Chapter 5

ENZYMES

STUDENTS' LEARNING OUTCOMES

After studying this chapter, the students will be able to:

- Identify the role and component parts of the active site of an enzyme.
- Differentiate among the three types of co-factors i.e., in organic ions, prosthetic group and co-enzymes, with examples.
- Explain the mechanism of enzyme action through the Induced Fit Model, including comparing it with Lock and Key Model.
- Explain enzyme catalysis with example of specific reactions.
- Define energy of activation and discuss through graph how an enzyme speeds up a reaction by lowering the energy of activation.
- Explain the effect of temperature on the rate of enzyme action with example of human and thermophilic bacteria
- Investigate the effect of pH on enzyme activity Compare the optimum pH of different enzymes like trypsin, pepsin, papain.
- Demonstrate that the concentration of enzyme affects the rate of enzyme action.
- Describe enzymatic inhibition, its types and its significance with examples.
- Name the molecules which act as inhibitors.
- Categorize inhibitors into competitive and non-competitive inhibitors.
- Explain feedback inhibition.
- Classify enzymes on the basis of the reactions catalyzed (oxidoreductases, transferases, hydrolases, isomerases, and ligases).
- Classify enzymes on the basis of the substrates they use (lipases, diastase, amylase, proteases etc.)

You know that the life of living organisms is a reflection of what is going on in their bodies. The sum of all chemical activities occurring in living organisms i.e., metabolism is regulated by enzymes.

5.1- ENZYMES

Enzymes may be defined as specific proteins that speed up specific chemical reactions by lowering the required activation energy, but are unaltered themselves in the process. Enzymes are also known as **biocatalysts**. Rates of enzyme-catalysed reactions may be 10^3 to 10^8 times greater than the rates of corresponding uncatalyzed reactions.

All cells do not have the same set of enzymes. The chemical reactions going on in red blood cells are very different from those going on within a nerve cell because red blood cells and nerve cells contain different sets of enzymes.

All enzymes are synthesized inside cells by ribosomes. After their synthesis, either they stay and work inside cell or they are secreted out to work at other sites.

A reaction that is catalysed by an enzyme and is completed in 30 minutes, would take one year to get completed without being catalysed by enzyme. Thus, we can say that without enzymes there would have been no life at all.

Inside cell, many enzymes are dissolved in cytoplasm; for example, the enzymes of glycolysis. Many are tightly bound to membranes of certain organelles, for example, the enzyme of Calvin cycle and Krebs cycle. Some enzymes are integral part of ribosomes; for example, the enzymes of protein synthesis.

Active Site of Enzyme

Enzymes are three-dimensional globular proteins. They are made of polypeptide chains that are coiled upon themselves. There is a small cleft or depression on the surface of globular enzyme molecule. It consists of only a few amino acids. This site is known as **active site**. It is the location at which catalysis occurs.

Some enzymes may prove harmful, if become active at wrong place. For example; pepsin is a protein digesting enzyme. It can destroy protein-made structures present inside cells where it is synthesized. That is why it is produced in inactive form (pepsinogen) and is secreted out of cells. When it reaches its target site of action, it is activated (pepsin).

The shape of active site of each enzyme is very specific. So, only a certain substrate molecule can fit into it. It is three-dimensional and bears a specific charge. Active site has two distinct regions i.e., **binding site** and **catalytic site**. Substrate molecule fits into binding site by weak chemical forces, such as hydrogen bonds. Catalytic site catalyses the reaction and substrate is transformed into products.

5.2- Cofactors And Coenzymes

Many enzymes use additional chemical components to aid in catalysis. These additional non-protein components are called **cofactors**. There are three kinds of cofactor: metal ions, prosthetic groups, and coenzymes.

The protein part of enzyme is called **apoenzyme** and complete enzyme including co-factor is called **holoenzyme**.

Many enzymes use **metal ions**, such as Ca^{+2}, Mg^{+2}, Mn^{+2}, Cu^{+2}, and Zn^{+2} as their cofactors. These metal ions change the non-functional active sites of enzymes into functional sites. The attachment of a cofactor also changes the shape of enzyme and allows it to combine with substrate (Figure 5.1).

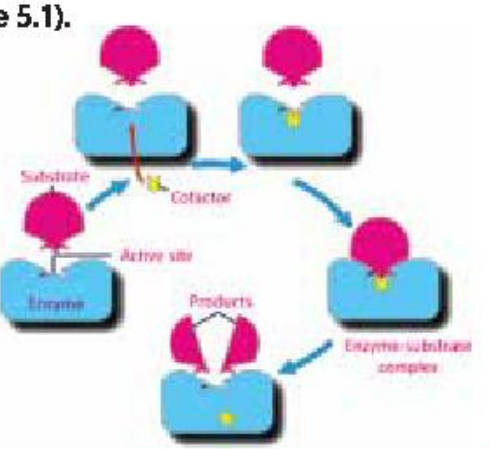

Figure 5.1: Cofactor, changing the shape of active site

Some cofactors form covalent bonds with enzyme and are known as **prosthetic groups**. Prosthetic group may be an organic compound e.g., hematin.

When the cofactor is a non-protein organic molecule and is loosely attached with enzyme, it is called a **coenzyme**. Coenzymes participate in enzyme-catalysed reactions, often by transporting electrons (hydrogen atoms), from one enzyme to another. Many vitamins (e.g., niacin and riboflavin) function as coenzymes. Some are part of coenzymes. The most important coenzyme in cell is the hydrogen acceptor nicotinamide adenine dinucleotide (NAD^+). When NAD^+ acquires a hydrogen atom from an enzyme, it reduces to NADH. The electron of hydrogen atom contains energy that NADH molecule carries. For example, when food is oxidized in cell, enzymes draw electrons from food molecules and transfer them to NAD^+, which reduces to NADH.

Many trace elements such as molybdenum and manganese, which are necessary for our health, are used by enzymes as cofactors.

5.3- Mechanism Of Enzyme Action

The speed of a chemical reaction depends on the amount of activation energy required to initiate it. **Activation energy** is the energy which works to destabilize existing chemical bonds. Enzymes bring reactants together in correct orientation or stress particular chemical bonds of reactants. Thus, they lower the activation energy required for new bonds to form and speed up the rate of reactions (Figure 5.2). Reactions proceed much faster than their normal speed.

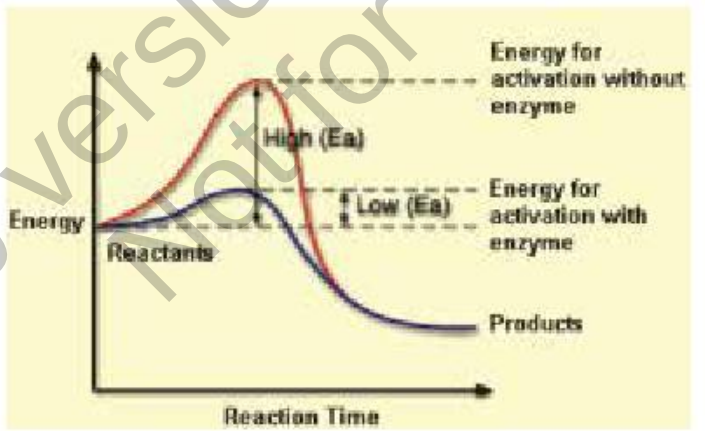

Figure 5.2: Enzymes lower the activation energy

The presence of enzymes does not affect the nature or properties of end products. For example, sucrose (substrate) will always be hydrolysed into glucose and fructose (products) whether sucrase (enzyme) is present or not.

Due to its specificity, an enzyme recognizes a specific substrate. The substrate binds with the active site of enzyme. In this way, an enzyme-substrate complex (ES complex) is formed and catalytic site is activated. The atoms of catalytic site stress and

destabilize particular bonds of substrate. So, activation energy is lowered. This action initiates the reaction and substrate is transformed into products. After it, enzyme detaches itself from the products, in an unaltered state. The mechanism of enzyme action can be summarised as follows:

$$E + S \longrightarrow ES \longrightarrow E + P$$

Enzyme Substrate — Enzyme-substrate complex — Enzyme Product

In complex metabolic pathways e.g., respiration, photosynthesis, protein synthesis etc., many enzymes act in a sequence and regulate the steps of pathway. The successive enzymes controlling these steps are present together along with their cofactors. The products from one enzyme's catalysis serve as substrate for the enzyme of next step and are transformed into next products. The series goes on and finally end products are formed that inhibit (through feedback) the first enzyme.

Models for Mechanism of Action of Enzymes

Lock-and-Key Model

In 1894 a German chemist **Emil Fischer** proposed lock-and-key model. According to this model, "as a specific key can open only a specific lock, in the same manner a specific enzyme can transform only one specific substrate into products". This model postulates that active site is a rigid structure and there is no modification or flexibility in it before, during or after the enzyme action (Figure 5.3).

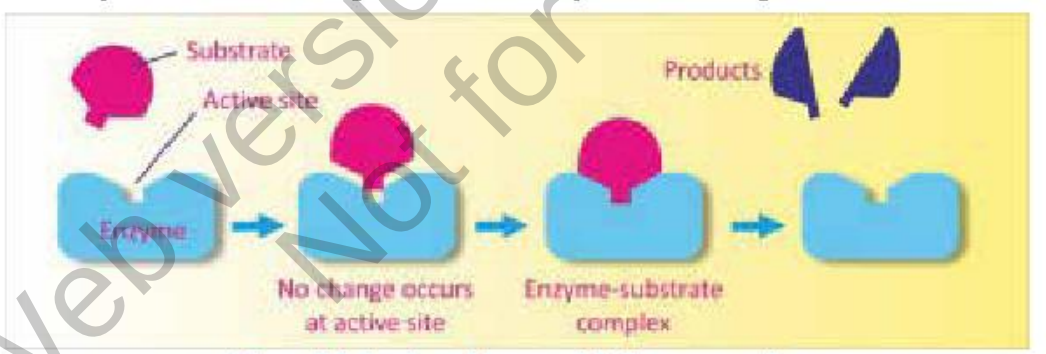

Figure 5.3: Lock-and-key model of enzyme action

Induced Fit Model

Later studies did not support lock-and-key model in all reactions. On the basis of new evidence, an American biochemist **Daniel Koshland**(1958) presented induced fit model. According to this model, "when a substrate combines with the binding site of an enzyme, it induces **changes** in enzyme structure. These changes enable the enzyme to perform its catalytic activity more effectively." This model postulates that active site is not a rigid structure and is capable of going under modification and flexibility, before the enzyme action (catalysis) starts (Figure 5.4).

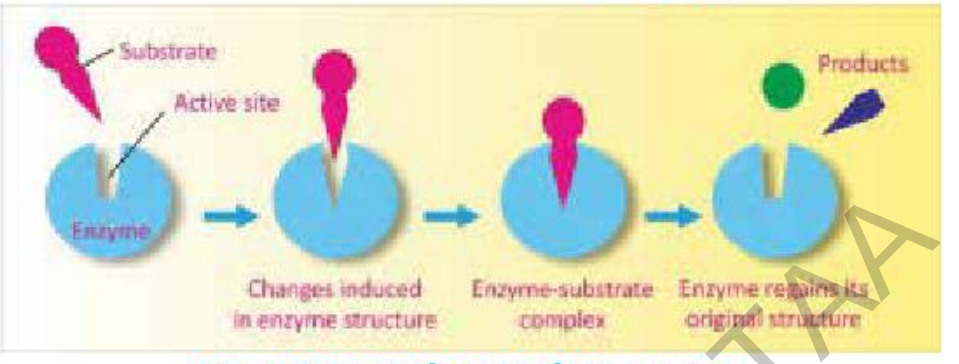

Figure 5.4: Induced-fit model of enzyme action

5.4- Factors Affecting The Rate Of Enzyme Action

Enzymes are very sensitive to the environment in which they work. The activity of an enzyme is affected by any change that alters its chemistry and its three-dimensional shape. Some of the factors that can affect the rate of enzyme action are being discussed next.

1. Temperature

The shape of a protein is determined by the hydrogen bonds and hydrophobic interactions that hold its polypeptide chains in particular position. Both the hydrogen bonds and hydrophobic interactions are easily disrupted by slight changes in temperature. Every enzyme works at its maximum rate at a specific temperature called its **optimum temperature**. The optimum temperature for human enzymes is 37 °C.

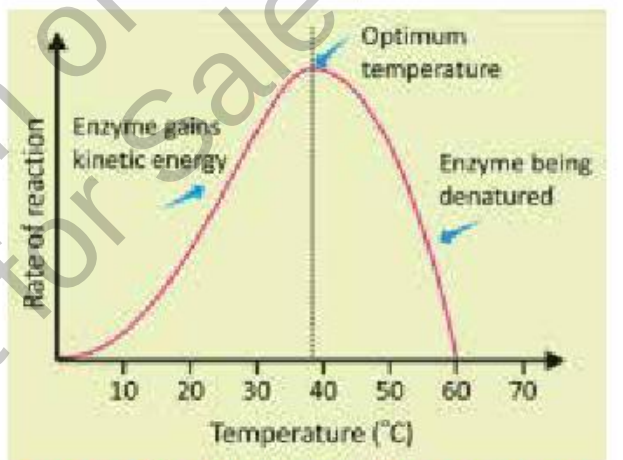

Figure 5.5: Effect of temperature on enzyme activity

When temperature fall below optimum temperature, the bonds that determine enzymes shape become less flexible. They do not permit the induced change in active sites that is necessary for enzyme action and so reaction rate is slow. When temperature is raised up to a certain limit, the heat adds in activation energy and so reactions are accelerated. Heat also provides kinetic energy to substrate and enzyme molecules. It causes them to move rapidly. Thus, they collide more frequently and reaction rate is increased. When temperature is raised well above

Thermophilic bacteria live in hot springs. They have proteins with stronger bonding between their polypeptide arms and can function at temperature of 70 °C or higher.

optimum temperature, the heat energy increases the vibrations of atoms of enzyme molecules. When vibrations become too violent, bonds cannot hold polypeptide chains in the proper position and globular structure of enzyme is lost. This phenomenon is known as **denaturation** of enzyme. It results in a rapid decrease in the rate of enzyme action and it may be blocked completely.

2. pH

All enzymes work at their maximum rate at a narrow range of pH. A slight change (increase or decrease) in this pH causes retardation in enzyme activity or blocks it completely. Every enzyme works its best at a specific pH, called its **optimum pH**. For example, **pepsin** is active in acidic medium (low pH) while **trypsin** shows its optimum activity in alkaline medium (high pH). Some enzymes like **papain** from green papaya work both in acidic and alkaline media.

In the globular structure of an enzyme, polypeptide chains are held by bonds between oppositely charged amino acids, such as glutamic acid (-) and lysine (+). These bonds are sensitive to hydrogen ion concentration. Any change in pH can change the ionization of amino acids at active site. Moreover, it may affect the ionization of substrate. Extreme change in pH can break the bonds in enzymes, resulting in enzyme denaturation.

Table: Optimum pH of important human enzymes

Enzyme	Optimum pH
Pepsin	1.5–1.6
Salivary amylase	4.6–5.2
Sucrase	6.2
Pancreatic amylase	6.7–7.0
Catalase	7.0
Urease	7.0
Trypsin	7.8–8.7
Pancreatic lipase	8.0
Arginase	10.0

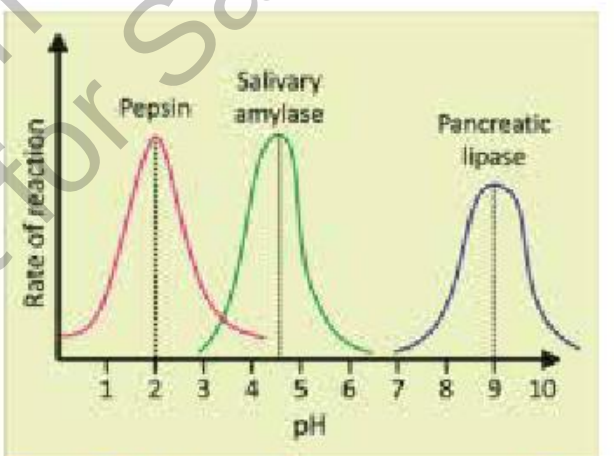

Figure 5.8: Optimum pH of some enzyme and effect of change of pH on enzyme activity

3. Enzyme Concentration

Enzymes are very efficient and a small number of enzyme molecules can catalyse reactions of large amount of substrate. The overall rate of enzyme-controlled reactions depends directly on the amount of enzyme present at a specific time (if substrate concentration is unlimited). When enzyme concentration increases, there are more enzyme molecules and more active sites. So, more substrate molecules bind with new active sites and are transformed into products. If enzyme concentration goes on

increasing but substrate concentration remains the same, no more substrate molecules will attach with enzymes. So, the rate of reaction stays constant and does not increase further (Fig.5.7).

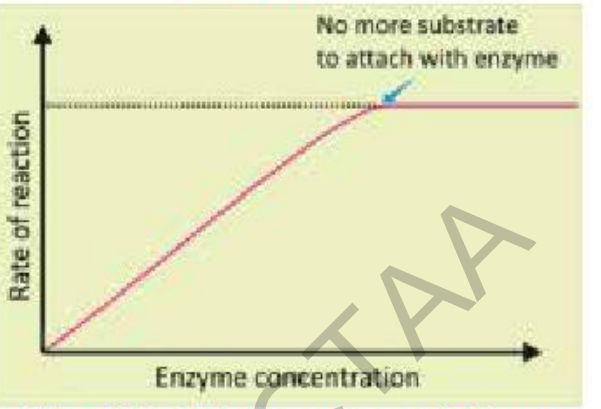

Figure 5.7: Effect of enzyme concentration on enzyme activity

4. Substrate Concentration

If there are enzyme molecules with vacant active sites, an increase in substrate concentration will increase the rate of reaction. If enzyme concentration is kept constant and the amount of substrate is increased, a point is reached where any further increase in substrate does not increase the rate of reaction any more.

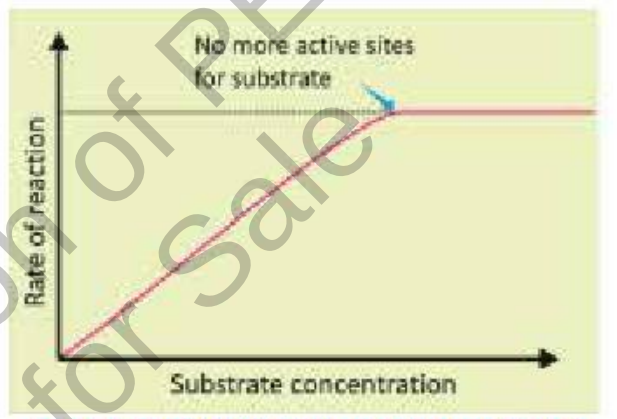

Figure 5.8: Effect of substrate concentration on enzyme activity

When enzyme molecules are free (at low substrate concentration) new substrate molecules bind with the available active sites and so more products are formed in the given time i.e., rate of enzyme action is increased. But when all active sites of enzymes are occupied (at high substrate concentration), any more substrate molecules do not find free active sites and so reaction rate does not increase (Fig.5.8)

5.5- ENZYME INHIBITION

A chemical that interferes and blocks an enzyme's activity is called an **Inhibitor**. Inhibitors attach with enzymes but are not transformed into products and thus block active sites temporarily or permanently. This phenomenon is known as enzyme **Inhibition**. The final products of complex enzymatic reactions also act as the inhibitors of the enzyme of the first step.

Inhibitors are often used as drugs, but they can also act as poisons. An example of an enzyme inhibitor being used as a drug is aspirin. It inhibits the enzymes that produce prostaglandin (that causes inflammation). Thus, aspirin suppresses pain and inflammation. The poison cyanide is an irreversible enzyme inhibitor that combines with copper and iron in the active site of enzyme cytochrome oxidase and blocks cellular respiration.

Types of Inhibitors

Competitive and non-competitive inhibitors

Two general classes of inhibitors are recognized; competitive and non-competitive inhibitors. A **competitive inhibitor** resembles the enzyme's substrate. It competes with substrate for the same binding site on enzyme. When competitive inhibitor is selected by binding site, it blocks active site and does not permit substrate from attaching. Thus, it prevents enzyme from acting (Figure 5.9).

Competitive inhibitors are used as antibiotics to kill bacteria. These inhibitor molecules are similar in structure to bacterial enzymes which are necessary for their life. The inhibitors bind and inhibit the enzymes of bacteria.

Figure 5.9: Competitive inhibition of an enzyme

The enzyme succinic dehydrogenase catalyses the oxidation of succinic acid to fumaric acid. Malonic acid has structural similarity with substrate (succinic acid). So, both of them compete for active site of enzyme. Malonic acid is selected by active site and thus blocks it.

A **non-competitive inhibitor** has no real structural similarity to substrate. So, it does not enter active site. Instead, it binds enzyme at other places. Its binding alters the shape of enzyme so that active site does not fit substrate and so enzyme is inhibited (Figure 5.10).

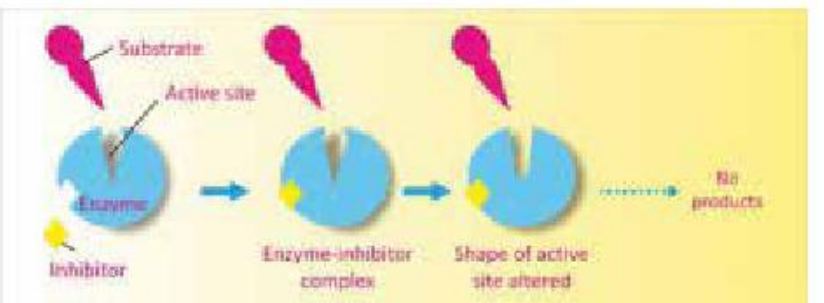

Figure 5.10: Non-competitive inhibition of an enzyme

For example; two substrates i.e., succinic acid and CoA react to form succinyl-CoA. This reaction is catalysed by enzyme succinyl-CoA synthetase. After its formation, the product i.e., succinyl CoA acts as a non-competitive inhibitor and binds with enzyme. Thus, enzyme is inhibited and no more succinyl-CoA is produced.

Reversible and Irreversible Inhibitors

The action of any inhibitor can be irreversible or reversible, depending upon the kind of bond formed between inhibitor and enzyme.

Irreversible Inhibitors make covalent bonds with enzyme. Such inhibitors cannot be released by dilution or dialysis or by increasing the concentration of substrate, for example, penicillin permanently disables the enzyme responsible for building bacterial cell walls.

Reversible inhibitors make weak bonds (e.g., hydrogen bonds) with enzyme. Such inhibitors can be released and the inhibition caused by them can be neutralized by increasing the concentration of substrate for example, malonate is a reversible inhibitor. It temporarily slows down the reaction by blocking the enzyme succinate dehydrogenase, which is involved in cellular respiration. This inhibition can be reversed when malonate is removed.

Significance of Enzyme Inhibition

Enzyme inhibition is crucial in various biological processes.

1. Enzyme inhibition plays a vital role in regulating metabolic pathways. By inhibiting specific enzymes, the rate of a metabolic reaction can be controlled.
2. Many drugs work as inhibitors. For example, antibiotics inhibit the enzymes of bacteria, while cancer drugs may inhibit enzymes involved in cell division.
3. Enzyme inhibitors are used to manage various medical conditions. For example, some inhibitors of enzymes involved in blood clotting are used as anticoagulants.
4. Some toxins and poisons inhibit important enzymes in the body. Understanding how these inhibitors affect enzymes can be critical in treating cases of poisoning.
5. Enzyme inhibitors serve as valuable tools in pharmaceutical research. They are used to study the function of specific enzymes, and potential drugs.

Enzyme inhibition is an important part of studying enzyme kinetics. It helps to understand the factors that influence enzyme activity.

Feedback Inhibition of Enzymes

We know that in metabolic pathways, the product of one reaction becomes the substrate for next reaction. At the end of pathway, a desired product is synthesized. In order to regulate the concentration of that product, pathway needs to be shut down. This is done through feedback inhibition. The final product of pathway acts as inhibitor. It reacts with some initial enzyme and changes its conformation. That

enzyme can no longer bind to its substrate. So, pathway closes and no more product is prepared (Figure 5.11).

For example, when a cell has a greater number of ATP than its requirement, ATP itself acts as a non-competitive inhibitor and blocks the enzyme that catalyses ATP synthesis.

Feedback Inhibition is the phenomenon where the product of a process controls the process itself, oftentimes limiting the production of more products.

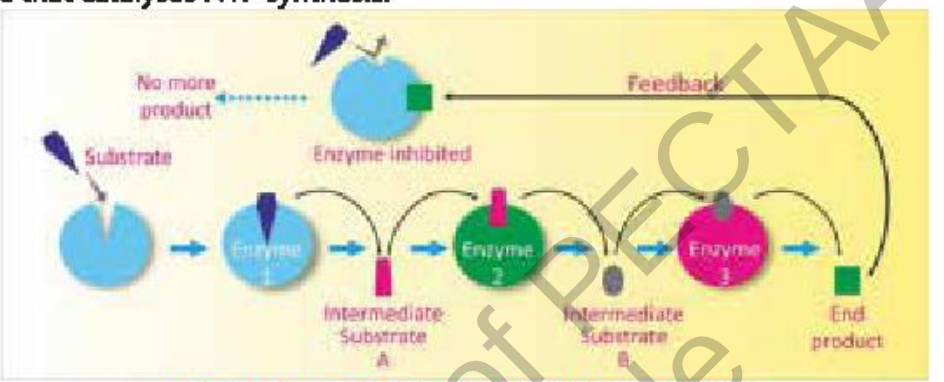

Figure 5.11: Feedback inhibition of enzyme action

5.6- CLASSIFICATION OF ENZYMES

Enzymes are classified on the basis of reactions they catalyse and also on the basis of substrates they use.

Classification on the Basis of Reactions

According to the general type of reaction, enzymes are classified into six classes.

1- Oxidoreductases: These enzymes catalyse the oxidation / reduction of their substrates. They add or remove H^+ ions or electrons from substrates. For example, **cytochrome oxidase** catalyses the oxidation of cytochrome.

2- Transferases: The enzymes of this class catalyse the transfer of a specific functional group (e.g., methyl, acyl, amino, or phosphate) from one substrate to another. For example, **hexokinase transfers** phosphate group from ATP to glucose.

3- Hydrolases: These enzymes catalyse hydrolysis reactions. They break their substrates into monomers by adding water. For example; **lipase, amylase, peptidase,** and other digestive enzymes catalyse the hydrolysis of food molecules.

4- Lyases: These enzymes catalyse non-hydrolytic addition or removal of groups (e.g., CO_2, NH_2 etc.) from substrates. For example, **pyruvate decarboxylase** removes CO_2 from pyruvic acid.

5- Isomerases: These enzymes catalyse the intra-molecular rearrangement i.e., one isomer is converted into another. For example, **hexose isomerase** converts glucose to fructose.

6- Ligases: These enzymes catalyse the reactions in which two molecules join by forming new C-C, C-N, C-O, or C-S bonds, using energy from ATP. For example, **polymerase** enzymes join monomers by using ATP.

Classification on the Basis of Substrates

Enzymes are also classified into following groups on the basis of their substrates.

1- Proteases: This group included the enzymes which catalyse the breakdown of proteins. For example, **pepsin** and trypsin enzymes catalyse the breakdown of large polypeptides into smaller polypeptides. Similarly, **aminopeptidases** further breakdown small polypeptides into dipeptides and erypsin breaks dipeptides into amino acids.

2- Lipases: These enzymes act upon lipids and catalyse their breakdown. For example, pancreatic **lipase** hydrolyses lipids into fatty acids and glycerol.

3- Carbohydrases: These enzymes act upon bigger carbohydrates and break them into smaller units. For example, **amylase** acts upon starch or glycogen and breaks them into maltose. **Cellulase** breaks cellulose into cellobiose (a disaccharide) or glucose. Similarly, **maltase** breaks down maltose into glucose, **sucrase** breaks sucrose into glucose and fructose, and **lactase** breaks lactose into glucose and galactose.

4- Nucleases: These enzymes act upon nucleic acids and catalyse their breakdown. For example, **RNAase, DNAase, ATPase** are responsible for the breakdown of RNA, DNA and ATP respectively.

Class	Reaction type	Important subclasses
1- Oxidoreductases	A + B ⇌ A + B (red, ox, ox, red)	Dehydrogenases Oxidases Reductases
2- Transferases	A B + C ⇌ A + C B	Phospho-transferases Amino-transferases Acyl-transferases
3- Hydrases	A B + H_2O ⇌ C + D	Peptidases Lipases Glycodsidases
4- Lyases	A ⇌ C + B	Decarboxylases Aldolases Synthases
5- Isomerases	A ⇌ A	Epimerases Mutases *cis trans* isomerases
6- Ligases	A + B + ATP ⇌ A B + ADP + i	C-C ligases C-O ligases C-N ligases

Figure 5.12: Enzyme classification on the basis of reactions

SECTION 1: MULTIPLE CHOICE QUESTIONS

1. What roles does nicotinamide adenine dinucleotide play in oxidative pathways?
   (a) Enzyme (b) Coenzyme (c) Prosthetic group (d) Inhibitor
2. The enzymes that catalyse the reactions in which two molecules are joined together by synthesis of new bonds, using energy from ATP, are placed in group;
   (a) Hydrolase (b) Ligase (c) Lyase d) Transferase
3. Which of the following is an example of hydrolases?
   (a) Lipase (b) Glycogen phosphorylase
   (c) Pyruvate decarboxylase (d) Cytochrome oxidase
4. Which of the following statements about enzymes is correct?
   (a) They increase the activation energy of a reaction.
   (b) They are consumed during the reaction.
   (c) They are specific in terms of the reactions they catalyse.
   (d) They always work optimally at high temperatures.
5. Enzyme B requires Zn'+ to catalyse the conversion of substrate X . The zinc is best identified as a(n):
   (a) Coenzyme (b) Activator (c) Substrate (d) Product
6. If an enzyme solution is saturated with substrate, the most effective way to obtain an even faster yield of products would be
   (a) Add more of the enzymes (b) Add more substrate
   (c) Add an allosteric inhibitor (d) Add a non-competitive inhibitor
7. How does a non-competitive inhibitor decrease the rate of an enzyme-catalysed reaction?
   (a) By binding the active site of the enzyme
   (b) By changing the shape of the enzyme
   (c) By changing the free energy change of the reaction
   (d) By acting as a coenzyme for the reaction
8. Which enzyme class is responsible for catalysing the addition of water to a substrate molecule?
   (a) Ligase (b) Lyase (c) Hydrolase (d) Isomerase

SECTION 2: SHORT QUESTIONS

1. Define enzyme and co-factor.
2. Differentiate between co-enzyme and prosthetic group.
3. What do you mean by hydrolases? Give two examples.
4. What is meant by activation energy?
5. Define feedback inhibition.
6. Give examples of competitive and non-competitive inhibitors.

7. What is optimum pH? Give optimum pH of three human enzymes.

SECTION 3: LONG QUESTIONS

1. Describe the structure of enzyme, explaining the role and component parts of the active site of an enzyme.
2. Differentiate among the three types of co-factors, by giving examples.
3. Explain the mechanism of enzyme action through Induced Fit Model, comparing it with Lock and Key Model.
4. Define activation energy and explain through graph how an enzyme speeds up a reaction by lowering activation energy.
5. Describe the effect of temperature on the rate of enzyme action.
6. Compare the optimum temperatures of enzymes of human and thermophilic bacteria.
7. Describe how the concentration of enzyme affects the rate of enzyme action.
8. Explain the effect of substrate concentration on the rate of enzyme action.
9. Describe enzymatic inhibition, its types and its significance.
10. Categorize inhibitors into competitive and non-competitive inhibitors.
11. Explain feedback inhibition.
12. Classify enzymes on the basis of the reactions catalysed.
13. Give examples of enzymes' naming according to substrates.

INQUISITIVE QUESTIONS

1. Does physical exercise involve anabolic processes, catabolic processes, or both? Give evidence for your answer.
2. If a chemical reaction could occur without an enzyme, why is it important to have one?
3. Construct and interpret graphs based on data about the effect of temperature, enzyme concentration and substrate concentration on the rate of enzyme action.
4. Identify the competitive and non-competitive inhibitors from a list of chemicals used in daily life.