PRE-MEDICAL

# BOTANY

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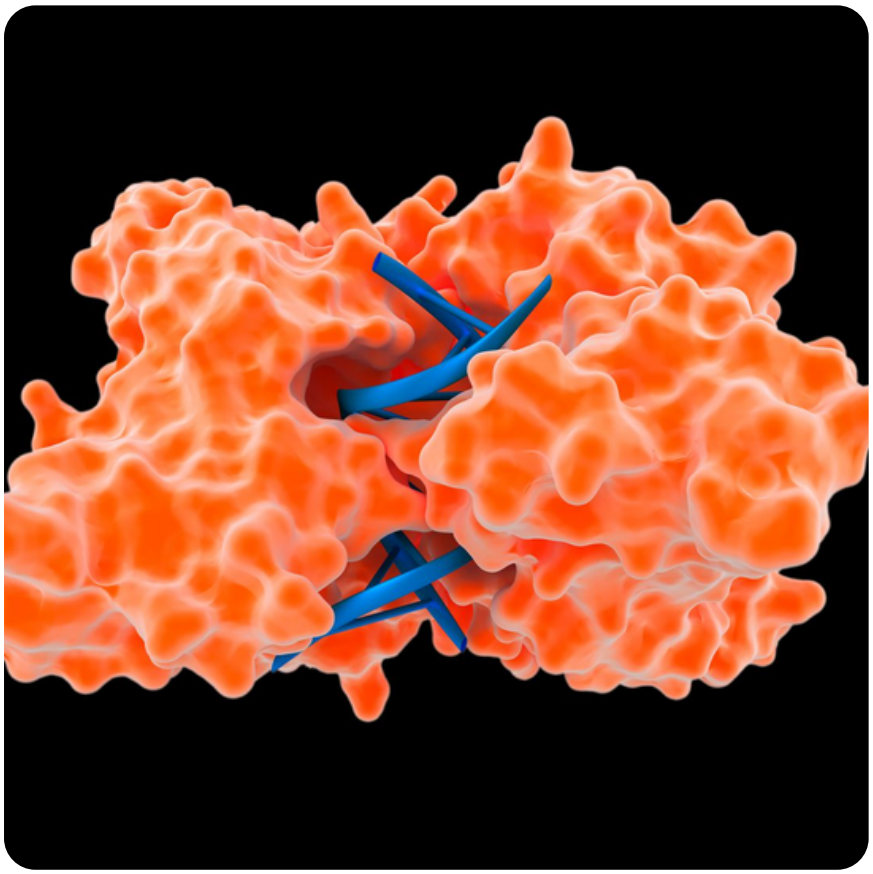


## STUDY MATERIAL

## Enzymes

ENGLISH MEDIUM

# Copyright Statement

**MELVIN CALVIN** born in Minnesota in April, 1911 received his Ph.D. in Chemistry from the University of Minnesota. He served as Professor of Chemistry at the University of California, Berkeley.

Just after world war II, when the world was under shock after the Hiroshima-Nagasaki bombings, and seeing the ill effects of radioactivity, Calvin and co-workers put radioactivity to beneficial use.

He along with J.A. Bassham studied reactions in green plants forming sugar and other substances from raw materials like carbon dioxide, water and minerals by labelling the carbon dioxide with $C^{14}$. Calvin proposed that plants change light energy to chemical energy by transferring an electron in an organised array of pigment molecules and other substances. The mapping of the pathway of carbon assimilation in photosynthesis earned him Nobel Prize in 1961.

The principles of photosynthesis as established by Calvin are, at present, being used in studies on renewable resource for energy and materials and basic studies in solar energy research.

# ENZYME

## 01. INTRODUCTION

- **Introduction**
- **Characteristics of Enzymes**
- **How do Enzymes bring about such-high rates of Chemical Conversions**
- **Nature of Enzyme Action**
- **Cofactors**
- **Classification & Nomenclature of Enzymes**
- **Factors Affecting Enzyme Activity**
- **Dynamic State of Body Constituents - Concept of Metabolism**
- **Metabolic Basis for Living**
- **The Living State**

- **Enzyme = En + Zyme**
  ⇓ ⇓
  **In + yeast**
- **Enzymes catalyse biochemical reactions in the cells.**
- **Buchner** discovered and isolated the enzyme **zymase** from yeast cells, while **Kuhne** coined the term enzyme.
- **J.B. Sumner** purified and crystalized **urease** enzyme from ***Canavalia*/Jack bean/Lobia plant.**

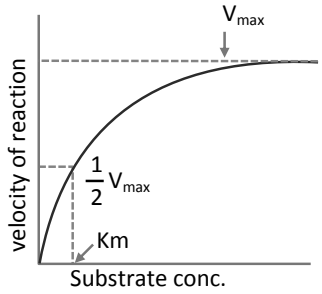
## 02. CHARACTERISTICS OF ENZYMES

- Almost all enzymes are protein. Though, there are **some nucleic acids** that behave like enzymes. These are called **ribozymes.**
- Enzymes are **colloidal substances,** which are macromolecules of amino acids and are synthesised by ribosomes under **genetic control.**
- Enzyme can be depicted by a **line diagram**. An enzyme like any protein has –
  **(a) Primary structure :** Amino acid sequence of the protein. It lacks active sites.
  **(b) Secondary structure :** It is a **helical structure** which also lacks active sites.
  **(c) Tertiary structure :** In this structure backbone of the protein chain folds upon itself, the chain criss-crosses itself and hence many crevices or pockets are made such pockets represent **active sites.**
  The catalytic structure of most of the enzymes are **tertiary** and **globular.**
  (d) **Quarternary structure :** Represented by isoenzymes and active sites are present.
- **Active site :** An active site of an enzyme is a crevice or pocket into which the substrate fits. Thus enzymes through their active site, catalyse reactions at a high rate.
- Enzymes are **very specific** to their substrate or reactions. They are required in very small amount to catalyse a reaction. Catalytic power of an enzyme depends upon –
  (a) Turn over number
  (b) Km constant
  **(a) Turn over number :** It is the number of substrate molecules converted into products per unit time by a molecule of enzyme. Thus, **catalytic power is directly proportional to turn over number. Carbonic anhydrase enzyme has turn over number 600000/sec.**

**(b) Km constant :** This was coined by **Michaelis** and **Menten.** It is the concentration of substrate at which rate of reaction attains half of its maximum velocity.

$$K_m \propto \frac{1}{2} V_{max}$$

**Catalytic power of an enzyme is inversely proportional to its Km value.**




**NCERT XI Page No. 157, Figure No. 9.7 (c)**

| Enzyme (Biocatalyst) | Inorganic catalyst |
|---|---|
| Enzymes are thermo-sensitive and get damaged at high temperatures (say above 40°C) | They work efficiently at high temperatures and high pressures. |

However, enzymes isolated from organisms who normally live under extremely high temperatures (eg. hot vents and sulphur springs), are stable and **retain their catalytic power even at high temperature.** Thermal stability is thus an important quality of such enzymes isolated from thermophilic organisms. e.g. taq polymerase.

**Uncatalysed reaction versus catalysed reaction**

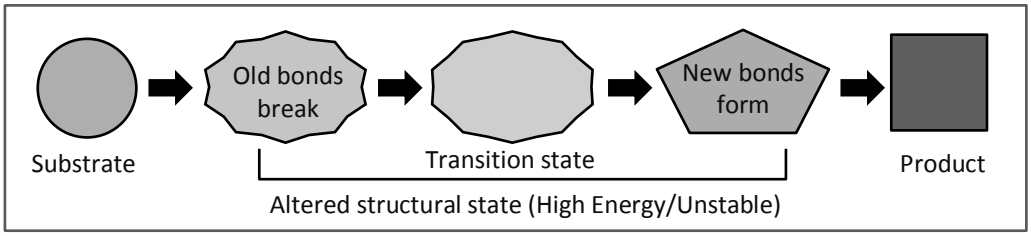
$$\underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Water}}{H_2O} \xrightleftharpoons{\text{Carbonic anhydrase}} \underset{\text{Carbonic acid}}{H_2CO_3}$$

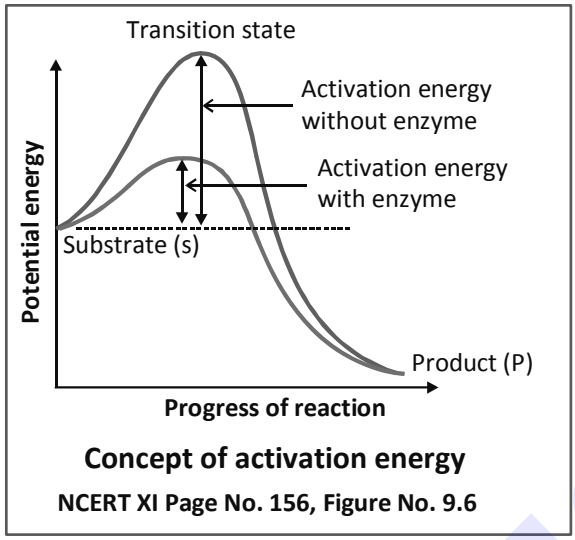
In the absence of an enzyme this reaction is very slow, with about 200 molecules of $H_2CO_3$ being formed in an hour. However, in the presence of **enzyme carbonic anhydrase** inside cytoplasm the reaction speeds dramatically with about **600,000 molecules being formed every second.** The enzyme has accelerated the reaction rate by **about 10 million times.**

## 03. HOW DO ENZYMES BRING ABOUT SUCH HIGH RATES OF CHEMICAL CONVERSIONS ?

The chemical or metabolic conversion refers to a **reaction.** The chemical which is converted into a product is called a **substrate**. Hence enzymes, i.e. proteins with three dimensional structures including an active site to convert a **substrate (S)** into a **product (P)**.

Symbolically, this can be depicted as : $S \rightarrow P$






**Concept of activation energy**

**NCERT XI Page No. 156, Figure No. 9.6**

**The y-axis represents the potential energy content.** The x-axis represents the progression of the structural transformation or states through the **'transition state'.** In above graph 'P' is at a lower level than 'S', thus this reaction is an **exothermic reaction.** (No need to supply energy in order to form the product.) However, whether it is an exothermic or spontaneous reaction or an endothermic or energy requiring reaction, the 'S' has to go through a much higher energy state or transition state.

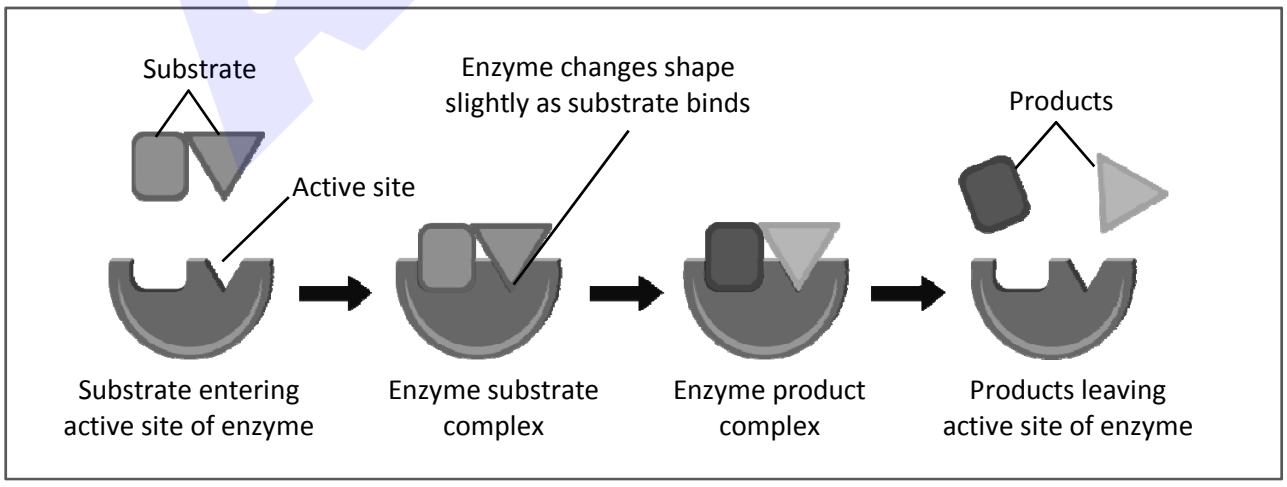
**"The difference in average energy content of 'S' from that of this transition state is called activation energy".**

Enzymes eventually bring down this energy barrier making the transition of 'S' to 'P' more easy.

## 04. NATURE OF ENZYME ACTION

Each enzyme (E) has a substrate (S) binding site in its molecular structure so that a highly reactive **enzyme-substrate complex (ES) is produced.** This complex is **short-lived** and dissociates into its product(s) P and the **unchanged enzyme** with an intermediate formation of the **enzyme-product complex (EP).** The formation of the ES complex is essential for catalysis.

**Enzyme + Substrate** $\rightleftharpoons$ **ES** $\longrightarrow$ **EP** $\longrightarrow$ **Enzyme + Product**



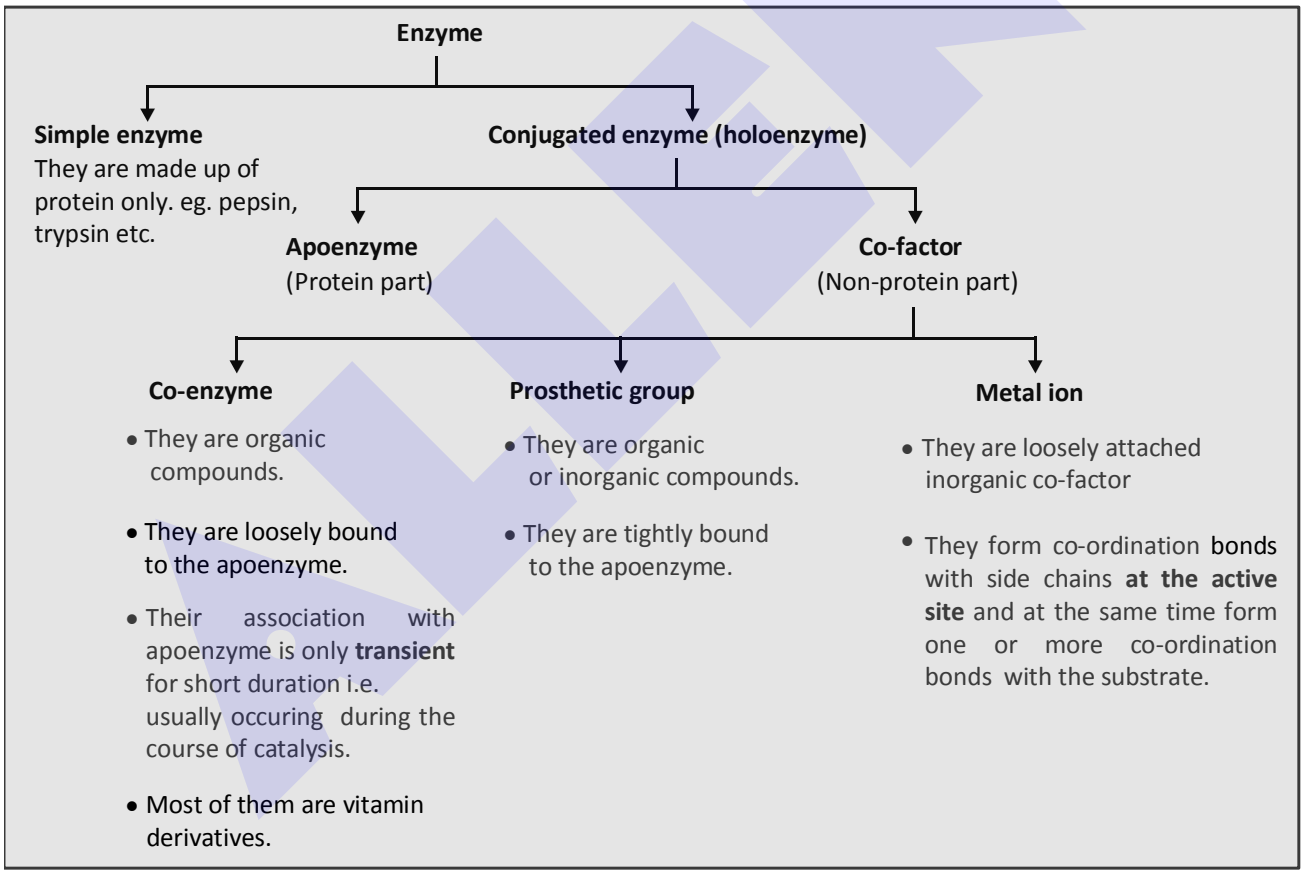
The catalytic cycle of an enzyme action can be described in the following steps:

- First, the **substrate binds to the active site of the enzyme,** fitting into the active site.
- The **binding of the substrate induces the enzyme to alter its shape, fitting more tightly** around the substrate.
- The **active site of the enzyme,** now in close proximity of the substrate **breaks or form the chemical bonds of the substrate** and the **new enzyme- product complex is formed.**
- The **enzyme releases the products** of the reaction and the free **enzyme is ready to bind to another molecule of the substrate** and run through the catalytic cycle once again.

## 05. COFACTORS

Enzymes are composed of **one or several polypeptide chains.** However, there are a number of cases in which **non-protein constituents called cofactors** are bound to the enzyme to make the enzyme catalytically active. In these instances, the **protein portion of the enzymes is called the apoenzyme and non protein portion is called the cofactor**.

Three kinds of cofactors may be identified: **co-enzymes, prosthetic groups** and **metal ions.**




**Important examples of coenzymes, prosthetic group and metal ions :**

- Co-enzyme nicotinamide adenine dinucleotide (NAD) and NADP contain the vitamin **niacin**.
- In **peroxidase** and **catalase**, **haem** is the prosthetic group and it is a part of the active site of the enzyme.
- Zinc (Zn) is a metal ion cofactor for the proteolytic enzyme **carboxypeptidase**.

## 06. CLASSIFICATION AND NOMENCLATURE OF ENZYMES

Thousands of enzymes have been discovered, isolated and studied. Most of these enzymes have been classified into different groups **based on the type of reactions they catalyse.** Enzymes are divided into **6 classes** each with 4-13 subclasses and named accordingly by a four-digit number.

**(I)** **Oxidoreductases/dehydrogenases :** Enzymes which catalyse oxidoreduction (oxidation-reduction) between two substrates i.e. **S** and **S'** e.g. cytochrome c oxidase, dehydrogenase etc.

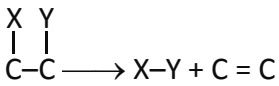
S reduced + S' oxidised $\longrightarrow$ S oxidised + S' reduced

**(II)** **Transferases :** Enzymes catalysing a transfer of a group, **G (other than hydrogen)** between a pair of substrate **S** and **S'**. e.g. transaminase, hexokinase etc.

S–G + S' $\longrightarrow$ S + S'–G

**(III)** **Hydrolases :** Enzymes catalysing hydrolysis of ester, ether, peptide, glycosidic, C–C, C–halide or P–N bonds. e.g. proteases, lipases, carbohydrases etc.

**(IV)** **Lyases :** Enzymes that catalyse removal of a group from substrate by **mechanisms other than hydrolysis** and leaving double bonds. e.g. aldolase.



**(V)** **Isomerases :** Includes all enzymes catalysing inter-conversion of optical, geometric or positional isomers. e.g. hexoisomerase, mutase etc.

**(VI)** **Ligases/synthase :** Enzymes catalysing the linking together of two compounds. Such enzymes catalyse joining of C–O, C–S, C–N, P–O etc. bonds. e.g. citrate synthase, DNA ligase etc.

## 07. FACTORS AFFECTING ENZYME ACTIVITY

The activity of an enzyme can be affected by a **change in the conditions which can alter the tertiary structure of the protein.** These include :

(1) Temperature
(2) pH
(3) Change in substrate concentration
(4) Inhibitor

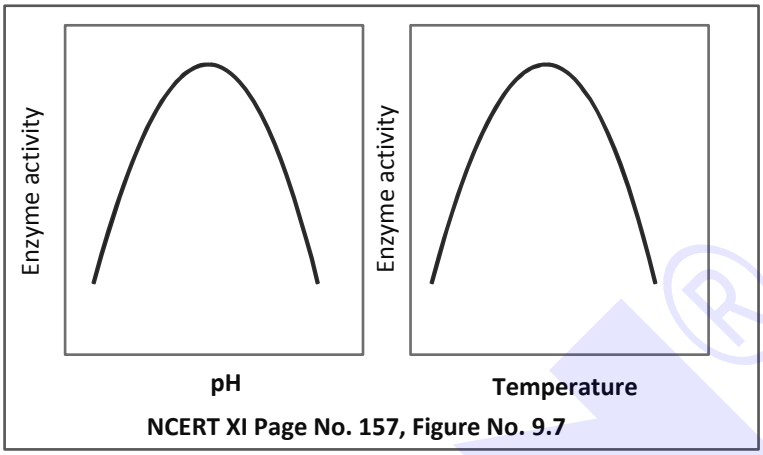
### (1) TEMPERATURE

Enzymes generally function in a **narrow range** of temperature. Each enzyme shows its highest activity at a particular temperature called the **optimum temperature.** Activity declines both below and above the optimum value. **Low temperature preserves the enzyme in a temporarily inactive state** whereas high temperature destroys enzymatic activity because **proteins are denatured by heat.**

**A general rule of thumb is that rate doubles or decreases by half for every 10°C change in either direction. Thus, value of $Q_{10}$ for enzymatic activities is 2.**

## (2) pH

Enzymes generally function in a **narrow range** of pH. Each enzyme shows its highest activity at a particular pH called the **optimum pH.** Activity declines both below and above the optimum value.



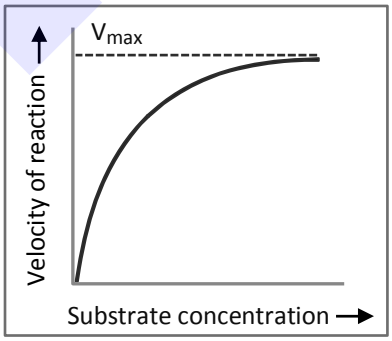

**NCERT XI Page No. 157, Figure No. 9.7**

## (3) SUBSTRATE CONCENTRATION

With the increase in substrate concentration, the velocity of the enzymatic reaction **rises at first.** The reaction ultimately reaches a maximum velocity ($V_{max}$)**.**

**This velocity is not exceeded by any further rise in concentration of the substrate.**

**Reason :** The enzyme molecules are fewer than the substrate molecules and after saturation of these enzyme molecules, there are no free enzyme molecules to bind with the additional substrate molecules.




**Change in substrate concentration**

**NCERT XI Page No. 157, Figure No. 9.7**

## (4) INHIBITORS/ENZYME INHIBITION

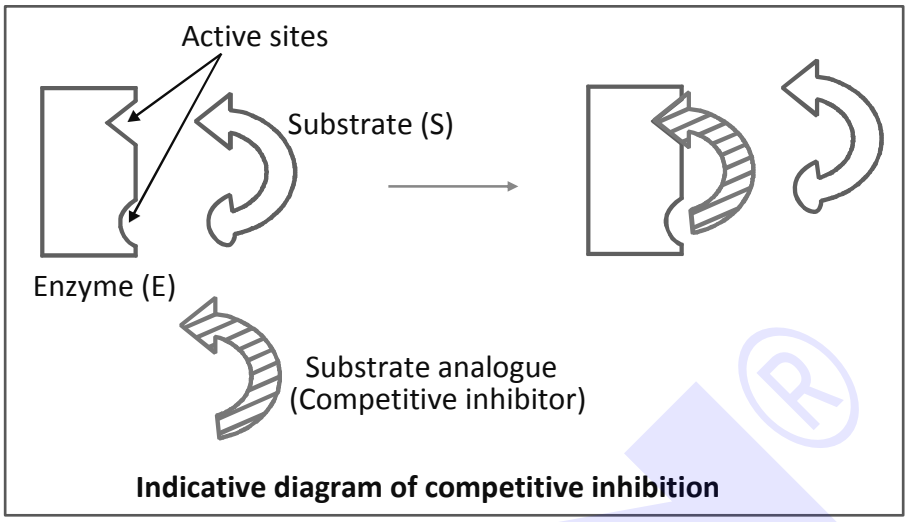
The activity of an enzyme is also sensitive to the presence of **specific chemicals that bind to the enzyme.** When the binding of the chemical shuts off (inhibits) enzyme activity, the process is called **inhibition** and the chemical is called an **inhibitor.**

**It is of two types :**

### (A) Competitive inhibition :

- When the **inhibitor closely resembles the substrate (substrate analogues)** in its molecular structure and **binds with active site** of enzyme leads to inhibition of enzyme activity.

- It is competitive as well as **reversible** because competitive inhibitor binds with active site of enzyme reversibly to form an enzyme-inhibitor complex.




**Indicative diagram of competitive inhibition**

**eg.** **(i)** **Step 8 of Krebs cycle** – Inhibition of succinate dehydrogenase by malonate. Malonate is structural analogue for substrate succinate.

**(ii)** Inhibition of folic acid synthesis by sulpha drugs.

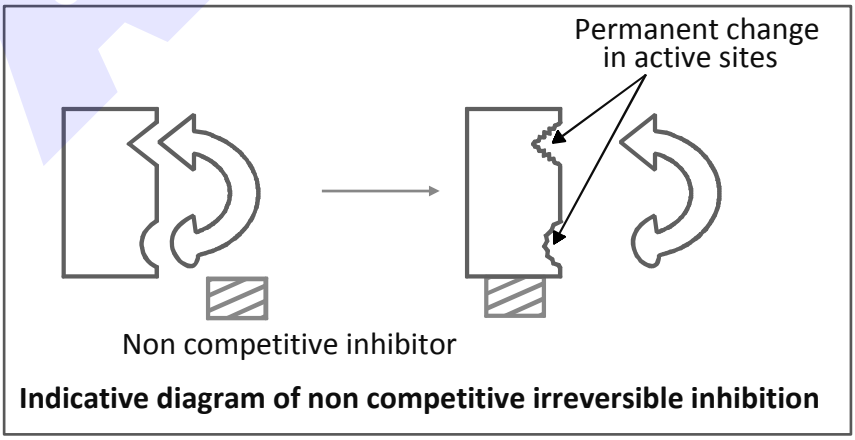
- In Bacteria, p-amino benzoic acid (PABA) is a **precursor of folic acid.** Bacteria requires folic acid for growth and multiplication. Sulpha drugs are structural analogues of p-amino benzoic acid, thus inhibits synthesis of folic acid. This competitive inhibition is often used in the controls of bacterial pathogens.

**(B) Non competitive inhibition :**

**It is again of two types :**

**(i) Non competitive irreversible :**

- When the inhibitor binds irreversibly with the **site other than active site**.

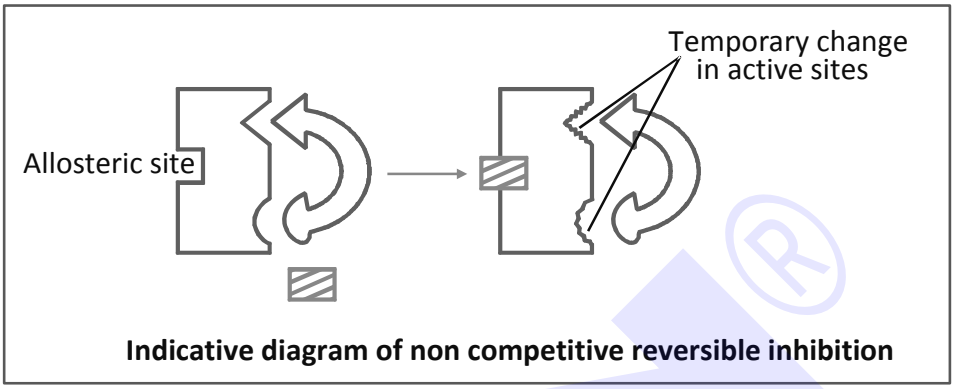



**Indicative diagram of non competitive irreversible inhibition**

**eg.** Cyanide binds with Cu center of cytochrome c oxidase.

**(ii) Non competitive reversible : (Mostly)**

- Some enzymes have **allosteric site** to control active site. This control is called **modulation.**
- Most of non competitive inhibitors bind reversibly with **allosteric site** and negatively change the configuration of active site. Such inhibition is called **non competitive reversible inhibition or negative allosteric modulation.**




**Indicative diagram of non competitive reversible inhibition**

**eg.** **Step 3 of glycolysis – Phosphofructokinase (PFK),** pacemaker enzyme of glycolysis is inhibited reversibly by excess of ATP (negative modulation).

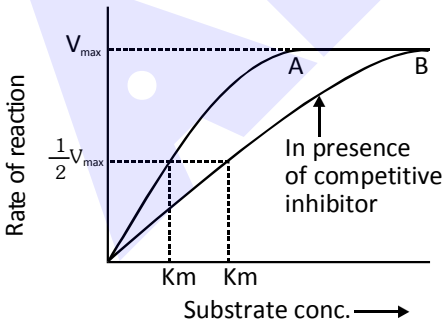
- Some allosteric enzymes are inhibited by the product of that biochemical reaction which is catalysed by them, such inhibition called **feedback inhibition** or **retro inhibition** or **product inhibition.**

**eg.** **Step 1 of glycolysis –**

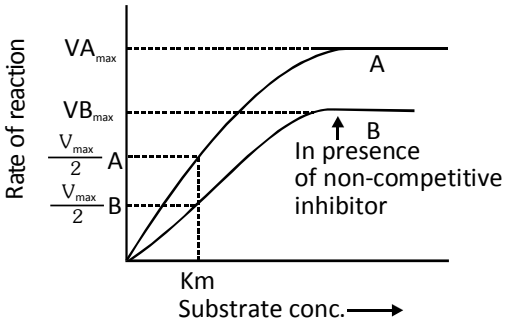
$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{++}]{\text{Hexokinase}} \text{Glucose} - 6 - \text{phosphate} + \text{ADP}$$

In above reaction the enzyme hexokinase is inhibited reversibly by excess of product (glucose-6-phosphate)

- In the presence of competitive inhibitor Km increases while $V_{max}$ remains unchanged.
- In the presence of non competitive inhibitor Km remains unchanged while $V_{max.}$ decreases. Km is not applicable for allosteric modulation.




**Effect of competitive inhibitor**




**Effect of non competitive inhibitor**

**Note : Allosteric site may also be used to increase enzymatic activity such phenomenon is called positive allosteric modulation. eg. Phosphofructokinase/PFK** activated by excess of AMP (positive modulation).

## 08. DYNAMIC STATE OF BODY CONSTITUENTS - CONCEPT OF METABOLISM

- All biomolecules have a turn over. This means that they are constantly being changed into some other biomolecules and also made from some other biomolecules. This breaking and making is through chemical reactions constantly occuring in living organisms. Together all these chemical reactions are called **metabolism**.
- Each of the metabolic reactions results in the transformation of biomolecules. A few examples for such metabolic transformations are; removal of $CO_2$ from amino acids making an amino acid into an amine, removal of amino group in a nucleotide base; hydrolysis of a glycosidic bond in a disaccharide, etc.
- Majority of these metabolic reactions do not occur in isolation but are always linked to some other reactions. In other words, metabolites are converted into each other in a series of linked reactions called metabolic pathways.
- These metabolic pathways are similar to the automobile traffic in a city. These pathways are either linear or circular. These pathways criss-cross each other, i.e., there are traffic junctions. Flow of metabolites through metabolic pathway has a definite rate and direction like automobile traffic. This metabolic flow is called the dynamic state of body constituents.
- There is no uncatalysed metabolic conversion in living systems. Even $CO_2$ dissolving in water, a physical process, is a catalysed reaction in living systems.

## 09. METABOLIC BASIS FOR LIVING

- Metabolic pathways can lead to a more complex structure from a simpler structure (for example, acetic acid becomes cholesterol) or lead to a simpler structure from a complex structure (for example. glucose becomes lactic acid in our skeletal muscle). The former cases are called biosynthetic pathways or anabolic pathways. The latter constitute degradation and hence are called catabolic pathways.
- Anabolic pathways, as expected, consume energy. Assembly of a protein from amino acids requires energy input. On the other hand, catabolic pathways lead to the release of energy. For example, when glucose is degraded to lactic acid in our skeletal muscle, energy is liberated.
- How do living organisms derive their energy? What strategies have they evolved? How do they store this energy and in what form? How do they convert this energy into work? All this study comes under a sub-discipline called **'Bioenergetics'.**

## 10. THE LIVING STATE

- Chemical compounds in a living organism, otherwise called metabolites, or biomolecules, are present at concentrations characteristic of each of them. For example, the blood concentration of glucose in a normal healthy individual is 4.2 m mol/L – 6.1 m mol/L, while that of hormones would be nanograms/mL.
- The most important fact of biological systems is that all living organisms exist in a steady-state characterised by concentrations of each of these biomolecules. These biomolecules are in a metabolic flux. Any chemical or physical process moves spontaneously to equilibrium.
- One should remember from physics that systems at equilibrium cannot perform work. As living organisms work continuously, they cannot afford to reach equilibrium. Hence the **living state is a non-equilibrium steady-state** to be able to perform work.
- Living process is a constant effort to prevent falling into equilibrium. This is achieved by energy input. Metabolism provides a mechanism for the production of energy. Hence the living state and metabolism are synonymous. Without metabolism there cannot be a living state.

## ★ Golden Key Points ★

- Almost all enzymes are protein. Though, there are **some nucleic acids** that behave like enzymes. These are called **ribozymes.**
- The **protein portion of the enzymes is called the apoenzyme and non protein portion is called the cofactor**.
- **Co-enzyme** are organic compounds. They are loosely bound to the apoenzyme.
- **Prosthetic group** are organic or inorganic compounds. They are tightly bound to the apoenzyme.
- **Metal ion** are loosely attached inorganic co-factor.
- **A general rule of thumb is that rate doubles or decreases by half for every 10°C change in either direction.**
- Competitive inhibition is often used in the controls of bacterial pathogens.

## BEGINNER'S BOX — ENZYME : INTRODUCTION TO THE LIVING STATE

**1.** The catalytic structure of most of the enzymes is :-
(1) quaternary and linear protein (2) secondary and helical protein
(3) primary and linear protein (4) tertiary and globular protein

**2.** Which of the following is/are correct ?
(1) Catalytic power of an enzyme is related to its Km value.
(2) Thermal stability can be a quality of some enzymes.
(3) Enzyme-substrate complex is a short lived structure.
(4) All of the above

**3.** Which of the following is a incorrect match?
(1) Enzyme – one or several polypeptide chains.
(2) Prosthetic group – only organic compound.
(3) Coenzymes – mostly vitamins.
(4) Metal ion – coordination bonds with active site.

**4.** Which of the following is an incorrect match?
(1) Lyases enzymes – breakdown without help of water.
(2) Low temperature – enzyme temporarily inactive.
(3) Competitive inhibition – binding of cyanide to cytochrome 'c' oxidase
(4) Hexokinase – feedback inhibition.

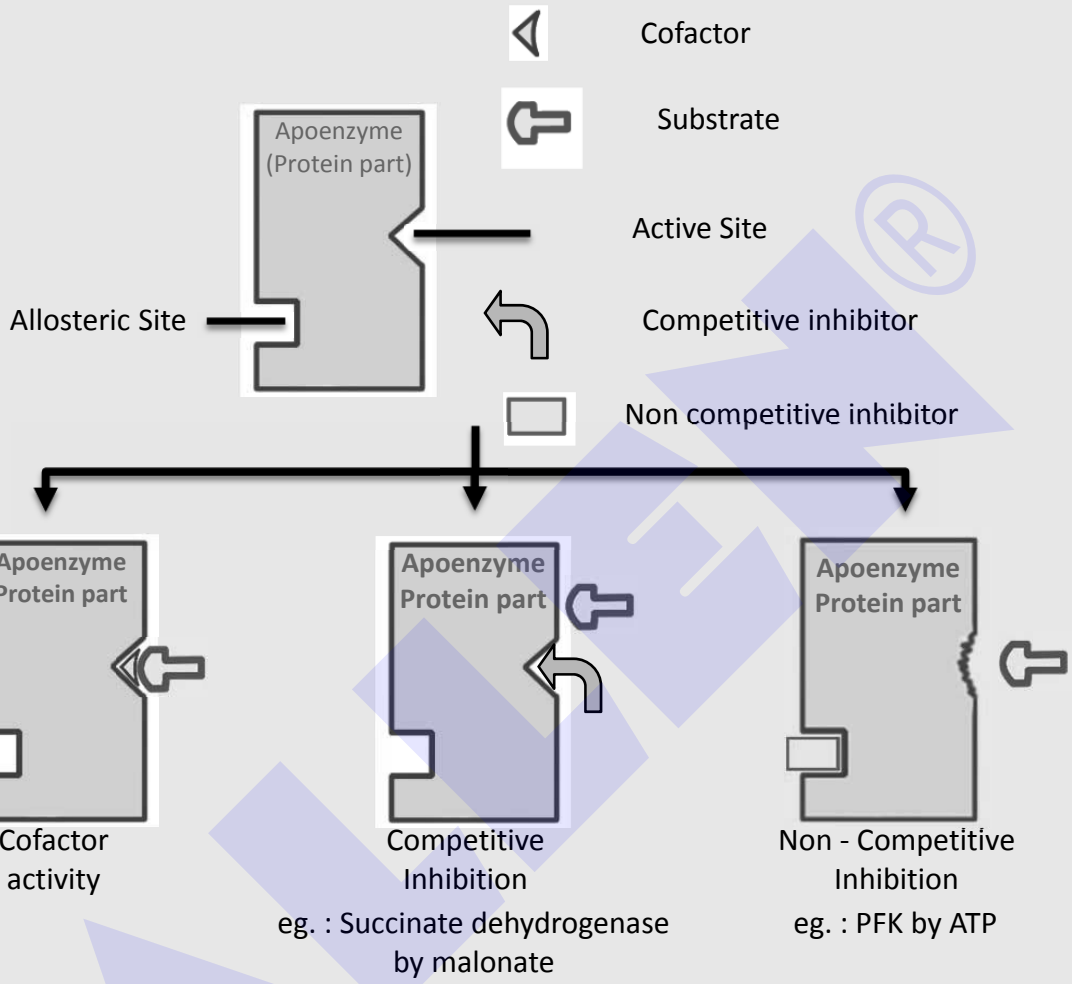
**5.** The blood concentration of glucose in a normal healthy person is :-
(1) 4.2 – 6.1 m mole/L (2) 1.1 – 1.5 m mole/L
(3) 10.5 – 19.5 m mole/L (4) 25 – 50 m mole/L

## BEGINNER'S BOX — ANSWER KEY

### ENZYME : INTRODUCTION TO THE LIVING STATE

| Que. | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Ans. | **4** | **4** | **2** | **3** | **1** |

ESSENCE




**Classification of Enzymes**

1. **Oxidoreductases/dehydrogenases: Catalyse the transfer H / e between two substrates.**
2. **Transferases: Catalyse the transfer of group other than hydrogen between two substrates.**
3. **Hydrolases : Breakdown of ester, ether, peptide, glycosidic, C-C, C-halide or P-N bonds with the help of water.**
4. **Lyases: Enzymes that catalyse removal of groups from substrates without help of water.**
5. **Isomerases: Includes all enzymes catalysing inter-conversion of optical, geometric or positional isomers.**
6. **Ligases: Enzymes catalysing the linking together of two compounds.**