

## Extensive Questions

**25. Write the properties of enzymes.**

**Ans:**

**Properties of enzymes:**

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

- (i) The enzymes increase the speed of chemical reaction by,  $10^6$ - $10^{14}$  times faster than the rate of uncatalyzed reaction.
- (ii) Enzymes are highly sensitive to pH and temperature changes of the system.
- (iii) Some enzymes require proper co-factor for proper activity.
- (iv) Enzymes lower the need of activation energy.
- (v) Enzymes are required in very small quantity for the reaction e.g. a very few enzymes can convert huge amount of substrate into products within very short time.
- (vi) Enzymes are either highly specific (sucrose can only digest sucrose) or slightly less specific that they can catalyze the related reaction. E.g. carbonic anhydrase can add  $O_2$  in hemoglobin and can control the formation of carbonic acid and bicarbonates in blood.
- (vii) Enzymes can work in vivo (living cells) as well as in vitro (glassware)
- (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 component 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.

### 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 the aldolase consists of glycine, histidine and alanine amino acids.

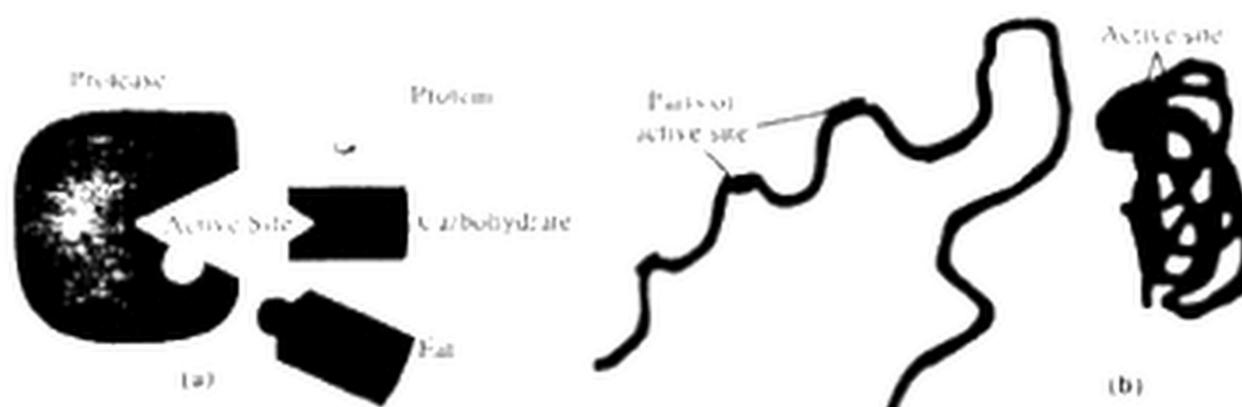
### Functional regions of active site:

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, sometimes 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 co-factors can be considered "helper molecules" that assist in biochemical transformations.

**Types of Cofactors:**

The cofactors may be organic or inorganic.

**Inorganic Cofactors:**

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



**Organic Cofactors:**

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

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



On the other hand, a prosthetic group is covalently bounded 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 enzyme 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 enzymes to start all over again.



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:

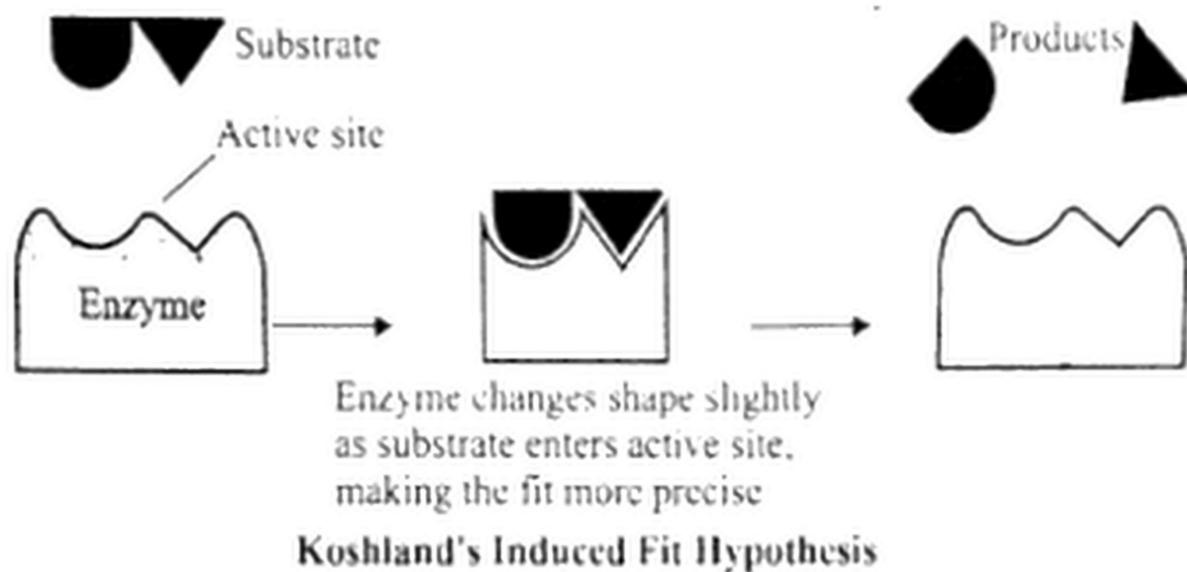
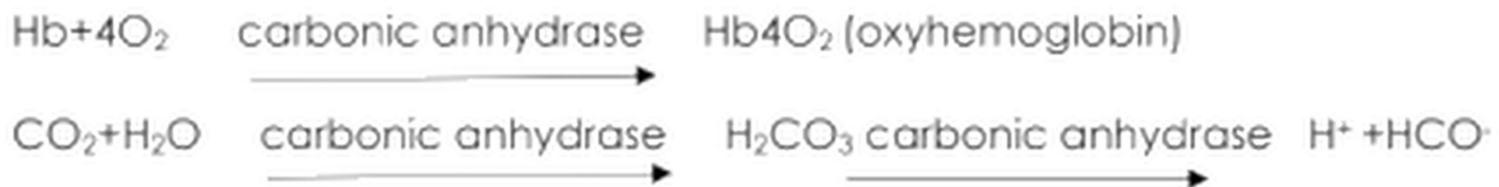
Koshland proposed **Induced fit model** in **1959**.

According to this model, the active site is flexible, therefore it is modified as the substrate interacts with enzyme. The amino acids which make up the active site is molded into a precise shape which enable an enzyme to perform its catalytic function more effectively. The change which is induced in the shape of active site is responsible for the change in the shape of substrate into product. As the reaction is completed the active site regains its original shape. This is the flexibility of active site which allow more than one type of related substrates to

be attached on the active site and therefore, an enzyme can carry on more than one type of related reactions.

The example is carbonic anhydrase which can add O<sub>2</sub> in hemoglobin as well as can control the formation of carbonic acid and bicarbonates in blood.

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



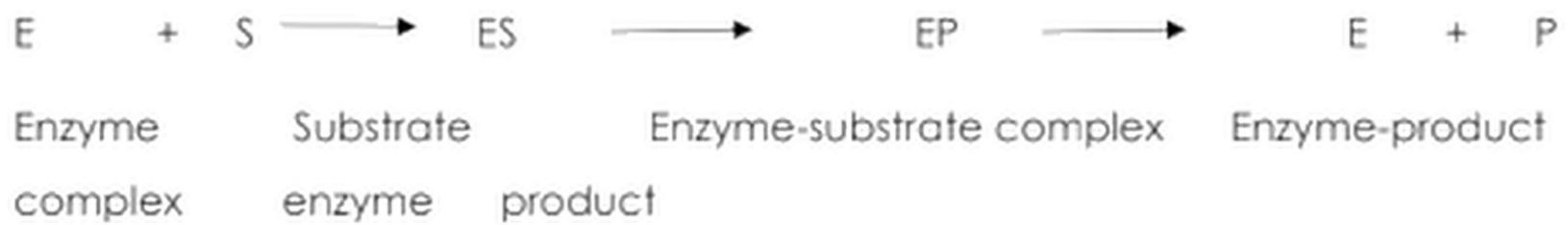
**29. Explain the mechanism of enzyme action through Lock and key model.**

**Ans:**

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### Models of Enzyme action:

#### Lock and key model:

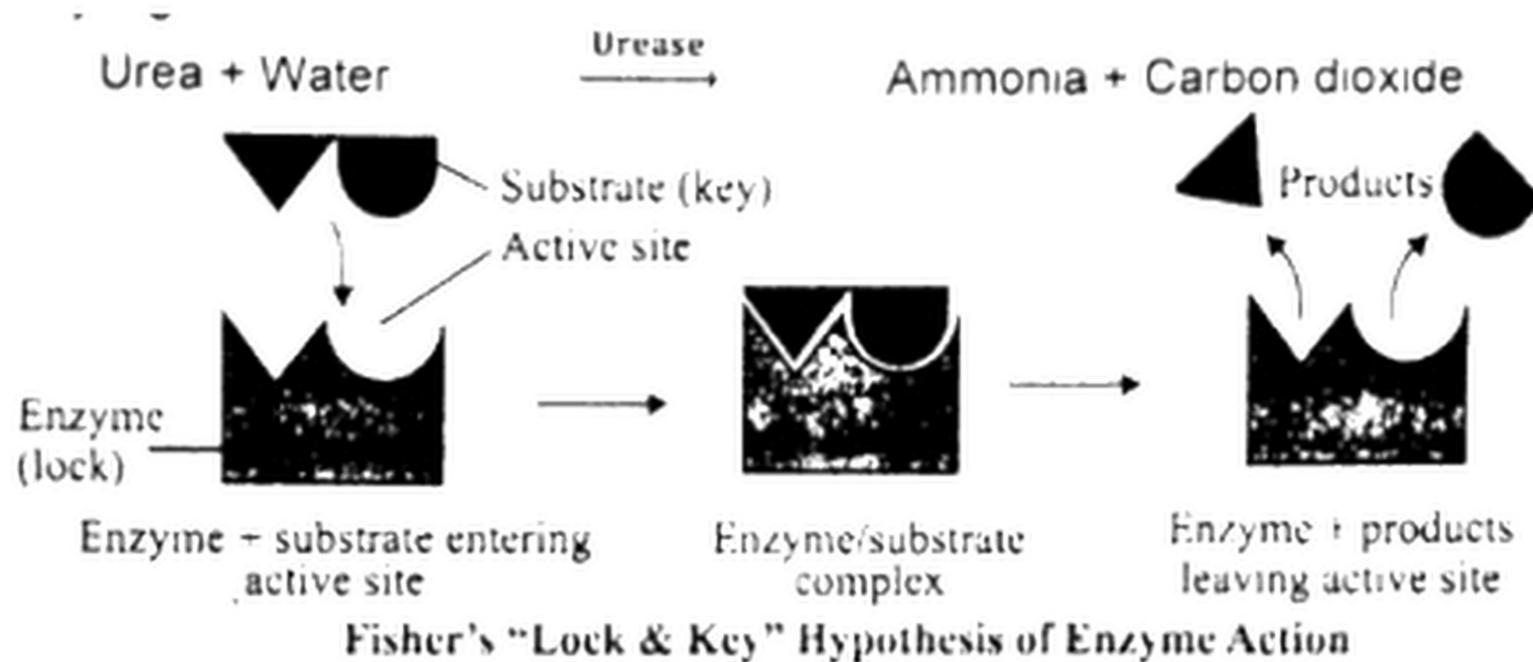
The mechanism of enzyme action can be explained by two different models. Emil Fischer proposed Lock and Key model in 1894.

According to this model active site, have definite structure and rigid shape. Shape of active site is complementary to the shape of substrate. Therefore, the substrate of a specific shape can bind to the active site. The active site remains change or unchanged during the reaction. Lock and key model assume that as 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 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, sucrose, maltase etc. The ability of enzyme to catalyze one specific

reaction is perhaps its most significant property. Although, many enzymes show broad range of specificity towards the substrate they catalyzed. When one enzyme can catalyze only one substrate and essentially no other, it is called absolute specificity. E.g. Urease



**30. Explain how enzymes catalyzes specific reaction.**

**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 enzymes 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 the 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, sucrose, maltase etc. The ability of enzyme to catalyze one specific reaction is perhaps its most significant property.
- iii- Although, many enzymes show broad range of specificity towards the substrate they catalyzed. When one enzyme can catalyze only one substrate and essentially no other, it is called absolute specificity. E.g. Urease
- iv- Urease is an enzyme that catalyzed 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.



#### Characteristics 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 substrate.
- 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 reactions that they catalyze.
- Enzymes do not affect the direction of the reactions 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 enzymes 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 reaction through a variety of mechanisms.

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

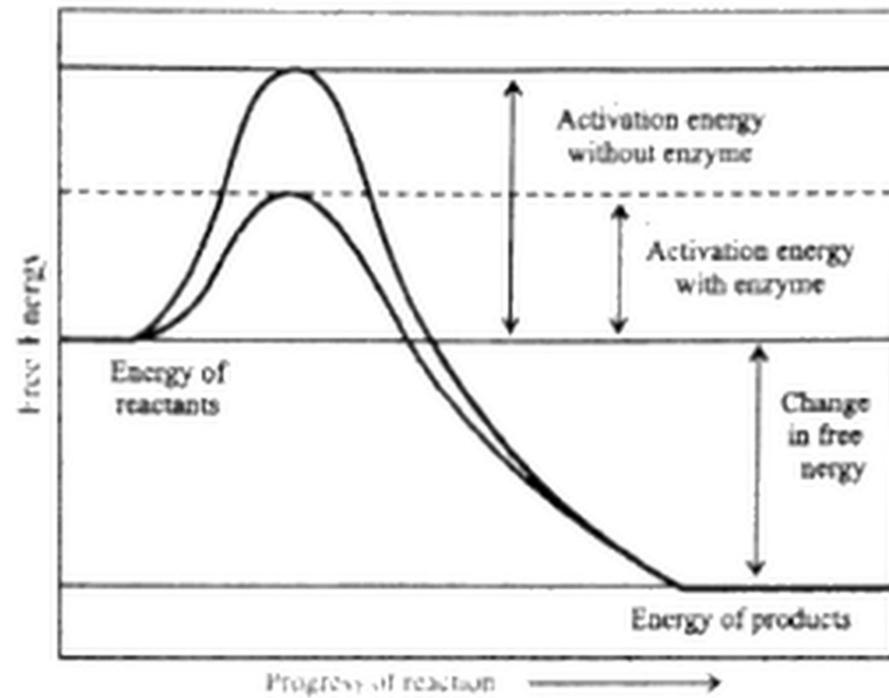
**Ans:**

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 activation energy. In non-living system, we use heat as energy of activation to increase the number of effective collisions 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 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 they 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 reaction  $B$ .  $AE$  complex then reacts with reactant  $B$  to form  $AB$  and enzyme  $E$  is released.



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 the 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:**

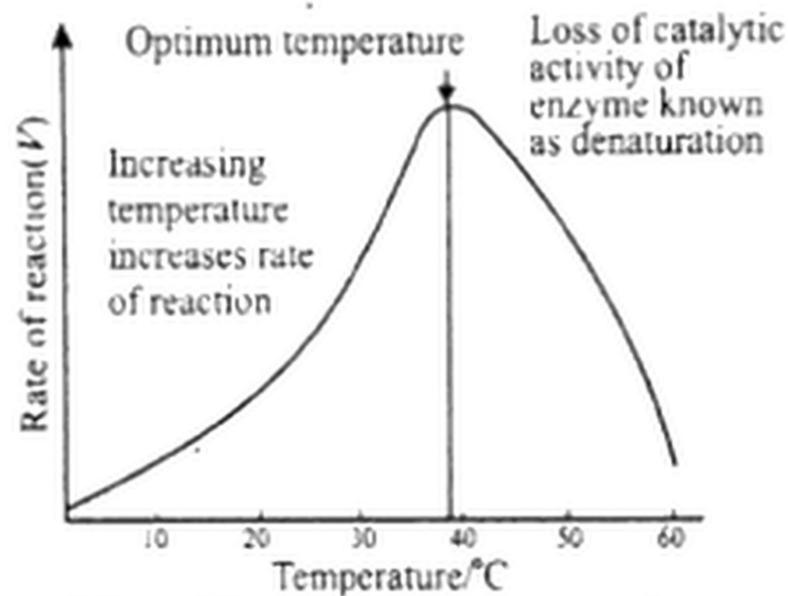
The temperature that promotes maximum activity is called an optimum temperature.

**Maximum temperature:**

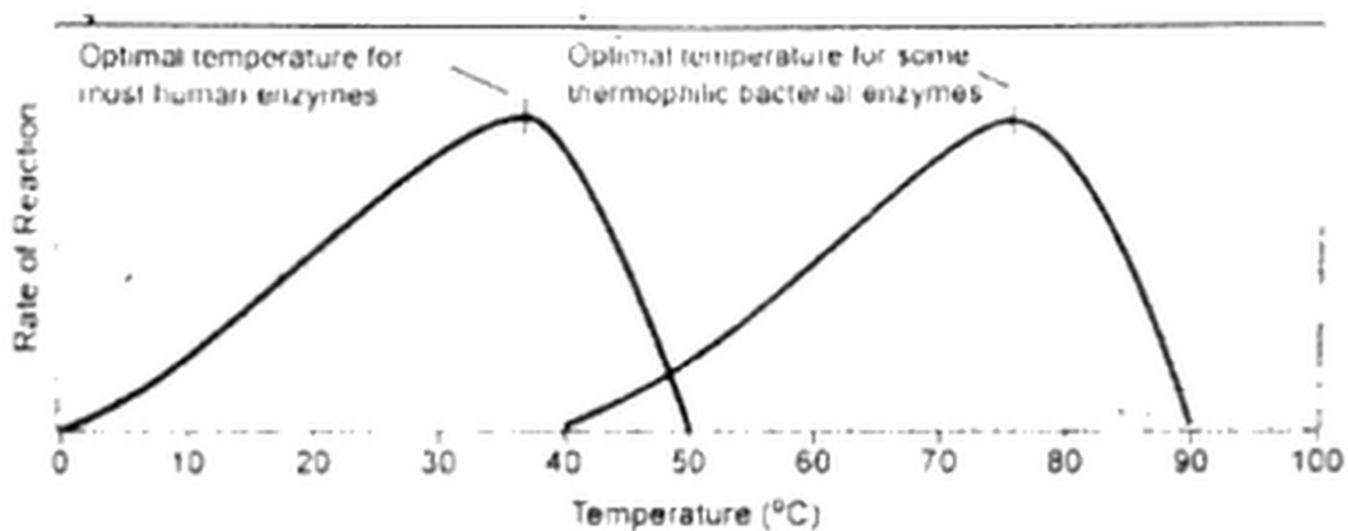
If the temperature is increased above this level, then a decrease in the rate of the reaction occurs despite the increasing frequencies of collision. This is because the secondary and tertiary structures of the enzymes have been disrupted and the enzyme is said to be denatured. The enzyme unfolds and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

**Minimum Temperature:**

All human enzymes have an 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.

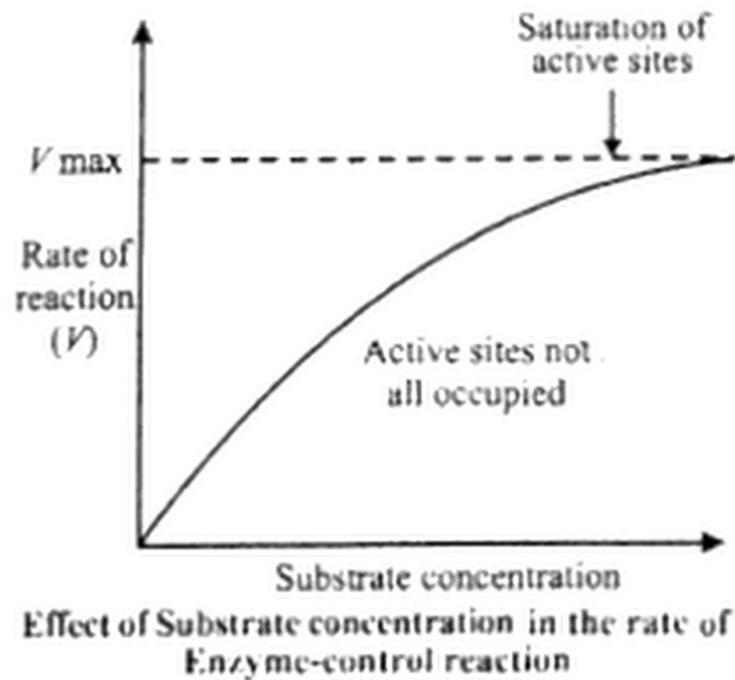
**34. Explain the effect of substrate concentration on the rate of enzyme action.**

**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.



**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 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 acts as enzyme inhibitors:**

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

**Types of inhibition:**

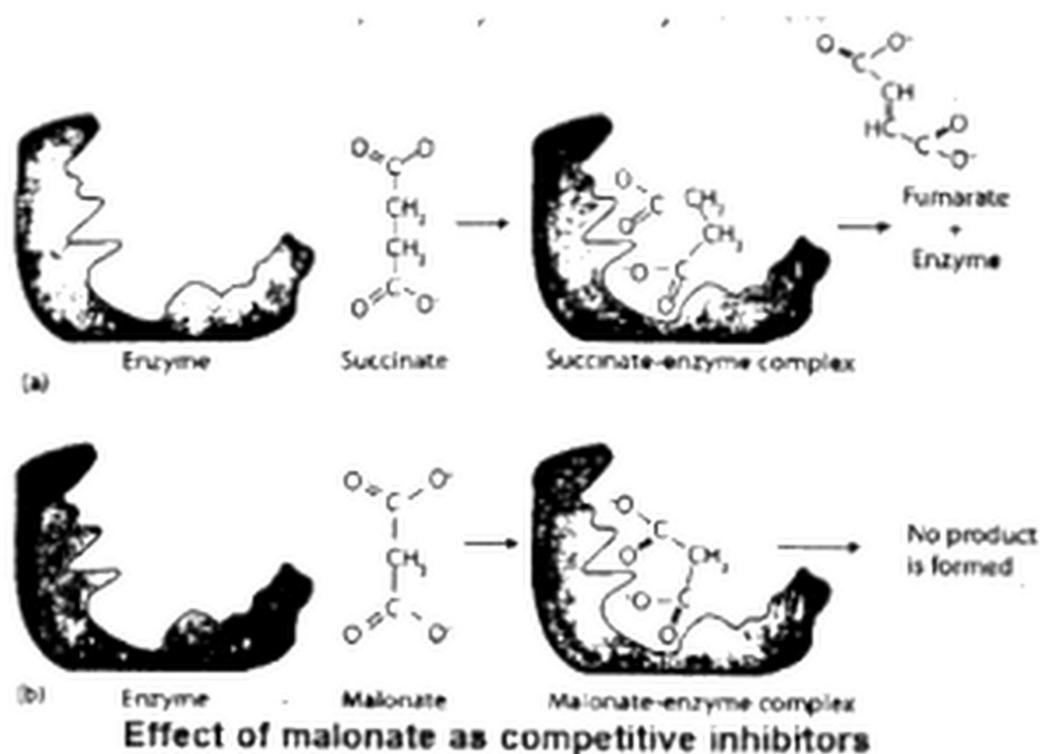
Inhibition can be competitive or non-competitive.

**Competitive inhibition:**

A type of enzyme inhibition in which enzymes 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 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 to 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 concentrations of the inhibitors, the active site will usually be occupied by the substrate. An example of the inhibitor is malonate. Succinate dehydrogenase that catalyzes the formation of fumarate from succinate is competitively inhibited by malonate.



### Importance of competitive enzyme inhibitors:

- (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 inhibitor:

In Non-competitive inhibition, inhibitor molecules bind to enzymes other than active site. The other site of enzyme is called allosteric site.

### Types of Non- competitive inhibitors:

#### Reversible non-competitive enzymes:

Reversible non-competitive enzymes inhibitors work not by preventing 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 Enzymes:**

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

**Examples:**

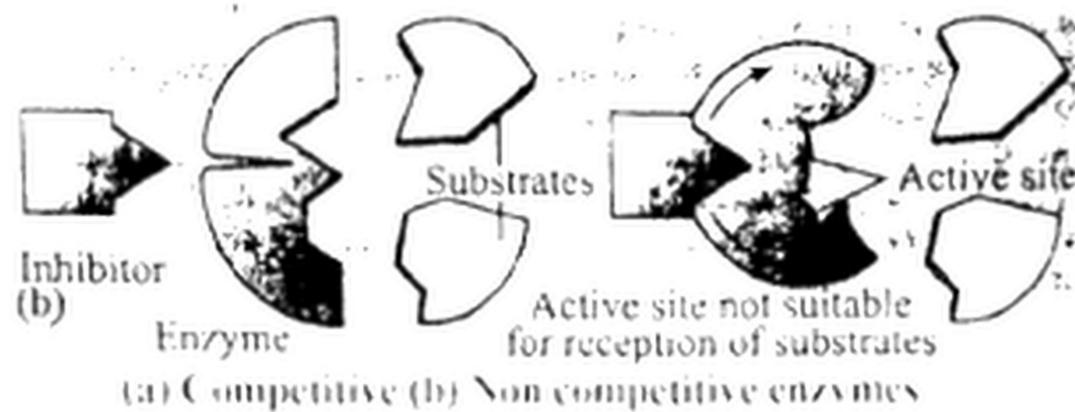
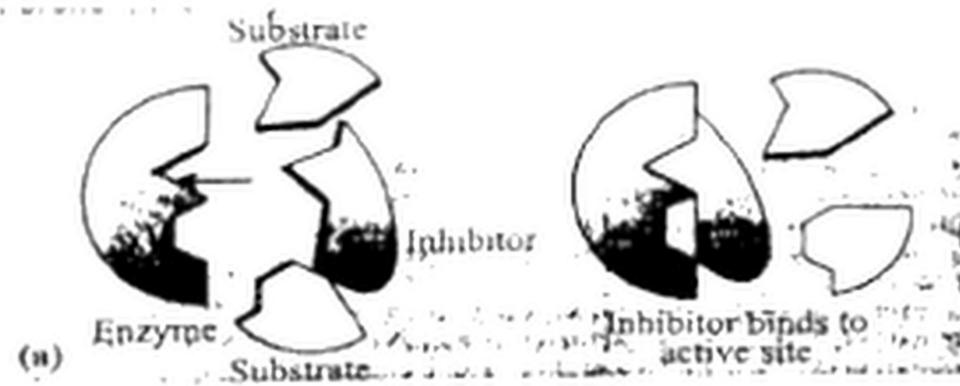
The examples of irreversible non-competitive inhibitors include cyanides and salts of heavy metals.

**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 prosthetic group.

**Ions of heavy metals:**

Ions of heavy metals such as mercury, silver and copper ( $\text{Hg}^{++}$ ,  $\text{Ag}^+$  and  $\text{Cu}^{++}$ ) 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.



### 36. Explain feedback mechanism with reference to enzymes.

**Ans:**

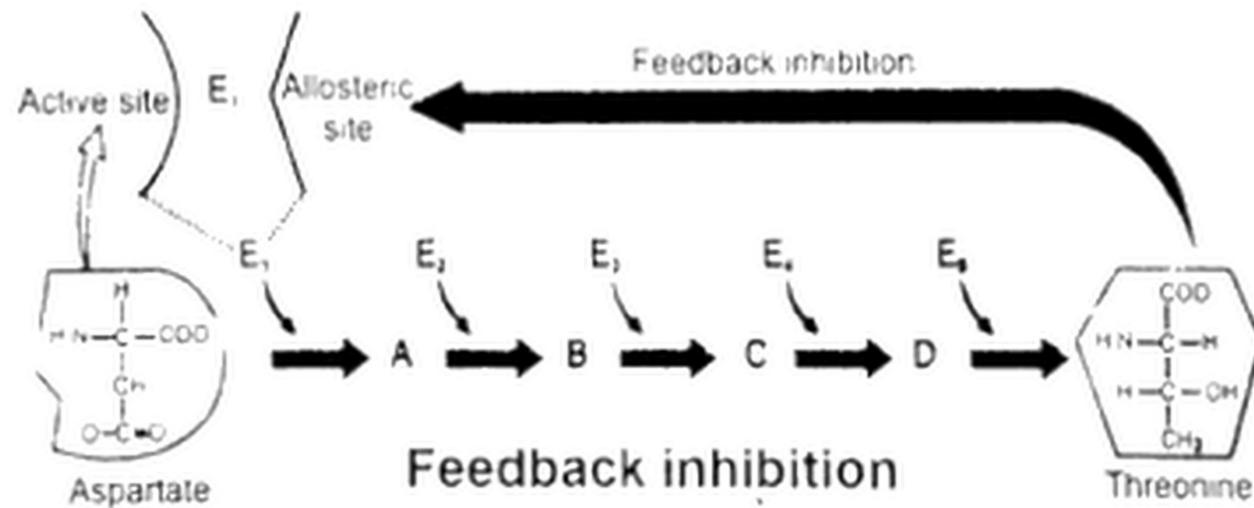
#### **Feedback mechanism:**

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 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 the enzymes based on reactions catalyzed.**

**Ans:**

**Classification of Enzymes:**

Based on reactions that enzymes catalyze, they have been classified into six groups.

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

**1-Oxidoreductases:**

The enzymes catalyze oxidation/reduction of their substrate and act by removing or adding electron or H<sup>+</sup> ions from or to the substrate

**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.

**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.

**Example:**

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 examples of hydrolases.

**4-Lyases:**

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

**Examples:**

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.

**Examples:**

Phosphohexos isomerase changes glucose 6-phosphate to fructose 6-phosphate.

**6-Ligases:**

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

**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	$A - B + C \rightleftharpoons A + C - B$
3.	hydrolases	$A - B + H_2O \rightleftharpoons A - H + B - OH$
4.	Lyases	$A - B \rightleftharpoons A + B$ (reverse reaction synthesis)
5.	Isomerases	$A - B - C \rightleftharpoons A - B - C$
6.	Ligases	$A + B + ATP \rightleftharpoons A - B + ADP + Pi$

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

Ans:

**Classification of Enzymes:**

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

**Proteases:**

These enzymes act upon proteins.

**Example:**

Pepsin and trypsin (both digest large 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 glycerol.

**Example:**

Pancreatic lipases.

**Carbohydrates:**

These enzymes cause breakdown of carbohydrates.

**Examples:**

- (a) Amylase (digest starch or glycogen into maltose)
- (b) Cellulase (digest cellulose into cellobiose, disaccharide)
- (c) Maltase (Digest maltose into glucose)
- (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 breakdown of DNA and RNA.

**Example:**

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

