CHAPTER-3

ENZYMES

Science Titbits

During the early nineteenth century, two French chemists, **Payen** and **Persoz** grounded up barley seeds in water to make a crude mixture that would digest starch. They gave the name **diastase** whatever it was that digested the starch.

Science Titbits

How are enzymes formed? Enzymes are proteins, so they are formed as per message or base sequence in DNA. Enzymes are synthesized by living cells but they retain their catalytic action even when extracted from cells, i.e., they can act in vitro. These days' enzymes are also being produced by recombinant DNA technology

Critical Thinking

Industrial pollution can change the pH of a pond, lake or river to make the water more acidic. How can this affect the metabolic pathways of the plants that live in water?

Ans: Acidic pH is much harmful for the metabolic pathways of animal and plants Different enzymes of plants are adopted at certain pH values. Change in pH adversely affects the functioning of these enzymes. It in turn affects the metabolic pathway controlled by these enzymes.

Science Titbits

Penicillin blocks the active site of an enzyme unique to bacteria. When penicillin is taken, bacteria die but human are unaffected.

Teacher's Point

Teachers would guide the students to construct and interpret graphs based on data about the effect of temperature, enzyme concentration and substrate concentration on the rate of enzyme action.

Critical Thinking

Suggest why substrate concentration has no effect on non-competitive inhibition?

Ans: **Non-competitive inhibitors** bind to the other sites, may **not** be active site, and reduces the activity but does **not affect** the binding of the **substrate**. Therefore, the extent of **inhibition** depends on the **concentration** of the **substrate**

OR

In non-competitive inhibition substrates do not compete with inhibitor. Here inhibitor molecules bind to enzyme other than active site and makes the enzyme inactive. Here active site is free but it cannot attache substrate molecules due to change in its shape. Hence increase in concentration of substrate has no effect on non-competitive inhibition

Skills: Analyzing

- **Identify the competitive and non-competitive inhibitors from the given list of chemical (consult any book of Biochemistry or Enzymology).**

Ans: **Competitive inhibitors:**

Antibodies, antimetabolites, penicillin, iodoacetate, melonate, CoA (high concentration)

Non-competitive inhibitors:

Acetaldehyde, Di-Isopropyl fluorophosphate (DFP- nerve gas), mercury, silver, copper, cyanide

Science Titbits

How are enzymes named?

(a) Enzymes are named by adding "ase" to the name of substrate they act, e.g. proteases, lipases etc. (b) Enzymes are named according to the types of reaction they catalyse, e.g. oxidases, reductases etc. (c) Enzymes are named by taking into consideration both the substrate acted upon and the type of reaction catalysed, e.g. DNA- polymerase, (d) Some enzymes are named as per substance synthesized, e.g. rhodonase catalyses synthesis of rhodonate from hydrochloric acid and sodium thiosulphate

Science, Technology and Society Connections

- **List the diagnostic uses of enzymes.**

(a) **Aldolase:** progressive muscular dystrophy, viral hepatitis and advanced cancer of the prostate

(b) **Creatine Phosphokinase:** damage to muscle cells.

(c) **Gamma-gSutarnyl Transpeptidase:** in assessing liver function.

(d) **Lactic Dehydrogenase:** in differentiating heart attack, anemia, lung injury, or liver disease

(e) **Lipase:** Damage to the pancreas.

Science, Technology and Society Connections

♦ **Venoms as enzyme inhibitors**

Snake venom is highly modified saliva that is produced by special glands of certain species of snakes. Snake venom is a combination of many toxins (proteins) and different enzymes, use for the purposes like increasing the prey's uptake of toxins. Snake venom inhibits cholinesterase to make the prey lose control of its muscles. Venom is an inhibitor for an essential enzyme cytochrome oxidase in the cells. There are three distinct type of venom that act on the body differently.

(1) Hemotoxic venoms act on the heart and cardiovascular system.

(2) Neurotoxic venom acts on the nervous system and brain.

(3) Cytotoxic venom has a localized action at the site of the bite. Venom occupies the active site of the enzyme or combining with the iron which may present in the prosthetic group or which may be required as an enzyme activator.

Activity

1. Performing of chemical test to demonstrate that enzymes are proteins.

2. Performing amylase test on starch with boiled amylase and un-boiled amylase in separate test tubes and confirmation through iodine test.

EXERCISE

MCQs

1. Select the correct answer.

(i) The catalytic activity of an enzyme is restricted to its small portion called

(A) active site (B) passive site
(C) regulation site (D) allosteric site

(ii) Which of the following has a coenzyme activity?

(A) NAD^+ (B) Ca^{++}
(C) both "a" and "b" (D) none of them

(iii) Non-competitive inhibitors react with enzymes at:

(A) active site (B) allosteric site
(C) both "a" and "b" (D) none of them

(d) **Lactic Dehydrogenase:** in differentiating heart attack, anemia, lung injury, or liver disease

(e) **Lipase:** Damage to the pancreas.

Science, Technology and Society Connections

♦ **Venoms as enzyme inhibitors**

Snake venom is highly modified saliva that is produced by special glands of certain species of snakes. Snake venom is a combination of many toxins (proteins) and different enzymes, use for the purposes like increasing the prey's uptake of toxins. Snake venom inhibits cholinesterase to make the prey lose control of its muscles. Venom is an inhibitor for an essential enzyme cytochrome oxidase in the cells. There are three distinct type of venom that act on the body differently.

(1) Hemotoxic venoms act on the heart and cardiovascular system.

(2) Neurotoxic venom acts on the nervous system and brain.

(3) Cytotoxic venom has a localized action at the site of the bite. Venom occupies the active site of the enzyme or combining with the iron which may present in the prosthetic group or which may be required as an enzyme activator.

Activity

1. Performing of chemical test to demonstrate that enzymes are proteins.

2. Performing amylase test on starch with boiled amylase and un-boiled amylase in separate test tubes and confirmation through iodine test.

EXERCISE

MCQs

1. Select the correct answer.

(i) The catalytic activity of an enzyme is restricted to its small portion called

(A) active site (B) passive site
(C) regulation site (D) allosteric site

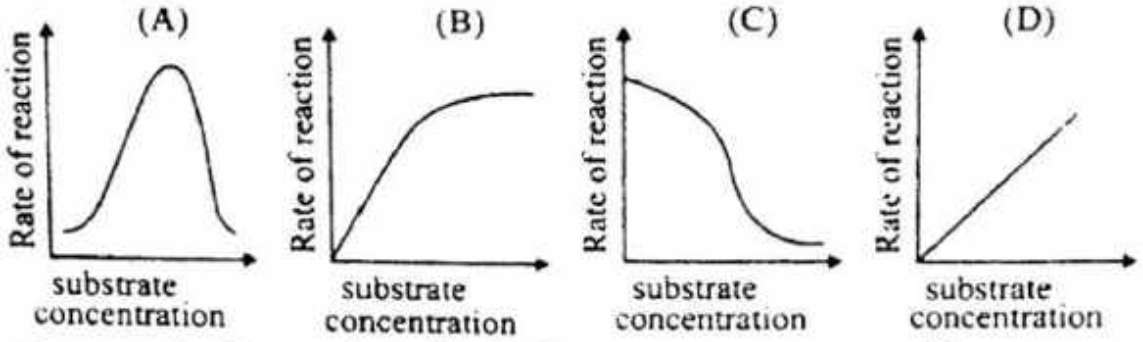
(ii) Which of the following has a coenzyme activity?

(A) NAD^+ (B) Ca^{++}
(C) both "a" and "b" (D) none of them

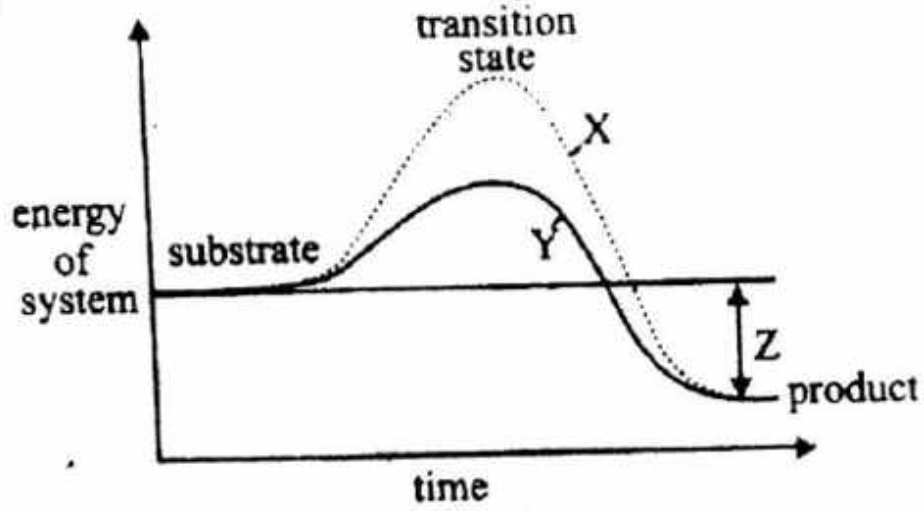
(iii) Non-competitive inhibitors react with enzymes at:

(A) active site (B) allosteric site
(C) both "a" and "b" (D) none of them

(iv) **Which graph shows the expected relationship between enzyme activity and substrate concentration?**



(v) **The graph shows the effect of an enzyme on a reaction.**



Which combination identifies X, Y and Z?

	X	Y	Z
A	catalyzed reaction	uncatalyzed reaction	activation energy
B	catalyzed reaction	uncatalyzed reaction	energy lost during reaction
C	uncatalyzed reaction	catalyzed reaction	energy gained by product
D	uncatalyzed reaction	catalyzed reaction	overall energy change

(vi) **Combination of apoenzyme and coenzyme produces**

(A) prosthetic group (B) holoenzyme
(C) enzyme (D) isoenzyme

(vii) **The specificity of enzyme is due to their**

(A) surface configuration (B) pH
(C) hydrogen bonding (D) high molecular weight

(viii) **An essential feature of a competitive inhibitor is its ability to**

(A) activate an operator gene
(B) combine with prosthetic group
(C) modify a substrate
(D) occupy an active site

(ix) **The reaction rate of salivary amylase with starch decreases as the concentration of chloride ions is reduced. Which of the following describe the role of the chloride ions?**

(A) allosteric inhibitors (B) cofactors
(C) coenzyme (D) competitive inhibitor

(x) How does an enzyme increase the rate of a reaction?

(A) by bringing the reacting molecules into precise orientation
(B) by increasing the rate of random collisions of molecules
(C) by shifting the point of equilibrium of the reaction
(D) by supplying the energy required to start the reaction

(xi) Many enzymes are secreted in inactive form to protect

(A) cell proteins (B) mitochondria
(C) cell membrane (D) cell DNA

(xii) Erypsin is an example of?

(A) carbohydrases (B) proteases
(C) lipases (D) nucleases

(xiii) Ribozymes consist of:

(A) only protein (B) protein + none protein part
(C) only RNA (D) none of them

Answers

(i) A	(ii) A	(iii) B	(iv) B	(v) C
(vi) B	(vii) A	(viii) D	(ix) B	(x) D
(xi) A	(xii) B	(xiii) C		

SHORT QUESTIONS

2. Write three Properties that are common to all enzymes.

Ans: The enzymes are organic catalysts. However, they have many properties of inorganic catalyst like

(i) enzymes increase the speed of chemical reaction, by 10^6-10^{14} times faster than the rate of the uncatalysed reaction.

(ii) Enzymes are highly sensitive to pH and temperature changes of the system.

(iii) Some enzymes require co-factor for proper activity.

(iv) Enzymes lower the need of activation energy.

OR (Second Answer)

1) They are made up of protein. **2)** They do not used up in reaction.
3) They speed up the reaction. **4)** They are specific in action.

3. What are ribozymes?

Ans: Ribozymes:

Ribozymes are the enzymes which consist of RNA and are found in ribosomes.

Example:

For example, peptidyl transferase is a ribozyme which controls polypeptide elongation during translation process.

4. What is the structure of enzyme?

Ans: Structure of Enzyme:

With exception of ribozymes, all the enzymes are globular proteins which are made up of one or more polypeptides.

(x) How does an enzyme increase the rate of a reaction?

(A) by bringing the reacting molecules into precise orientation
(B) by increasing the rate of random collisions of molecules
(C) by shifting the point of equilibrium of the reaction
(D) by supplying the energy required to start the reaction

(xi) Many enzymes are secreted in inactive form to protect

(A) cell proteins (B) mitochondria
(C) cell membrane (D) cell DNA

(xii) Erypsin is an example of?

(A) carbohydrases (B) proteases
(C) lipases (D) nucleases

(xiii) Ribozymes consist of:

(A) only protein (B) protein + none protein part
(C) only RNA (D) none of them

Answers

(i) A	(ii) A	(iii) B	(iv) B	(v) C
(vi) B	(vii) A	(viii) D	(ix) B	(x) D
(xi) A	(xii) B	(xiii) C		

SHORT QUESTIONS

2. Write three Properties that are common to all enzymes.

Ans: The enzymes are organic catalysts. However, they have many properties of inorganic catalyst like

(i) enzymes increase the speed of chemical reaction, by 10^6-10^{14} times faster than the rate of the uncatalysed reaction.

(ii) Enzymes are highly sensitive to pH and temperature changes of the system.

(iii) Some enzymes require co-factor for proper activity.

(iv) Enzymes lower the need of activation energy.

OR (Second Answer)

1) They are made up of protein. **2)** They do not used up in reaction.
3) They speed up the reaction. **4)** They are specific in action.

3. What are ribozymes?

Ans: Ribozymes:

Ribozymes are the enzymes which consist of RNA and are found in ribosomes.

Example:

For example, peptidyl transferase is a ribozyme which controls polypeptide elongation during translation process.

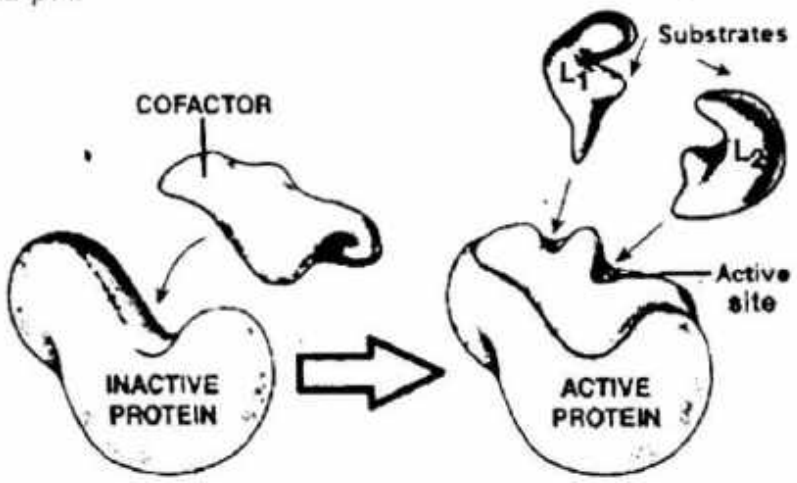
4. What is the structure of enzyme?

Ans: Structure of Enzyme:

With exception of ribozymes, all the enzymes are globular proteins which are made up of one or more polypeptides.

Structure of Enzyme:

Many enzymes consist of a protein and a non-protein (called the cofactor) The proteins in enzymes are usually **globular**. The intra- and intermolecular bonds that hold proteins in their secondary and tertiary structures are disrupted by changes in temperature and pH.



Structure of enzyme

5. What is active site of enzyme? Write its shape and function.

Ans: Active Site:

In biology, the **active site** is the region of an **enzyme** where substrate molecules bind and undergo a chemical reaction. The **active site** consists of residues that form temporary bonds with the substrate (binding **site**) and residues that catalyse a reaction of that substrate (catalytic **site**).

Shape of Enzymes and Components of an Active Site:

Majority of enzymes which are protein in nature can have molecular weights ranging from about 10,000 to over 1 million. Such enzymes have tertiary or quaternary structures.

Functions of an Active Site:

The catalytic activity of an enzyme is located in its **active site** which is a specific charge bearing, three dimensional cavity. The substrate (the reactant which is to be converted into product) molecule is attached to the active site by non-covalent interactions like hydrogen bonding and hydrophobic interactions

Components of an Active Site:

Active site consists of 3-12 amino acids which may be scattered in the polypeptide but are brought together in a particular fashion due to secondary and tertiary folding of the protein molecule, e.g., the active site for aldolase consists of glycine, histidine, and alanine amino acids.

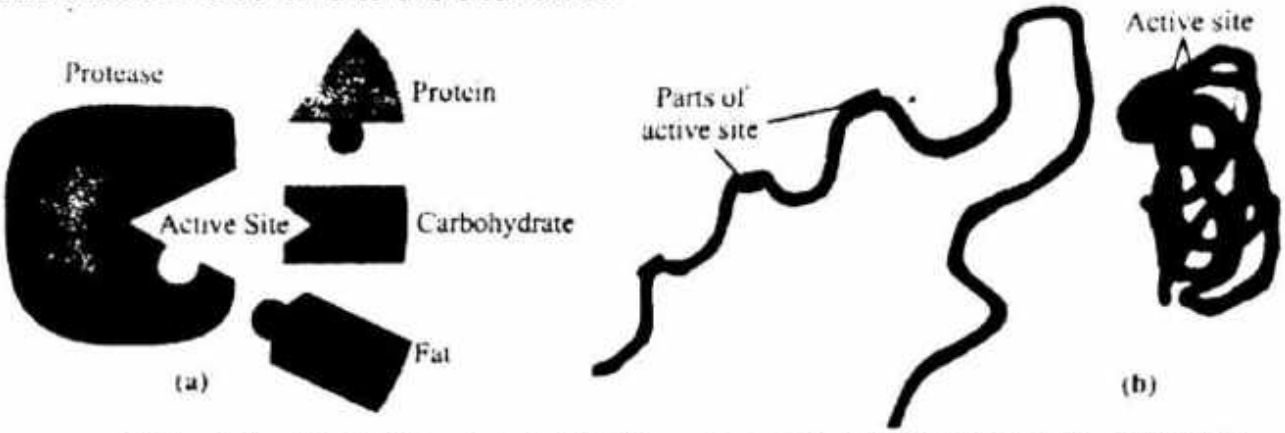
Functional Regions of Active Site:

An active site consists of two functional regions, i.e., binding site and catalytic site.

Some amino acids have active site which makes bonds with substrate constitute the **binding site** while the other amino acids which cause conversion of substrate into product (catalysis) constitute the **catalytic site.**

Shape of an Active Site:

The shape of active site is designed according to the substrate therefore only a particular substrate can attach to the active site, however, sometime related substrate can also bind to the active site.



Active Site: (a) Which substrate fits the active site? (b) Grouping of amino acids of a polypeptide during the formation of tertiary structure to produce an active site.

6. What is cofactor? Explain.

Ans: Cofactor:

Enzymes consist of a non-protein called the **cofactor**.

OR (Second Answer)

A **cofactor** is a non-protein chemical compound or metallic ion that is required for a protein's **biological** activity to happen. These proteins are commonly enzymes, and **cofactors** can be considered "helper molecules" that assist in biochemical transformations.

7. Explain the enzyme pepsin which does not require cofactor.

Ans: The enzymes which do not require cofactor can also show active and inactive states.

Pepsin:

Pepsin is an example of such enzyme. It is secreted by gastric gland from stomach wall in an inactive state, the pepsinogen. In this state, it has an additional polypeptide fragment attached to its active site which does not allow the binding of substrate, hence it remains inactive. When pepsinogen is exposed in HCl (as in stomach cavity) the additional polypeptide fragment is removed and as a result inactive (apoenzyme) pepsinogen is changed into its active (holoenzyme) form, the **pepsin**.

8. Explain inorganic cofactor.

Ans: Inorganic Cofactor:

The inorganic cofactors are different metallic ions such as Fe^{++}, Mg^{++}, Cu^{++}, Zn^{++}, etc. These are only attached to the enzymes when substrate gets bind i.e., they are detachable cofactors. Such cofactors are also called **activators**.

$$\text{Glucose + ATP} \xrightarrow{\text{Hexokinase + } Mg^{+2}} \text{Glucose-6-phosphate + ADP}$$

9. Explain organic cofactor.

Ans: Organic Cofactor:

The organic cofactors are either co-enzymes or prosthetic groups. The coenzymes are the derivatives of vitamins.

For example ATP, NAD^+, FAD^+ are common coenzymes. Like inorganic cofactors they are also attached to the enzymes when substrate gets bind i.e., they are also detachable cofactors.

$$\text{Ethyl alcohol} \xrightarrow{\text{Alcohol dehydrogenase + NAD}} \text{Acetaldeevde + } NADH_2$$

10. What is prosthetic group? Give an example.

Ans: Prosthetic Group:

A **prosthetic group** is covalently bonded part of an enzyme which is permanently attached to enzyme and does not detach after the completion of reaction.

Example: An iron containing porphyrin ring attached to some enzymes like cytochromes is the example of prosthetic group

11. What is the mechanism of enzyme action?

Ans: Mechanism of Enzyme Action:

In an enzyme-catalysed reaction, the substrate first binds to the active site of the enzyme to form an **enzyme-substrate (ES) complex,** then the substrate is converted into **product** while it is attached to the enzyme (EP **complex),** and finally the product is released, thus allowing the enzyme to start all over again.

$$\underset{\text{enzyme}}{E} + \underset{\text{substrate}}{S} \longrightarrow \underset{\text{enzyme-substrate complex}}{ES} \longrightarrow \underset{\text{enzyme-product complex}}{EP} \longrightarrow \underset{\text{enzyme}}{E} + \underset{\text{product}}{P}$$

Mechanism of Enzyme Action

Actually, the enzyme can make the local conditions inside the active site quite different from those outside (such as pH, water concentration, charge), so that the reaction is more likely to happen. For example, if a substrate is to be split, a bond might be stretched by the enzyme, making it more likely to break.

12. What is the role of free energy of activation in a chemical reaction?

Ans: Role of free energy of activation in a chemical reaction:

About 1,000 chemical reactions are being carried out in a cell at any time Energy of activation required for such a large number of reactions cannot be provided by living system.

The living system works in isothermal condition; the excited state of molecules or reactants is achieved by biochemical process. Enzyme (E) reacts with reactant (A) to form an AE transitional complex. The energy level of AE complex reaches to the energy level of reactant B. AE complex then reacts with reactant B to form AB and enzyme (E) is released.

$$A + E \longrightarrow AE\ Complex + B \longrightarrow AB + E$$

Enzyme does decrease the energy of activation by changing energy dependent process to energy independent process. Thus the energy of activation is energy required to break the existing bonds and begin the reaction. An enzyme greatly reduces the activation energy necessary to initiate a chemical reaction

13. List the external conditions which affect rate of enzyme reaction.

Ans: Factors Affecting the Rate of Enzymatic Action:

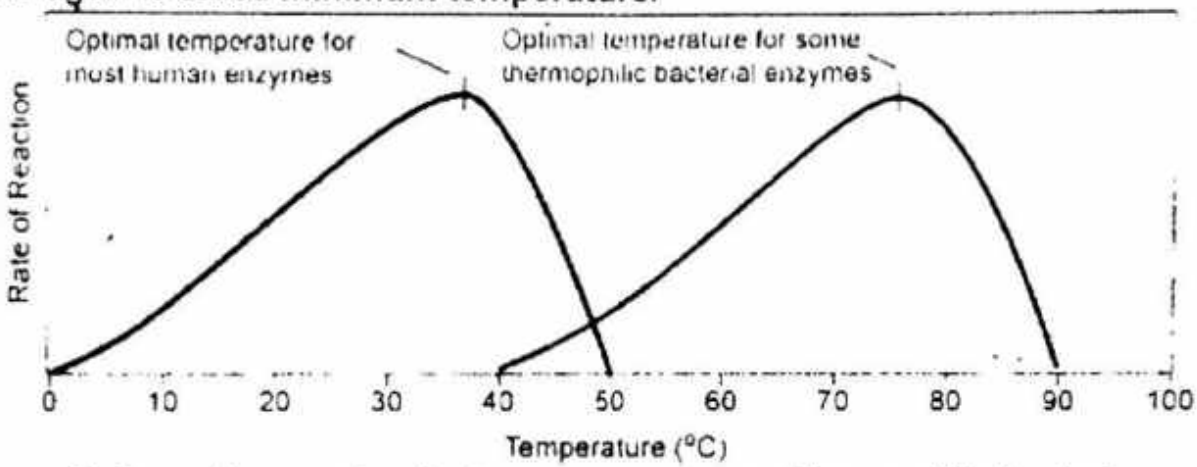
The rate of enzymatic reaction is measured by the amount of substrate changed or amount of product formed, during a period of time.

The external conditions which affect rate of enzyme reactions are

i. Temperature
ii. pH
iii. Concentration of enzyme
iv. Substrate concentration

14. Compare the optimum temperatures of enzymes of human and thermophilic bacteria.

Ans: All human enzymes have a optimum temperature of about 37-38°C, but bacteria living in hot springs may have an optimum temperature of 70°C or higher. Such enzymes have been used in biological washing powders for high temperature washes. If temperature is reduced to near or below freezing point, enzymes are inactivated, not denatured. They will regain their catalytic influence when higher temperatures are restored. This temperature where an inactive enzyme becomes active again is called **minimum temperature.**



Optimum temperature for human enzymes and thermophilic bacteria

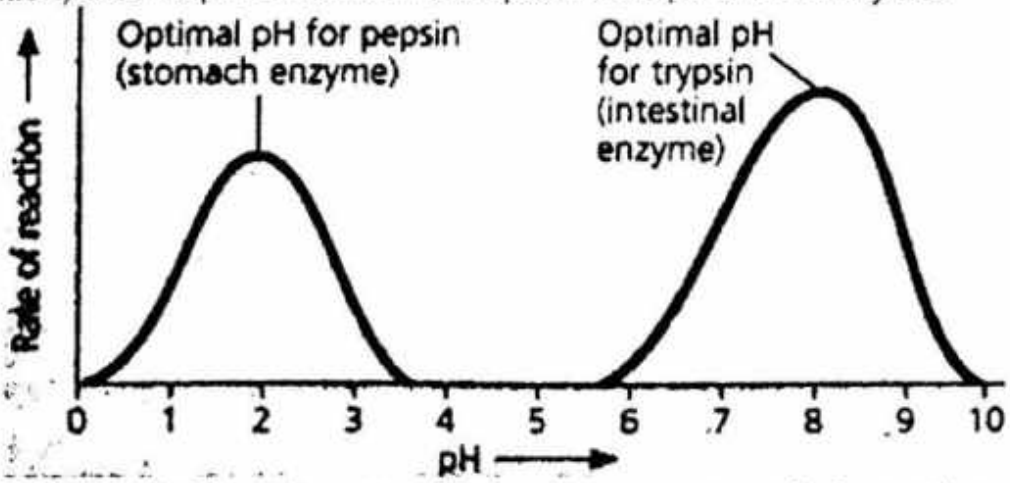
15. Describe the range of pH at which human enzymes function.

Ans: pH:

Every enzyme functions most effectively over a particular pH range. This narrow range of pH at which the maximum rate of reaction is achieved is called **optimum pH.**

Effect of pH:

Enzyme conformation is sensitive to pH changes because pH influences the charges on the amino acid side chains that are involved in maintaining tertiary and quaternary structure of enzyme. Slight change in optimum pH of an enzyme causes ionization of amino acid of the enzyme therefore, they become inactive temporarily. On the other hand, extreme changes in optimum pH alter the ionic charge of the acidic and basic groups of enzyme and therefore disrupt the ionic bonding (denaturation) that helps to maintain the specific shape of the enzyme.



Effect of pH on the rate of an enzyme – controlled reaction

The optimum pH values for most enzymes fall in the range of pH 6-8, but there are exceptions. Some enzymes like papain from green papaya act both in acidic and alkaline media. Protein digesting enzyme pepsin is active in acidic medium at pH 2 and trypsin is inactive at this pH but shows maximum activity in alkaline medium at pH 8

16. What are enzyme inhibitors? Name the molecules which act as enzyme inhibitors.

Ans: Enzyme Inhibition:

The phenomenon in which an enzyme fails to catalyze a reaction is called **enzyme inhibition** and the molecules which react with enzyme but are not converted into desired products are called **enzyme inhibitors**.

In general, the enzyme inhibition is a normal part of the regulation of enzyme activity within cells but sometimes when external factors cause enzyme inhibition; it may become dangerous for life.

Molecules Act as Enzyme Inhibitors:

The molecules which act as inhibitors include poisons, cyanides, antibodies, antimetabolites, penicillin, sulpha drugs etc. Inhibition may be competitive or noncompetitive.

17. What is the importance of competitive enzyme inhibitors?

Ans: Importance of Competitive Enzyme Inhibitors:

The **importance** of competitive inhibitors is:

(a) It supports lock and key hypothesis.

(b) It shows that substances which are similar to substrate are not acted upon by enzymes.

(c) Competitive inhibitors are used as drugs in the control of bacterial pathogens. Antibiotics known as sulphonamides are used to combat bacterial infection.

18. Describe cyanides as irreversible non-competitive inhibitor.

Ans: Irreversible Non-Competitive Inhibitor:

An irreversible non-competitive enzyme inhibitor destroys enzyme by altering its shape so that the substrate cannot bind to the active site. The examples of irreversible non-competitive inhibitors include cyanides and salts of heavy metals.

Cyanides:

Cyanides are potent poisons of living organism because they can kill an organism by inhibiting cytochrome oxidase essential for cellular respiration. They block the action of these enzymes by combining with iron which may be present in the prosthetic group.

19. Describe ions of heavy metals as irreversible non-competitive inhibitor.

Ans: Irreversible Non-Competitive Inhibitor:

An irreversible non-competitive enzyme inhibitor destroys enzyme by altering its shape so that the substrate cannot bind to the active site. The examples of irreversible non-competitive inhibitors include cyanides and salts of heavy metals.

Ions of Heavy Metals:

Ions of heavy metals such as mercury, silver and copper (Hg^{++}, Ag^{+}, and Cu^{++}) combine with thiol (-SH) groups in the enzyme breaking the disulphide bridges. These bridges are important in maintaining tertiary structure. When these bridges are broken, the enzyme becomes denatured and inactive.

20. List the diagnostic uses of enzymes.

Ans: Diagnostic uses of enzymes:

(a) Aldolase: progressive muscular dystrophy, viral hepatitis and advanced cancer of the prostate

(b) Creatine Phosphokinase: damage to muscle cells

(c) Gamma-gSutarnyl Transpeptidase: in assessing liver function.

(d) Lactic Dehydrogenase: in differentiating heart attack, anemia, lung injury, or liver disease.

(e) Lipase: Damage to the pancreas.

21. Describe venoms as enzyme inhibitors.

Ans: Venoms as enzyme inhibitors:

Snake venom is highly modified saliva that is produced by special glands of certain species of snakes. Snake venom is a combination of many toxins (proteins) and different enzymes use for the purposes like increasing the prey's uptake of toxins. Snake venom inhibits cholinesterase to make the prey lose control of its muscles. Venom is an inhibitor for an essential enzyme cytochrome oxidase in the cells.

There are three distinct type of venom that act on the body differently.

(1) Hemotoxic venoms act on the heart and cardiovascular system.

(2) Neurotoxic venom acts on the nervous system and brain.

(3) Cytotoxic venom has a localized action at the site of the bite. Venom occupies the active site of the enzyme or combining with the iron which may present in the prosthetic group or which may be required as an enzyme activator

22. Describe/Explain briefly:

carbonic anhydrase, active site, holoenzyme, apoenzyme, pepsinogen, cofactors, prosthetic group, non-regulatory enzyme, allosteric enzymes, energy of activation, enzyme inhibitors, reversible inhibition, reversible non-competitive enzyme, irreversible non-competitive enzyme, oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, proteases, lipases, carbohydrases, nucleases.

Ans: Carbonic Anhydrase:

Carbonic anhydrase is an enzyme that assists rapid inter-conversion of carbon dioxide and water into **carbonic** acid, protons and bicarbonate ions. This enzyme was first identified in 1933, in red blood cells of cows. Since then, it has been found to be abundant in all mammalian tissues, plants, algae and bacteria. This ancient enzyme has three distinct classes (called alpha, beta and gamma carbonic anhydrase).

OR

Carbonic anhydrase which can add O_2 in haemoglobin as well as can control the formation of carbonic acid and bicarbonates in blood.

Active Site:

In biology, the **active site** is the region of an **enzyme** where substrate molecules bind and undergo a chemical reaction. The **active site** consists of residues that form temporary bonds with the substrate (binding **site**) and residues that catalyse a reaction of that substrate (catalytic **site**).

OR

The **active site** refers to the specific region of an **enzyme** where a substrate binds and catalysis takes place or where chemical reaction occurs. It is a structural element of protein that determines whether the protein is functional when undergoing a reaction from an **enzyme**.

Holoenzyme:

An enzyme which requires a cofactor becomes active only if the cofactor is combined with it. Such an active enzyme is called **holoenzyme**.

Apoenzyme:

If the cofactor is not available the remaining protein part of enzyme becomes catalytically inactive and is called **apoenzyme**.

Pepsinogen:

Pepsin is an example of such enzyme. It is secreted by gastric gland from stomach wall in an inactive state, the pepsinogen. In this state, it has an additional polypeptide fragment attached to its active site which does not allow the binding of substrate, hence it remains inactive. When pepsinogen is exposed in HCl (as in stomach cavity) the additional polypeptide fragment is removed and as a result inactive (apoenzyme) pepsinogen is changed into its active (holoenzyme) form, the **pepsin**.

OR

Pepsinogen, is released by the chief cells in the stomach wall, and upon mixing with the hydrochloric acid of the gastric juice, **pepsinogen** activates to become **pepsin**.

Cofactor:

A **cofactor** is a non-protein chemical compound or metallic ion that is required for a protein's **biological** activity to happen. These proteins are commonly enzymes, and **cofactors** can be considered "helper molecules" that assist in biochemical transformations.

Prosthetic Group:

A **prosthetic group** is covalently bonded part of an enzyme which is permanently attached to enzyme and does not detach after the completion of reaction.

Example: An iron containing porphyrin ring attached to some enzymes like cytochromes is the example of prosthetic group.

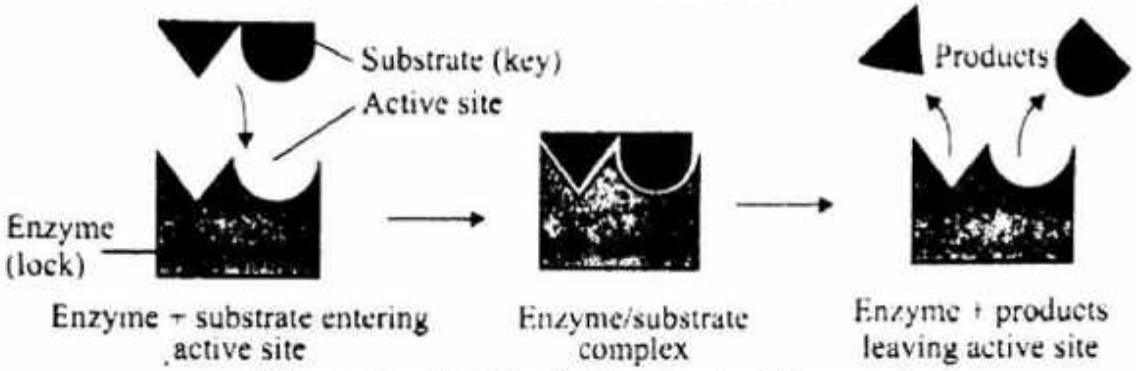
Non-regulatory Enzyme:

The mechanism of enzyme action can be explained with the help of two different models. **Emil Fischer** proposed **Lock and key model** in 1894.

According to this model the active site of the enzyme has definite shape and rigid structure. Shape of active site is complementary to the shape of substrate. Therefore, a particular substrate can only bind to the active site. The active site remains unchanged during or after the reaction. Lock and key model assumes that like a particular key opens a particular lock, a specific **enzyme (key)** acts upon a particular **substrate (lock).** Actually, the notched portion of the key is equivalent to the active site on the enzyme. It reflects that enzymes are highly specific in their action and each enzyme can carry out only one particular reaction. The enzymes, which work according to this model, are called **non-regulatory enzymes**.

However, this model is exercised by a very small number of enzymes, for example sucrase, maltase and etc. The ability of enzyme to catalyze one specific reaction is perhaps its most significant property. Although, many enzymes show a broad range of specificity towards the substrate they catalyze. When one enzyme can catalyze only one substrate and essentially no others it is called **absolute** specificity e.g. urease.

Urea + Water $\xrightarrow{\text{Urease}}$ Ammonia + Carbon dioxide



Fisher's "Lock & Key" Hypothesis of Enzyme Action

Allosteric Enzymes:

Koshland proposed **Induced fit model** in 1959.

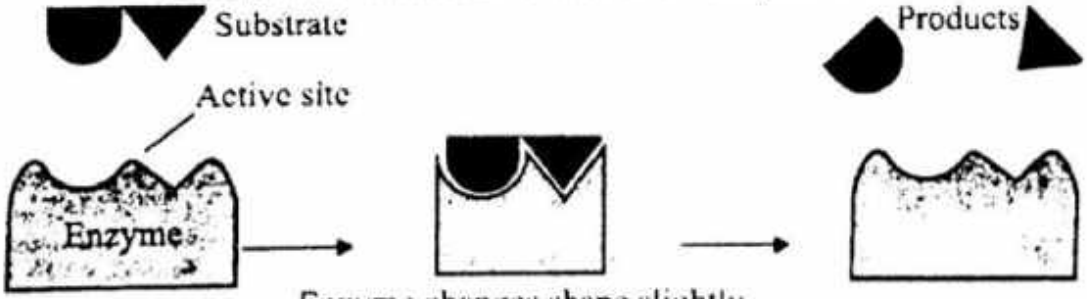
According to this model the active site is flexible; therefore, it is modified as the substrate interacts with enzyme. The amino acids, which makeup the active site are molded into a precise shape which enables the enzyme to perform its catalytic function more effectively. The change which is induced in the shape of active site is responsible for the conversion of substrate into product. As the reaction is completed the active site regains its original shape. This is the flexibility of active site which allows more than one type of related substrates to be attached on active site and therefore, an enzyme can carry out more than one type of related reactions The example is carbonic anhydrase which can add O_2 in haemoglobin as well as can control the formation of carbonic acid and bicarbonates in blood.

Enzymes, which follow the induced fit mechanism, are called **regulatory** or **allosteric enzymes** for example hexokinase.

$Hb + 4O_2 \xrightarrow{Carbonic\ anhydrase} Hb4O_2$ (Oxyhaemoglobin)

$CO_2 + H_2O \xrightarrow{Carbonic\ anhydrase} H_2CO_3 \xrightarrow{Carbonic\ anhydrase} H^+ + HCO^-$

Koshland's "Induced Fit" model of enzyme action



Enzyme changes shape slightly as substrate enters active site, making the fit more precise

Koshland's Induced Fit Hypothesis

Energy of Activation:

Molecules do not react with one another unless they are activated in some way. The energy that must be added to cause molecules to react with one another is called the **energy of activation.**

Enzyme Inhibitors:

The phenomenon in which an enzyme fails to catalyze a reaction is called **enzyme inhibition** and the molecules which react with enzyme but are not converted into desired products are called **enzyme inhibitors**.

Reversible Inhibition:

A type of enzyme inhibition in which enzyme activity is blocked by the presence of a chemical that compete with the substrate for binding to the active site is called **competitive inhibition.** Usually a competitive inhibitor is structurally similar to the normal substrate and so fits into the active site of the enzyme. However, it is not similar enough to substitute fully for the normal substrate in the chemical reaction and the enzyme cannot attack it to form reaction products. Competitive inhibition is usually temporary, and the inhibitor eventually leaves the enzyme hence it is also called **reversible inhibition.**

This means that the level of inhibition depends on the relative concentrations of substrate and inhibitor, since they are competing for places in enzyme active sites. Therefore, if the concentration of the substrate is increased relative to the concentration of the inhibitor, the active site will usually be occupied by the substrate. An example of inhibitor is **malonate.** Succinate dehydrogenase that catalyzes the formation of fumarate from succinate is competitively inhibited by malonate.

Reversible Non-Competitive Enzyme:

Reversible non-competitive enzyme inhibitors work not by preventing the formation of enzyme-substrate complexes, but by preventing the formation of enzyme-product complexes. So they prevent the substrate to be converted into product.

Example: Feedback inhibition is an example of reversible non-competitive enzyme inhibition.

Irreversible Non-Competitive Enzyme:

On the other hand, an irreversible non-competitive enzyme inhibitor destroys enzyme by altering its shape so that the substrate cannot bind to the active site.

Example: The examples of irreversible non-competitive inhibitors include cyanides and salts of heavy metals.

Oxidoreductases:

These enzymes catalyze oxidation/reduction of their substrate and act by removing or adding electron or H^+ ions from or to the substrate.

Example: Cytochrome oxidase oxidizes cytochrome.

Transferases:

These enzymes catalyze the transfer of specific functional group other than hydrogen from one substrate to another. The chemical group transferred in the process is not in a free state.

Example: For example **hexokinase** transfers a phosphate group from ATP to glucose

Hydrolases:

These enzymes bring about the breakdown of large complex organic molecules into smaller ones by adding water (hydrolysis) and breaking the specific covalent bonds.

Examples: Examples are proteolytic enzymes which breakdown proteins into peptones and peptides such as **pepsin, renin** and **trypsin.** Other digestive enzymes that work in digestive tract are also the examples of hydrolases.

Lyases:

These enzymes catalyze the breakdown of specific covalent bonds and removal of groups without hydrolysis.

Example: For example **histidine decarboxylase** breaks the covalent bonds between carbon atoms in histidine forming carbon dioxide and histamine.

Isomerases:

These enzymes bring about intra-molecular rearrangement of atoms in the molecules and thus forming one isomer from another

Example: For example **phosphohexose isomerase** changes glucose 6-phosphate to fructose 6- phosphate.

Ligases:

These enzymes bring about joining together of two molecules. The energy is derived by hydrolysis of ATP.

Example: For example **polymerases** are responsible for linking monomers into a polymer such as DNA or RNA.

Proteases: These enzymes act upon proteins.

Examples are: pepsin and **trypsin** (both digest large polypeptides into small polypeptides or peptones), **aminopeptidases** and **carboxypeptidases** (both digest peptones into dipeptides) and **erypsin** (digest dipeptides into amino acids)

Lipases: These enzymes hydrolyze lipids into fatty acids and glycerols.

Examples: Examples are **pancreatic lipases.**

Carbohydrases:

These enzymes cause breakdown of carbohydrates.

Examples are:

(a) amylase (digest starch or glycogen into maltose)
(b) cellulase (digest cellulose into cellubiose, a disaccharide)
(c) maltase (digest maltose into glucoses)
(d) diastase (acts on starch)
(e) sucrase (digest sucrose into glucose and fructose)
(f) lactase (digest lactose into galactose and glucose)

Nucleases:

These are involved in the breakdown of DNA and RNA.

Examples are:

(a) RN Aases (digest RNA into ribonucleotides)
(b) DNAases (digest DNA Into deoxyribo nucleotides).
(c) AT Pases (cause hydrolysis of ATP in muscles etc.)

23. Define:

(a)	**Metabolism**	**(b)**	**Enzymes**
(c)	**active site of an enzyme**	**(d)**	**cofactor**
(e)	**coenzyme**	**(f)**	**apoenzyme**
(g)	**holeoenzyme**	**(h)**	**prosthetic group**
(i)	**non-regulatory enzymes**	**(j)**	**regulatory enzymes**
(k)	**feedback inhibition**	**(l)**	**energy of activation**
(m)	**reversible inhibitors**	**(n)**	**allosteric site**
(o)	**absolute specificity of enzymes**		

Ans: (a) Metabolism:

The sum of all the chemical reactions going on in a cell is known as **metabolism.**

(b) Enzymes:

Enzymes which are biological catalysts and therefore they speed up the biochemical reaction without being consumed. Without enzymes reactions are possible but they would proceed at very slow speed that will have no significance for life.

(c) Active Site of an Enzyme:

In biology, the **active site** is the region of an **enzyme** where substrate molecules bind and undergo a chemical reaction. The **active site** consists of residues that form temporary bonds with the substrate (binding **site**) and residues that catalyse a reaction of that substrate (catalytic **site**).

(d) Cofactor:

A **cofactor** is a non-protein chemical compound or metallic ion that is required for a protein's **biological** activity to happen. These proteins are commonly enzymes, and **cofactors** can be considered "helper molecules" that assist in biochemical transformations.

(e) Coenzyme:

A coenzyme is an organic non-protein compound that binds with an enzyme to catalyze a reaction. Coenzymes are often broadly called cofactors, but they are chemically different. A coenzyme cannot function alone, but can be reused several times when paired with an enzyme.

(f) Apoenzyme:

If the cofactor is not available the remaining protein part of enzyme becomes catalytically inactive and is called **apoenzyme**.

(g) Holeoenzyme:

An enzyme which requires a cofactor becomes active only if the cofactor is combined with it. Such an active enzyme is called **holoenzyme**.

(h) Prosthetic Group:

A **prosthetic group** is covalently bonded part of an enzyme which is permanently attached to enzyme and does not detach after the completion of reaction. An iron containing porphyrin ring attached to some enzymes like cytochromes is the example of prosthetic group.

(i) Non-regulatory Enzymes:

Lock and key model assumes that like a particular key opens a particular lock, a specific **enzyme (key)** acts upon a particular **substrate (lock).** Actually, the notched portion of the key is equivalent to the active site on the enzyme. It reflects that enzymes are highly specific in their action and each enzyme can carry out only one particular reaction. The enzymes, which work according to this model, are called **non-regulatory** enzymes.

(j) Regulatory Enzymes:

A **regulatory enzyme** is an **enzyme** in a biochemical pathway which, through its responses to the presence of certain other biomolecules, regulates the pathway activity. This is usually done for pathways whose products may be needed in different amounts at different times, such as hormone production.

OR

"In a multi-step enzymatic process, there will be one enzyme which will be responsible for the overall rate of that process". This critical rate limiting enzyme is called the regulatory enzyme. Regulatory enzyme shows enhanced or decreased catalytic activities in response to other molecules (signals) in the cells.

(k) Feedback Inhibition:

The activity of almost every enzyme in a cell can be regulated by its product. When the activity of an enzyme is inhibited by its own product, it is called **feedback inhibition**.

(l) Energy of Activation:

Molecules do not react with one another unless they are activated in some way. The energy that must be added to cause molecules to react with one another is called the **energy of activation.**

(m) Reversible Inhibitors:

A type of enzyme inhibition in which enzyme activity is blocked by the presence of a chemical that compete with the substrate for binding to the active site is called **competitive inhibition.** Usually a competitive inhibitor is structurally similar to the normal substrate and so fits into the active site of the enzyme. However, it is not similar enough to substitute fully for the normal substrate in the chemical reaction and the enzyme cannot attack it to form reaction products. Competitive inhibition is usually temporary, and the inhibitor eventually leaves the enzyme hence it is also called **reversible inhibition.**

(n) Allosteric Site:

In non-competitive inhibition the inhibitor molecule binds to an enzyme other than active site. The other binding site of enzyme is called **allosteric site**.

(o) Absolute Specificity of Enzymes:

When one enzyme can catalyze only one substrate and essentially no others it is called **absolute specificity** e.g. urease.

24. Write the difference between:

(a) binding site and catalytic site of an enzyme
(b) apoenzyme and holoenzyme
(c) prosthetic group and coenzyme
(d) inorganic cofactor and organic cofactor
(e) lock and key model and Induced fit model of enzyme action
(f) competitive and noncompetitive enzyme inhibitors
(g) reversible non-competitive enzyme inhibitors and irreversible non- competitive enzyme inhibitors.

Ans: (a) binding site and catalytic site of an enzyme:

The **active site** consists of residues that form temporary bonds with the substrate (binding **site**) and residues that catalyse a reaction of that substrate (catalytic **site**). An active site consists of two functional regions, i.e., binding site and catalytic site. Some amino acids have active site which makes bonds with substrate

constitute the **binding site** while the other amino acids which cause conversion of substrate into product (catalysis) constitute the **catalytic site.**

(b) apoenzyme and holoenzyme:

An enzyme which requires a cofactor becomes active only if the cofactor is combined with it. Such an active enzyme is called **holoenzyme**

If the cofactor is not available the remaining protein part of enzyme becomes catalytically inactive and is called **apoenzyme**

OR (Second Answer)

Difference between Holoenzyme and Apoenzyme	
Holoenzyme	**Apoenzyme**
Holoenzyme is an active enzyme consisting of an apoenzyme bound to its cofactor	Apoenzyme is the protein component which lacks its cofactor.
Cofactor:	
Holoenzyme is bound to its cofactor	Apoenzyme is the enzyme component without the cofactor
Activity:	
Holoenzyme is catalytically active	Apoenzyme is catalytically inactive.
Completeness:	
Holoenzyme is complete and can initiate the reaction	Apoenzyme is incomplete and cannot initiate the reaction.
Examples:	
DNA polymerase, RNA polymerase are examples of holoenzym	Aspartate transcarbamoylase is an example for apoenzyme.

(c) prosthetic group and coenzyme:

The organic cofactors are either co-enzymes or prosthetic groups. The coenzymes are the derivatives of vitamins.

For example ATP, NAD^+, FAD^+ are common coenzymes. Like inorganic cofactors they are also attached to the enzymes when substrate gets bind i e they are also detachable cofactors

$$\text{Ethyl alcohol} \xrightarrow{\text{Alcohol dehydrogenase + NAD}} \text{Acetaldeevde + } NADH_2$$

On the other hand a **prosthetic group** is covalently bonded part of an enzyme which is permanently attached to enzyme and does not detach after the completion of reaction. An iron containing porphyrin ring attached to some enzymes like cytochromes is the example of prosthetic group.

OR (Second Answer)

Difference between Prosthetic Group and Coenzyme:	
Prosthetic group	**Coenzyme**
Prosthetic group is a type of a helper molecule which is a nonproteinaceous compound that helps enzymes to perform their functions	Coenzyme is a specific kind of cofactor molecule which is an organic molecule that helps enzymes to catalyze chemical reactions
Bond with Enzymes:	
They bind tightly or covalently with enzymes to aid enzymes.	They bind loosely with the active site of the enzyme to help catalytic function

Composition:	
Prosthetic groups are metal ions, vitamins, lipids, or sugars	Coenzymes are vitamins, vitamin derivatives or nucleotides
Main Function:	
Prosthetic group mainly provides a structural property to the enzyme.	Coenzyme mainly provides a functional property to the enzyme.
Removal from the Enzyme:	
Prosthetic groups cannot be easily removed from the enzymes.	Coenzymes can be easily removed from the enzymes.
Examples:	
Examples include flavin nucleotides and heme.	Examples include AMP, ATP, coenzyme A, FAD, and NAD^+, S-adenosyl methionine

(d) inorganic cofactor and organic cofactor:

The cofactor may be inorganic or organic molecules. The inorganic cofactors are different metallic ions such as Fe^{++}, Mg^{++}, Cu^{++}, Zn^{++}, etc. These are only attached to the enzymes when substrate gets bind i.e., they are detachable cofactors. Such cofactors are also called activators.

$$\text{Glucose + ATP} \xrightarrow{\text{Hexokinase + } Mg^{+2}} \text{Glucose-6-phosphate + ADP}$$

The organic cofactors are either co-enzymes or prosthetic groups. The coenzymes are the derivatives of vitamins.

For example ATP, NAD^+, FAD^+ are common coenzymes Like inorganic cofactors they are also attached to the enzymes when substrate gets bind i.e., they are also detachable cofactors.

$$\text{Ethyl alcohol} \xrightarrow{\text{Alcohol dehydrogenase + NAD}} \text{Acetaldeevde + } NADH_2$$

OR (Second Answer)

	Cofactor (inorganic)	Coenzyme (organic)
Definition	It is a non-protein chemical compounds that are bound tightly or loosely to an enzyme (protein).	It is defined as small, organic, non-protein molecules, which carry chemical groups between enzymes.
Characteristics	These are inorganic substances.	These are organic substances.
Function	It assists in biological transformations.	It aids or helps the function of an enzyme.
Type	They are chemical compounds.	They are chemical molecules.
Bound	It is tightly bound to an enzyme.	It is loosely bound to an enzyme.
Action	They act on catalyst to increase the speed of the reaction.	They act as carries to the enzymes.
Example	Metal Ions like Zn++, K+ and Mg++, etc.	Vitamins, Biotin, Coenzyme A, etc.

(e) lock and key model and Induced fit model of enzyme action:

Lock and Key model of enzyme action	Induced fit model of enzyme action
1. Active site is a single entity.	**1.** Active site is made of two components.
2. There is no separate catalytic group.	**2.** A separate catalytic group is visualized
3. Active site is static.	**3.** In contact with substratum, the buttressing group undergoes conformational change.
4. Development of transition state is not considered.	**4.** It considers the development of transition state before the reactants undergo change.
5. It does not visualize the weakening of substrate bonds.	**5.** Catalytic group is believed to weaken the substrate bonds by nucleophile and electrophilic attack.
6. It does not explain the mechanism of non- activity in case of competitive inhibitor.	**6.** It gives a mechanism for non-action over competitive inhibitor

(f) competitive and noncompetitive enzyme inhibitors:

Competitive inhibitors	Non-competitive inhibitors
A competitive inhibitor will block the enzyme's active site (ie. it will occupy **the same space** as the natural substrate, blocking it from being catalyzed).	A non-competitive inhibitor will bind to the enzyme **somewhere other than the active site** of the enzyme; an allosteric site. This will change the shape of the enzyme such that the natural substrate may or may not be able to bind to the enzyme's active site, but the enzyme won't be able to complete the chemical reaction necessary
Antibodies, antimetabolites, penicillin, iodoacetate, melonate, CoA (high concentration).	Acetaldehyde Di-Isopropyl fluorophosphate (DFP- nerve gas), mercury, silver, copper, cyanide.

OR (Second Answer)

Competitive Inhibition	Non-competitive inhibition
1. The structure of inhibition molecule is similar to that of the substrate.	**1.** The Structure of the inhibitor molecule is entirely different.
2. The inhibitors get attached to the active site of the enzyme.	**2.** The inhibitor forms complex at a point other than the active site.
3. It competes with the substrate molecule or for the enzyme.	**3.** It does not complete with the substrate.
4. It does not alter the structure of the enzyme	**4.** It alters the structure of the enzyme in such a way that the substrate may get attached to the active site but products are not formed

5. The reaction can be reversed by increasing the substrate concentration	**5.** The reaction goes on decreasing as more and more inhibitors contact the enzyme till saturation is reached.
6. Example: Sulpha drugs given to bacteria complete with para-amino benzoic acid (PABA) and folic acid synthesis is Inhibited.	**6. Example:** Cyanide combines with the prosthetic group of cytochromo oxidase and inhibits the election transport chain

(g) reversible non-competitive enzyme inhibitors and irreversible non-competitive enzyme inhibitors:

Reversible Non-Competitive Enzyme Inhibitors:	Irreversible Non-Competitive Enzyme Inhibitors:
Reversible non-competitive enzyme inhibitors work not by preventing the formation of enzyme-substrate complexes, but by preventing the formation of enzyme-product complexes. So they prevent the substrate to be converted into product.	On the other hand, an irreversible non-competitive enzyme inhibitor destroys enzyme by altering its shape so that the substrate cannot bind to the active site.
Example: Feedback inhibition is an example of reversible non-competitive enzyme inhibition.	**Example:** The examples of irreversible non-competitive inhibitors include cyanides and salts of heavy metals.

EXTENSIVE QUESTIONS

25. Write the properties of enzymes.

Ans: Properties of Enzymes:

The enzymes are organic catalysts. However, they have many properties of inorganic catalyst like

(i) enzymes increase the speed of chemical reaction, by 10^6-10^{14} times faster than the rate of the uncatalysed reaction.

(ii) Enzymes are required in very small quantity for the reaction, e.g., a very few enzymes can convert huge amount of substrate into products with in very short time.

(iii) Enzymes are highly sensitive to pH and temperature changes of the system.

(iv) Enzymes are either highly specific (sucrase can only digest sucrose) or slightly less specific that they can catalyze the related reaction, e.g., carbonic anhydrase can add O_2 in haemoglobin and can control the formation of carbonic acid and bicarbonates in blood

(v) Enzymes can work in *vivo* (living cells) as well as in *vitro* (glassware).

(vi) Some enzymes require co-factor for proper activity

(vii) Enzymes lower the need of activation energy

(viii) Enzymes only speed up a reaction and do not affect the equilibrium of the reaction.

5. The reaction can be reversed by increasing the substrate concentration	**5.** The reaction goes on decreasing as more and more inhibitors contact the enzyme till saturation is reached.
6. Example: Sulpha drugs given to bacteria complete with para-amino benzoic acid (PABA) and folic acid synthesis is Inhibited.	**6. Example:** Cyanide combines with the prosthetic group of cytochromo oxidase and inhibits the election transport chain

(g) reversible non-competitive enzyme inhibitors and irreversible non-competitive enzyme inhibitors:

Reversible Non-Competitive Enzyme Inhibitors:	Irreversible Non-Competitive Enzyme Inhibitors:
Reversible non-competitive enzyme inhibitors work not by preventing the formation of enzyme-substrate complexes, but by preventing the formation of enzyme-product complexes. So they prevent the substrate to be converted into product.	On the other hand, an irreversible non-competitive enzyme inhibitor destroys enzyme by altering its shape so that the substrate cannot bind to the active site.
Example: Feedback inhibition is an example of reversible non-competitive enzyme inhibition.	**Example:** The examples of irreversible non-competitive inhibitors include cyanides and salts of heavy metals.

EXTENSIVE QUESTIONS

25. Write the properties of enzymes.

Ans: Properties of Enzymes:

The enzymes are organic catalysts. However, they have many properties of inorganic catalyst like

(i) enzymes increase the speed of chemical reaction, by 10^6-10^{14} times faster than the rate of the uncatalysed reaction.

(ii) Enzymes are required in very small quantity for the reaction, e.g., a very few enzymes can convert huge amount of substrate into products with in very short time.

(iii) Enzymes are highly sensitive to pH and temperature changes of the system.

(iv) Enzymes are either highly specific (sucrase can only digest sucrose) or slightly less specific that they can catalyze the related reaction, e.g., carbonic anhydrase can add O_2 in haemoglobin and can control the formation of carbonic acid and bicarbonates in blood

(v) Enzymes can work in *vivo* (living cells) as well as in *vitro* (glassware).

(vi) Some enzymes require co-factor for proper activity

(vii) Enzymes lower the need of activation energy

(viii) Enzymes only speed up a reaction and do not affect the equilibrium of the reaction.

26. Explain the role and component parts of the active site of an enzyme.

Ans: Shape of Enzymes and Components of an Active Site:

Majority of enzymes which are protein in nature can have molecular weights ranging from about 10,000 to over 1 million. Such enzymes have tertiary or quaternary structures.

Catalytic Activity of an Enzyme:

The catalytic activity of an enzyme is located in its **active site** which is a specific charge bearing, three dimensional cavity. The substrate (the reactant which is to be converted into product) molecule is attached to the active site by non-covalent interactions like hydrogen bonding and hydrophobic interactions.

Components of an Active Site:

Active site consists of 3-12 amino acids which may be scattered in the polypeptide but are brought together in a particular fashion due to secondary and tertiary folding of the protein molecule, e.g., the active site for aldolase consists of glycine, histidine, and alanine amino acids.

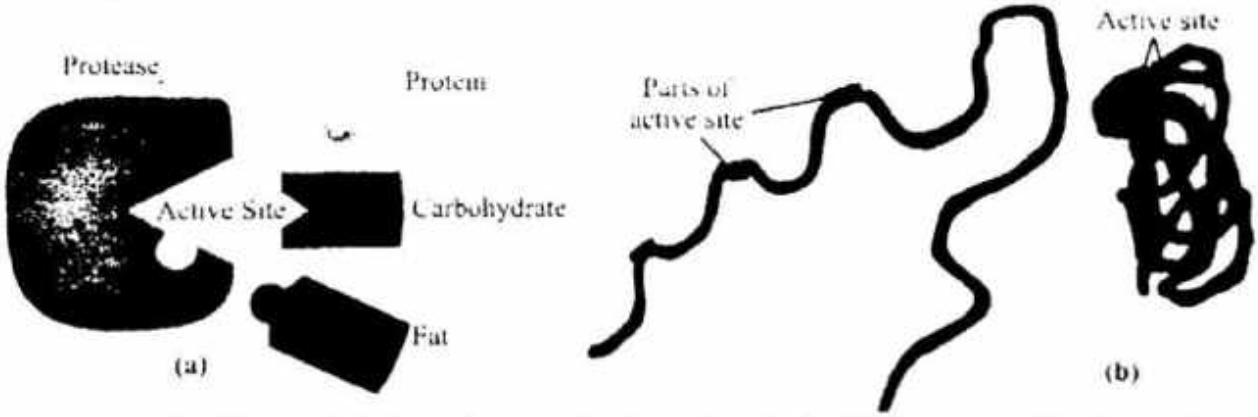
Functional Regions of Active Site:

An active site consists of two functional regions, i.e., binding site and catalytic site.

Some amino acids have active site which makes bonds with substrate constitute the **binding site** while the other amino acids which cause conversion of substrate into product (catalysis) constitute the **catalytic site**.

Shape of an Active Site:

The shape of active site is designed according to the substrate therefore only a particular substrate can attach to the active site, however, sometime related substrate can also bind to the active site.



Active Site: (a) Which substrate fits the active site? (b) Grouping of amino acids of a polypeptide during the formation of tertiary structure to produce an active site.

27. What are cofactors? Describe the two types of cofactors by giving examples.

Ans: Cofactors:

A **cofactor** is a non-protein chemical compound or metallic ion that is required for a protein's **biological** activity to happen. These proteins are commonly enzymes, and **cofactors** can be considered "helper molecules" that assist in biochemical transformations.

Types of Cofactors:

The cofactor may be inorganic or organic molecules.

i. Inorganic Cofactors:

The inorganic cofactors are different metallic ions such as Fe^{++}, Mg^{++} Cu^{++} Zn^{++} etc. These are only attached to the enzymes when substrate gets bind i.e., they are detachable cofactors. Such cofactors are also called **activators**.

$$\text{Glucose + ATP} \xrightarrow{\text{Hexokinase + } Mg^{+2}} \text{Glucose-6-phosphate + ADP}$$

ii. Organic Cofactors:

The organic cofactors are either co-enzymes or prosthetic groups. The coenzymes are the derivatives of vitamins.

For example ATP, NAD^+, FAD^+ are common coenzymes. Like inorganic cofactors they are also attached to the enzymes when substrate gets bind i.e. they are also detachable cofactors.

$$\text{Ethyl alcohol} \xrightarrow{\text{Alcohol dehydrogenase + NAD}} \text{Acetaldeevde + } NADH_2$$

On the other hand a **prosthetic group** is covalently bonded part of an enzyme which is permanently attached to enzyme and does not detach after the completion of reaction. An iron containing porphyrin ring attached to some enzymes like cytochromes is the example of prosthetic group.

28. Explain the mechanism of enzyme action through induced fit model.

Ans: Mechanism of Enzyme Action:

In an enzyme-catalysed reaction, the substrate first binds to the active site of the enzyme to form an **enzyme-substrate (ES) complex,** then the substrate is converted into **product** while it is attached to the enzyme (EP **complex),** and finally the product is released thus allowing the enzyme to start all over again.

$$\underset{\text{enzyme}}{E} + \underset{\text{substrate}}{S} \longrightarrow \underset{\text{enzyme-substrate complex}}{ES} \longrightarrow \underset{\text{enzyme-product complex}}{EP} \longrightarrow \underset{\text{enzyme}}{E} + \underset{\text{product}}{P}$$

Mechanism of Enzyme Action

Actually, the enzyme can make the local conditions inside the active site quite different from those outside (such as pH, water concentration, charge), so that the reaction is more likely to happen. For example, if a substrate is to be split, a bond might be stretched by the enzyme, making it more likely to break.

Models of Enzyme Action:

Induced fit model of enzyme action:

Koshland proposed **Induced fit model** in 1959

According to this model the active site is flexible, therefore, it is modified as the substrate interacts with enzyme. The amino acids, which makeup the active site are molded into a precise shape which enables the enzyme to perform its catalytic function more effectively. The change which is induced in the shape of active site is responsible for the conversion of substrate into product. As the reaction is completed the active site regains its original shape. This is the flexibility of active site which allows more than one type of related substrates to be attached on active site and therefore, an enzyme can carry out more than one type of related reactions.

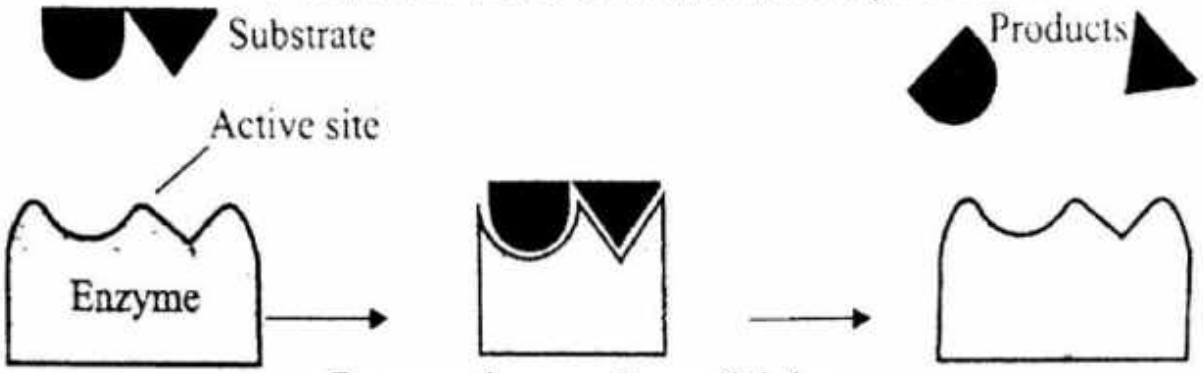
The example is carbonic anhydrase which can add O_2 in haemoglobin as well as can control the formation of carbonic acid and bicarbonates in blood.

Enzymes, which follow the induced fit mechanism, are called **regulatory** or allosteric **enzymes** for example hexokinase.

$Hb + 4O_2 \xrightarrow{Carbonic\ anhydrase} Hb4O_2$ (Oxyhaemoglobin)

$CO_2 + H_2O \xrightarrow{Carbonic\ anhydrase} H_2CO_3 \xrightarrow{Carbonic\ anhydrase} H^+ + HCO^-$

Koshland's "Induced Fit" model of enzyme action



Enzyme changes shape slightly as substrate enters active site, making the fit more precise

Koshland's Induced Fit Hypothesis

29. Explain the mechanism of enzyme action through lock and key model.

Ans: Mechanism of Enzyme Action:

In an enzyme-catalysed reaction, the substrate first binds to the active site of the enzyme to form an **enzyme-substrate (ES) complex**, then the substrate is converted into **product** while it is attached to the enzyme (EP **complex**), and finally the product is released, thus allowing the enzyme to start all over again

$$\underset{\text{enzyme}}{E} + \underset{\text{substrate}}{S} \longrightarrow \underset{\text{enzyme-substrate complex}}{ES} \longrightarrow \underset{\text{enzyme-product complex}}{EP} \longrightarrow \underset{\text{enzyme}}{E} + \underset{\text{product}}{P}$$

Mechanism of Enzyme Action

Actually, the enzyme can make the local conditions inside the active site quite different from those outside (such as pH, water concentration, charge), so that the reaction is more likely to happen. For example, if a substrate is to be split, a bond might be stretched by the enzyme, making it more likely to break.

Models of Enzyme Action:

Lock and Key model of enzyme action:

The mechanism of enzyme action can be explained with the help of two different models. **Emil Fischer** proposed **Lock and key model** in 1894

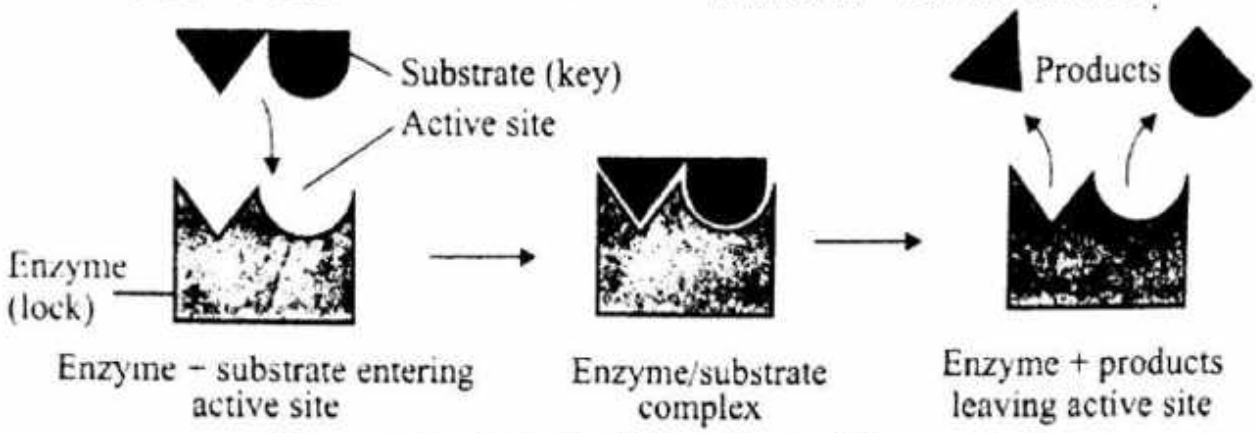
According to this model the active site of the enzyme has definite shape and rigid structure. Shape of active site is complementary to the shape of substrate.

Therefore, a particular substrate can only bind to the active site. The active site remains unchanged during or after the reaction. Lock and key model assumes that like a particular key opens a particular lock, a specific **enzyme (key)** acts upon a particular **substrate (lock).** Actually, the notched portion of the key is equivalent to the active site on the enzyme. It reflects that enzymes are highly specific in their action and each enzyme can carry out only one particular reaction. The enzymes, which work according to this model, are called **non-regulatory** enzymes.

However, this model is exercised by a very small number of enzymes, for example sucrase, maltase and etc. The ability of enzyme to catalyze one specific

reaction is perhaps its most significant property. Although, many enzymes show a broad range of specificity towards the substrate they catalyze. When one enzyme can catalyze only one substrate and essentially no others it is called **absolute** specificity e.g., urease.

Urea + Water $\xrightarrow{\text{Urease}}$ Ammonia + Carbon dioxide



Fisher's "Lock & Key" Hypothesis of Enzyme Action

30. Explain how an enzyme catalyzes specific reactions.

Ans: Enzymes are highly specific both in the reactions that they catalyze and in their choice of reactants, which are called substrates. An enzyme usually catalyzes a single chemical reaction or a set of closely related reactions.

They are of vital importance for life because most of the chemical reactions in cells and tissues are catalyzed by enzymes. Without enzyme action, those reactions would not occur or would not happen with the required speed for the biological processes in which they are involved.

Enzymes are specific in following ways:

i. It reflects that enzymes are highly specific in their action and each enzyme can carry out only one particular reaction.

ii. The enzymes, which work according to this model, are called **non-regulatory enzymes**. However, this model is exercised by a very small number of enzymes, for example sucrase, maltase and etc. The ability of enzyme to catalyze one specific reaction is perhaps its most significant property.

iii. Although, many enzymes show a broad range of specificity towards the substrate they catalyze. When one enzyme can catalyze only one substrate and essentially no others it is called **absolute** specificity e.g., urease.

Urea + Water $\xrightarrow{\text{Urease}}$ Ammonia + Carbon dioxide

iv. Urease is an enzyme that catalyzes the conversion of urea to ammonia and carbon dioxide. Certain bacteria that convert urea to ammonia as part of the nitrogen cycle contain this enzyme.

$$\underset{\text{urea}}{CH_4N_2O} + \underset{\text{water}}{H_2O} \xrightarrow{\text{urease}} \underset{\text{ammonia}}{2NH_3} + \underset{\text{carbon dioxide}}{CO_2}$$

The conversion of urea and water into ammonia and carbondioxide, one of many biochemical reactions catalyzed by enzymes.

Ureas enzyme catalyzed conversion of urea to ammonia

Characteristic of Enzyme Catalyzes:

- Almost all biochemical processes are catalyzed by enzymes
- As almost all Enzymes are proteins, their ability to catalyze reactions is attributable to their primary, secondary, tertiary, and quaternary structures.
- Enzymes have a high degree of specificity for their substrates.
- Enzymes accelerate chemical reactions tremendously.
- Enzymes can function in aqueous solution under mild conditions, which are unlike the conditions that are frequently needed in organic chemistry.
- Enzymes are effective in minute amounts because they are not used up in the reaction that they catalyze.
- Enzymes do not affect the direction of the reaction but make the reaction reach equilibrium sooner.
- Both synthesis and decomposition of molecules in a living system normally proceed too slowly to be useful to metabolic survival. However, the presence and activity of enzymes speed up those to support life activities of cellular metabolism.
- Enzymes make up a substantial portion of the total protein of the cell. A typical cell contains about 3000 different kinds of enzyme molecules and many copies of each kind.
- Within a cell, chemical reactions take place within a narrow temperature and pH range. This is possible because enzymes generally lower the activation energy of a reaction through a variety of mechanisms.

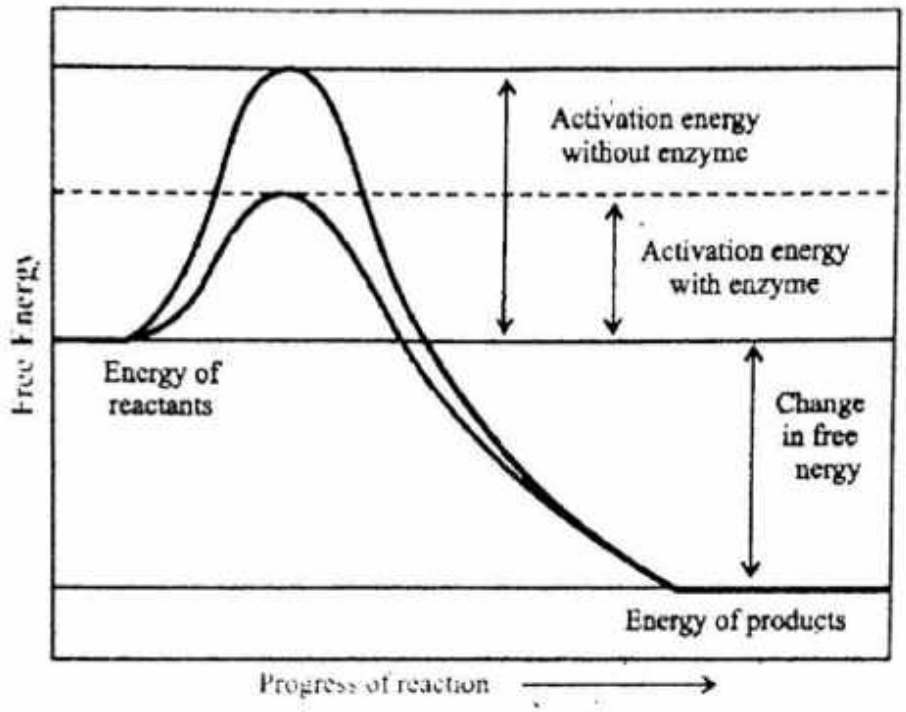
31. Explain through graph how an enzyme speeds up reaction by lowering the energy of activation.

Ans: Molecules do not react with one another unless they are activated in some way.

Energy of Activation:

The energy that must be added to cause molecules to react with one another is called the **energy of activation.** In nonliving system we use heat as energy of activation to increase the number of effective collision between molecules. In living systems large amount of heat cannot be used as energy of activation.

Why? All living cells and organisms are mainly composed of thermolabile (temperature sensitive) protein t molecules. About 1,000 chemical reactions are being carried out in a cell at any time. Energy of activation required for such a large number of reactions cannot be provided by living system.



Energy of Activation: Enzymes speed the rate of chemical reactions because the lower the amount of energy required to activate the reactants

The living system works in isothermal condition; the excited state of molecules or reactants is achieved by biochemical process. Enzyme (E) reacts with reactant (A) to form an AE transitional complex. The energy level of AE complex reaches to the energy level of reactant B. AE complex then reacts with reactant B to form AB and enzyme (E) is released.

A + E ⟶ AE Complex + B ⟶ AB + E

Enzyme does decrease the energy of activation by changing energy dependent process to energy independent process. Thus the energy of activation is energy required to break the existing bonds and begin the reaction. An enzyme greatly reduces the activation energy necessary to initiate a chemical reaction.

32. Describe the effect of temperature on the rate of enzyme action.

Ans: Temperature:

Heating increases molecular motion. Thus the molecules of the substrate and enzyme move more quickly, so probability of a reaction to occur is increased. Increasing temperature affect the rate of reaction in such a way that an increase of just 10°C in the existing temperature doubles the rate of reaction but this effect remains up to a certain limit.

Optimum Temperature:

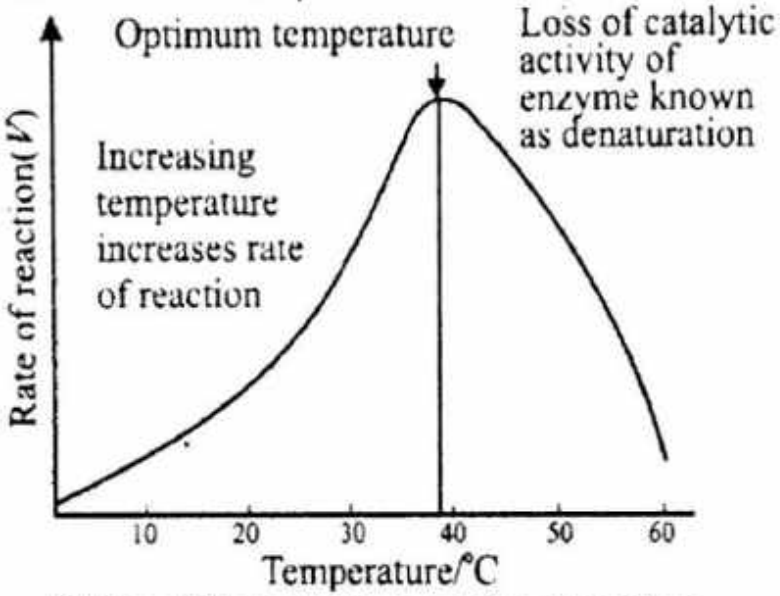
The temperature that promotes maximum activity is called an **optimum temperature.**

Maximum Temperature:

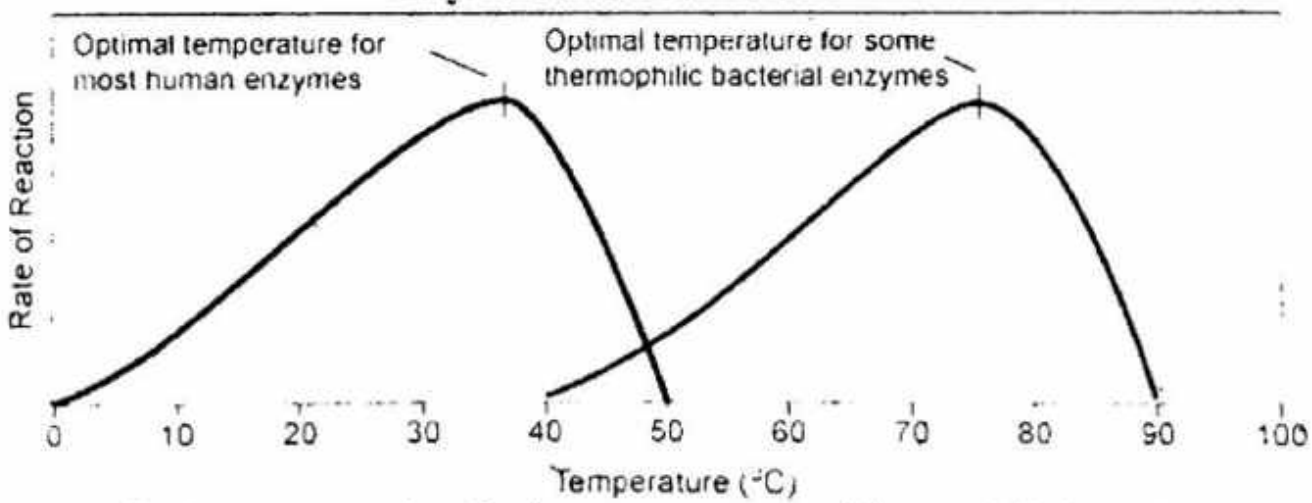
If the temperature is increased above this level, then a decrease in the rate of the reaction occurs despite the increasing frequencies of collision. This is because the secondary and tertiary structures of the enzyme have been disrupted and the enzyme is said to be denatured. The enzyme unfolds and the precise structure of the active site is gradually lost. This temperature which causes denaturation of enzyme is called **maximum temperature.**

Minimum Temperature:

The bonds which are most sensitive to temperature change are hydrogen bonds. All human enzymes have a optimum temperature of about 37-38°C. but bacteria living in hot springs may have an optimum temperature of 70°C or higher Such enzymes have been used in biological washing powders for high temperature washes. If temperature is reduced to near or below freezing point, enzymes are inactivated, not denatured. They will regain their catalytic influence when higher temperatures are restored. This temperature where an inactive enzyme becomes active again is called **minimum temperature**.



Effect of Temperature on the rate of an Enzyme Controlled Reaction



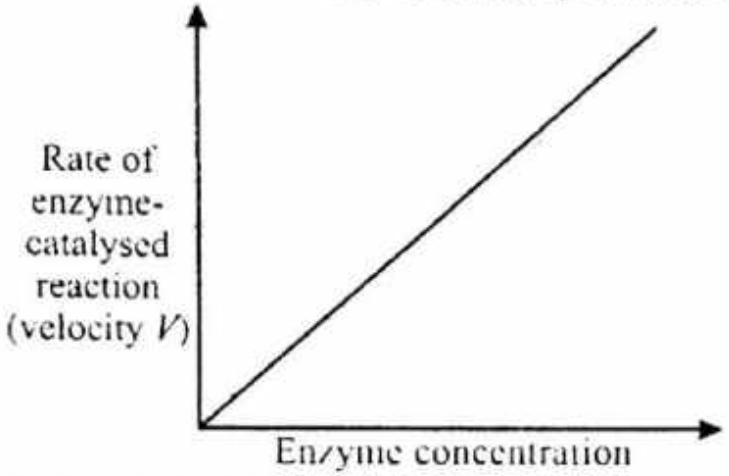
Optimum temperature for human enzymes and thermophilic bacteria

33. Describe how the concentration of enzyme affects the rate of enzyme action.

Ans: Enzyme Concentration:

Provided that the substrate concentration is maintained at a high level (unlimited availability), and other conditions such as pH and temperature are kept constant, the rate of reaction becomes directly proportional to the enzyme concentration. If there is only one enzyme in the system it can convert hundreds of substrates into products but it takes more time. By increasing concentration of

enzyme, numbers of active sites become more available and the rate of conversion of substrate into product becomes fast. Such effect persists till the equilibrium state (when concentration of enzyme and substrate become equal), after that further increase in enzyme concentration will have no effect upon rate of reaction.

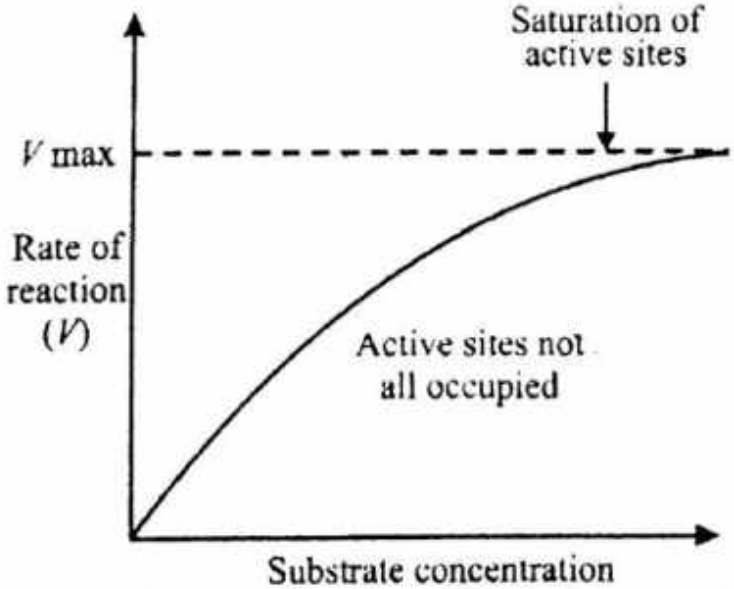


Relationship between Enzyme concentration and the rate of an Enzyme-controlled reaction

34. Explain the effect of substrate concentration on the rate of enzyme action.

Ans: Substrate Concentration:

When other conditions such as pH and temperature are - kept constant and the enzyme concentration is maintained at a higher level (unlimited availability), the increase in substrate concentration (S) increases the velocity (V) of the enzymatic reaction at first. The reaction ultimately reaches a maximum velocity at equilibrium state. The rise in V is decreased progressively with further increase in S. The reaction does not increase by any further rise in substrate concentration. This happens because all the active sites of enzyme molecules are occupied by the substrates (saturation) and no enzyme is left free to bind with additional molecules of the substrate.



Effect of Substrate concentration in the rate of Enzyme-control reaction

35. Describe enzymatic inhibition, its types and its significance.

Ans: Enzyme Inhibition:

The phenomenon in which an enzyme fails to catalyze a reaction is called **enzyme inhibition** and the molecules which react with enzyme but are not converted into desired products are called **enzyme inhibitors**. In general, the enzyme inhibition is a normal part of the regulation of enzyme activity within cells but sometimes when external factors cause enzyme inhibition, it may become dangerous for life.

Molecules Act as Inhibitors:

The molecules which act as inhibitors include poisons, cyanides, antibodies, antimetabolites, penicillin sulpha drugs etc.

Types of Inhibition:

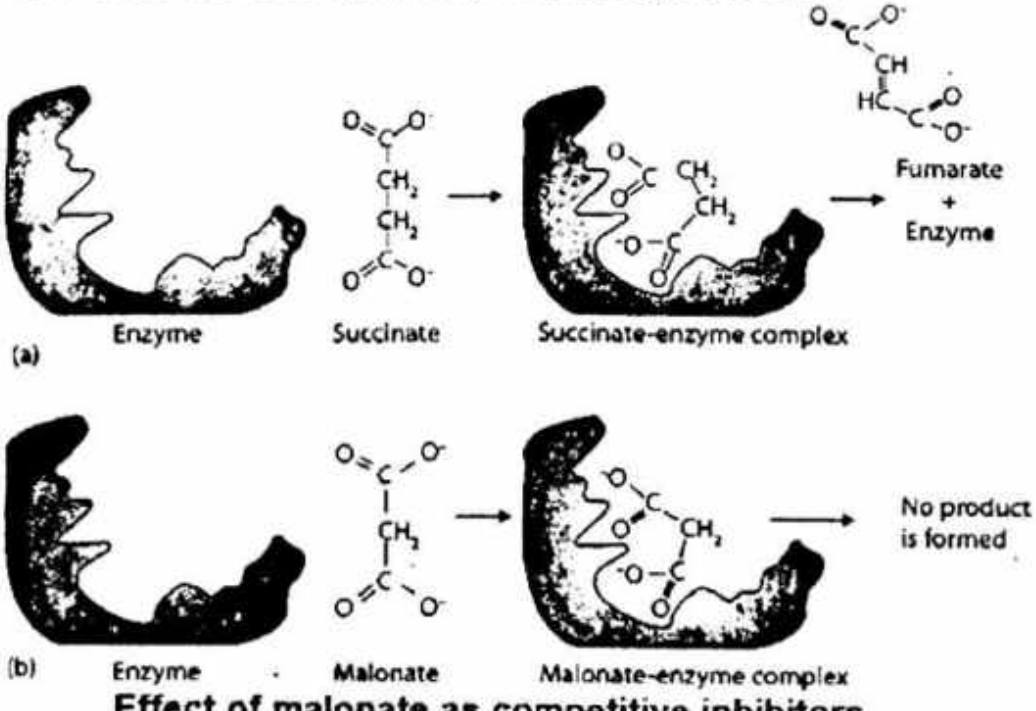
Inhibition may be competitive or noncompetitive.

Competitive Inhibition:

A type of enzyme inhibition in which enzyme activity is blocked by the presence of a chemical that compete with the substrate for binding to the active site is called **competitive inhibition.** Usually a competitive inhibitor is structurally similar to the normal substrate and so fits into the active site of the enzyme. Howéver, it is not similar enough to substitute fully for the normal substrate in the chemical reaction and the enzyme cannot attack it to form reaction products.

Reversible Inhibition:

Competitive inhibition is usually temporary, and the inhibitor eventually leaves the enzyme hence it is also called **reversible inhibition.** This means that the level of inhibition depends on the relative concentrations of substrate and inhibitor, since they are competing for places in enzyme active sites. Therefore, if the concentration of the substrate is increased relative to the concentration of the inhibitor, the active site will usually be occupied by the substrate. An example of inhibitor is **malonate.** Succinate dehydrogenase that catalyzes the formation of fumarate from succinate is competitively inhibited by malonate.



Effect of malonate as competitive inhibitors

Importance of Competitive Inhibitors:

The **importance** of competitive inhibitors is

(a) It supports lock and key hypothesis

(b) It shows that substances which are similar to substrate are not acted upon by enzymes.

(c) Competitive inhibitors are used as drugs in the control of bacterial pathogens. Antibiotics known as sulphonamides are used to combat bacterial infection.

Non-Competitive Inhibitors:

In non-competitive inhibition the inhibitor molecule binds to an enzyme other than active site. The other binding site of enzyme is called **allosteric site**

Types of Non-Competitive Inhibitors:

The non-competitive inhibitors inactivate the enzyme temporarily (reversible inhibition) or they denature the enzyme permanently (irreversible inhibition).

Reversible non-competitive Enzyme Inhibitors:

Reversible non-competitive enzyme inhibitors work not by preventing the formation of enzyme-substrate complexes, but by preventing the formation of enzyme-product complexes. So they prevent the substrate to be converted into product.

Example:

Feedback inhibition is an example of reversible non-competitive enzyme inhibition.

Irreversible Non-Competitive Enzyme Inhibitors:

An **irreversible non-competitive** enzyme inhibitor destroys enzyme by altering its shape so that the substrate cannot bind to the active site.

Example:

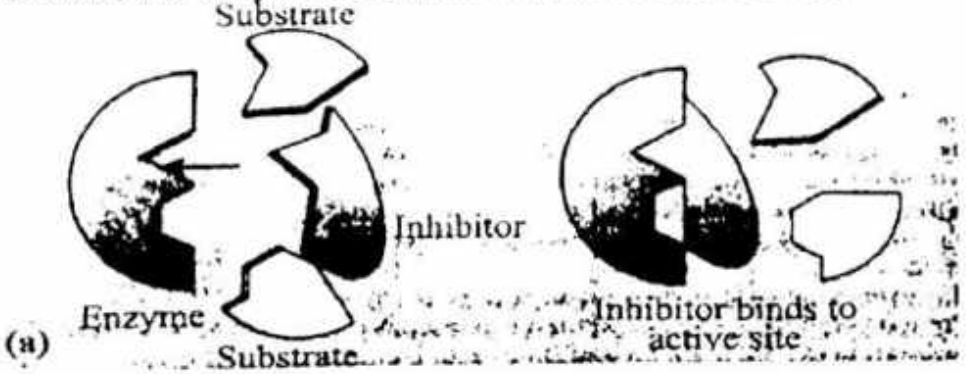
The examples of irreversible non-competitive inhibitors include cyanides and salts of heavy metals.

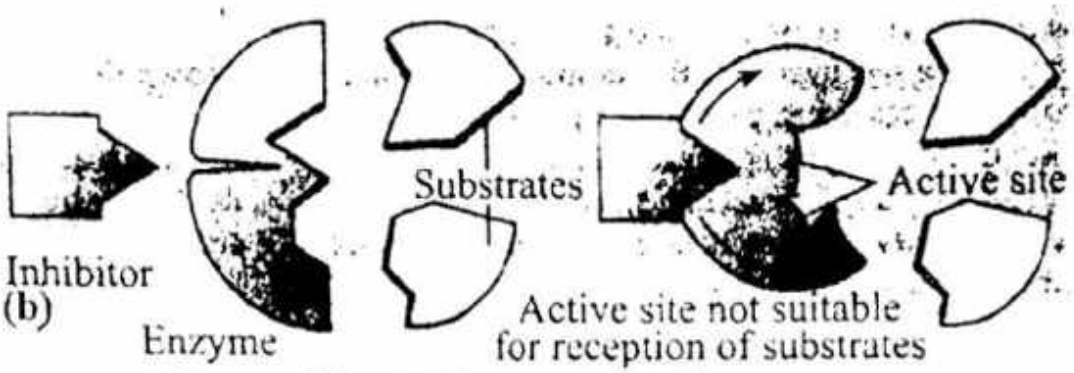
Cyanides:

Cyanides are potent poisons of living organism because they can kill an organism by inhibiting cytochrome oxidase essential for cellular respiration. They block the action of these enzymes by combining with iron which may be present in the prosthetic group.

Ions of Heavy Metals:

Ions of heavy metals such as mercury, silver and copper (Hg^{++}, Ag^{+} and Cu^{++}) combine with thiol (-SH) groups in the enzyme breaking the disulphide bridges. These bridges are important in maintaining tertiary structure. When these bridges are broken, the enzyme becomes denatured and inactive.





(a) Competitive (b) Non competitive enzymes

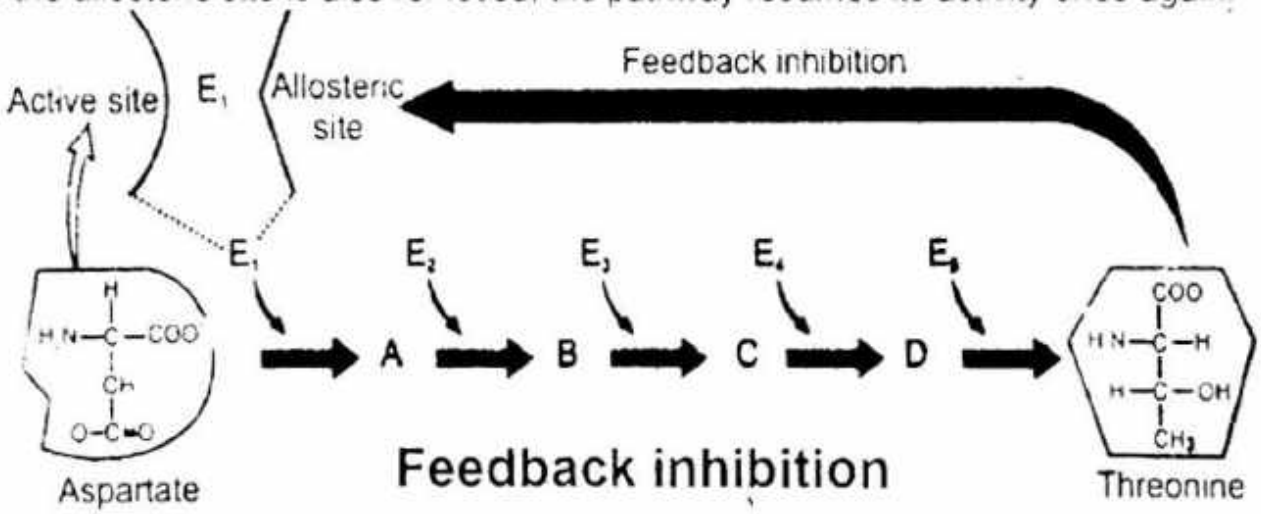
36. Explain feedback mechanism with reference to enzymes.

Ans: Feedback Inhibition:

The activity of almost every enzyme in a cell can be regulated by its product. When the activity of an enzyme is inhibited by its own product, it is called feedback inhibition. This is a type of reversible non-competitive inhibition.

This phenomenon is a part of normal regulatory mechanism and usually happens during the regulation of metabolic pathways.

For example, the amino acid aspartate becomes the amino acid threonine by a sequence of five enzymatic reactions. When threonine, the end product of this pathway, is present in excess, it binds to an allosteric site on enzyme 1 on this pathway and then the active site is no longer able to bind aspartate. When all the threonine is consumed in cellular events, the threonine molecule which is attached to the allosteric site is also removed, the pathway resumes its activity once again.



37. Classify enzymes on the basis of reactions catalyzed.

Ans: Classification of Enzymes:

Enzymes can be classified either on the basis of reaction types that they catalyze or on the basis of substrate which are acted upon by the enzyme.

Classification based upon reaction type:

A systematic nomenclature and classification of enzymes based on reaction types and reaction mechanism was given by International Union of Biochemistry in 1961.

On that basis all the enzymes have been classified into six groups.

1.	Oxidoreductases	2.	Transferases	3.	Hydrolases
4.	Lyases	5.	Isomerases	6.	Ligases

1. Oxidoreductases:

These enzymes catalyze oxidation/reduction of their substrate and act by removing or adding electron or H^+ ions from or to the substrate. For example **cytochrome oxidase** oxidizes cytochrome.

2. Transferases:

These enzymes catalyze the transfer of specific functional group other than hydrogen from one substrate to another. The chemical group transferred in the process is not in a free state, for example **hexokinase** transfers a phosphate group from ATP to glucose.

3. Hydrolases:

These enzymes bring about the breakdown of large complex organic molecules into smaller ones by adding water (hydrolysis) and breaking the specific covalent bonds. Examples are proteolytic enzymes which breakdown proteins into peptones and peptides such as **pepsin, renin** and **trypsin**. Other digestive enzymes that work in digestive tract are also the examples of hydrolases.

4. Lyases:

These enzymes catalyze the breakdown of specific covalent bonds and removal of groups without hydrolysis. For example **histidine decarboxylase** breaks the covalent bonds between carbon atoms in histidine forming carbon dioxide and histamine.

5. Isomerases:

These enzymes bring about intra-molecular rearrangement of atoms in the molecules and thus forming one isomer from another. For example **phosphohexose isomerase** changes glucose 6- phosphate to fructose 6-phosphate.

6. Ligases (Synthetases):

These enzymes bring about joining together of two molecules. The energy is derived by hydrolysis of ATP. For example **polymerases** are responsible for linking monomers into a polymer such as DNA or RNA.

Table: Classification of enzymes based upon reaction type

Sr. No	Enzyme Class	General Scheme of Reaction
1.	Oxidoreductases	$A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}$
2.	Transferases	$A - B + C \longrightarrow A + C - B$
3.	Hydrolases	$A - B + H_2O \longrightarrow A - H + B - OH$
4.	Lyases	$A - B \rightleftharpoons A + B$ (reverse reaction syntheses)
5.	Isomerases	$A - B - C \rightleftharpoons A - C - B$
6.	Ligases (synthetases)	$A + B + ATP \longrightarrow A - B + ADP + P_i$

38. Classify enzymes on the basis of the substrate they use.

Ans: Classification of Enzymes:

Enzymes can be classified either on the basis of reaction types that they catalyze or on the basis of substrate which are acted upon by the enzyme.

Classification based upon substrate:

Enzymes can be classified on the basis of substrates they use. Some of the examples are proteases, lipases, carbohydrases and nucleases

1. **Proteases:**

These enzymes act upon proteins. Examples are: **pepsin** and **trypsin** (both digest large polypeptides into small polypeptides or peptones), **aminopeptidases** and **carboxypeptidases** (both digest peptones into dipeptides) and **erypsin** (digest dipeptides into amino acids)

2. **Lipases:**

These enzymes hydrolyze lipids into fatty acids and glycerols. Examples are **pancreatic lipases.**

3. **Carbohydrases:**

These enzymes cause breakdown of carbohydrates. Examples are:

(a) amylase (digest starch or glycogen into maltose)
(b) cellulase (digest cellulose into cellubiose, a disaccharide)
(c) maltase (digest maltose into glucoses)
(d) diastase (acts on starch)
(e) sucrase (digest sucrose into glucose and fructose)
(f) lactase (digest lactose into galactose and glucose)

4. **Nucleases:**

These are involved in the breakdown of DNA and RNA. Examples are:

(a) RN Aases (digest RNA into ribonucleotides)
(b) DNAases (digest DNA into deoxyribo nucleotides).
(c) AT Pases (cause hydrolysis of ATP in muscles etc.)