

Unit 5: Enzymes

Long Questions

DESCRIPTIVE QUESTIONS

ENZYME

Q1. Describe the structure of enzymes, explaining the role and component parts of the active site.

Ans. Definition: Enzymes may be defined as specific globular proteins that speed up specific chemical reactions by **lowering the required activation energy**, but remain unaltered 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.

Structure: Enzymes are three-dimensional globular proteins. They are made of polypeptide chains that are coiled upon themselves.

Active site: 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.

Shape: 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**.

Regions: Active site has two distinct regions i.e., **binding site** and **catalytic site**.

(i) **Binding site:** Substrate molecule fits into **binding site** by weak chemical forces, such as hydrogen bonds.

(ii) **Catalytic site:** It catalyses the reaction and substrate is transformed into products

COFACTORS AND COENZYMES

Q2. Differentiate among the three types of co-factors by giving examples.

Ans. Cofactors: Many enzymes use additional chemical components to aid in catalysis. These additional non-protein components are called **cofactors**.

Types: There are three kinds of cofactors: **metal ions**, **prosthetic groups** and **coenzymes**.

(i) **Metal ions:** 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

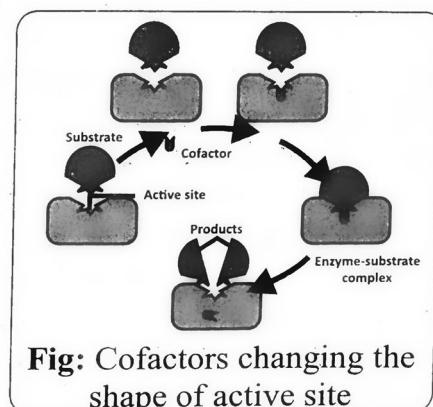


Fig: Cofactors changing the shape of active site

★ **Note:** All questions and paragraphs marked with a star ★ are explanations of the Box Information provided in the textbook.

enzymes into functional sites. The attachment of a cofactor also changes the shape of enzyme and allows it to combine with substrate.

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

(ii) **Prosthetic Groups:** Some cofactors form **covalent bonds** with enzyme and are known as **prosthetic groups**. Prosthetic group may be an organic compound e.g., **hematin**. Hematin also known as heme, is a part of hemoglobin and myoglobin, it acts as prosthetic group in enzymes like catalases and peroxidases.

(iii) **Coenzyme:** 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** (vitamin B₃) and **riboflavin** (vitamin B₂)) function as coenzymes. Some are part of coenzymes. The most important coenzyme in cell is the hydrogen acceptor **Nicotinamide Adenine Dinucleotide (NAD⁺)**.

Role of NAD⁺ in a cell

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.

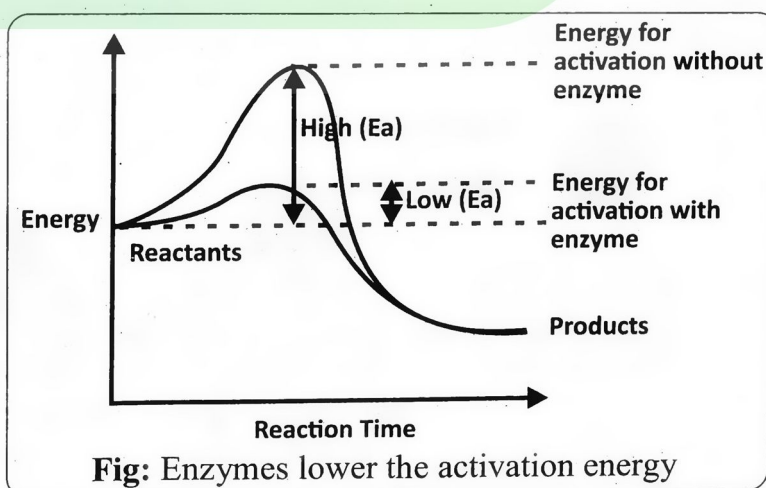
Q3. Define activation energy and Explain through graph, how an enzyme speeds up a reaction by lowering activation energy?

Ans. 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**.

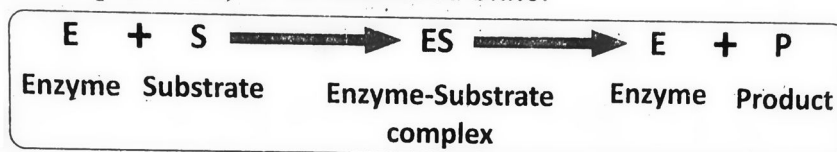
Effect of enzymes on activation energy: Enzymes bring reactants together in correct orientation or stress particular chemical bonds of reactants. Thus, they lower the activation energy required for new bonds to form and speed up the rate of reactions. Reactions proceed much faster than their normal speed.

Effect on End Product: 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.

Enzymes Substrate Complex: 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

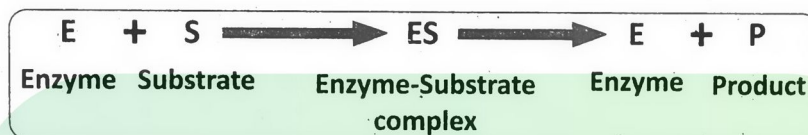


action initiates the reaction and substrate is transformed into products. After it, enzyme detaches itself from the products, in an unaltered state.



Q4. Explain the mechanism of enzyme action through induced fit model. Also compare it with lock and key model of enzyme action.

Ans. Enzyme-substrate complex: 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 summarized as follows.

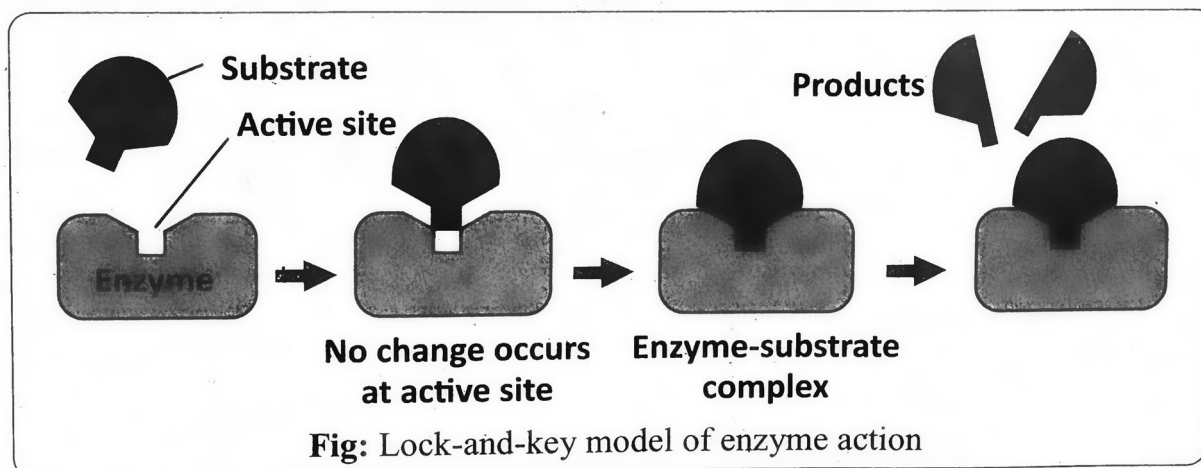


Models for Mechanism of Action of Enzymes

1. Lock and key Model

History: 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”.

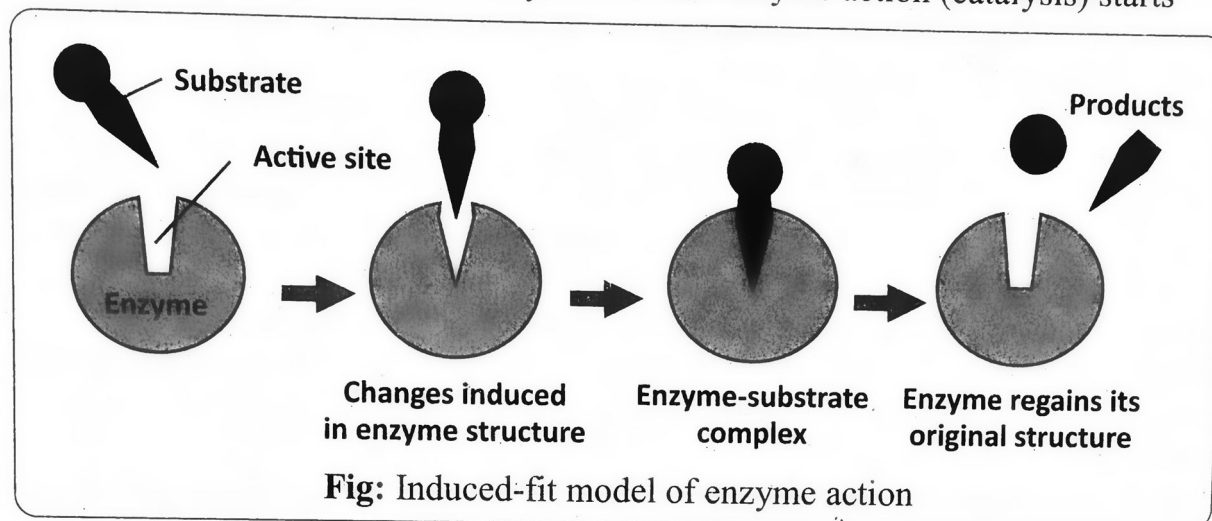
Postulates: This model postulates that active site is a **rigid structure** and there is no **modification** or flexibility in it before, during or after the enzyme action



2. Induced Fit Model (Modified form of Lock and Key Model)

History: Later studies did not support lock-and-key model in all reactions. On the basis of new evidence, an American biochemist **Daniel Koshland** in 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.”

Postulates: 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



Comparison of induced fit model with lock and key model

Active site: According to **lock and key model** active site is a **rigid** structure but according to **induced fit model** it is **flexible**.

Enzyme regulation: Lock and key model is followed by **non-allosteric enzymes**, while induced fit model is followed by **allosteric enzymes**.

Reactions: Lock and key model is usually for **simple reactions** (e.g., one step reactions) while induced fit model is usually for **complex reactions** allowing feedback regulation.

FACTORS AFFECTING THE RATE OF ENZYME ACTION

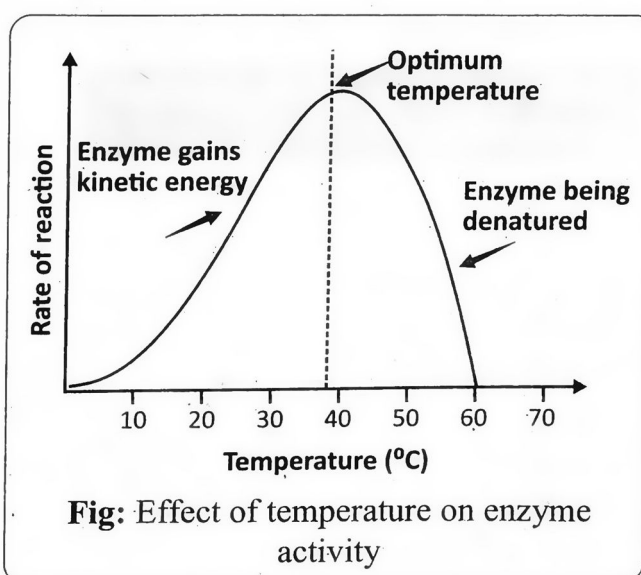
Q5. Describe the effect of temperature on the rate of enzyme action. / Compare the effect of temperature on enzymes of human and thermophilic bacteria.

Ans. Effect of Temperature on Enzyme Activity in Humans

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.

Optimum Temperature: Every enzyme works at its maximum rate at a specific temperature called its optimum temperature. e.g., The optimum temperature for human enzymes is **37 °C**.

At low Temperature: When temperature falls **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.



At High Temperature: 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.

Denaturation: When temperature is raised well above 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.

★ Effect of Temperature on Enzyme Activity in Thermophilic Bacteria

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. So, they are more thermostable than human enzymes.

Q6. How pH affects the rate of enzyme action? Write the optimum pH of various human enzymes.

Ans. Effect of pH on Enzyme Activity

All enzymes work at their maximum rate at a narrow range of pH.

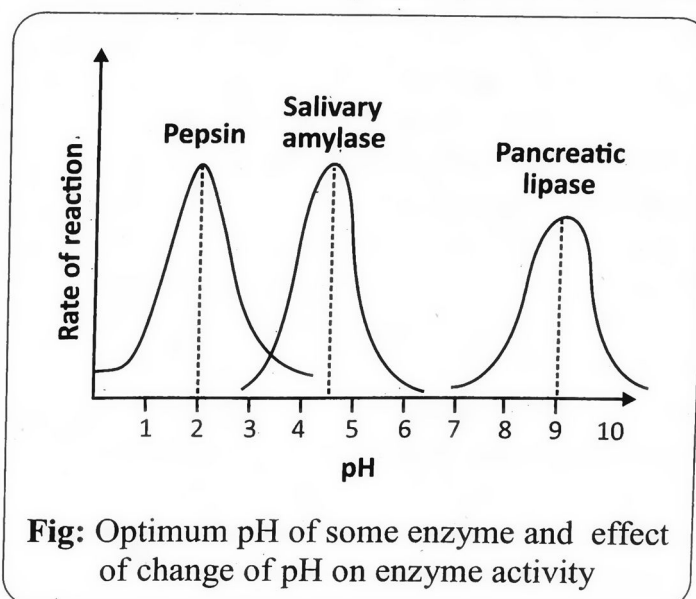
Optimum pH: Every enzyme works at its best at a specific pH, called its optimum pH. For example, **pepsin** is active in acidic medium (low pH) while **trypsin** shows its optimum activity in alkaline medium (high pH). Some enzymes like **papain from green papaya** work both in acidic and alkaline media.

Effect of changed pH: 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.

- A **slight change** (increase or decrease) in this **pH** causes retardation in enzyme activity or blocks it completely.
- **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

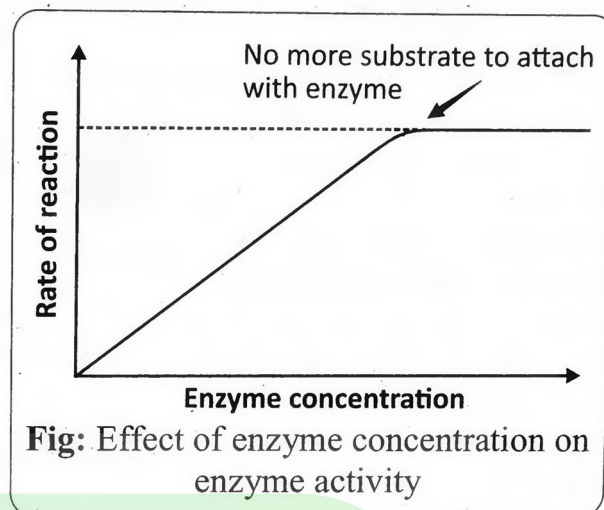


Q7. Describe how the concentration of enzyme affects the rate of enzyme action?

Ans. Enzymes are very **efficient** in low concentrations 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).

Effect of increase in Enzyme Concentration:

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 be available to attach with enzymes. So, the rate of reaction stays constant and does not increase further.



Q8. Explain the effect of substrate concentration on the rate of enzyme action?

Ans. If there are enzyme molecules with vacant active sites, an increase in substrate concentration will increase the rate of reaction.

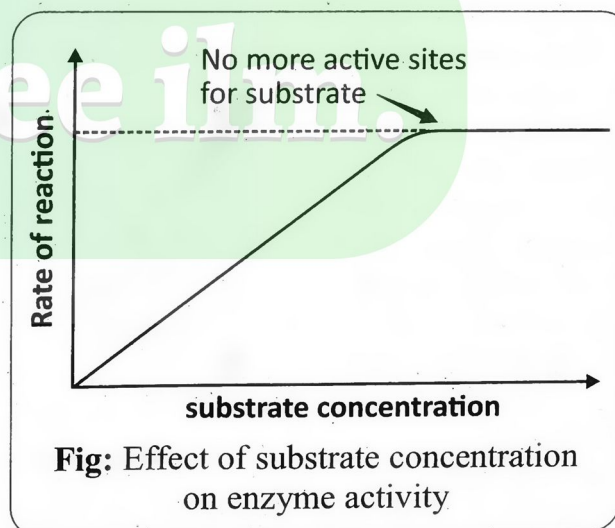
At Constant Enzyme Concentration: If enzyme concentration is kept **constant** and the amount of substrate is increased, a point is reached where any further increase in substrate does not increase the rate of reaction anymore.

Low substrate concentration

- 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.

At High Substrate Concentration

- 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 and this point is called **saturation**.



ENZYMATIC INHIBITION

Q9. Describe Enzymes inhibition. / Describe enzymatic inhibition, its types and its significance.

Ans. Inhibitor: A chemical that interferes and blocks an enzyme's activity is called an inhibitor.

Inhibition: In this process 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 (allosteric inhibition).

Types of Inhibitors: Two general types of inhibitors are recognized competitive and non-competitive inhibitors.

1. Competitive: A competitive inhibitor **resembles** structurally with the enzyme's substrate. It competes with substrate for the same binding site on enzyme.

Example: 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.

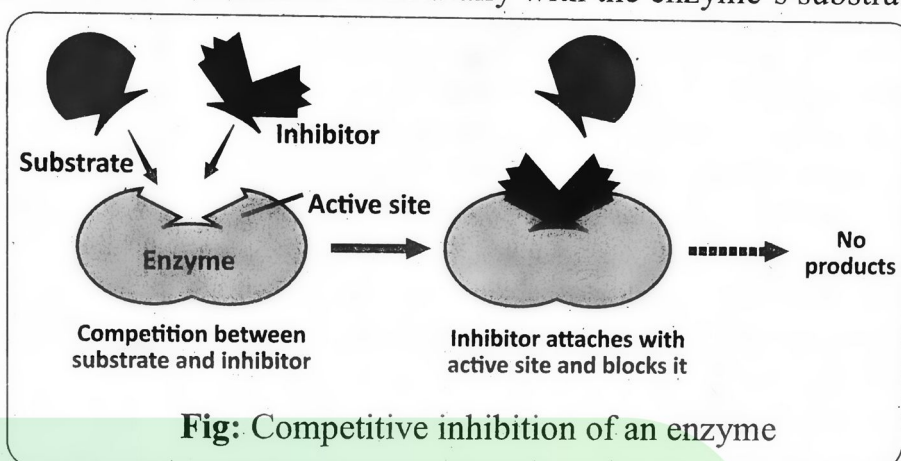


Fig: Competitive inhibition of an enzyme

Malonic acid is selected by active site and thus blocks it.

2. Non-competitive: 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.

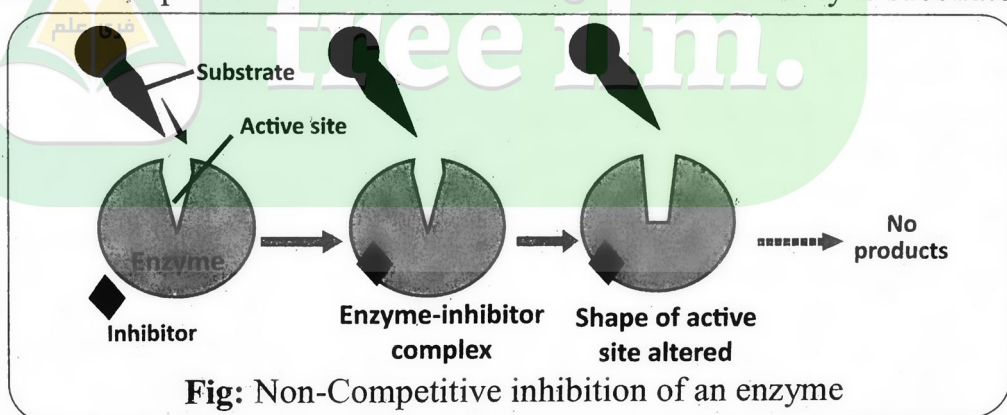


Fig: Non-Competitive inhibition of an enzyme

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.

3. 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 of succinate. 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.

4. Significance of Enzyme inhibition

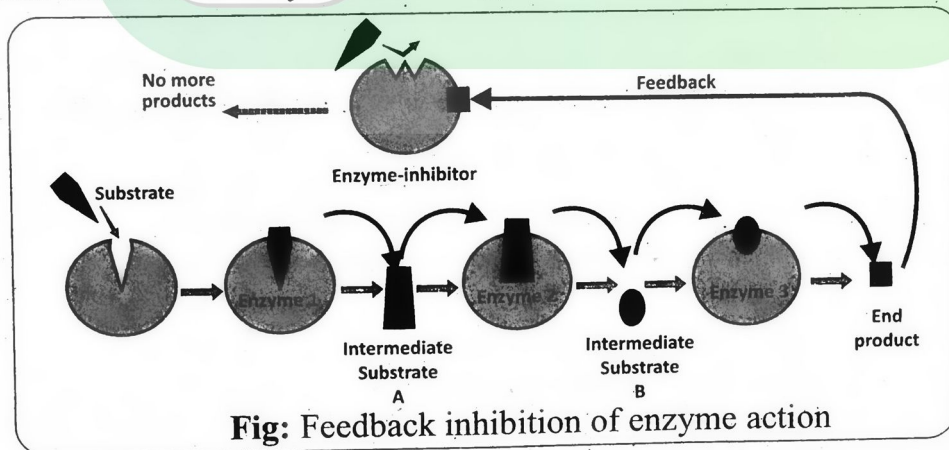
Enzyme inhibition is crucial in various biological processes.

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

Q10. Explain feedback inhibition of enzymes.

Ans. Definition: Feedback inhibition is the phenomenon where the product of a process controls the process itself, oftentimes limiting the production of more products.

Feedback Inhibition of Enzymes: In metabolic pathways, the product of one reaction becomes the substrate for the 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.

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.

CLASSIFICATION OF ENZYMES

Q11. Classify enzymes on the basis of reactions catalyzed.

Ans. 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 to removal of groups (e.g., CO₂, NH₂ etc.) from substrates.

For example, **pyruvate decarboxylase** removes CO₂ 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, like DNA polymerase join nucleotides.

Q12. Classify enzymes on the basis of substrates. Give examples of enzymes naming according to substrates.

Ans. 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

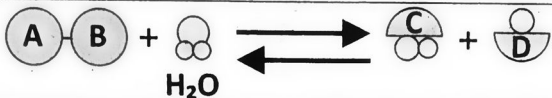
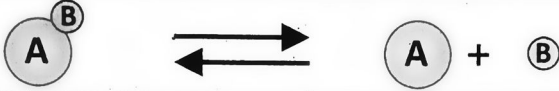
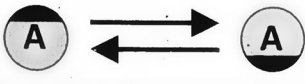
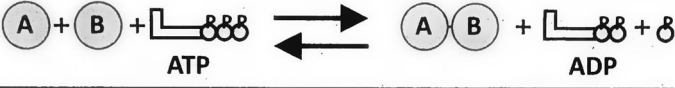
- Amylase** acts upon starch or glycogen and breaks them into maltose.
- Cellulase** breaks cellulose into **cellobiose** (a disaccharide) or glucose.
- Maltase** breaks down maltose into glucose.
- Sucrase** breaks sucrose into glucose and fructose.
- Lactase** breaks lactose into glucose and galactose.

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.

Q13. Classify enzymes in form of table on the basis of reactions they catalyze. Also write their important subclasses.

Ans. Enzymes are classified into following groups on the basis of reaction they catalyze.

Class	Reaction type	Important Subclasses
1. Oxidoreductases		Dehydrogenases Oxidases Reductases
2. Transferases		Phospho-transferases Amino-transferases

3. Hydrolyses		Acyl-transferases Peptidases Lipases Glycosidases
4. Lyases		Decarboxylases Aldolases Synthases
5. Isomerases		Epimerases Mutases <i>cis trans</i> isomerases
6. Ligases		C-C ligases C-O ligases C-N ligases



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