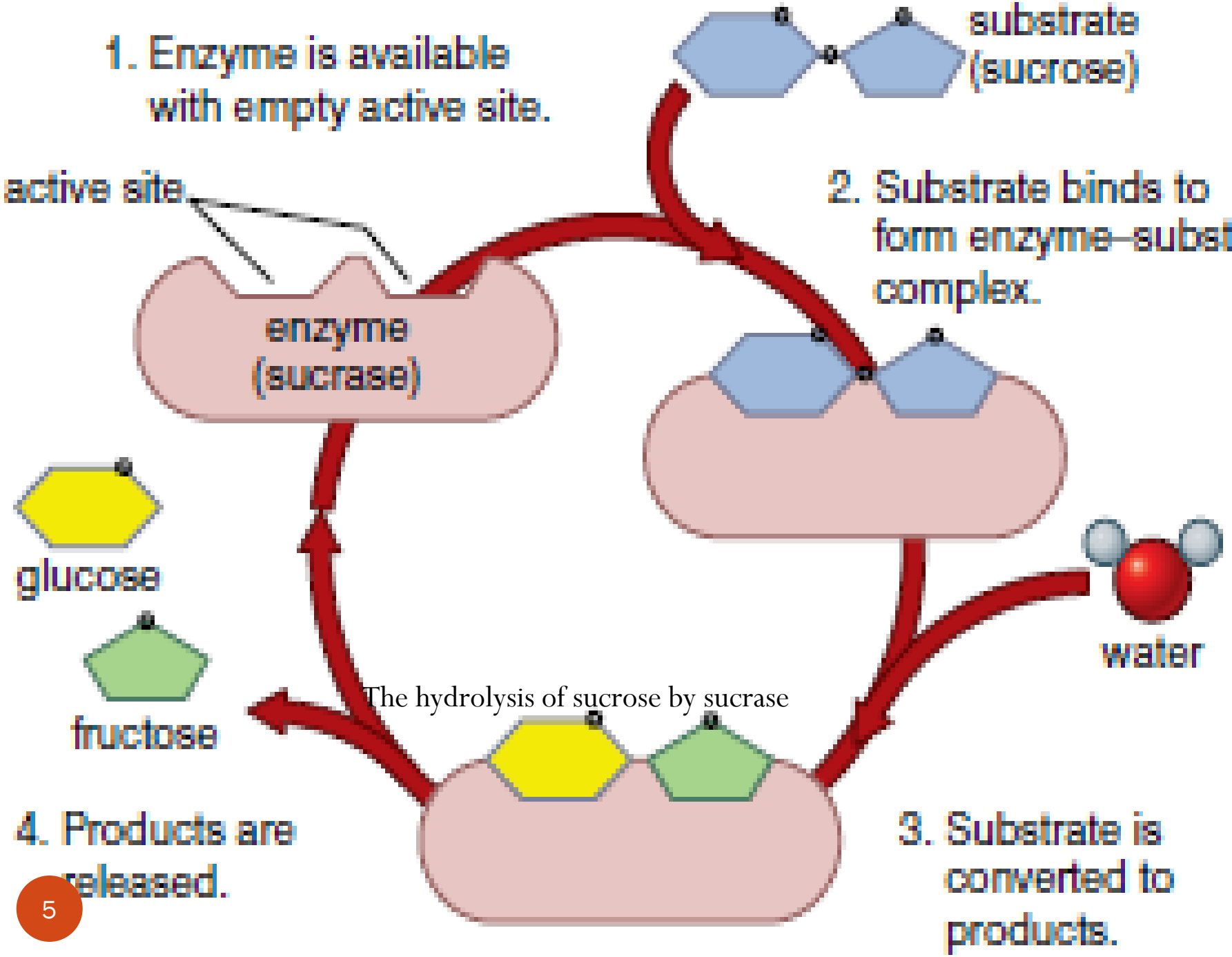


***Figure 3.1** The human lipase enzyme*





The hydrolysis of sucrose by sucrase

- An enzyme is a globular protein with a uniquely shaped active site; it acts as a biological catalyst for a specific reaction, but remains unaltered by the reaction.

- *active site* the part of an enzyme molecule that binds with its substrate so that the enzyme can catalyse the chemical reaction

- *substrate* a substance upon which an enzyme acts in a biochemical reaction

- e*nzyme-substrate complex* the intermediate formed, temporarily, when an enzyme binds to its substrate.

E+S_____ESC_____E+P

- *catalyst* a substance that speeds up a chemical reaction and remains unchanged at the end of the reaction
- Activation energy?????

What are the properties of enzymes?

I. They are all *proteins(globular)*

II. They are *biological catalysts*: they speed up a reaction with out being used up, so they can be used over and over again.

III. They are *specific*: they catalyse one reaction only.

IV. A *small amount* of enzyme can bring about a *change in a large amount of its substrate.*

V . Enzymes are *affected by pH and temperature*.

- They can be destroyed by **excessive heat**.
- They are also affected by the concentration of their substrate and the presence of certain substances that act as inhibitors.

What are catalysts?

- A catalyst is a substance that speeds up a reaction; the reaction itself is unaltered. There is no overall change to:

a) the nature of the products

b) the energy change that takes place during the reaction the catalyst itself

- Enzymes allow biochemical reactions inside cells to take place quickly, at a temperature that will not damage the structure of the cell.

Why are enzymes specific?

- Because of the conformation of the active site (the way in which it is shaped), only a certain substrate or combination of substrates can bind with it.
- Because only one substrate (or substrate combination) can bind, there is only one possible reaction that can be catalysed.

- Not all biological catalysts are proteins. Recently, it has been shown that some RNA molecules can catalyse some biological reactions.

How are enzymes affected by pH and by temperature?

- Temperature affects enzyme action in two ways:

a) a higher temperature gives the enzyme molecules (and their substrate molecules) more kinetic energy; they move around faster and form more enzyme-substrate complexes.

b. a higher temperature affects the chemical bonds holding the tertiary structure of the enzyme in place (particularly those in the active site); as more and more of these bonds break, the shape of the active site changes and it can no longer bind with its substrate.

- PH affects the enzyme molecule in a similar way to high temperatures.
- A pH that is too low (too acid) or too high (too alkaline) will cause charges on the active site to alter and cause the active site to lose its conformation.
- The substrate cannot bind and so the reaction is no longer catalysed.

How do we name and classify enzymes?

- Different enzymes are named in different ways.

1. Most commonly enzymes are named by adding *'ase'* to part of the name of the substrate. For example, *lipase* (lipid hydrolysing enzyme), *sucrase* (sucrose hydrolysing enzyme.
2. Sometimes the enzymes are named on the basis of the ***reaction that they catalyse***. For example, ***polymerase (aids in polymerisation*** - joining similar units together), ***dehydrogenase (removal of hydrogen atoms or ions).***

Name of enzyme	Reaction catalysed
Lipase	Hydrolysis of lipids
ATPase	Hydrolysis of ATP
Succinate dehydrogenase	Removal of hydrogen ions from succinate (during respiration)
DNA polymerase	Joining of nucleotides to form DNA
Pepsin	Digestion of proteins in the stomachsof mammals

3 . Some enzymes have been named based on the *source* from which they were first identified. For example, *papayin from papaya*.

- ✓ Others are named according to where they act. For example, *intestinal protease acts on proteins in the intestine.*

4 . The names of some enzymes end with **'in',** indicating that **they are basically proteins**. For example, **pepsin, trypsin,** etc.

- ✓ These enzymes usually have alternative names that tell you rather more about them. For example, the alternative name for pepsin is gastric protease. This tells you that it acts on proteins and it does so in the stomach.

- Because of the varied ways in which enzymes had been named,biologists at the Enzyme Commission decided to produce a systematic way of naming enzymes, based on the ways in which **the enzymes act.**
- ***Enzyme Commission*** body set up to produce a systematic way of naming enzymes.
- Accordingly, enzymes are generally classified on the basis of **the type of reactions that they catalyse**.
- Six groups of enzymes can be recognised on this basis.

Class	Reaction catalysed	Examples
1. Oxidoreductases	Transfer of hydrogen and oxygen atoms or electrons from one substrate to another	Dehydrogenases oxidases
2. Transferases	Transfer of a specific group (a phosphate or methyl, etc.) from one substrate to another	Transaminase kinases
3. Hydrolases	Hydrolysis of a substrate(**hydrolytic)**	Esterases Digestive enzymes
4. Isomerases	Change of the molecular form of the substrate	Phosphohexoisomerase Fumerase
5. Lyases	**Nonhydrolytic** removal of a group or addition of a group to a substrate	Aldolases Decarboxylases
6.Ligases (Synthetases)	Joining of two molecules by the formation of new bonds	Citric acid synthetase

- Each class of enzymes contains several different, but related, subclasses.
- Each subclass is further divided into sub-subclasses. Within the sub-subclasses, each enzyme has a number.
- So, in the systematic naming of enzymes, an enzyme will have a 'name' such as **EC 3.4.11.1.**
- Each part of the description tells us something about the enzyme:

EC 3.4.11.1.

- EC stands for Enzyme Commission
- the first number shows to which of the six main classes the enzyme belongs
- the second figure indicates a subclass
- the third figure gives **a sub-subclass**
- the fourth figure is the **serial number of the enzyme** in its sub-subclass.

✓ to see how to name enzymes please vivit the web site www.chemqmul.ac.uk/iubmb/enzyme/

- Enzyme EC 3.4.11.1 is:

a. **a hydrolase** - all the enzymes in class 3 hydrolyse some kind of bond.

b. **a peptidase** - all the enzymes in subclass 4 of class 3 are peptidases and hydrolyse peptide bonds

c. **an amino-peptidase** - all the enzymes in sub-subclass 11 of sub-class 4 are amino-peptidases; they hydrolyse peptide bonds at the amino end of a polypeptide chain

d. **leucyl-amino-peptidase** - this particular amino-peptidase is number 1 of this sub-subclass

Table 3.3 Some industrial uses of enzyme technology

Sector	Application area	Benefits
Dairy	• Biochymosin to produce cheese • Lactase to produce lactose-free milk	• Supplies of natural rennet from calves livers are limited • Lactose-intolerant people suffer fewer cramps
Detergents	• Use of proteases, lipases and amylases in biological washing powders • Use of proteases and amylases in dishwasher detergents	• Many biological stains are removed efficiently at low temperatures (saving energy) • Remove food particles at lower temperatures and require fewer bleaching products to be added
Textiles	• Proteases to remove hair and lipases to degrease animal hides • Use of cellulase to 'bio-polish' cotton fabrics • Use of cellulase to 'bio-stone' denim	• Process is carried out much quicker than by traditional methods • Produces a smoother and glossier finish • The enzyme gives the 'stone-washed' effect much more easily
Food processing	• Pectinase to process fruit juice • Inverase to produce liquid-centre chocolates	• Clarifies fruit juice • Sucrose paste in the chocolate is made liquid by injection of the enzyme

Pulp and paper	• Amylases used in starch conversion • Use of xylanase enzymes in pre-bleaching the pulp • Use of esterases in control of 'stickies' (glues introduced during paper recycling)	• Reduces the quantity of starch in the paper and improves quality • Produces a whiter paper • Stickies would otherwise clog the machinery and reduce the quality of the paper
Medicine	• Glucose oxidase in clinistix strips, tests for glucose • Liver enzymes • Pulmozyme to treat cystic fibrosis	• Allows easy diagnosis of diabetes by testing urine • Testing for high levels of these in the blood confirms liver damage • Reduces viscosity (stickiness) of mucus
Pharma-ceutical	• Streptokinase to dissolve clots of heart-attack patients • Production of abacavir sulphate is controlled by enzymes	• Restores blood supply to area of heart muscle • Abacavir sulphate is an important anti-AIDS drug

- Using enzymes in industry can help the environment.
- Because enzymes allow some manufacturing processes to be carried out at lower temperatures, less carbon dioxide is produced in raising the temperature of the reactants.

How do enzymes act as catalysts?

- Catalysts speed up chemical reactions.
- In order for molecules to react, they must have sufficient energy.
- This energy to start off the reaction is called **activation energy (or E_a)**.
- Imagine a reaction in which substance A reacts with substance B to form substance AB. We can write an equation for this as: $A + B \longrightarrow AB$
- The reactant must 'climb an activation energy hill' before anything happens.

- Under normal conditions, very few molecules of A and B have sufficient kinetic energy to 'climb the activation energy hill', so the reaction proceeds slowly.
- A catalyst lowers the activation energy required for the reaction.
- More reactant molecules can meet this lower energy requirement and so the reaction proceeds more quickly.
- Because the enzyme molecule is unaltered by the reaction, it can be used over and over, and so a small amount of enzyme can affect a large amount of substrate.

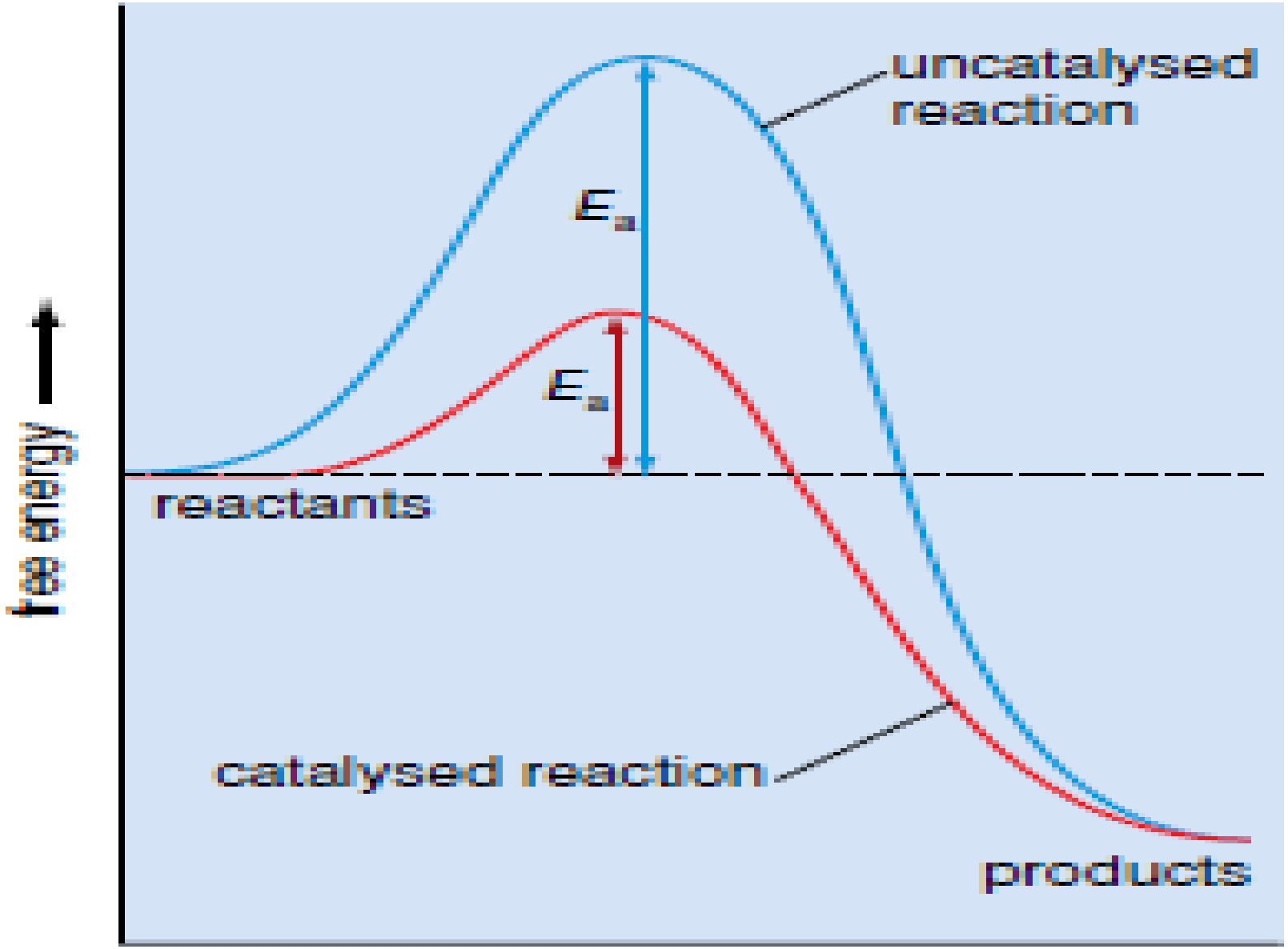


Figure 3.8 Activation energy for an uncatalysed reaction and the same reaction with a catalyst

How do enzymes lower activation energy?

- There are two models of enzyme action;

a) the lock-and-key model, first proposed in **1894** by a **German biochemist** named **Fischer** and

b) the **induced-fit** model, proposed in **1958 by Koshland.**

- Both of these models suggest that the enzyme catalyses the reaction by lowering the activation energy.
- However, they differ in the way that they explain how this happens.
- In particular, they differ in explaining how the substrate binds to the active site of the enzyme

- **activation energy** the energy required to start off a chemical reaction.
- **lock-and-key model** proposes that the shapes of the substrate molecules are complementary to that of the active site.
- **induced-fit model** the active site and substrate do not complement each other but the binding of substrate molecules produces a change in shape in the active site, allowing the substrate to fit the active site.

I. The lock-and-key model

- This model proposes that the shapes of the substrate molecules are complementary to that of the active site, rather like the shape of a key is complementary to that of the lock it fits.
- A useful way of thinking of complementary shapes is to think of an egg sitting in an egg cup.
- The egg can sit inside the egg cup because the shapes are complementary.
- One egg cannot sit inside another egg because the shapes are the same.
- The complementary substrate molecule binds with the active site of the enzyme to form the enzyme-substrate complex

- The complex causes the reactants to enter a transition state in which the activation energy of the reaction is lowered.
- The reaction takes place and the products formed are released.
- The lock-and-key model of enzyme action suggests that the enzyme lowers the activation energy by providing an alternative pathway for the reaction.



❖For example

I. For a non-catalysed pathway:

Reactant A + Reactant B → Product

II .Enzyme-catalysed pathway:

Reactant A + Reactant B + enzyme → Intermediate

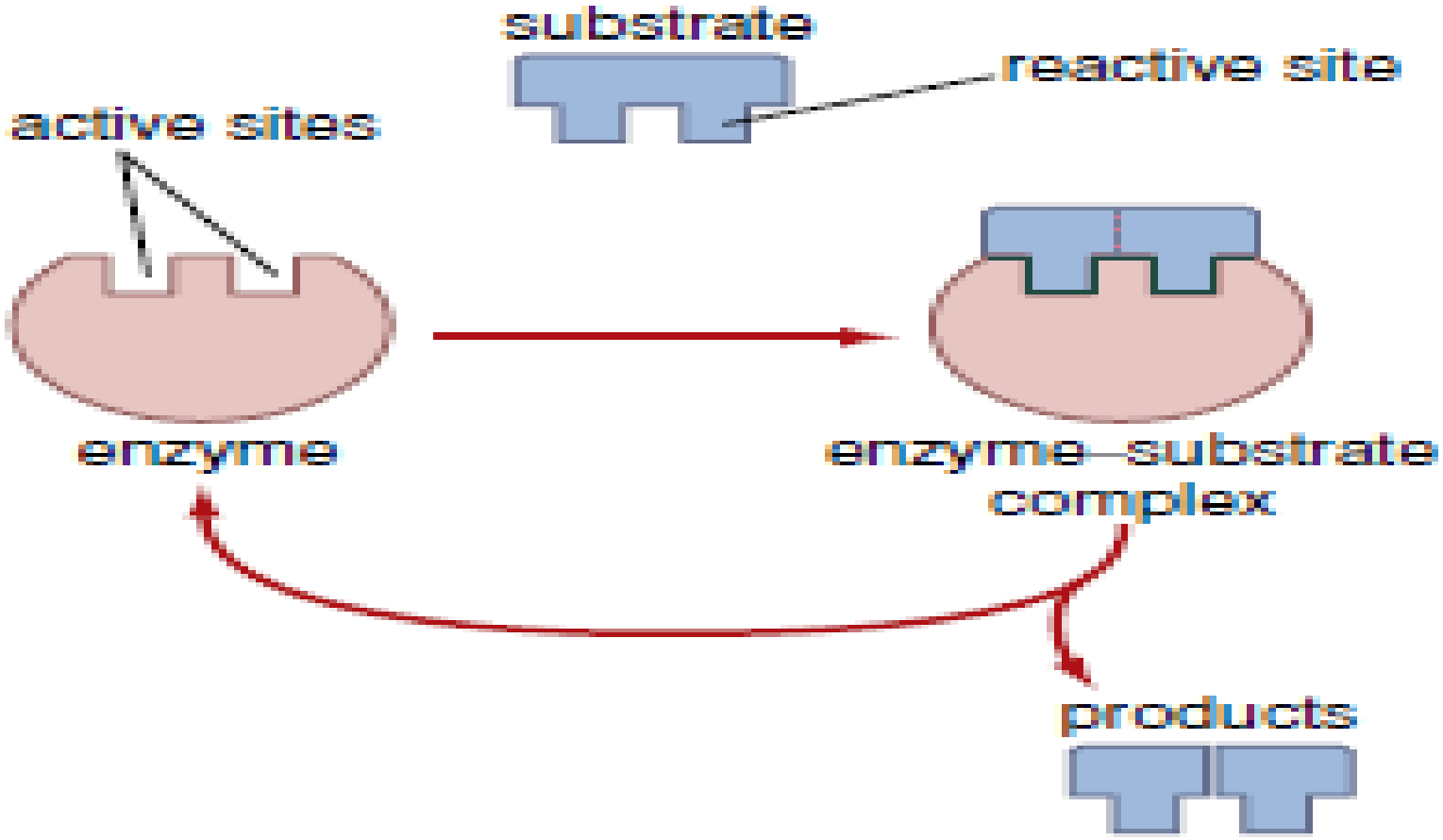
→ Product AB + enzyme

- This model sees the enzyme-substrate complex as the intermediate, which is part of a pathway that requires less energy than the normal pathway.
- However, a weakness of this model is that it does not explain how the intermediate reduces activation energy.

II.The induced-fit model

- This model suggests that the active site and the substrate **aren't naturally complementary** in shape, but the binding of substrate molecules produces a conformational change (change in shape)in the active site.
- This allows the substrate and active site to bind fully.
- The conformational change also puts the substrate molecules under **tension**, so they enter a 'transition state' and are able to react because of the lowered activation energy.

- In the transition state,bonds in the reactants are put under strain and break more easily and rejoin with other bonds to form the products. The products formed leave the active site.



- Most biologists now prefer the induced-fit model over the lock-and-key model as it explains other properties of enzymes, such as enzyme inhibition, in a more complete manner than the lock-and-key model.
- The rate of a chemical reaction is the rate at which reactants are converted into products.
- In the case of an enzyme-controlled reaction, this is determined by how many molecules of substrate bind with enzyme molecules to form enzyme-substrate complexes.

- The number of molecules of reactants that form enzyme-substrate complexes with each molecule of an enzyme, per second, is the **turnover rate** of the enzyme.
- **turnover rate** the number of molecules of reactants that form enzyme-substrate complexes with each molecule of an enzyme, per second

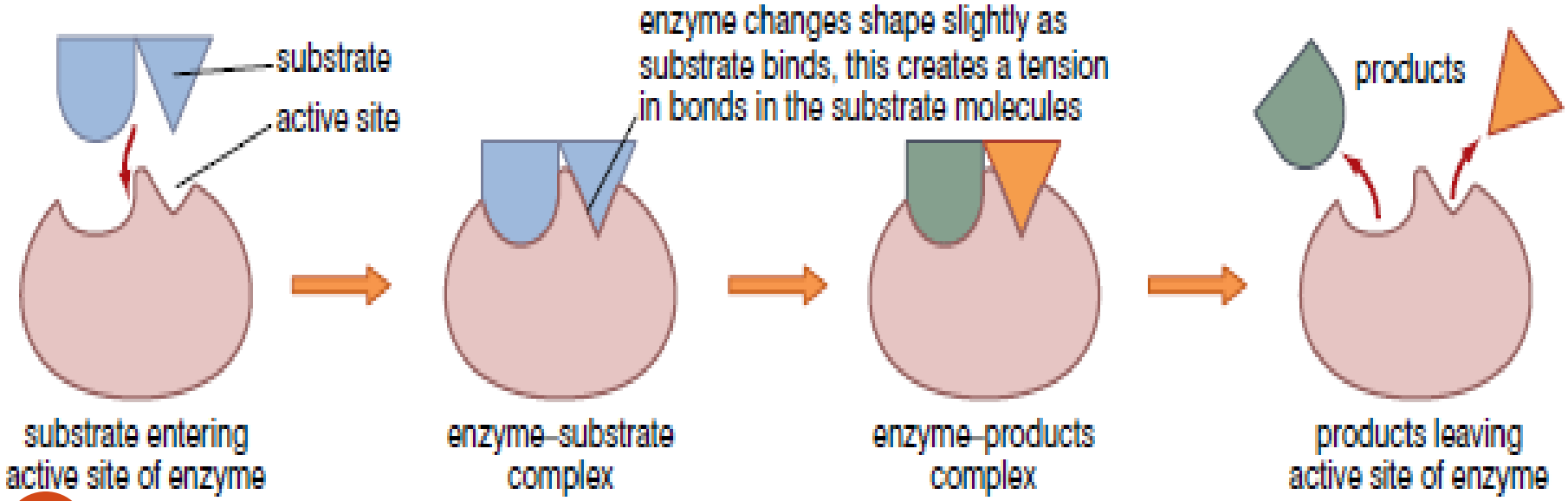
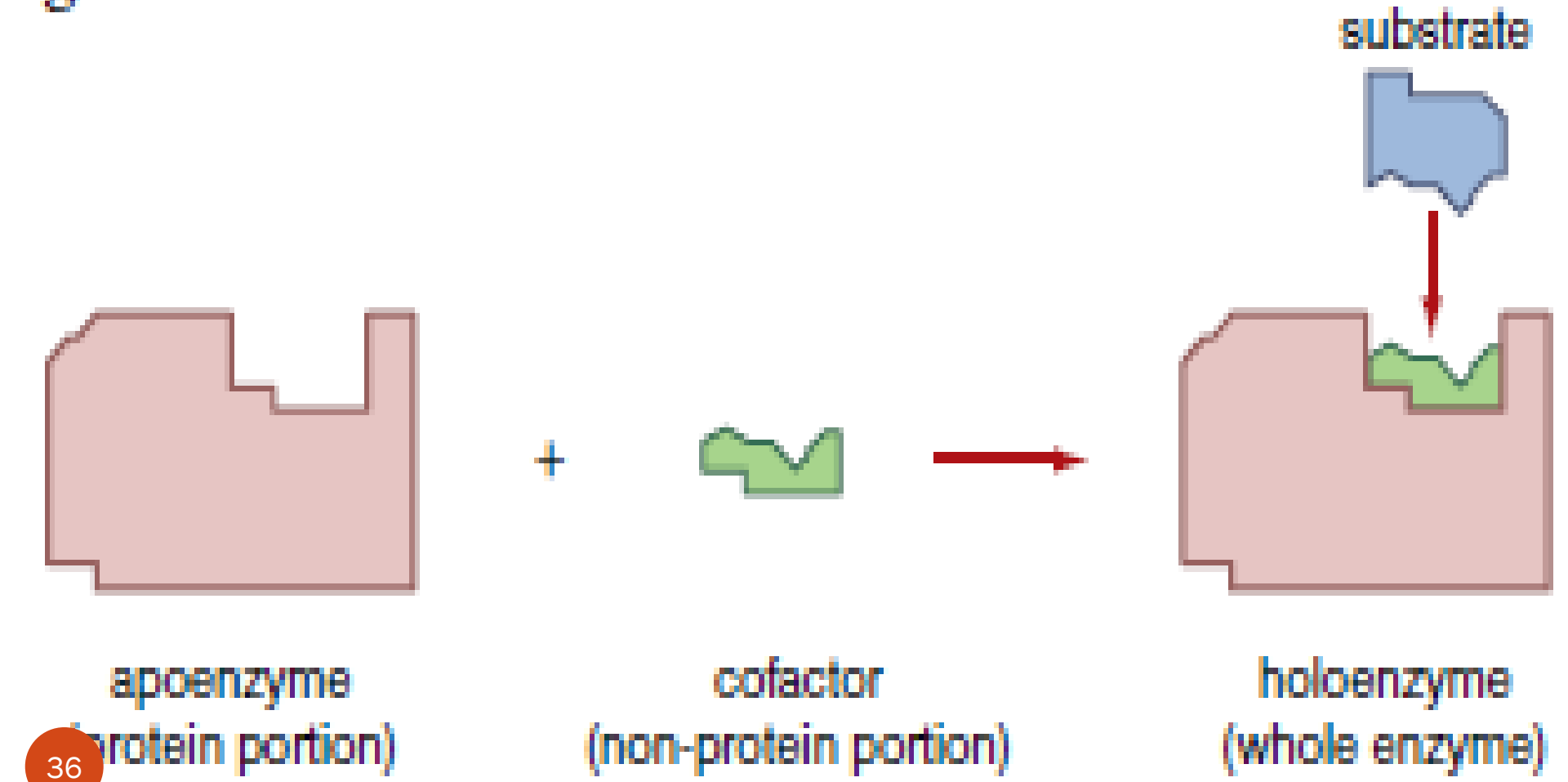


Figure 3.11 The induced-fit model of enzyme action

Why do some enzymes need cofactors?

- Sometimes an active enzyme isn't just a single molecule, but is made from two molecules, neither of which has enzymic activity without the other.
- The two parts are the **apoenzyme** and the **cofactor**.
- ***Apoenzyme*** - a protein that combines with a cofactor, to form an active enzyme.
- The protein is inactive on its own.
- ***Cofactor*** - a small non-protein particle essential for the activity of some enzymes.
- The cofactor combines with the apoenzyme to produce an active enzyme.

- Where an active enzyme molecule comprises an *apoenzyme* and a cofactor, the whole is sometimes referred to as the *holoenzyme*.



- Cofactors include:

I. Coenzymes

II. mineral ions

- **Coenzymes** are organic molecules and many are derived from vitamins.
- They bind with the enzyme to give *catalytic activity*

***Table 3.5** Common coenzymes and their functions*

Coenzyme	Vitamin	Enzyme	Function
Nicotinamide adenine dinucleotide (NAD)	Niacin	Oxidoreductase in respiration	Oxidation or hydrogen transfer in respiration
Flavin adenine dinucleotide (FAD)	Riboflavin	Oxidoreductase in respiration	Oxidation or hydrogen transfer in respiration

- Some enzymes can only function in the presence of certain mineral ions.
- These bind loosely with the enzyme to give it its catalytic activity.

Table 3.6 Enzymes that require mineral ions as cofactors

Enzyme	Mineral ion	Function
Carbonic anhydrase	Zinc ions (Zn^{++})	Causes CO_2 to react with water to form hydrogen carbonate
Alcohol dehydrogenase	Zinc ions (Zn^{++})	Oxidises alcohol
Cytochrome ...dase	Copper ions (Cu^{++} or Cu^{+})	Transfers electrons to oxygen during respiration

Factors affecting the functions of enzymes

- The turnover rate and, therefore, the activity of the enzyme are influenced by a number of external factors, including:

a. Temperature
b. PH
c. substrate concentration
d. the presence of inhibitors

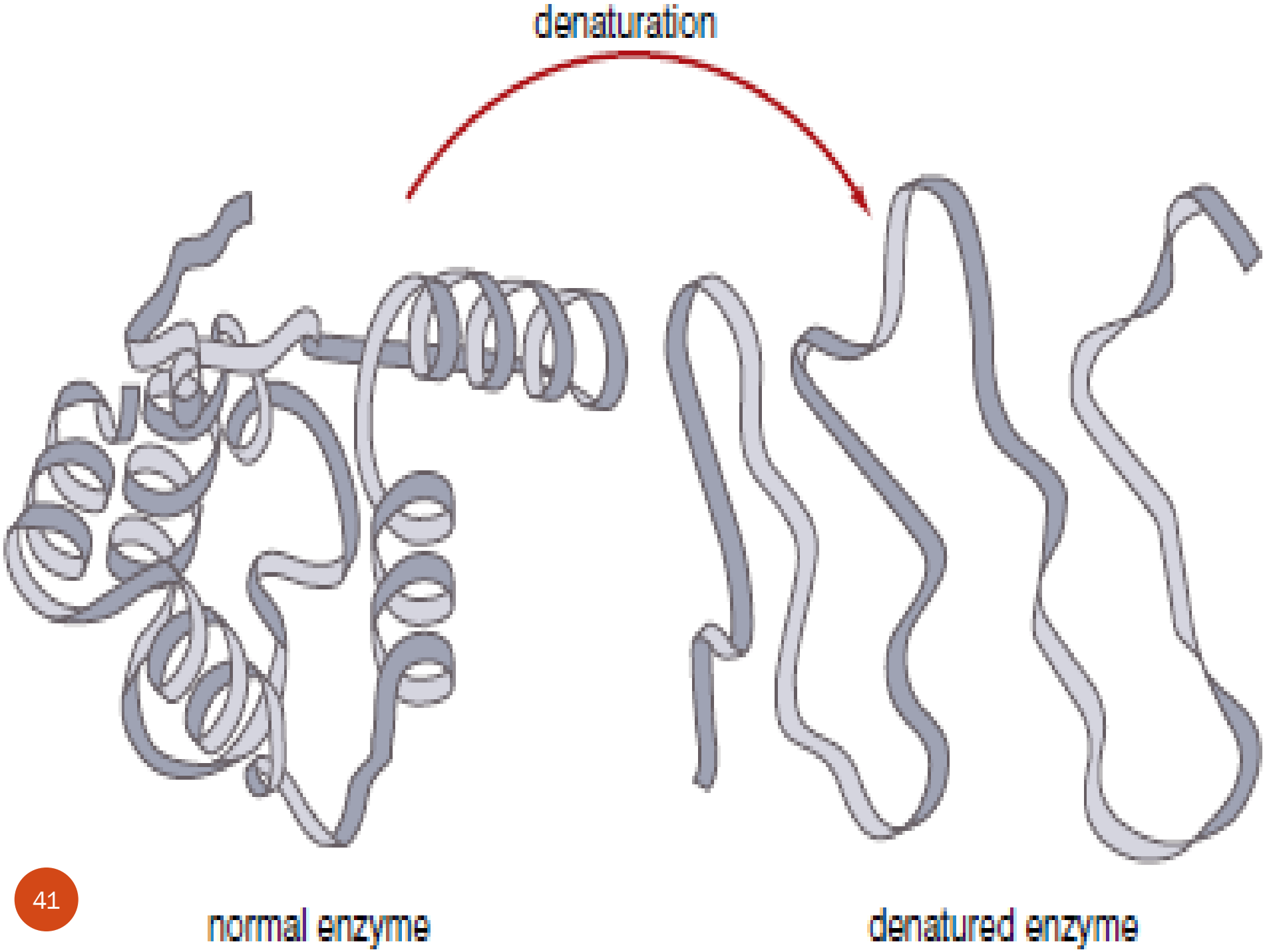
A.TEMPRATURE

- When the temperature is raised, particles are given more kinetic energy. This has two main effects:

A. 'Free' particles move around more quickly. This increases the probability that a substrate particle will collide with an enzyme molecule.

B. Particles within a molecule vibrate more energetically.

- ✓ This puts strain on the bonds that hold the atoms in place. Bonds begin to break and, in the case of an enzyme, the shape of the molecule, and the active site in particular, begin to change.
- ✓ The enzyme begins to lose its tertiary structure and denature


denaturation
normal enzyme
denatured enzyme

- The activity of an enzyme at a given temperature is a balance between these two effects.
- If the raised temperature results in little denaturation but a greatly increased number of collisions, the activity of the enzyme will increase.
- If the higher temperature causes significant denaturation then, despite the extra collisions, the activity of the enzyme will probably **decrease.**
- The temperature at which the two effects just balance each other is the **optimum temperature** for that enzyme.

- Any further increase in temperature will cause increased denaturation that will outweigh the effects of extra collisions.
- A decrease in temperature means that fewer collisions will occur.

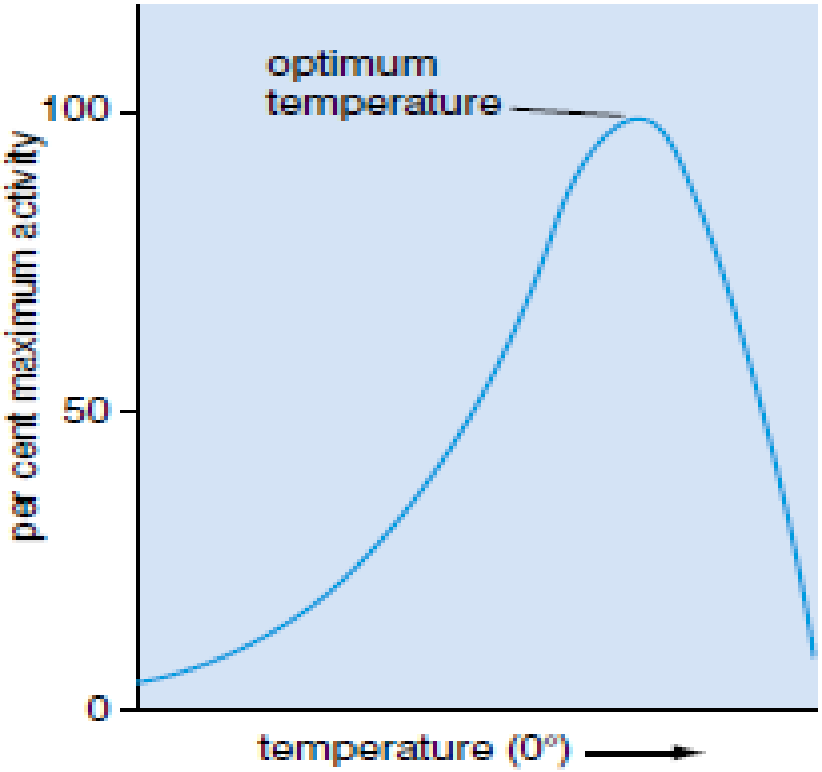


Figure 3.14 The effect of temperature on enzyme activity

B. The PH value

- The pH scale is a measure of the hydrogen ion concentration of a solution or other liquid system.
- The pH scale ranges from 0 to 14.
- Solutions with a pH of less than 7 are acidic, those with a pH of more than 7 are alkaline and a solution with a pH of exactly 7 is neutral.
- The majority of enzymes in most mammals function most efficiently within the pH range **6.0-8.0,** although the optimum pH of pepsin is between **pH 1.0 and pH 3.0.**
- Significant changes in pH can affect an enzyme molecule by:

a. breaking ionic bonds that hold the tertiary structure in place; this leads to denaturation of the enzyme molecule

b. altering the charge on some of the amino acids that form the active site; this makes it more difficult for substrate molecules to bind

- These effects occur if the pH becomes either more acidic or more alkaline.

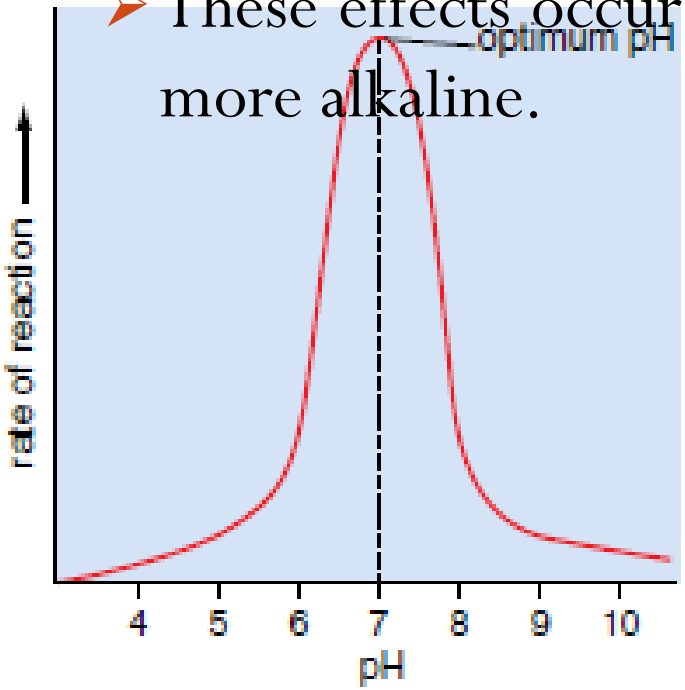
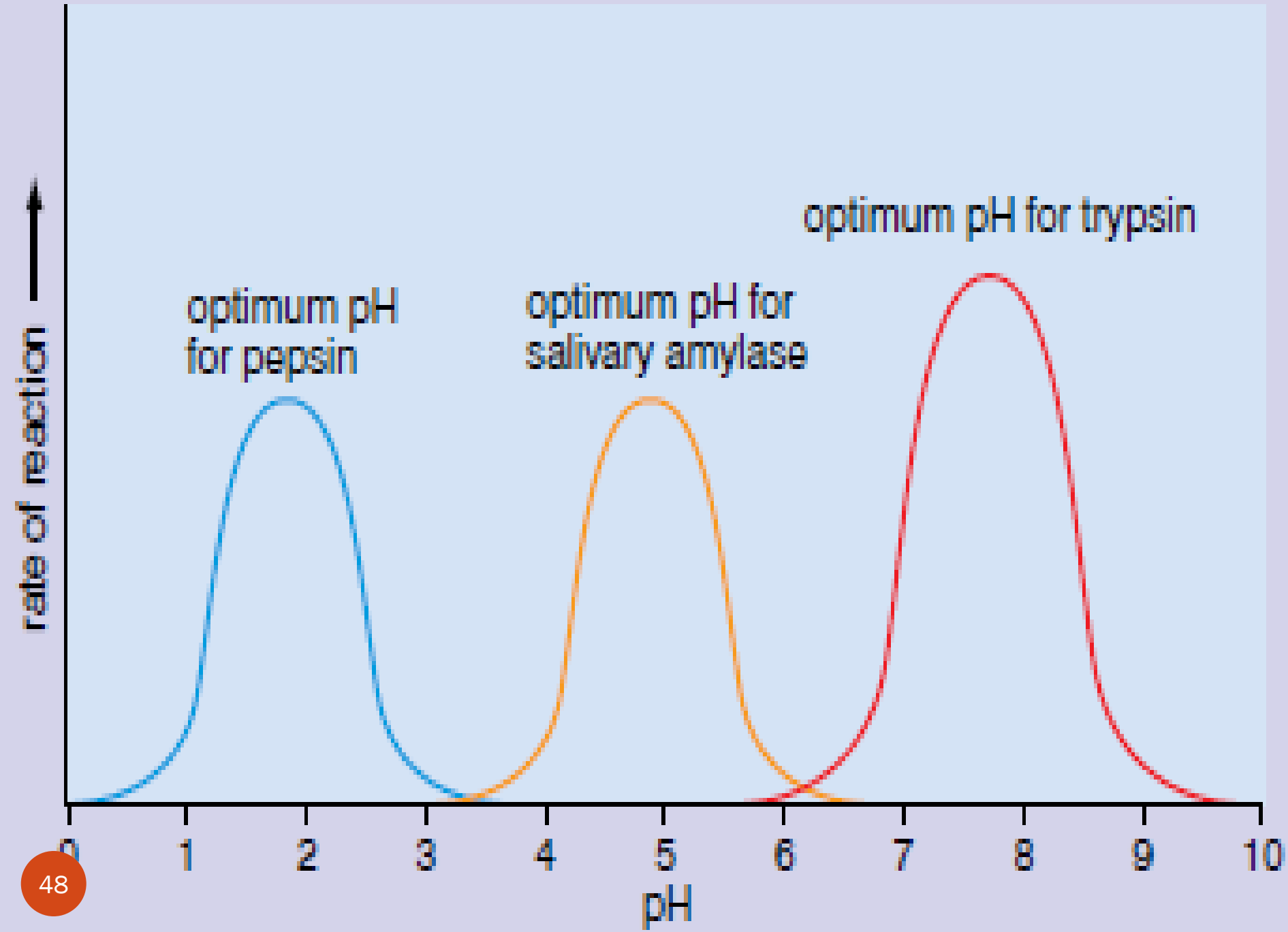


Figure 3.15 The effect of pH on enzyme activity

- pH **0** represents the highest H^+concentration and is the **most acid.**
- A **pH 1.0** solution has **one-tenth (0.1)** of this H^+ concentration;
- a pH 4 solution has one ten-thousandth (0.0001).
- **pH 14** represents the **lowest H+** concentration and is the most alkaline.
- pH 7 is neutral

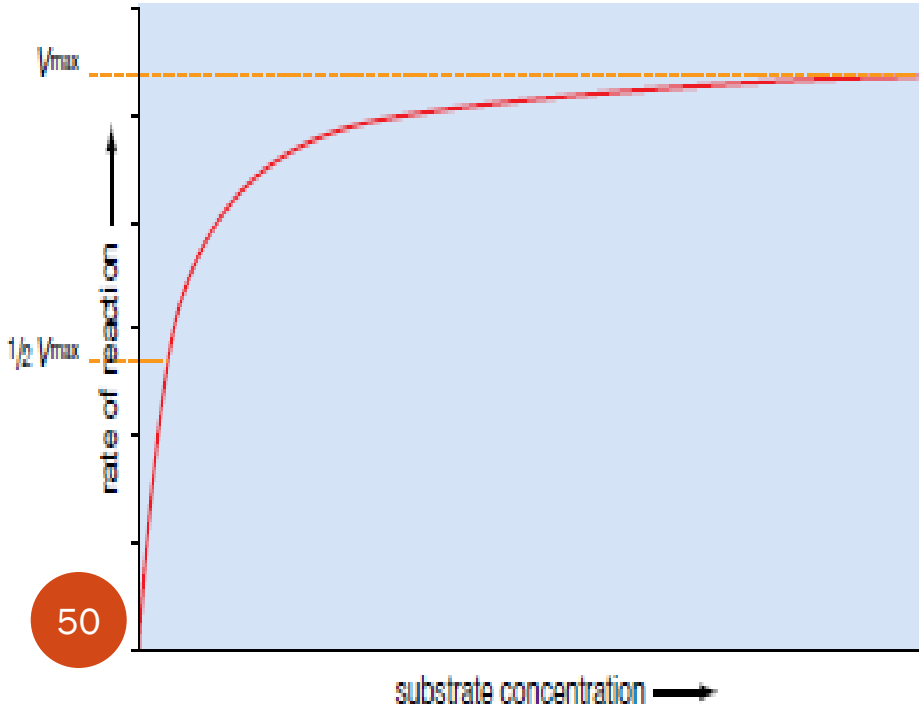
- The pH of the intestinal tract ofhumans changes from one regionto the next.
- The pH in the mouth varies from being slightly alkaline(pH 7.5) to quite acidic (pH 5.0)depending on whether or not we have eaten and also what we have eaten.
- The pH in the stomach can be as low as pH 1.5, whereas the pH of the small intestine is slightly alkaline at pH 7.5.
- Digestive enzymes from the different regions have optimum pHs that reflect the region in which they are secreted


optimum pH for trypsin
optimum pH for pepsin
optimum pH for salivary amylase
rate of reaction
0
1
2
3
4
5
6
7
8
9
10
pH

C.The concentration of the substrate

- The activity of an enzyme depends on the number of substrate molecules per second that bind to form enzyme-substrate complexes.
- So the number of substrate molecules present must have an effect.
- A small number of substrate molecules means few collisions and so only a few enzyme-substrate complexes form.
- Increasing the concentration of the substrate means more collisions and more enzyme-substrate complexes.
- So, the overall rate of reaction is increased.

- Eventually, because of the high substrate concentration, each enzyme molecule could be working at maximum turnover - that is, each active site is binding with substrate molecules all the time and there is no 'spare capacity' in the system.
- Increasing the substrate concentration beyond this point will have no effect on the activity of the enzyme because all the active sites are occupied all the time.



- **denature** the alteration of the tertiary structure of a protein; in living cells this is reversible
- **optimum temperature** temperature at which an enzyme works most efficiently
- **pH scale** measure of the hydrogen ion concentration of a solution.

D. Enzyme concentration

- Assuming a constant large supply of substrate molecules, each enzyme molecule will work at maximum turnover.
- Therefore, the reaction rate will be directly proportional to the number of enzyme molecules - the concentration of the enzyme.
- Increasing the concentration will increase the reaction rate.
- However, increasing the concentration of the enzyme will not increase the activity of the enzyme.
- Each enzyme molecule will be working at maximum turnover, so the activity of the enzyme is likely to remain constant.

Other substances affect enzyme activity

- Inhibitors are substances that bind to enzymes and prevent them from forming enzyme-substrate complexes and, as a result, stop, or slow down, the reaction.
- There are two main types of inhibitors:

A. **irreversible inhibitors**, and

B. **reversible inhibitors**.

- Irreversible inhibitors bind **strongly** to enzymes, usually by a covalent bond, permanently altering the structure of the enzyme molecule and inactivating it.

- The painkiller aspirin is an example of an irreversible inhibitor.
- It binds with the enzyme **cyclo-oxidase-2**, which is an important enzyme in producing *prostaglandins* which give the sensation of pain.
- ***Reversible inhibitors*** bind to enzymes only weakly and the bond that holds them breaks easily releasing the inhibitor.
- This allows the enzyme to become ***active again***.
- There are two main kinds of reversible inhibitors:

a. **competitive inhibitors , and**
b. **non-competitive inhibitors**

Competitive inhibitors

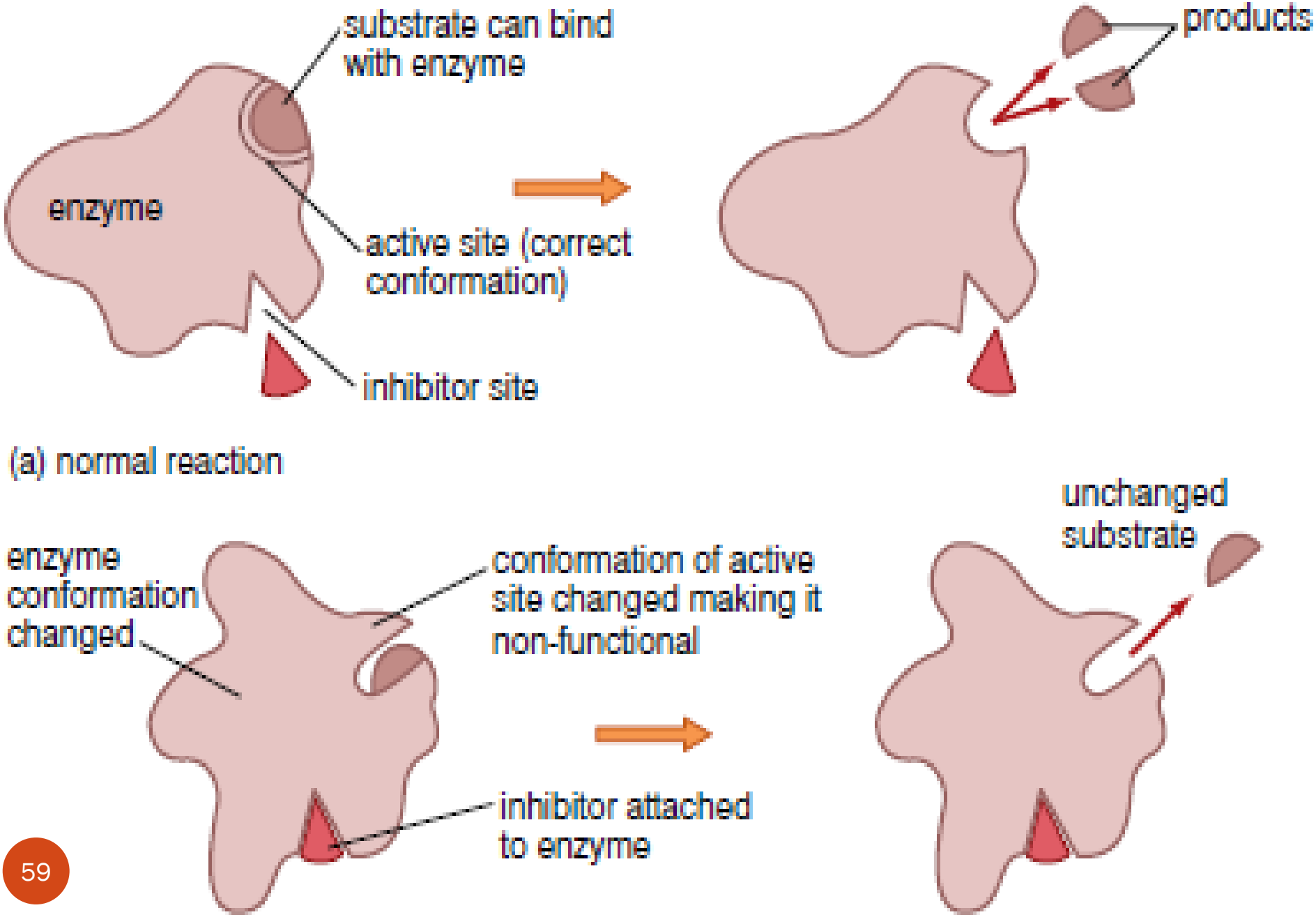
- Competitive inhibitors have molecules with shapes that are complementary to all, or part, of the active site of an enzyme.
- They are often similar in shape to the substrate molecules.
- They can bind with the active site and prevent substrate molecules from binding.
- The binding is only temporary and the competitive inhibitor is quickly released.
- A competitive inhibitor blocks the active site so substrate molecules cannot bind.

- The overall effect on the rate of reaction depends on the relative concentrations of substrate and inhibitor molecules.
- Each molecule of competitive inhibitor can inhibit(temporarily) one enzyme molecule - but only if it can collide with the enzyme molecule and bind with the active site.
- To do this, it must compete with the substrate molecules for the active site - hence the name, competitive inhibitor.
- **competitive inhibitor** is a molecule that inhibits enzyme activity by competing with the substrate for the active site.

- The painkiller ibuprofen acts as a competitive inhibitor of the enzyme cyclo-oxidase-2, competing with the precursors of prostaglandins, which are the substrate of cyclo-oxidase-2.
- The metabolic poison **cyanide** acts as a competitive inhibitor of the enzyme **cytochrome oxidase**, an important enzyme in the release of energy in respiration.

Non-competitive inhibitors

- Non-competitive inhibitors do not compete for the active site.
- Instead, they bind to another part of the enzyme called the ***allosteric site***.
- This produces a conformational change in the part of the enzyme molecule that includes the active site.
- Because of this, the active site is a different shape and can no longer bind with the substrate to catalyse the reaction.


substrate can bind with enzyme
enzyme
active site (correct conformation)
inhibitor site
products
(a) normal reaction
enzyme conformation changed
conformation of active site changed making it non-functional
inhibitor attached to enzyme
unchanged substrate

- The effectiveness of a non-competitive inhibitor is in no way affected by the concentration of the substrate.
- Suppose there are enough inhibitor molecules to bind with the allosteric sites of 80% of the enzyme molecules.
- 80% of the enzyme molecules will be inhibited irrespective of the number of substrate molecules and the reaction rate will drop to 20% of maximum.
- Non-competitive inhibitors are particularly important in regulating metabolic pathways in cells.

How do inhibitors control enzyme activity in living cells?

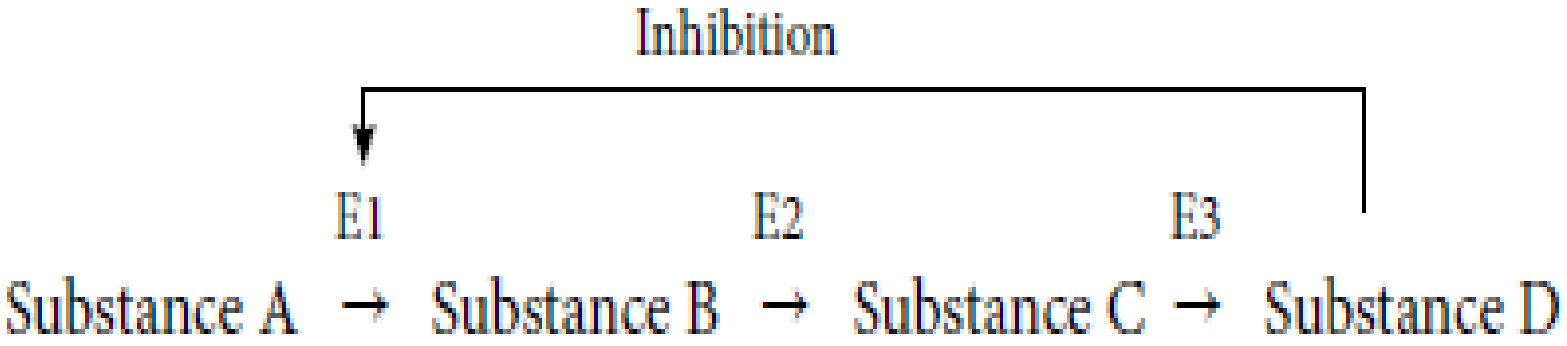
- Many substances are produced in cells as a result of a metabolic pathway which can be represented as:

E1 E2 E3

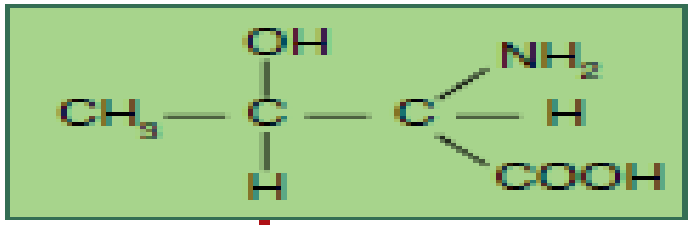
- *Substance A* → *Substance B* → *Substance C* → *Substance D*
- E1, E2 and E3 are enzymes catalysing the reactions.
- All the reactions in this sequence are enzyme controlled.
- Therefore, inhibition of any of these enzymes will interrupt the process.

- However, the main function of this pathway is to produce substance D for use by the cell.
- If the requirement for substance D in the cell decreases, then the concentration of D will increase.
- This is at least inefficient (producing something that is not being used) and may be potentially harmful because high concentrations could be toxic.

- Such reaction sequences are often controlled by end-product inhibition.
- The end product (D) inhibits the enzyme controlling the first stage of the reaction sequence, as shown in the diagram



End-product inhibition



threonine

enzyme 1 threonine deaminase

intermediate A

enzyme 2

intermediate B

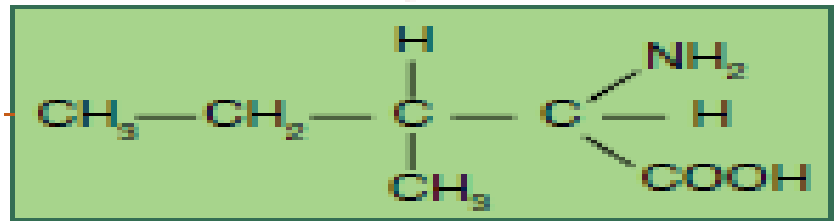
enzyme 3

intermediate C

enzyme 4

intermediate D

enzyme 5

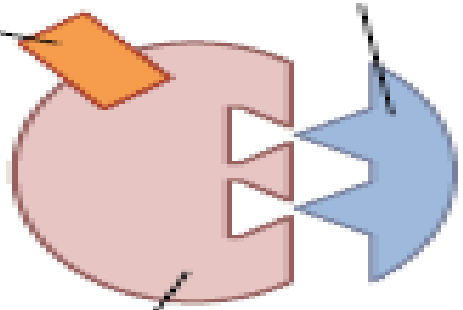


isoleucine

high levels of isoleucine allosteric inhibitor

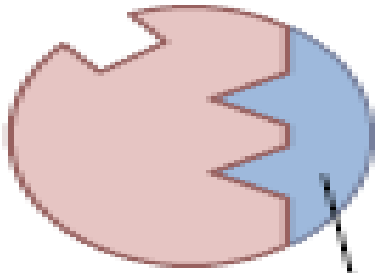
isoleucine (allosteric inhibitor)

substrate (threonine) cannot bind



enzyme (threonine deaminase)

low levels of isoleucine



substrate (threonine) able to bind

- Substance D normally acts as a non-competitive inhibitor, which prevents the enzyme E1 from catalysing the reaction that converts A to B.
- As a result, the entire reaction sequence is halted(temporarliy stopped).
- When the inhibition is removed, the metabolic pathway can start again. Sometimes, a decrease in the concentration of D is sufficient to remove the inhibition.
- In other cases, the inhibitor must be removed by a substance known as an activator.

- Enzymes are often kept inactive in a cell by an inhibitor and are activated by a specific activator only when they are required
- The above figure shows an example of a metabolic pathway controlled by end-product inhibition.
- In this pathway, the amino acid threonine is converted to another amino acid, isoleucine.
- Isoleucine acts as a non-competitive (allosteric) inhibitor.
- High concentrations of isoleucine inhibit more and more of the enzyme catalysing the first reaction in the pathway, until the pathway is completely inhibited


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HAPPY
CHRISTMAS