PHYSIOLOGY HONOURS (CBCS) SEMESTER-I
(MODULE-CC-1)

Prepared by: Dr. Madhumita Debnath
Asst. Professor in Physiology, Dr. K.L. Bhattacharyya College

Introduction

The living cell is the site of tremendous biochemical activity called **metabolism**. Enzyme facilitate life processes in essentially all life-forms from viruses to man. Enzymes act as **life catalysts**, substances that **accelerate the rate of a chemical reaction**. Enzymes **do not initiate reactions** that would not naturally occur but they accelerate any reaction that is already underway. **Enzymes enable the reaction to take place more rapidly at a safer, relatively low temperature that is consistent with living systems.** Enzymes are neither consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions. Enzymes are known to catalyze about 4,000 biochemical reactions.

- Enzymes can be defined as **colloidal, thermo-labile, organic catalysts synthesized by living cells**.
- They **catalyze thermodynamically possible reactions** both inside and outside of a cell by changing covalent bonds in specific substances called 'substrates'.

Chemical nature of Enzymes:

1. Enzymes are **protein** in nature. Some are simple proteins. Others are conjugated proteins.
2. Some proteins have single polypeptide chain in molecule. They are called **monomeric enzymes**. E.g., DNA polymerase-I in E.Coli.
3. Some are made of more than one polypeptide chains and are called **oligomeric enzymes**. Eg., Pyruvate kinase, lactate dehydrogenase etc. Oligomeric enzymes may be homo-dimeric(citrate synthase), hatero-dimeric (succinate thiokinase), hatero-tetrameric (isocitrate dehydrogenase) etc.
4. Sometimes several enzyme activities are located in different sites of a single macromolecule known as **multienzyme system**. They lose their catalytic activities if separated. They usually catalyze different consecutive reactions of a metabolic pathway. Eg., Fatty acid synthase, pyruvate dehydrogenase etc.

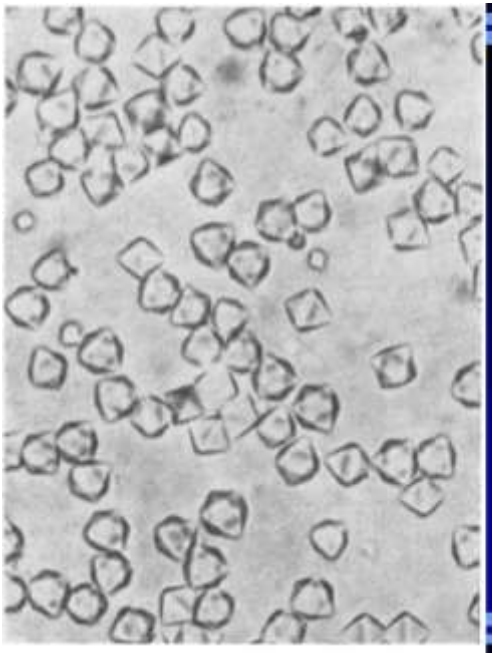
3) Sometimes **few RNA molecules exhibit enzymatic activities**. They are called **ribozymes.** The well-established natural ribozymes known to date are the hairpin, hammerhead, Hepatitis delta virus (HDV), Varkud Satellite, and *glmS* ribozymes, which form the classes of **small ribozymes**, as well as the group I and II introns, the ribosome, spliceosome, and RNase P, which are classified as **large ribozymes.** Ribozymes catalyze reactions such as RNA splicing, RNA cleavage and protein synthesis.

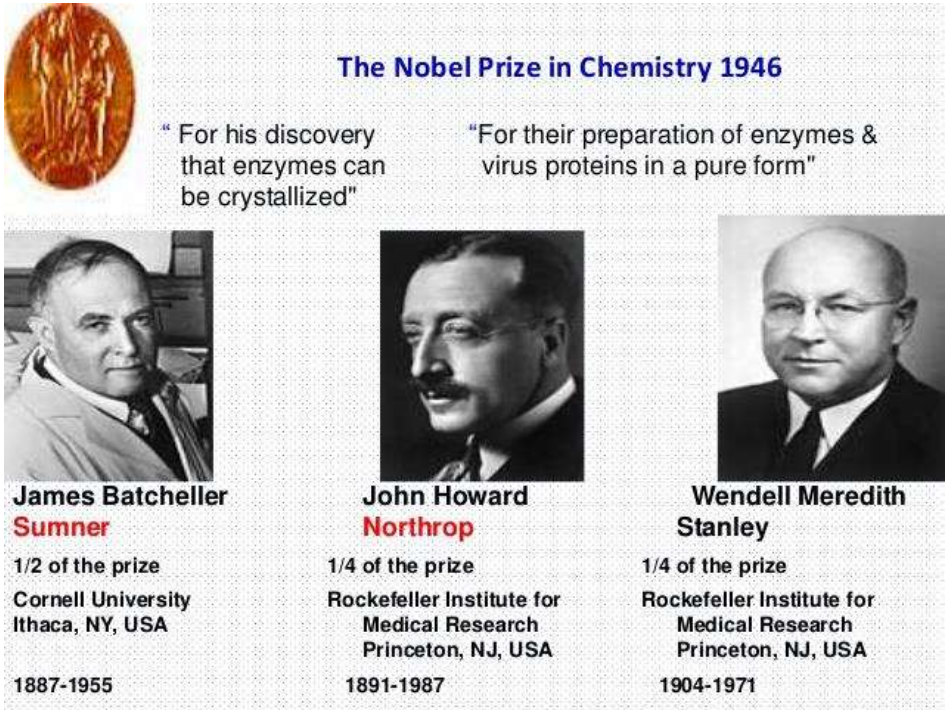
4) **Abzymes (antibody-enzyme)** is an antibody that expresses catalytic activity. A single molecule of an abzyme is capable of catalyzing the destruction of thousands of target molecules. These catalytic antibodies are selected from monoclonal antibodies generated by immunizing mice with haptens that mimic the transition states of enzyme-catalyzed reactions. For example, the 28B4 abzyme catalyzes periodate oxidation of *p*-nitrotoluene-methyl sulfide to sulfoxide.

HISTORY OF EVOLUTION OF ENZYME:

- The existence of enzymes has been known for well over a century. Biological catalysis was first recognized and described in the **early 1800s**, in studies of the digestion of meat by secretions of the stomach and the conversion of starch into sugar by saliva and various plant extracts.
- Not until 1897 was it shown by German chemist **Edward Buchner** that cell-free extracts of yeast could ferment sugars to alcohol and carbon dioxide, Buchner denoted his preparation as **zymase**.
- In **1876, William Kuhne** proposed that the name **'enzyme'** be used as the new term. The word itself means 'in yeast' and is derived from the Greek **'en' meaning 'in',** and **'zyme' meaning 'yeast'** or 'leaven'.
- It was not until **1926,** however, that the first enzyme was obtained in pure form, a feat accomplished by American biochemist **James B. Sumner** of Cornell University. Sumner was able to isolate and crystallize the enzyme **urease from the jack bean**. His work was to earn him the **1947 Nobel Prize.**

The crystallization of **urease** isolated from jack bean (Canavalia ensiformis) seeds by **James B. Sumner, in 1926**, demonstrated the proteinaceous nature of enzymes, a discovery laureated with the Nobel Prize in Chemistry in **1946.**

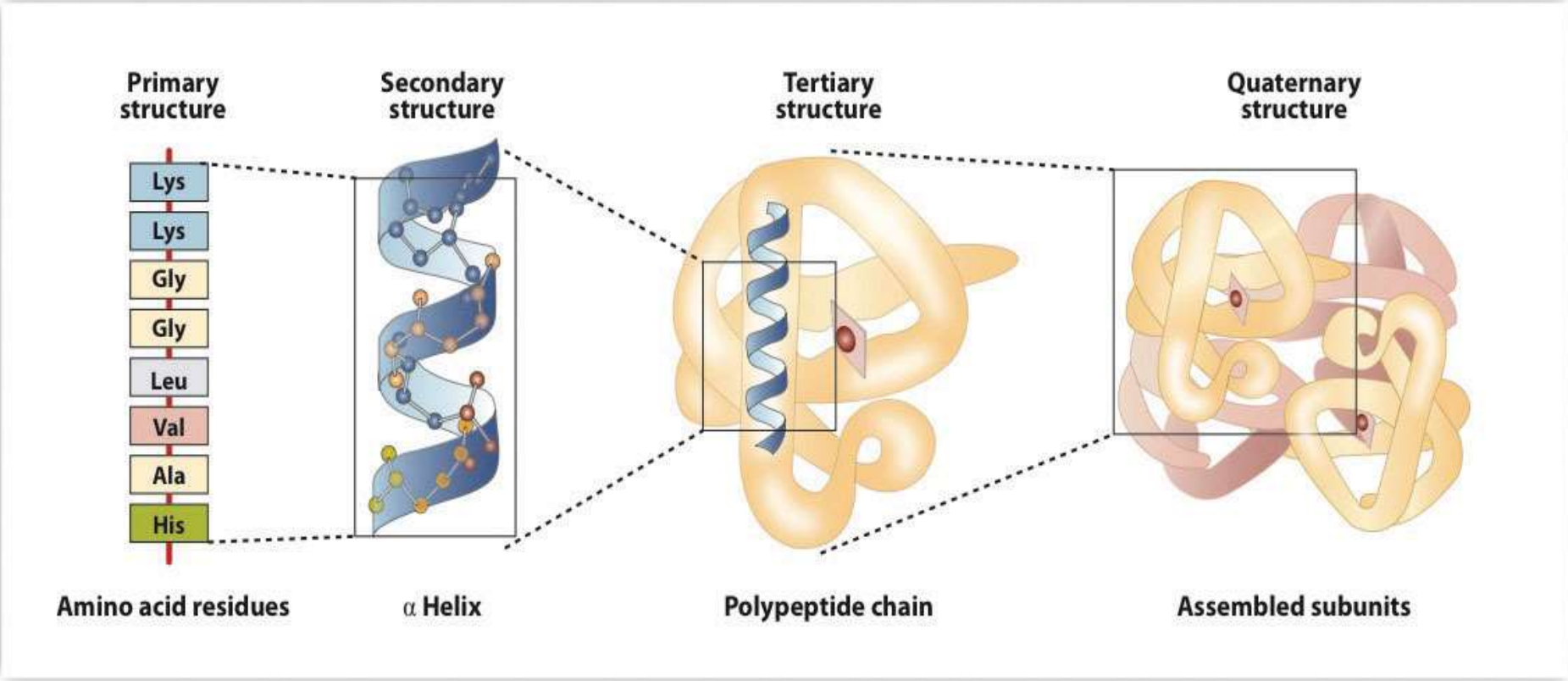




Structure of enzymes:

Enzymes are proteins Like all proteins, enzymes are composed mainly of the 20 naturally occurring amino acids. The structures of enzymes can be elucidating by the physical methods such as Spectroscopic methods, x-ray crystallography, and more recently, multidimensional NMR methods. On the basis of arrangement of amino acids, enzyme structure can be classified into following types:

1. Primary structure:
This amino acid sequence of the peptide chains is the primary structure of the enzyme.
2. Secondary structure:
Secondary structure is due to the interaction of amino acids with each other in
the same chain of protein. As a result the protein chain can fold up to generate α-helix or β-sheet.
3. Tertiary structure:
The arrangement of secondary structure elements and amino acid side chain interactions result in folded structure of the protein (3D structure). So that specific contacts are made between amino acid side chains and between backbone groups.
4. Quaternary structure and domains:
Many enzymes consist of more than one polypeptide chain (or subunit) that aggregate to confer catalytic activity. In some enzymes the subunits are identical, in others they differ in sequence and structure. This description of subunit arrangement in such enzymes is called the quaternary structure.


Primary structure
Secondary structure
Tertiary structure
Quaternary structure
Lys
Lys
Gly
Gly
Leu
Val
Ala
His
Amino acid residues
α Helix
Polypeptide chain
Assembled subunits

Comparison of Enzyme with Inorganic Catalyst:

SIMILARITIES AND DIFFERENCES BETWEEN ENZYMES AND CATALYSTS

SIMILARITIES

- Both are needed in minute quantities
- Both accelerate the rate of a reaction, but cannot initiate one
- Both of them bring about a decrease in the activation energy
- Both of them temporarily combine with the substrate molecule
- Both of them do not undergo any change in their composition and hence, can be used again and again
- Both of them do not alter the nature and quantity of the end products
- The reaction accelerated by both of them is reversible

DIFFERENCES

- Enzymes are complex proteins while catalysts are simple inorganic molecules
- Enzymes have a very high molecular weight while catalysts have a low molecular weight
- Enzymes catalyse only biological reactions
- Enzymes catalyse specific types of reactions while catalysts have a wide range
- Enzymes are significantly affected by temperature and pH, but not so in the case of catalysts
- Enzymes are regulated by specific substances called cofactors, but not so in the case of catalysts
- Enzymes can be easily inactivated but not so in the case of catalysts

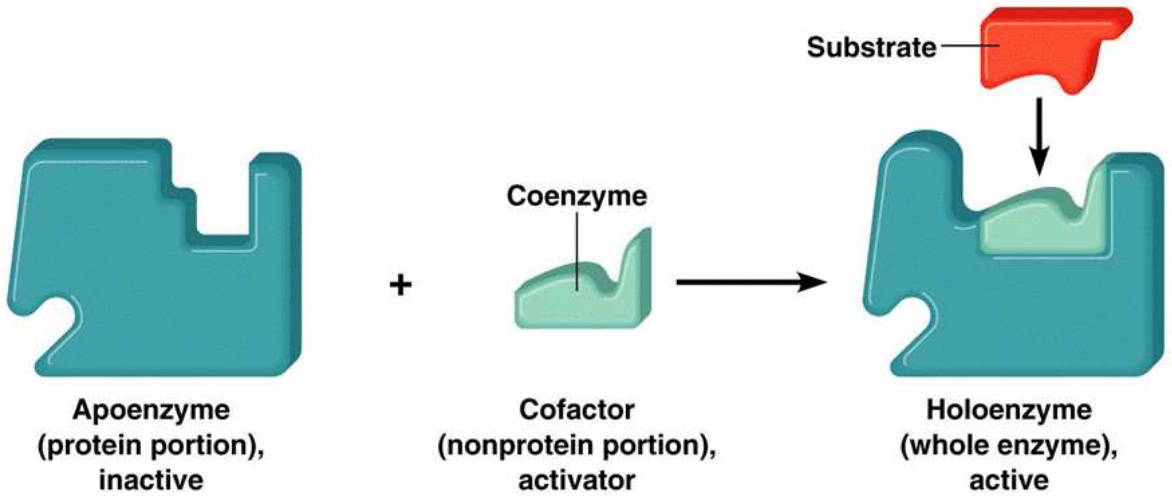
APAOENZYME AND HOLOENZYME

Most of the enzymes, except catalytic RNAs or ribozymes, are proteins. Some enzymes also require a non-protein part for their activity. Based on these, enzymes are categorised into two types: **Simple enzymes** (only made up of proteins, e.g. trypsin, pepsin, etc.) and **Conjugate enzymes or holoenzymes** .

1. Conjugate enzymes (Holoenzymes) consist of a protein as well as non-protein part essential for the activity. The protein part of the holoenzyme is known as **apoenzyme**, which is inactive. The non-protein part is called a **cofactor** and is necessary for the catalytic function of the enzymes.
2. So, **holoenzyme** is an active enzyme-cofactor complex, i.e. an apoenzyme attached to a cofactor.

Apoenzyme (Inactive) + Cofactor ⇌ Holoenzyme (Active).

Example of holoenzyme includes catalase, alcohol dehydrogenase, pyruvate kinase, ETC.



COENZYME, COFACTORS & PROSTHETIC GROUP

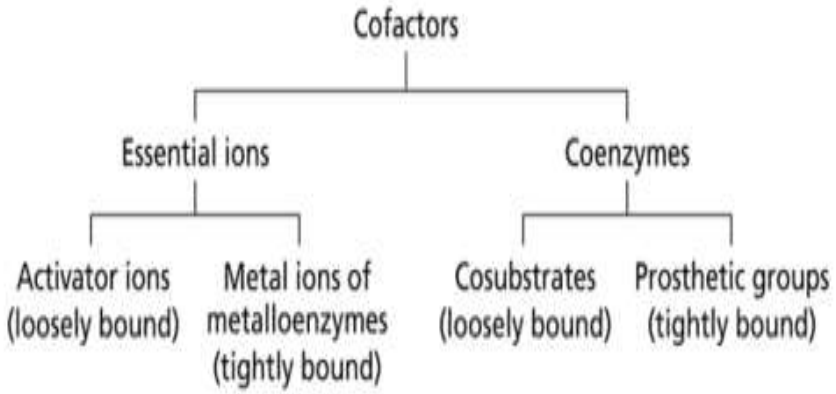
The terms, **cofactor, coenzyme and prosthetic group** have been defined through different perspectives.

1. A **cofactor** is any factor essentially required for enzyme activity or protein function. They can be **inorganic metal ions** or **organic factor.**

2. A **coenzyme** is an **organic cofactor which is directly involved in enzyme catalyzed reaction**. These molecules often sit at the active site of an enzyme and aid in recognizing, attracting, or repulsing a substrate or product. They are intermediate carriers of an atom or group of atoms, allowing a reaction to occur. Coenzymes **cannot function on their own** and require the presence of an enzyme. They are loosely bound to enzyme. The B vitamins serve as coenzymes for a number of enzymes.

   Example- coenzyme A, acetyl coenzyme A, cellular redox coenzymes: NAD^+ , NADH $NADP^+$ and NADPH etc.

(A cofactor which is not directly involved in enzyme catalysis, or only associated with the function of a non-catalytic protein is not a coenzyme.)



A **prosthetic group** is a non-protein factor that remains **covalently associated with the enzyme (strongly bound)** and required for a particular enzyme function. Prosthetic groups **permanently bond** with a protein. Prosthetic groups help proteins bind other molecules, act as structural elements or act as charge carriers.

An example of a prosthetic group is **heme** in hemoglobin, myoglobin, and cytochrome, **TPP, PLP Biotin, FAD** etc.

(The prosthetic group can be termed a coenzyme if it is directly involved in catalytic reaction or a cofactor if it is not involved in enzyme catalyzed reaction but functionally essential to the enzyme.

Coenzyme	Prosthetic group
Cofactors that are loosely bound to proteins or enzymes	Cofactors that are tightly bound or stably associated with the enzymes
Small organic molecule	Can be metal ions or small organic molecules
Non- covalently bound	Covalently bound
Facilitates the biological transformation of the enzyme	Assists the functioning of the enzyme
Can be easily removed from the enzyme	Difficult to remove from the enzyme
Ex: Coenzyme A, biotin, folic acid, Vitamin B12	Ex: Metal ions such as Co, Mg, Cu, Fe and organic molecules such as FAD and biotin

With help of the analogy, classify each of the molecules described below as a/an:
a. inorganic cofactor,
b. coenzyme,
c. prosthetic group. More than one choice may apply

1) Succinate dehydrogenase (SDH) is a Krebs cycle enzyme. FAD is a vitamin derivative that is covalently bonded to SDH. _____

2) Two magnesium ions bind non-permanently to DNA polymerase and are needed for catalysis of DNA synthesis during replication and DNA repair. _____

3) For the blood transport protein hemoglobin to perform its function, it requires an iron atom as part of a tightly bound, non-polypeptide organic molecule called heme. _____

Solutions

1) **b. and c.** FAD is an organic molecule related to nucleotides and is considered a prosthetic group because it is covalently bonded to SDH.

2) a. Inorganic magnesium ions are essential for catalytic activity of DNA polymerase but are not covalently attached to the enzyme.

3) b and c. Heme has a ring-shaped structure that binds an iron atom in its center. It is made from four organic molecules called pyrroles (C4H4NH). Heme is a prosthetic group because it is permanently associated with each polypeptide subunit of hemoglobin.

cofactor	enzyme or protein
Zn++	carbonic anhydrase
Zn++	alcohol dehydrogenase
Fe+++ or Fe++	cytochromes, hemoglobin
Fe+++ or Fe++	ferredoxin
Cu++ or Cu+	cytochrome oxidase
K+ and Mg++	pyruvate phosphokinase

Vitamins of B complex group acting as co-enzymes

vitamins	active form (co-enzyme)
Thiamine Vitamin B 1	TPP (thiamine pyrophosphate)
Riboflavin Vitamin B 2	FMN, FAD
Niacin Vitamin B 3	NAD,NADH
Pantothenic acid Vitamin B 5	component of coenzyme A
Pyridoxine Vitamin B 6	PLP (pyridoxal phosphate)
Biotin	Biotin
Folic acid	THF (Tetrahydrofolate)
Cobalamine Vitamin B 12	cobamide

Conjugated proteins

Class	Prosthetic group	Example
Lipoproteins	Lipids	B1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme(iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate Dehydrogenase
Metalloproteins	Iron	Frettin
	zinc	Alcohol Dehydrogenase
	Calcium	Calmodullin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

Table 6.2 Enzyme cofactors

Cofactor	Enzyme*
Coenzyme†	
Thiamine pyrophosphate (TPP)	Pyruvate dehydrogenase
Flavin adenine nucleotide (FAD)	Monoamine oxidase
Nicotinamide adenine dinucleotide (NAD^+)	Lactate dehydrogenase
Pyridoxal phosphate (PLP)	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
6′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase

Specificity of enzymes:

Enzymes are the most remarkable and highly specialized proteins, **they have a high degree of specificity for their substrates**, and they accelerate chemical reactions tremendously. In general, four types of behavior can be described:

1. **Absolute specificity** - Catalyze **only one reaction**. Example: a) Uricase, which acts only on uric acid. b) Arginase, which acts only on arginine. c) Carbonic anhydrase, which acts only on carbonic acid. d) Lactase, which acts on lactose

2. **Group specificity** – Catalyzes a **particular type of functional group**, which can occur in a variety of substrate. Example: a) Trypsin is an endopeptidase that hydrolyzes central peptide bonds in which the amino group belongs to basic amino acids e.g. arginine, lysine and histidine. b) Chymotrypsin is an endopeptidase that hydrolyzes central peptide bonds in which the carboxyl group belongs to aromatic amino acids.

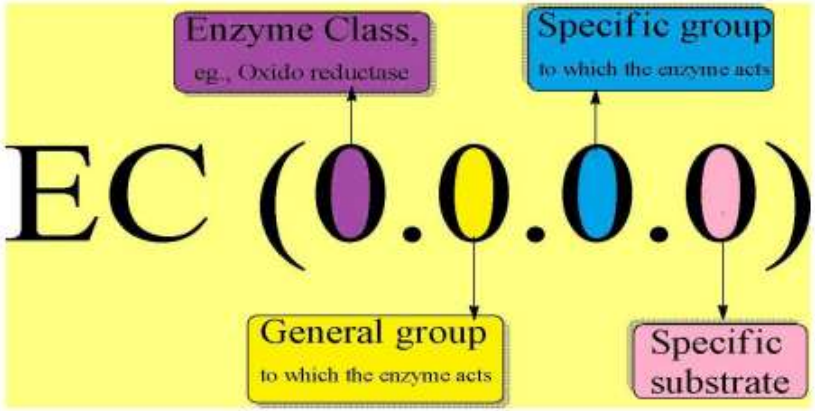
3. **Linkage specificity** - Catalyzes a **particular type of chemical bond** regardless of the rest of the molecular structure. Example: a) Amylase, which acts on α 1-4 glycosidic, bonds in starch, dextrin and glycogen. b) Lipase that hydrolyzes ester bonds in different triglycerides.

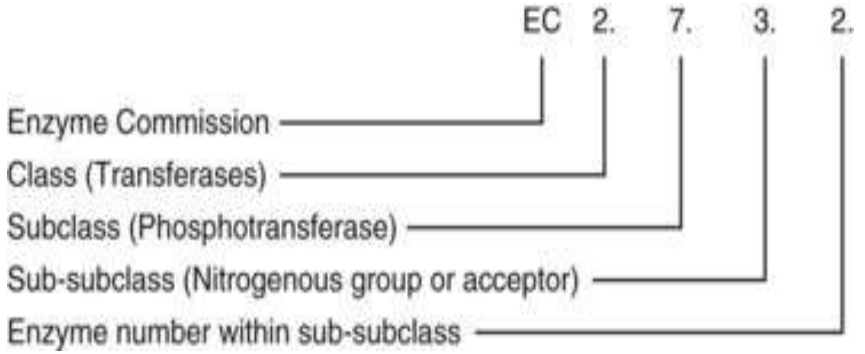
4. **Stereochemical specificity** - the enzyme will act on a **particular steric or optical isomer**. Example: a) L amino acid oxidase acts only on L amino acids. b) D amino acid oxidase acts only on D amino acids.

EC nomenclature and Classification of Enzyme:

1. The **Enzyme Commission number** (EC number) is a **numerical classification** scheme for enzymes, based on the chemical reactions they catalyze.
2. Rules for the enzyme nomenclature were prepared by **IUB (International Union of Biochemistry)** in **1964.** Under IUB system each enzyme has a name and a unique identification number. This unique identification number is named as 'Enzyme Commission Numbe' or **'E.C.Number'.**

3. Every E.C. number consists of the letters 'EC' followed by four numbers separated by dots. Those numbers represent a progressively **finer classification** of the enzyme. EC numbers contain four digits, for example **E.C. a.b.c.d.**





4. For example, the **tripeptide aminopeptidases** have the code **'EC 3.4.11.4'**,
   EC 3 enzymes are hydrolases (enzymes that use water to break up some other molecule)
   EC 3.4 are hydrolases that act on peptide bonds
   EC 3.4.11 are those hydrolases that cleave off the amino-terminal amino acid from a polypeptide
   EC 3.4.11.4 are those that cleave off the amino-terminal end from a tripeptide

EC numbers

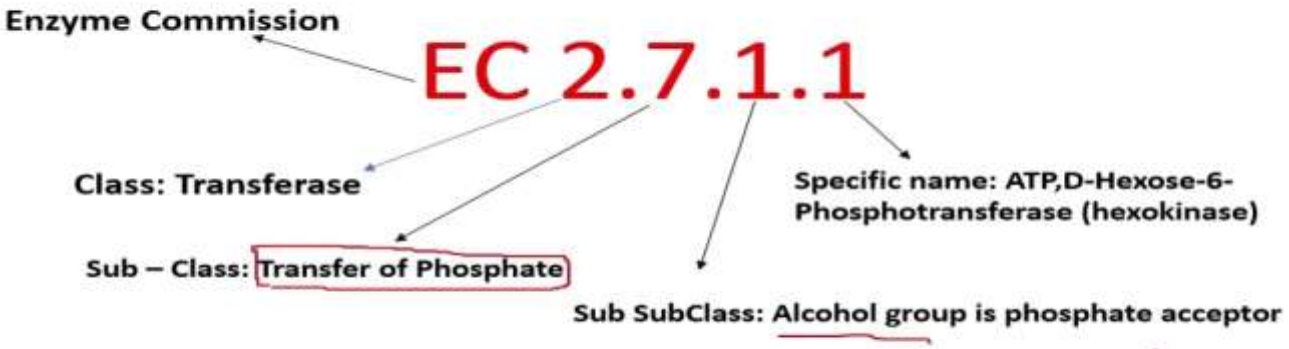
EC numbers are four digits, e.g. EC a.b.c.d, where

- "a" is the **class**,
- "b" is the **subclass**,
- "c" is the sub-subclass, and
- "d" is the **sub-sub-subclass**.

- The "b" and "c" digits describe the reaction, while the "d" digit is number of enzymes of the same function based on the actual substrate in the reaction.

CHEM TECH

HEXOKINASE



CLASSIFICATION OF ENZYMES:

Following are the 7 classes (previously 6 classes) of enzymes according to the recent system of nomenclature.

EC 1. OXIDOREDUCTASES: – They catalyze the transfer of hydrogen or oxygen atoms or electrons from one substrate to another, also known by trivial names like oxidases, dehydrogenases, or reductases. These are 'redox' reactions, so, an electron donor/acceptor is also required to complete the reaction. Typical reaction includes-

$$AH+B \rightarrow A+BH(\textbf{reduced})$$

$$A + O \rightarrow AO\ (\textbf{oxidized})$$

EC 2.TRANSFERASES: They catalyze group transfer reactions. Transferases are enzymes transferring a group, *e.g.* a methyl group, phosphate group, glycosyl group, from one compound (generally regarded as donor) to another compound (generally regarded as acceptor).Eg., Transaminase, kinase etc. These are of the general form-

$$A\text{-}X + B \leftrightarrow BX + A$$

EC 3. HYDROLASES: They catalyze hydrolytic reactions. These enzymes catalyse the hydrolytic cleavage of C-O, C-N, C-C and some other bonds, including phosphoric anhydride bonds. Examples Include lipases, esterases, peptidases /proteases etc. These reactions are of the general form:

$$A\text{-}B + H2O \leftrightarrow B\text{-}OH + HA$$

EC 4. LYASES: They catalyze non-hydrolytic addition or removal of functional groups from substrates, often creating a double bond in the product or cause addition of function groups across a double bond. C-C, C-N, C-O or C-S bonds may be cleaved. Includes decarboxylases, aldolases , synthases etc. Typical reaction includes-

$$X\text{-}A + B\text{-}Y \rightarrow A{=}B + X\text{-}Y$$

EC 5. ISOMERASES: They catalyze intra-molecular rearrangement in a substrate causing isomerization. According to the type of isomerism, they may be called racemases, epimerases, cis-trans-isomerases, isomerases, tautomerases, mutases or cyclo-isomerases etc. Typical reaction include-

$$ABC \rightarrow BCA$$

EC 6. LIGASES: They catalyzes the synthesis of various bonds (mostly C-X bonds), coupled with the breakdown or hydrolysis of a diphosphate bond in ATP or a similar triphosphate. Typical reaction include-

$$X + Y + ATP \rightarrow XY + ADP + P_i$$

EC 7: TRANSLOCASES: This is a newly added major class in the nomenclature system in August, 2018. They catalyze the movement of ion or molecule across membranes or their separation within membranes. Several of these involve the hydrolysis of ATP and had been previously classified as ATPases, although the hydrolytic reaction is not their primary function.

EC No.	*Reaction type*	*General example of the reaction type*	
EC 1	Oxidoreductase reactions	$X–H + Y \rightarrow X + Y–H$	(reduction)
		$X + 1/2O_2 \rightarrow XO$	(oxidation)
EC 2	Transferase reactions	$X–CH_3 + Y \rightarrow X + Y–CH_3$	(transfer)
EC 3	Hydrolase reactions	$X–Y + H_2O \rightarrow X–OH + Y–H$	(splitting)
EC 4	Lyase reactions	$X–CO_2–Y \rightarrow X–Y + CO_2$	(removal/addition)
EC 5	Isomerase reactions	$X–Y–X \rightarrow Y–X–X$	(rearrangement)
EC 6	Ligase reactions	$X + Y + ATP \rightarrow X–Y + ADP + Pi$	(joining)

MECHANISM OF ENZYME ACTION:

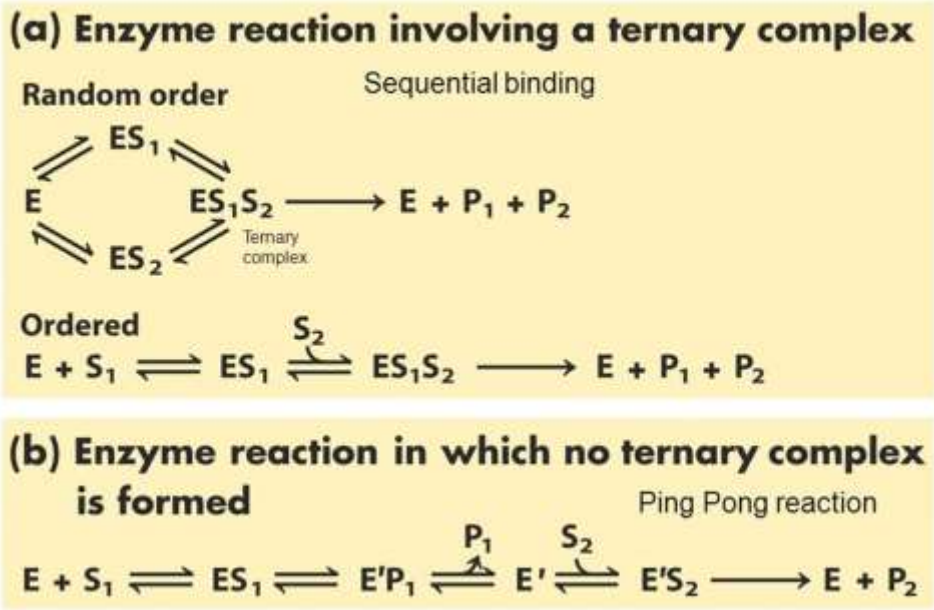
1. When an enzyme binds its substrate, it forms an **enzyme-substrate complex.**
2. There are four important types of interaction that hold the substrate in a defined orientation and form an enzyme-substrate complex (ES complex): **hydrogen bonds, van der Waals interactions, hydrophobic interactions and electrostatic force interactions.**
3. Enzyme catalyzed reaction can be a **single-substrate or a bi-substrate reaction**. If it is a bi-substrate reaction it can be either single displacement or double-displacement type.

•**Single substrate reaction**: **E + S→ ES complex → E+ P**

•**Bi-substrate reaction:**

In **single displacement** type, the enzyme and both substrates come together to form a common ternary complex. In ordered binding, substrate 1 must bind before substrate 2 can bind productively.

In **double displacement** type, enzyme substrate complex forms, a product leaves the complex. The altered enzyme forms a second complex with another substrate molecule, and the second product leaves, regenerating the original enzyme. This is also called a **Ping-Pong** mechanism.



ACTIVE SITES OF ENZYME:

The region of the enzyme surface which combines with the substrate to form the enzyme-substrate complex and at which the transformation of the substrate to products occurs is called the **active site of the enzyme.**

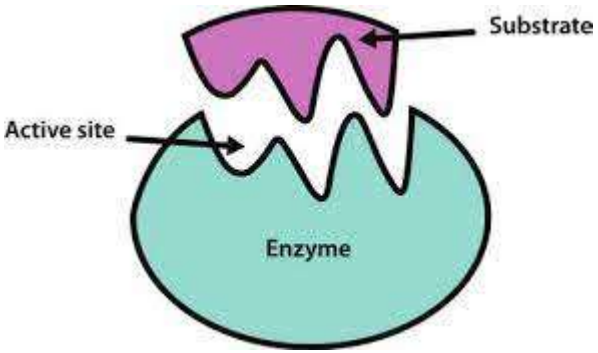
The exact chemical nature of the active site is not known. **It has been proposed that the active site is formed by the tertiary folding of the amino acid chains** of the enzyme protein and **possesses active groups capable of interacting with the specific groups of the substrate.**

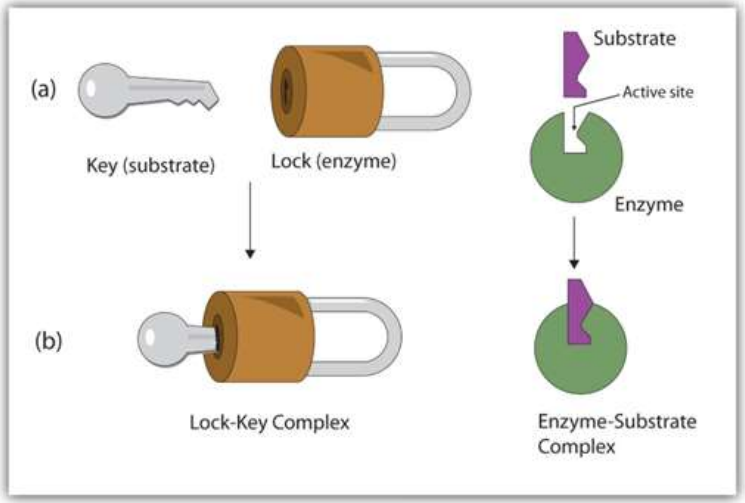
Two hypothesis:

1) Lock and Key hypothesis: A **substrate can fit into the active site of an enzyme only if the site has a conformation complementary to its own, just as a lock can be compatible only with its own key having a complementary groove cutting**.

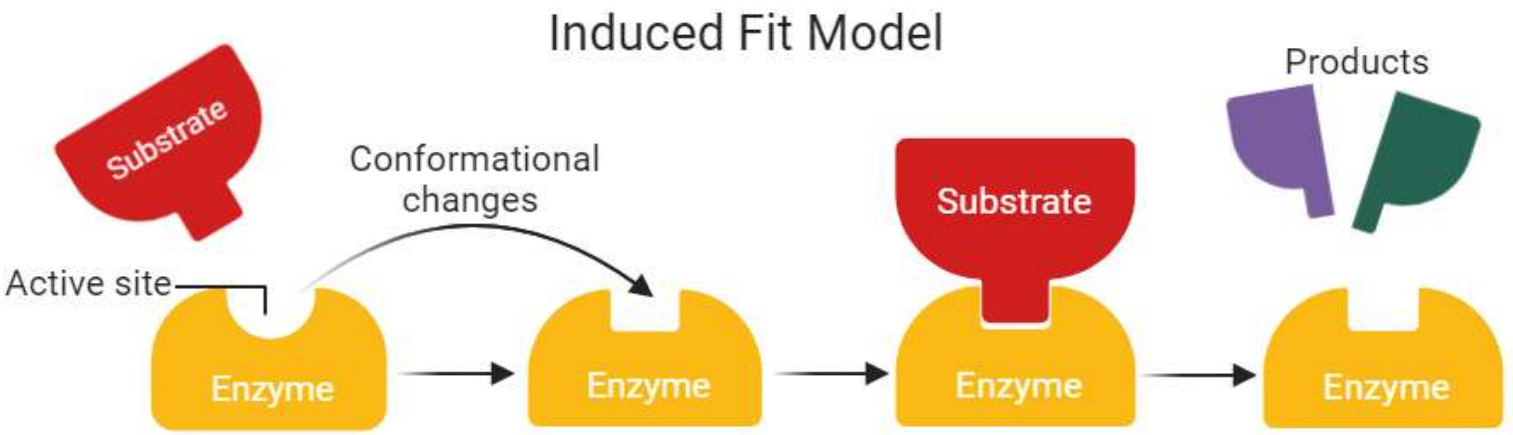
It can explain cases of absolute substrate specificity in which an enzyme acts on a specific substrate only.

It cannot explain: i) the cases of relative substrate specificity in which the same enzyme catalyses different reactions of a related group of substrates. Or ii) the cases of reaction specificity in which the same enzyme catalyses a specific type of reaction of different substrates.





2) **Induced Fit hypothesis:** Koshland in 1958, first proposed the induced-fit model, this suggests that the enzyme active site is **conformationally fluid**. **It is said that the active site of an enzyme is not rigid and pre-shaped; instead, it is flexible and elastic so that it can be changed or modified suitably if need arises.** According to the model, the contact with the substrate **induces some configurational changes** in the active site of the enzyme so that its new configuration is perfectly matching with that of the substrate. The substrate can thus fit into the active site perfectly to form the enzyme-substrate complex. Once the products are derived from it, the active site of the enzyme reverts to its original configuration. The induced fit model can **explain the enzyme specificity** better than the lock and key hypothesis.

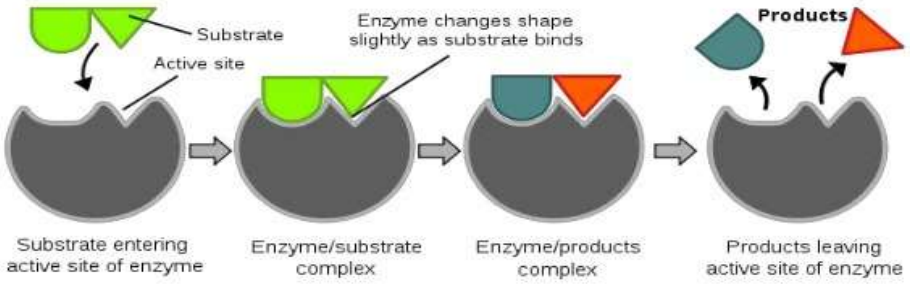


MECHANISM OF ENZYME ACTION:

1. When an enzyme binds its substrate, it forms an **enzyme-substrate complex**. This complex **lowers the activation energy of the reaction** and promotes its rapid progression.

2. Enzymes brings substrates together in an **optimal orientation,** lining up the atoms and bonds of one molecule with the atoms and bonds of the other molecule. This can contort the substrate molecules and facilitate bond-breaking.
3. The active site of an enzyme also creates an **ideal environment**, such as a slightly acidic or non-polar environment, for the reaction to occur.
4. The enzyme will always return to its original state at the completion of the reaction. One of the important properties of enzymes is that they **remain ultimately unchanged** by the reactions they catalyze. After an enzyme is done catalyzing a reaction, it releases its products.

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$



ACTIVATION ENERGY:

1. Energy in biological systems is described in terms of free energy, G. In **a reaction coordinate diagram,** the **free energy of the system is plotted against the progress of the reaction**. In its normal stable form or **ground state**, any molecule contains a specific amount of free energy.

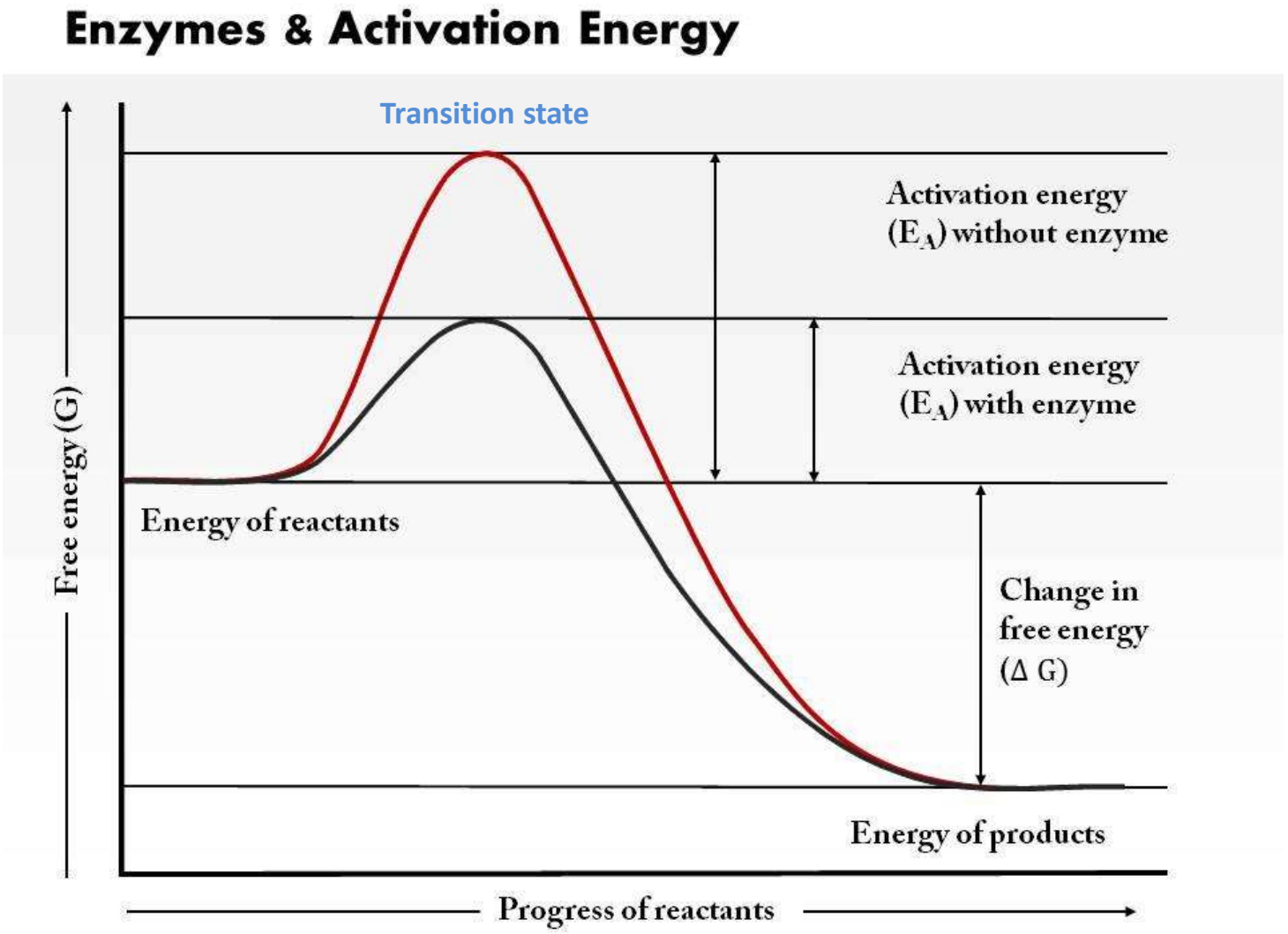
2. There exists an **energetic barrier** between S (substrate) and P(product) that represents the energy required for alignment of reacting groups, bond rearrangements and other changes needed for the reaction to occur in either direction.

2. **To undergo reaction, the molecules must overcome this barrier or "energetic hill" and therefore must be raised to a higher energy level**. At the top of energy hill is a point at which decay to the S or P state is equally probable. This is called the **transition state.**

3. The **transition state** is not a chemical species with any significant stability and should not be confused with a reaction intermediate (such as ES or EP). It is **simply a fleeting molecular moment in which events such as bond breakage, bond formation, and charge development have proceeded to the precise point at which decay to either substrate or product is equally likely.**

4. The difference between the energy levels of the ground state and the transition state is called the **Gibbs free energy of activation** or simply **activation energy** (symbolized by $\Delta G^{\neq}$). Higher activation energy corresponds to a slower reaction.

5. Reaction rates can be increased by either raising the temperature, or by adding an enzyme. **An enzyme enhance reaction rates by lowering activation energies**.

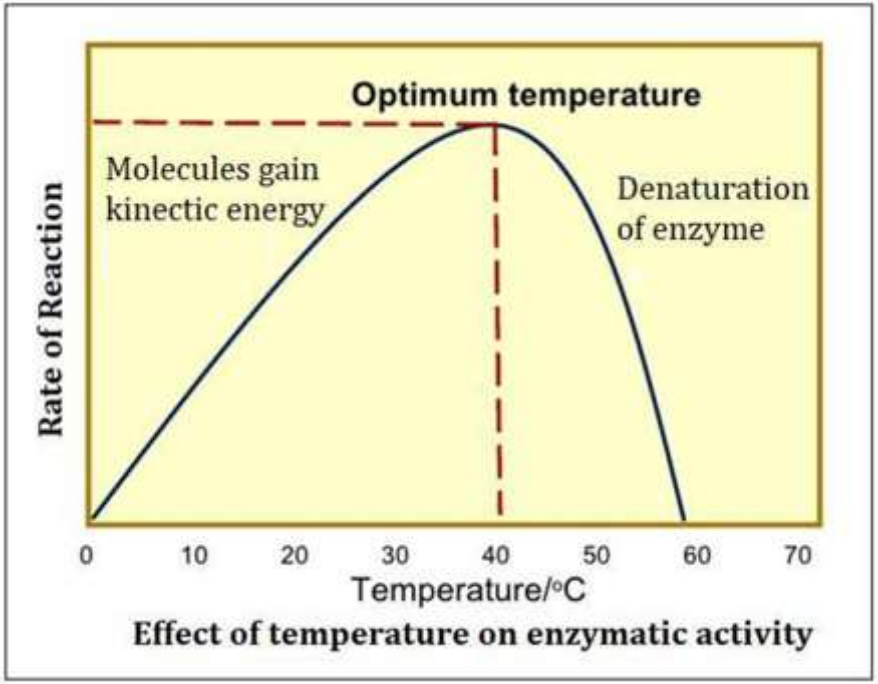


REACTION COORDINATE DIAGRAM

FACTORS AFFECTING ENZYME ACTIVITY:

1. Effect of temperature:

- The rate of an enzyme catalyzed reaction **increases with the increase in temperature up to a maximum and then falls.**
- When a graph is plotted between temperature versus enzyme activity, a bell-shaped curve is obtained.
- The temperature at which the maximum rate of reaction occurs is called the **enzyme's optimum temperature.** The optimum temperature is different for different enzymes; but for most of the enzymes it is between 40°C-45°C.
- Majority of enzymes in the human body have an **optimum temperature of around 37°C (98.6°F)** and are denatured or degraded at extreme temperatures. However, few enzymes like Taq DNA polymerase present in thermophilic bacteria, Thermus aquaticus, venom phosphokinase and muscle adenylate kinase are active even at 100°C.
- Increasing temperature **increases the kinetic energy** that molecules possess, leading to more random collisions between molecules per unit time. Since enzymes catalyze reactions by randomly colliding with substrate molecules, increasing temperature increases the rate of reaction, forming more product. As temperatures increases it leads to denaturation; a molecular arrangement which causes a loss of the active sites of the enzyme surfaces and results in a loss of efficiency.



Effect of temperature on enzymatic activity

Temperature Coefficient (Q_{10})

The amount the rate of reaction increases when the temperature is raised by $10^{o}C$ is known at the **temperature coefficient (Q_{10})**

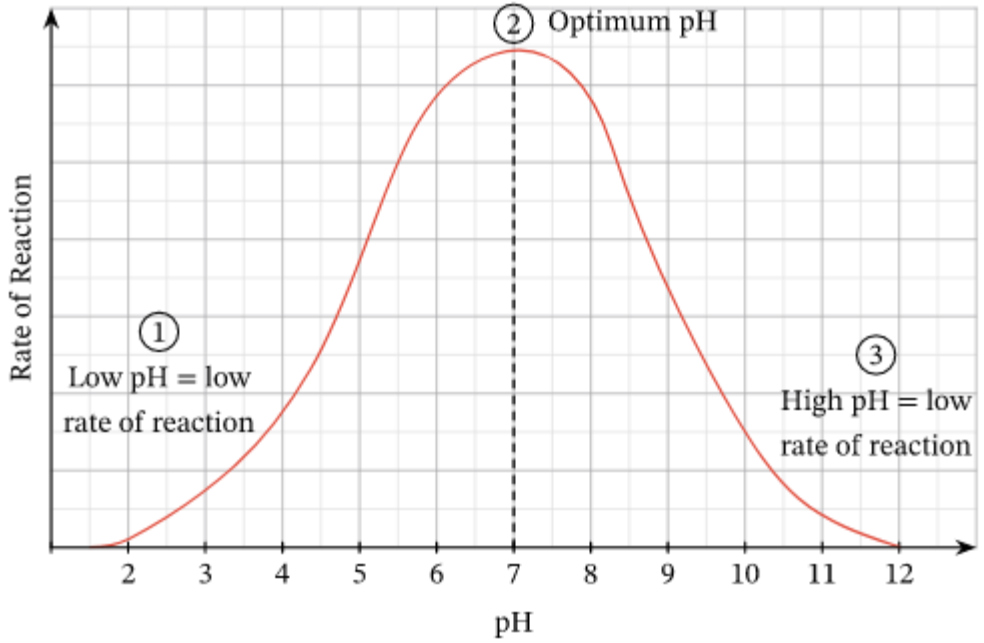
Equation:

$$Q_{10} = \frac{\text{rate of reaction at } (T + 10)^{o}C}{\text{rate of reaction at } T\ ^{o}C}$$

If Q_{10} is 2, the rate of reaction doubles for every 10^{o} rise in temperature

2. Effect of pH:

- Enzyme activity is also affected by pH.
- A plot of enzyme activity against pH results in a bell shaped curve. Each enzyme has its unique optimum pH at which the rate of reaction is greatest. The optimum pH is the pH at which the activity of a particular enzyme is at maximum. Below and above the optimum pH, the enzyme activity is much lowered and at extreme pH, the enzyme becomes totally inactive.
- Many enzymes of higher organisms show optimum reaction rate around neutral pH (6-8). However, there are several exceptions such as pepsin (pH 1-2), acid phosphatases (pH 4-5) and alkaline phosphatases (pH 10-11).
- Reduction in efficiency caused by changes in the pH is due to changes in the degree of ionization of the substrate and enzyme.

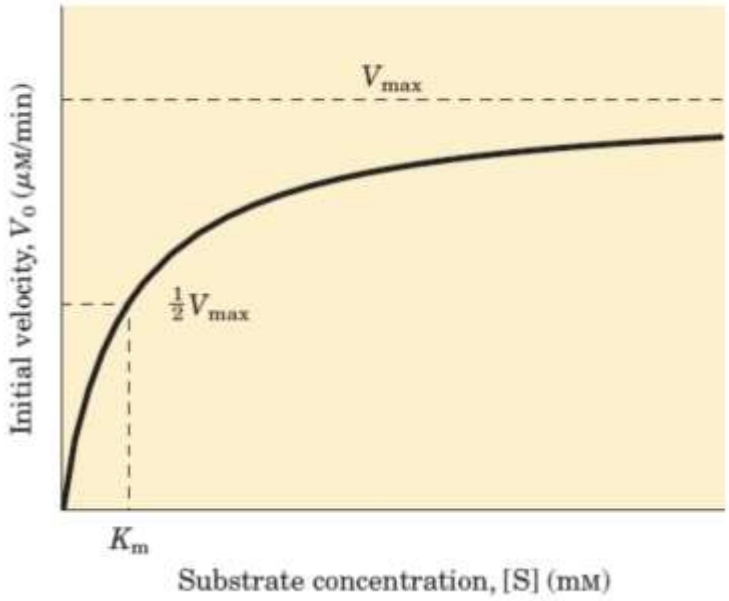


3. Effect of substrate concentration:

- The substrate concentration also influences enzyme activity.

  E+ S = ES complex = E + P

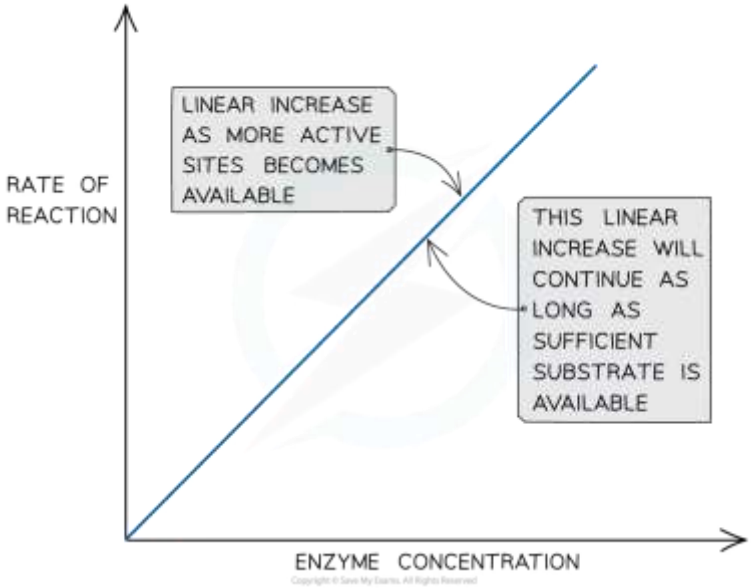
- The **velocity of enzyme-catalyzed reaction** (v) is expressed in **micromoles of substrate converted to product per minute**. In kinetic experiment, as the substrate concentration [S} increases from a very low level, the rate of reaction (v) also increases. In this phase, the rate is known as **initial velocity (V_0).**
- When the [S} increases further, the rate of rise slows down progressively. This is because the more substrate molecules will interact with enzyme molecules, the more products will be formed.
- Ultimately at a very high substrate concentration the V_0 reaches a **maximum level (V_{max})**. Beyond this point, further increase in [S] will have no effect on the velocity. This change is reflected in the following hyperbolic curve.
- This **'hyperbolic saturation kinetics'** shows that **'enzyme is the limiting factor**. 'At a very [S] , all enzyme molecules become saturated and work at their maximum possible rate. T Vmax represents the maximum reaction rate possible in the presence of excess substrate

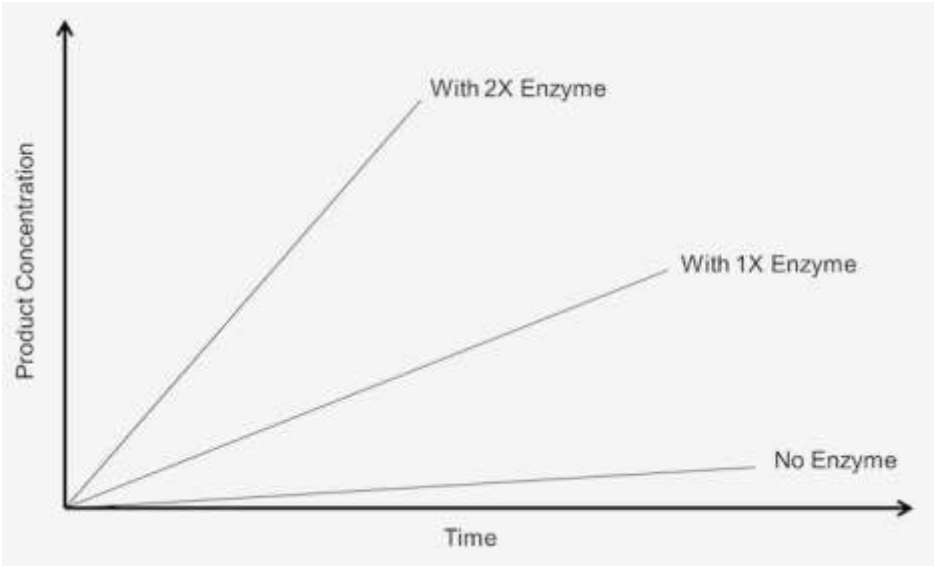


Effect of [S] on initial velocity (V_0) of enzyme catalysed reaction.

4. Effect of enzyme concentration:

An enzyme molecule binds its substrate (s), catalyzes a reaction, and releases the product (s). Thus the more enzyme is available, the more quickly the substrate can be converted into product. In general, as enzyme concentration increases, there is a proportional increase in reaction rate.





UNIT OF ENZYME ACTIVITY

- The **enzyme unit (U)** is that amount of enzyme that catalyses the conversion of 1 micromole of substrate per minute under standard conditions. The International Union of Biochemistry (I.U.B.) adopted enzyme unit as unit of enzyme activity in 1964. But it was discouraged in favour of the katal since the minute is not an SI unit.

- One **katal (kat)** is the amount of enzyme that catalyses 1 mole of substrate to product per second, so 1 kat = 6 X 10^7 U.

MICHAELIS-MENTEN KINETICS

What is enzyme kinetics?

Kinetic study of an enzyme includes the measuring of the rate of enzyme catalyzed reaction at different substrate and enzyme concentration.

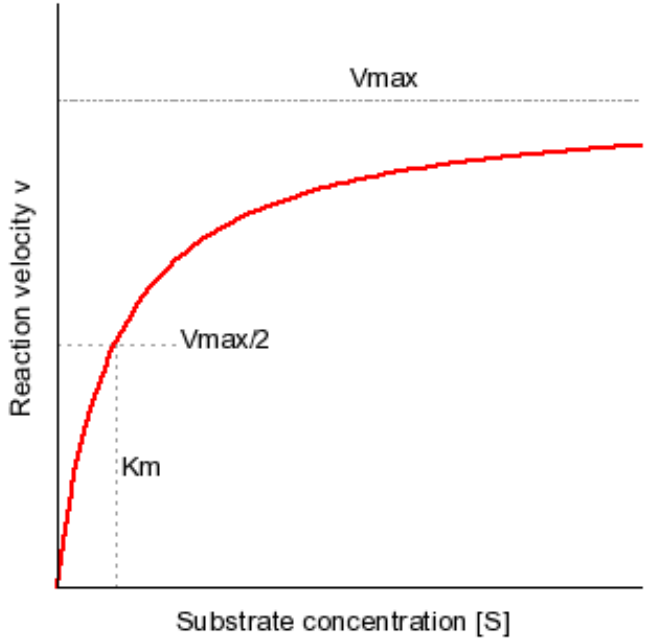
'Michaelis-Menten kinetics' is the simplest and best known model of enzyme kinetics as explained by **Leonor Michaelis and Maud Menten in 1913.** The model takes the form of an **equation** which describes the relationship between reaction rate (V) and substrate concentration [S] in a enzyme-catalyzed reaction. The equation is as follows:

$$V = \frac{V\text{max}\,[S]}{K\text{m}+[S]}$$

Where, **V**= velocity of reaction, **[S]** = substrate concentration, V**max** = maximum velocity and K**m** = Michaelis constant.

Graphical representation of M-M equation:

- M-M equation takes the form of an **hyperbola** when reaction velocity (V) is plotted against substrate concentration [S].
- In a kinetics experiment, when the [S] is very low, the rate of reaction (V) rises linearly. This rate of enzymatic reaction measured at this point is known as **Initial velocity (V_0).**
- When the [S] is increased further, the rate of rise in V_0 gradually slows down.
- Ultimately at a very high [S], V_0 reaches a **maximum level. (V_{max}).**
- Beyond this point, V_0 cannot increase any further even after addition of more substrate.
- Thus, the graph for M-M equation become hyperbolic in nature.



Leonor Michaelis
1875–1949

German biochemist and physician

Maud Menten
1879–1960

Canadian medical scientist

Michaelis constant (Km):

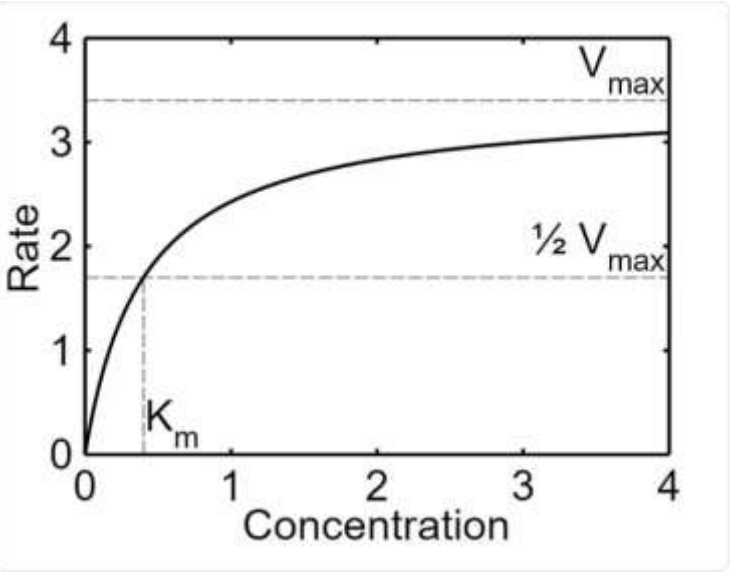
In Michaelis-Menten equation, if $\boldsymbol{V_0}$ is equal to ½ $\boldsymbol{V_{max}}$, then, we can write,

$$\frac{\boldsymbol{Vmax}}{\mathbf{2}} = \frac{V\text{max}\,[S]}{K\text{m}+[S]}$$

Or, Km+[S] = 2[S]

Or, Km = [S]

Therefore, Km is the '**molar concentration of the substrate at which ½ maximum velocity is obtained**'. Km is expressed as moles/L and its value ranges between 10^{-6} to 10^{-1}M.



Significance of Km:

1. Km indicates **substrate-affinity of the enzyme** and inversely related to it. The lower is the km , the higher is the affinity of the enzyme for its substrate.
2. Km has the dimension of concentration (mol/L). Its value ranges between $10^{-5}\ to\ 10^{-2}\ \frac{moles}{Litre}$.
3. Km is specific and constant for a given enzyme under defined condition of pH, temperature and time.
4. Km for an enzyme **varies from substrate to substrate**. For example, hexokinase enzyme has Km value in a decreasing order for mannose, D-glucosamine, and D-glucose.
5. Km values are **different for isoenzymes**. Isozymes are different enzymes catalysing same reactions on identical substrates. For example, hexokinase I,II,III and IV have different Km values for glucose.

The M-M equation: $V = \frac{V\text{max}\,[S]}{K\text{m}+[S]}$

1. **When [S] is much less than Km ,**The term Km + [S] ≈ Km.

Then, $V_0 \approx \frac{V\text{max}\,[S]}{K\text{m}}$

Or,$V_0 \approx \left(\frac{V\text{max}}{K\text{m}}\right)$ **X [S]**

Since Vmax and Km, both are constants, their ratio is also a constant. Therefore, when [S] is considerably below Km, the initial velocity is directly proportional to [S]. The rate of reaction is then said to be **'first order'** with respect to substrate.

2. **When [S] is much greater than Km ,** the term Km + [S] ≈[S].

Then, $V_0 \approx \frac{V\text{max}\,[S]}{[S]}$

Or, $V_0 \approx V\text{max}$

Thus, when [S] greatly exceeds Km, the reaction velocity is maximal (Vmax) and remain unaffected by further increases in the substrate concentration[S]. The rate of reaction becomes independent of substrate concentration, and is said to be '**zero order'** with respect to substrate.

Significance of Vmax:

1. Vmax is the reaction velocity of an enzymatic reaction when the binding site of an enzymes are saturated with substrates. It is directly proportional to [E]. **Vmax=** $K_2[E]_T$

2. It represents the **'turnover number'** (Kcat) of the enzyme., which is the number of substrate molecules converted into product by an enzyme per unit time when the enzyme is fully saturated with the substrate.
   Vmax= $K_{cat}[E]_T$
   or, K_{cat} **= Vmax /** $[E]_T$
   For example, carbonic anhydrase has a turnover number of 400,000 to 600,000 per second. It means, that each carbonic anhydrase enzyme can produce up to 600,000 molecules of product (bicarbonate ions) per second when fully saturated.

The Catalytic Efficiency of enzyme:

Catalytic efficiency of enzymes is best expressed in terms of the ratio of two kinetic constants **Kcat/Km**. This value is used to rank enzymes. A high value means that an enzyme binds tightly to a substrate (small Km) with a fast reaction.

Limitation of M-M equation:

1. For drawing the first segment of hyperbolic curve, one need to measure the initial velocity (V_0) at a very low substrate concentration, which is **practically very difficult**.

2. Similarly at the plateau phase (flat segment), one has to use a very high substrate concentration to obtain maximum velocity (V_{max})**.** At this point, the measurement of V_{max} becomes not only difficult but also **practically impossible.**

3. **Extrapolation** in hyperbolic curve is practically difficult.

4. Sometimes, when the experimentally observed V_0 values of are plotted against respective [S], the plotted points become **too scattered** and drawing of the hyperbola becomes practically impossible.

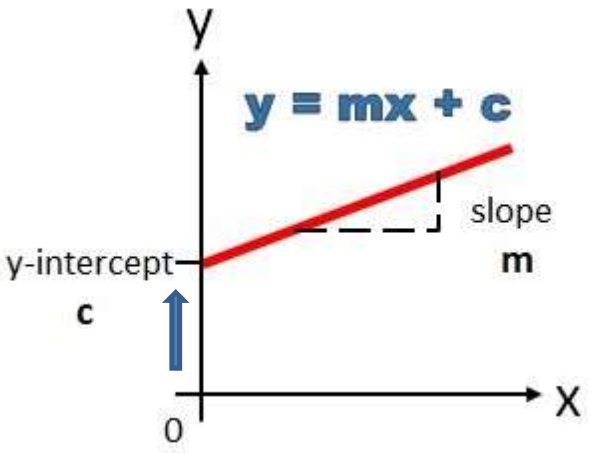
To remove all these difficulties, the **hyperbolic curve is linearly transformed .**

Lineweaver-Burk Double Reciprocal Plot:

(Linear transformation of M-M equation)

The M-M equation: $V = \frac{V\text{max}\,[S]}{K\text{m}+[S]}$

Let us remember the equation of straight-line: y=mx + c



Lineweaver and Burk in 1934 transformed M-M equation into an equation for **straight line.**

On inverting M-M equation,

$$\frac{1}{V_0} = \frac{K\text{m}+[S]}{V\text{max}\,[S]} = \frac{K\text{m}}{V\text{max}\,[S]} + \frac{[S]}{V\text{max}\,[S]}$$

Or, $\frac{1}{V_0} = \frac{K\text{m}}{V\text{max}} \times \frac{1}{[S]} + \frac{1}{V\text{max}}$

If $\frac{1}{V_0}$ is plotted against $\frac{1}{[S]}$, a straight line is obtained, called **'Lineweaver –burk plot" or 'Double reciprocal plot'**. This equation is similar to the equation of a straight line (y= mx + c).

Here, the slope of the line = $\frac{K\text{m}}{V\text{max}}$

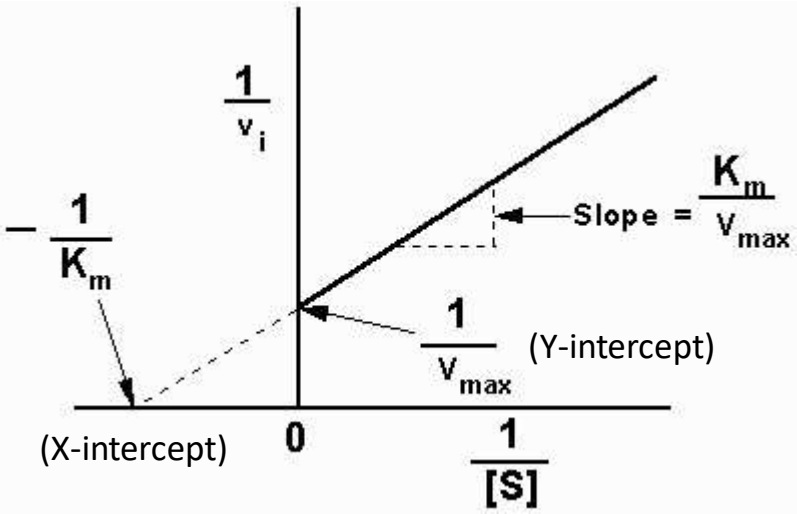
And y-intercept = $\frac{1}{V\text{max}}$

This line can be extrapolated to the x-axis.

We know that, at x-axis, y=0. Therefore, $\frac{1}{V_0}$ **=0,**

Therefore, $\frac{1}{[S]} = -\frac{1}{Km}$

So, the x-intercept = $-\frac{1}{Km}$



Lineweaver-Burk Plot

Significance of Double–Reciprocal plot:

1. The L-B double reciprocal plot can be used for an **accurate determination of Km and Vmax** in an experiment. Km can be determined graphically either from the value of x-intercept or from the values of slope and y-intercept. The Vmax also can be determined graphically from the reciprocal of y-intercept.
2. The L-B plot can be used for evaluation of different types of **enzyme inhibitions.**
3. The L-B plot is used for evaluation of **allosteric modulation.**
4. The L-B plot can also be used for evaluation for determination of **concentration of active enzymes** in a sample.
5. The L-B plot is **much easier to draw** in comparison to hyperbolic curve and can be **easily extrapolated**. This plot can be drawn accurately even if some plotted points are scattered.

ENZYME INHIBITIONS

1. Enzyme inhibition means a decrease in the catalytic activity of the enzyme. Inhibition occurs when some low MW compound (called inhibitors) bind to enzyme.
2. Enzyme inhibition can be either reversible or irreversible.
   - In **reversible inhibition**, the enzyme binds to an inhibitor by weak, non-covalent bonds, which are easily dissociable. **Upon dialysis, enzyme activity can be restored**.
   - In **irreversible inhibition**, enzymes binds to inhibitor by strong covalent bonds. Therefore the enzyme cannot be easily dissociated from the inhibitor upon dialysis. So, the original enzyme activity cannot be restored. It leads to **permanent loss of enzyme action**. Example- nerve gas, penicillin etc.

TYPES OF ENZYMES INHIBITION

COMPETITIVE INHIBITION:

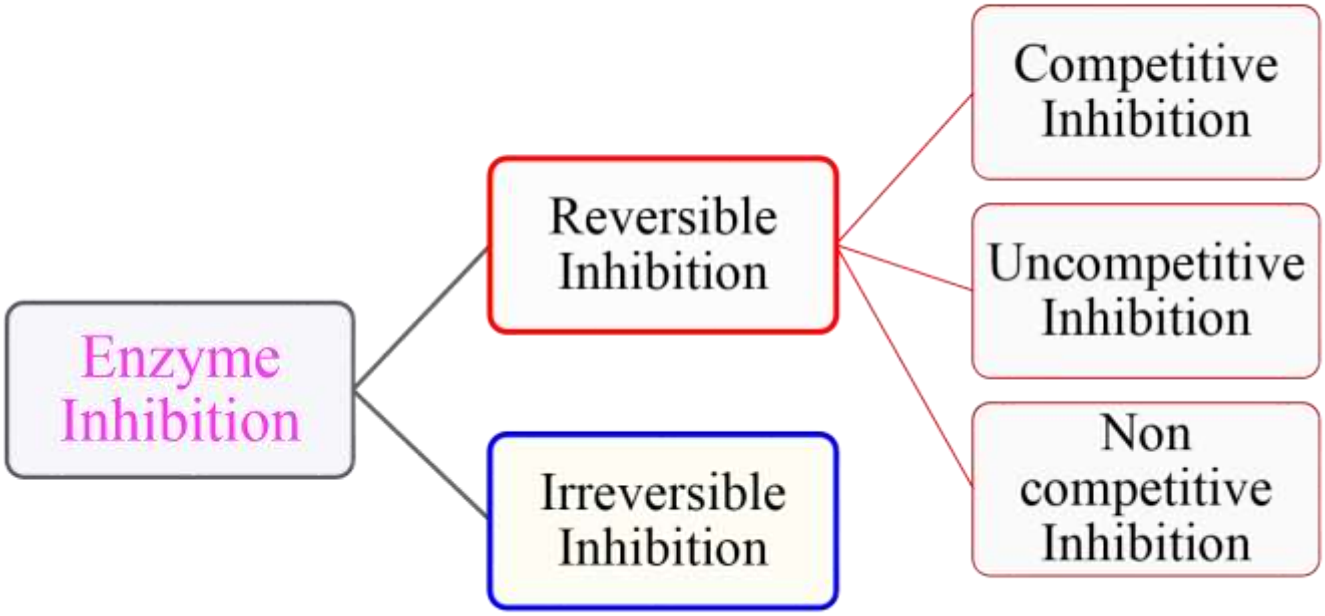
- Inhibitor (I) bears **close structural similarity with substrates** (S), and competes with substrate for active site.
- Reversible inhibition.
- Example- inhibition of succinate dehydrogenase enzyme by malonate (inhibitor) which has close structural similarity with substrate (succinate).
- **Substrate affinity is lowered. (Rise in Km value).**

NON-COMPETITIVE INHIBITION

- Inhibitor bears **no structural similarity with substrate**. Inhibitors bind to a site other than active sites.
- Inhibitor can bind to both free enzyme and ES complex.
- Usually reversible.
- Example- ferro-chelatase enzyme is inhibited by lead (inhibitor)
- **Only Vmax is lowered.**

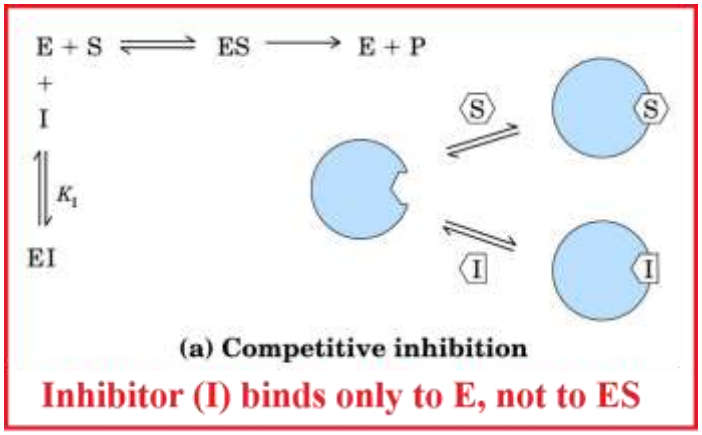
UN-COMPETITIVE INHIBITION

- Inhibitor is **not structurally similar to substrate**. (Rare)
- It doesn't bind to free enzyme. But only binds to ES complex. This binding enhances substrate affinity. This cause a fall in release of product.
- Reversible
- Example- inhibition of Aryl sulfatase by hydrazine.
- **Both Km and Vmax is lowered (Substrate affinity increases)**


Enzyme Inhibition
Reversible Inhibition
Irreversible Inhibition
Competitive Inhibition
Uncompetitive Inhibition
Non competitive Inhibition

COMPETITIVE INHIBITION:

1. In this kind of inhibition, the inhibitor (I)competes with the substrate(S) for binding to the active site of an enzyme When the inhibitor occupies the active site, it forms an enzyme-inhibitor complex (EI).



2. **Change in kinetics:** Competitive inhibitors <u>increases the Km of an enzyme</u>. Because the inhibitor binds reversibly, the substrate can compete and force out all inhibitors at a very high substrate concentration. Thus a competitive inhibitor <u>does not change the Vmax of an enzyme</u>, only changes Km.

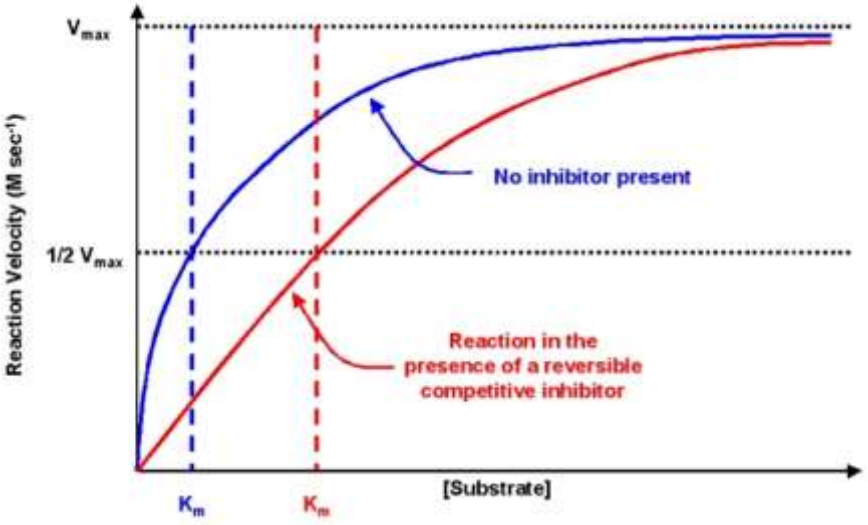
The apparent or new km value under competitive inhibition is: Km (1+ $\frac{[I]}{Ki}$)

Where, [I] = molar concentration of inhibitor
and ki= dissociation constant for E-I complex.

(i) Therefore, M-M equation changes accordingly:

Original equation: $V = \frac{Vmax\,[S]}{Km+[S]}$

Under competitive inhibition: $V = \frac{Vmax\,[S]}{Km\,(1+\frac{[I]}{Ki})+[S]}$



(ii) The equation for Lineweaver–Burk (Double –reciprocal) Plot also changes accordingly.

Original L-B equation: $\frac{1}{V_0} = \frac{Km}{Vmax} X \frac{1}{[S]} + \frac{1}{Vmax}$

Where, the slope = $\frac{Km}{Vmax}$

y-intercept = $\frac{1}{Vmax}$

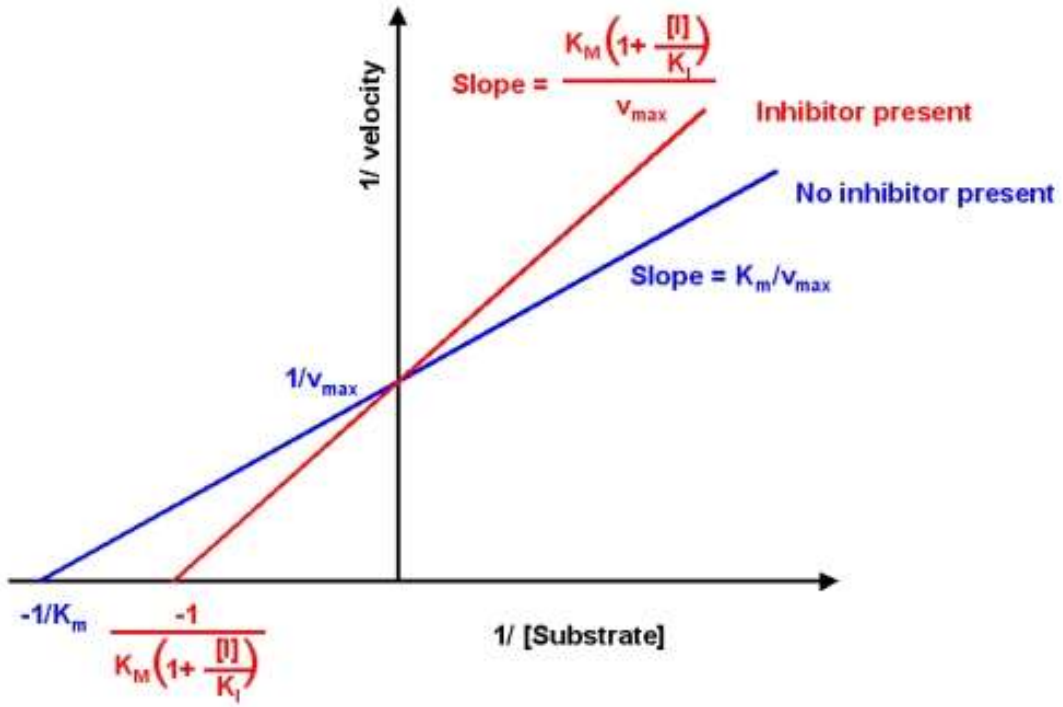
x-intercept = $-\frac{1}{Km}$

L-B equation under competitive inhibition: $\frac{1}{V_0} = \frac{Km(1+\frac{[I]}{Ki})}{Vmax} X \frac{1}{[S]} + \frac{1}{Vmax}$

Where, new slope= $\frac{Km(1+\frac{[I]}{Ki})}{Vmax}$ (Increased)

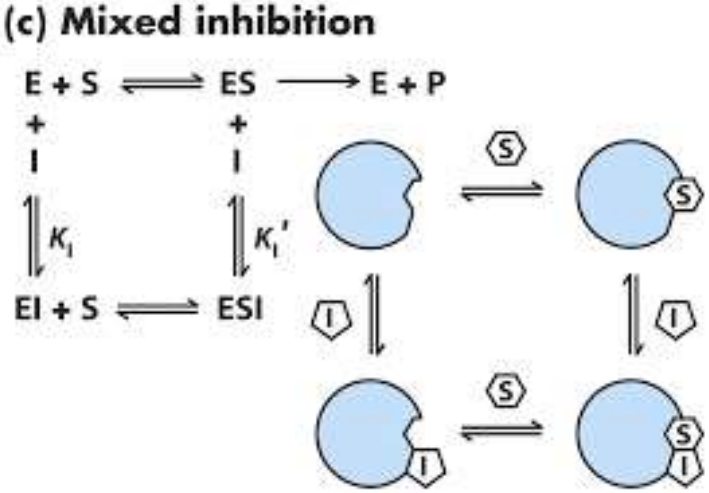
New y-intercept= $\frac{1}{Vmax}$ (unchanged)

new x-intercept = $-\frac{1}{Km(1+\frac{[I]}{Ki})}$ (decreased)



NON- COMPETITIVE INHIBITION:

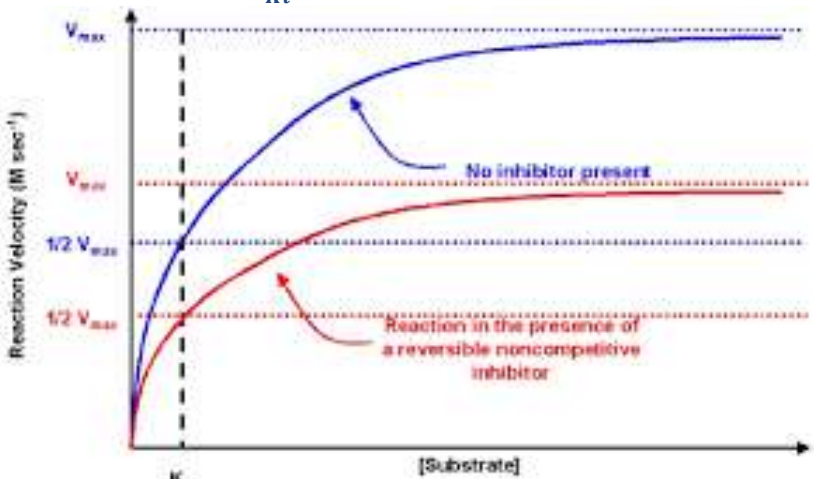
1. In this kind of inhibition, the inhibitor (I) doesn't bear any structural similarity with the substrate (S). Inhibitors usually binds to a site other than active site of than of the enzyme. Therefore, the inhibitor can bind both to the free enzyme (E) or the enzyme substrate complex (ES) the binding somehow distort the 3-D structure of the enzyme which slows down the catalytic activity of the enzyme. The inhibitor can not be totally removed by simply increasing the substrate concentration.



2. **Change in kinetics:** Non-Competitive inhibitors do not alter the Km of an enzyme. But the original velocity of reaction can never be restored by simply increasing the [S]. As a result, maximum velocity (Vmax) is lowered.

The apparent or new Vmax value under non-competitive inhibition is: $\frac{Vmax}{(1+\frac{[I]}{Ki})}$

Where, [I] = molar concentration of inhibitor
and ki= dissociation constant for E-I complex.



(i) Therefore, M-M equation changes accordingly:

Original equation: $V = \frac{Vmax\,[S]}{Km+[S]}$

Under competitive inhibition: $V = \frac{Vmax\,[S]}{(1+\frac{[I]}{Ki})\,(Km+[S])}$

(ii) The equation for Lineweaver–Burk (Double –reciprocal) Plot also changes accordingly.

Original L-B equation: $\frac{1}{V_0} = \frac{Km}{Vmax} \times \frac{1}{[S]} + \frac{1}{Vmax}$

Where, the slope = $\frac{Km}{Vmax}$

y-intercept = $\frac{1}{Vmax}$

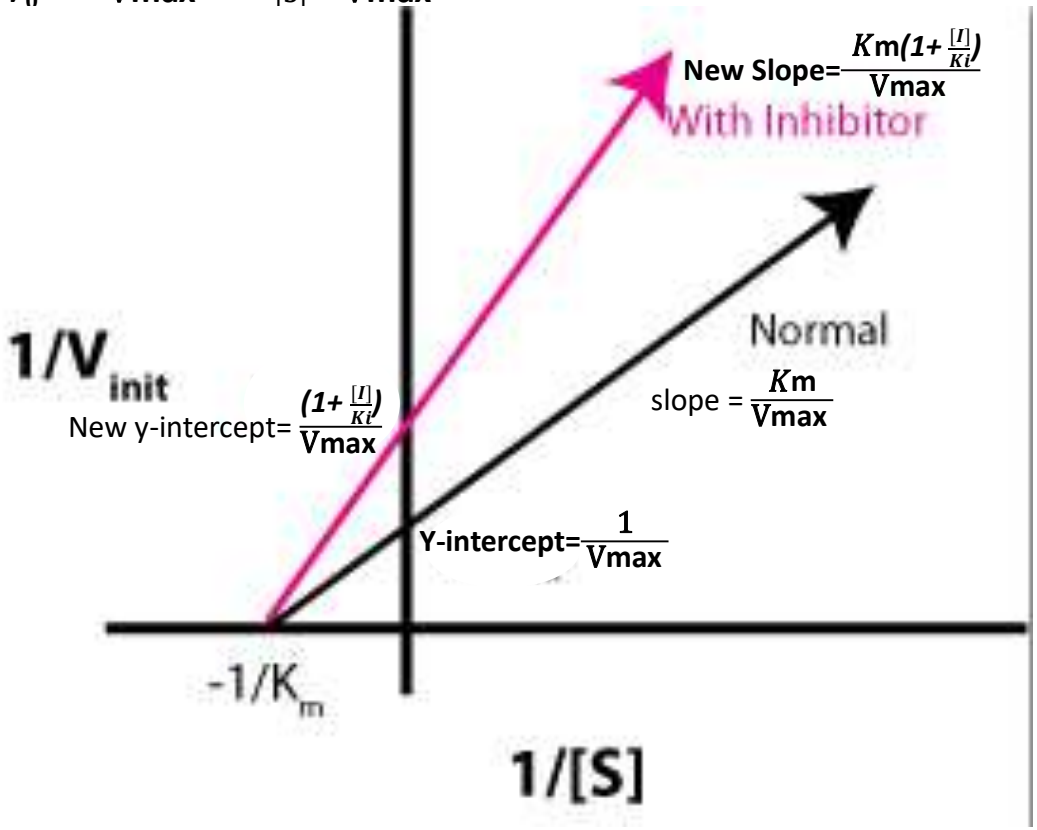
x-intercept = $-\frac{1}{Km}$

L-B equation under non-competitive inhibition: $\frac{1}{V_0} = \frac{Km\,(1+\frac{[I]}{Ki})}{Vmax} \times \frac{1}{[S]} + \frac{(1+\frac{[I]}{Ki})}{Vmax}$

Where, new slope= $\frac{Km(1+\frac{[I]}{Ki})}{Vmax}$ (Increased)

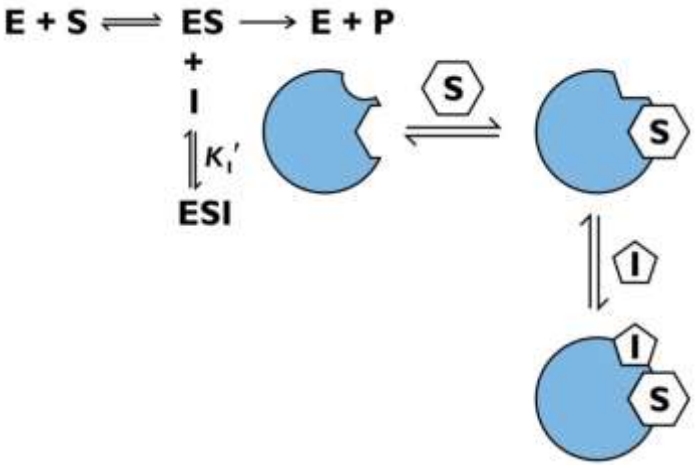
New y-intercept= $\frac{(1+\frac{[I]}{Ki})}{Vmax}$ (Increased)

new x-intercept = $-\frac{1}{Km}$ (unchanged)



UN-COMPETITIVE INHIBITION:

1. In this kind of inhibition, the inhibitor (I) doesn't bear any structural similarity with the substrate (S). Inhibitors do not bind to the free enzyme. They only bind to the ES complex. Binding of inhibitor distorts the 3-D structure of the active site of the enzyme in such a way that substrate-affinity of the enzyme increases . This causes a fall in the release of product. Here also, the inhibitor can not be totally removed by simply increasing the substrate concentration.



2. **Change in kinetics:** In Un-Competitive inhibition, substrate affinity of the enzyme increases, which is reflected by a fall in Km. The original Vmax is also lowered.

The apparent or new Km value under Un-competitive inhibition is: $\frac{Km}{(1+\frac{[I]}{Ki})}$

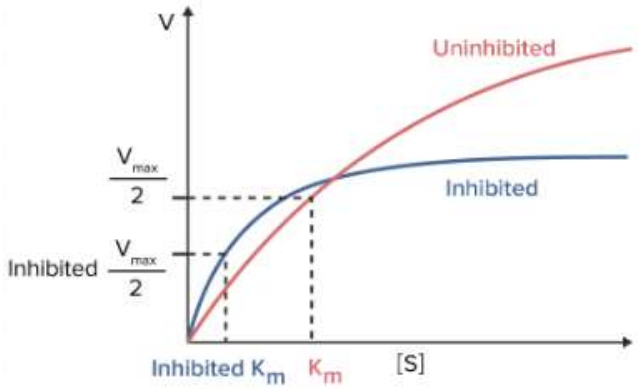
The apparent or new Vmax value under Un-competitive inhibition is: $\frac{Vmax}{(1+\frac{[I]}{Ki})}$

Where, [I] = molar concentration of inhibitor
and ki= dissociation constant for E-I complex.

(i) Therefore, M-M equation changes accordingly:

Original equation: $V = \frac{Vmax\,[S]}{Km+[S]}$

Under Un-competitive inhibition: $V = \frac{Vmax\,[S]}{(Km+[S]\ (1+\frac{[I]}{Ki})}$



(ii) The equation for Lineweaver–Burk (Double –reciprocal) Plot also changes accordingly.

Original L-B equation: $\frac{1}{V_0} = \frac{Km}{Vmax} X \frac{1}{[S]} + \frac{1}{Vmax}$

Where, the slope = $\frac{Km}{Vmax}$

y-intercept = $\frac{1}{Vmax}$

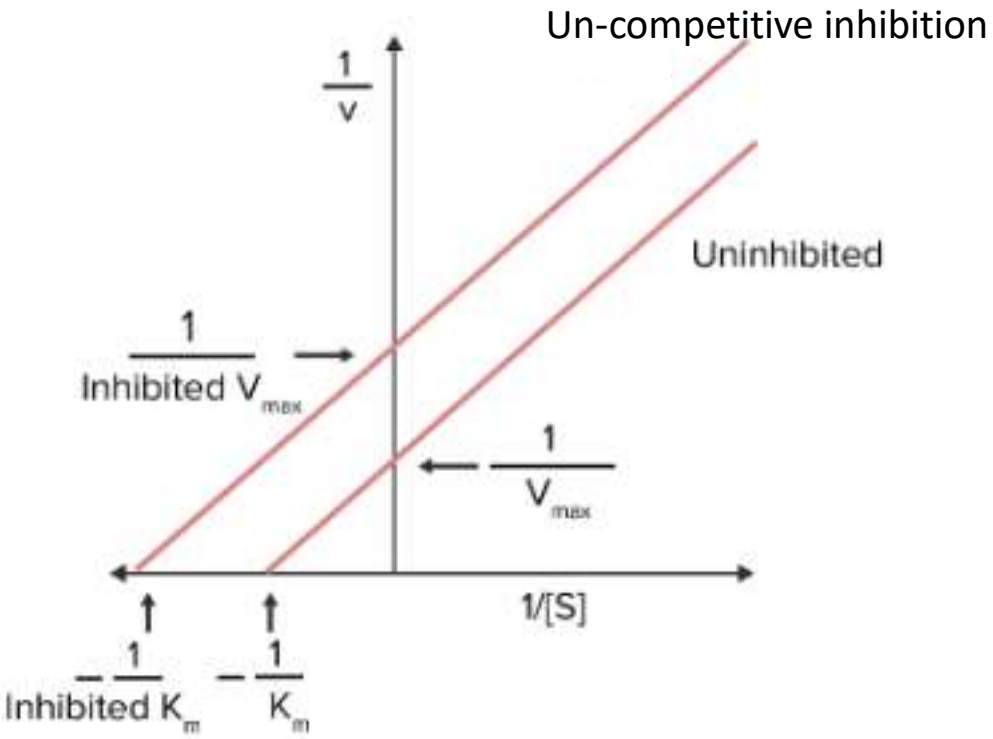
x-intercept = $-\frac{1}{Km}$

L-B equation under Un-competitive inhibition: $\frac{1}{V_0} = \frac{Km}{Vmax} X \frac{1}{[S]} + \frac{(1+\frac{[I]}{Ki})}{Vmax}$

Where, new slope= $\frac{Km}{Vmax}$ (Unchanged)

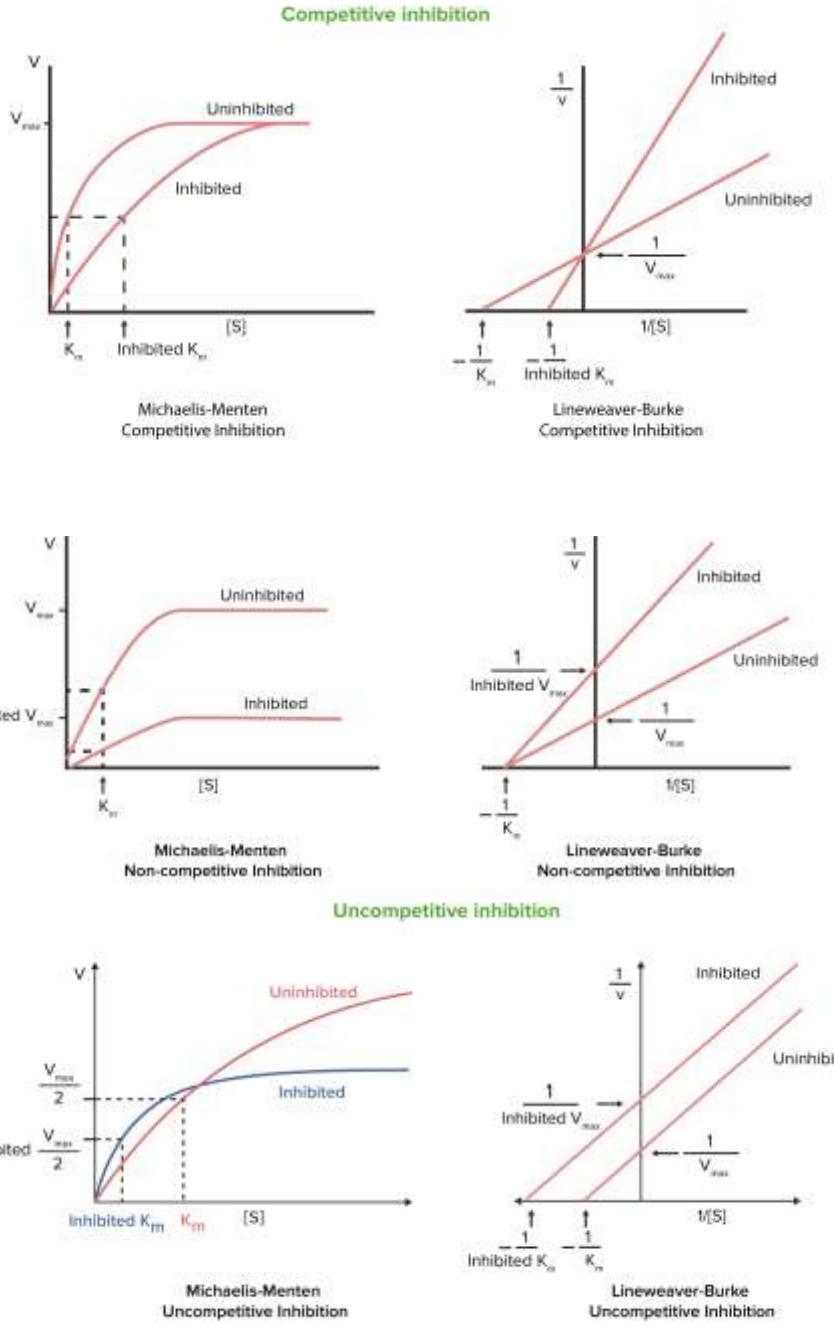
New y-intercept= $\frac{(1+\frac{[I]}{Ki})}{Vmax}$ (Increased)

new x-intercept = $-\frac{(1+\frac{[I]}{Ki})}{Km}$ (Increased)



Summary of kinetics of enzyme inhibition:

Inhibition	Km	Vmax
COMPETITIVE	Raises Km ↑	No change
NON-COMPETITIVE	No change	Fall in Vmax ↓
UN-COMPETITIVE	Fall in Km ↓	Fall in Vmax ↓



Difference between competitive and non-competitive inhibition

Competitive inhibition

- **example:** succinate dehydrogenase is inhibited by malonate
- substrate and inhibitor are chemically similar & have same shape
- inhibitor binds to active site
- inhibitor does not change the shape of the active site
- increases in substrate concentration reduce the inhibition

Non-competitive inhibition

- **example:** pyruvate kinase is inhibited by alanine
- substrate and inhibitor are chemically not similar & have different shape
- inhibitor binds away from the active site i.e. at allosteric site
- inhibitor changes the shape of the active site
- increases in substrate concentration do not affect the inhibition

	Irreversible inhibitors	Reversible inhibitors
Type of bonds with E	Inhibitors bind **covalently** with enzyme	Inhibitors bind **non-covalently** with enzyme
Removal	Cannot be removed by dialysis or other way	Can be removed by dialysis
Activity Restoration	Permanently modify the active site residues(functional group) which the enzyme become inactive.	Removal of the inhibitor restores enzyme activity

TYPE OF INHIBITOR	K_m VALUE	V_{max} VALUE	BINDING SITE	SUBSTRATE RESEMBLANCE
Competitive	Increases	Unchanged	Active Site ONLY	Yes
Non-Competitive	Unchanged	Decreases	Free Enzyme OR E-S Complex	No
Uncompetitive	Decreases	Decreases	E-S Complex ONLY	No

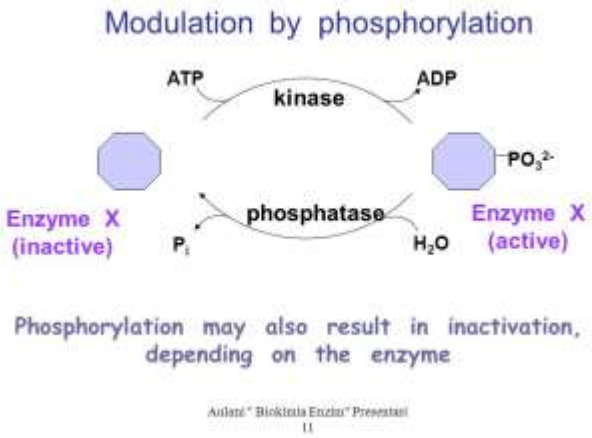
REGULATION OF ENZYME ACTIVITIES

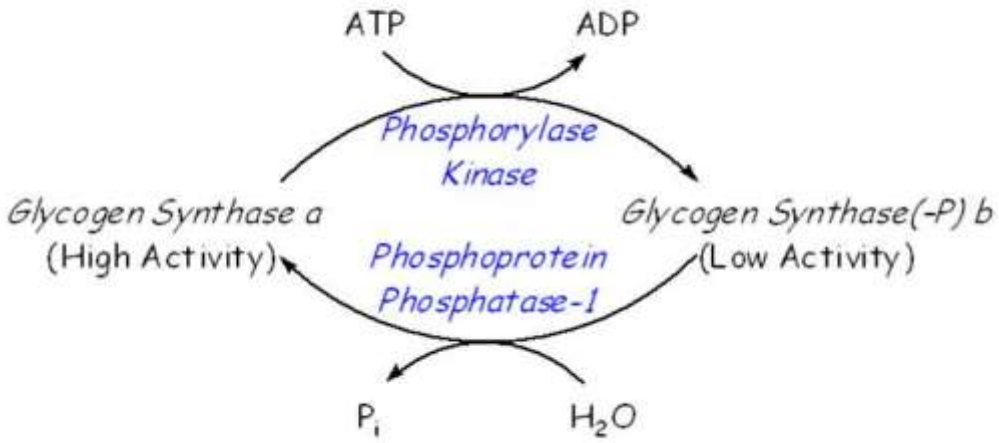
ALLOSTERIC MODULATION | **COVALENT MODIFICATION**

COVALENT MODIFICATION:

1. Many enzymes are regulated via covalent modification.
2. It involves addition of some functional groups (like **phosphoryl, adenylyl, acetyl. methyl, amide, carboxy, sulphate etc**.) on to the enzyme via **covalent attachment**. Their binding causes a conformational change in the enzyme which causes an alteration in the activity of the enzyme.
3. Regulation by covalent modification is slower than allosteric modulation.
4. Over 500 different types of covalent modification have been found in proteins.
5. It is of two **types- reversible and irreversible.**

Reversible covalent modification- Reversible addition/removal of functional groups on to the enzyme regulates enzyme activity. Phosphorylation/ dephosphorylation is the most common form of it. Example- **Glycogen synthase. It is phosphorylated and inactivated by a protein kinase, and, dephosphorylated and activated by a protein phosphatase.**



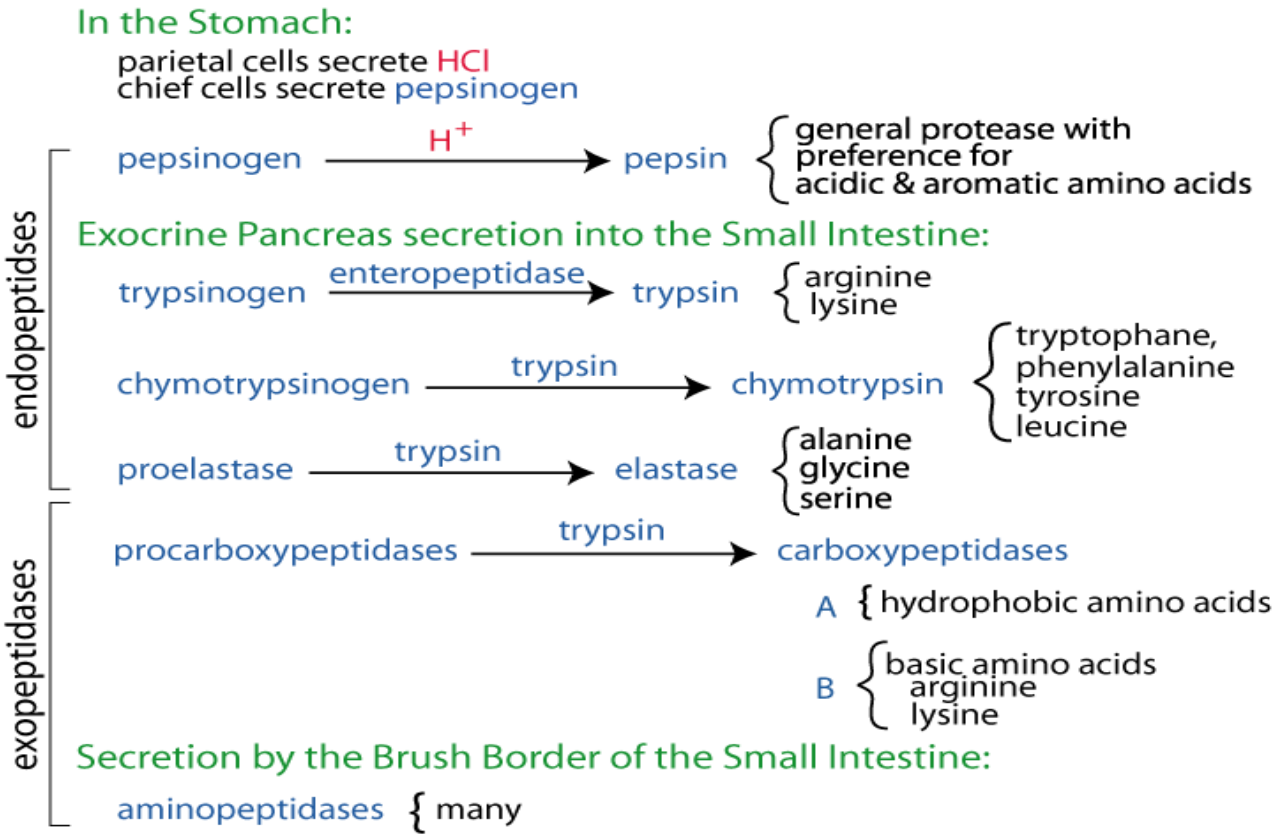


Irreversible covalent modification:

Some proteins/ enzymes are synthesized in inactive forms. They are called **zymogens or pro-enzymes**. They are activated when small length of protein is cleaved off from one end by proteases. This causes an irreversible change in the tertiary structure of the enzyme to generate the active enzyme. **Activated enzyme can not be reconverted back to the original inactive form**.

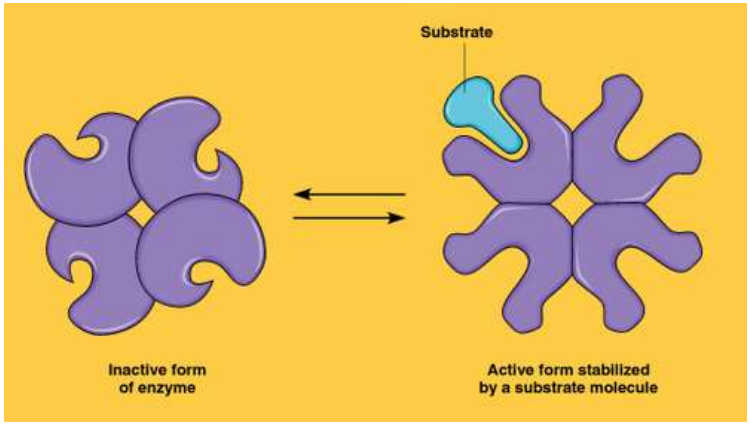
$$\text{Zymogen} \xrightarrow[pH]{Specific\ proteases} \text{Active enzyme} + \text{Masking substance}$$

Example:



ALLOSTERIC MODULATION

1. Activity of some enzymes is either enhanced or decreased by binding of some **low-mw ligands** to a specific site which is different from the active site of the enzyme. Such low-MW ligands are called **'allosteric modulators'.** (Greek word 'allo' means 'other' and 'stereos' mean 'site'). They bind to a specific site of the enzyme called **'allosteric site'** by reversible covalent interactions. Such binding causes a conformational change in the enzyme and alters its activity.
2. Allosteric modulator may be a positive modulator (**allosteric activator**) or a negative modulator (**allosteric inhibitor**) depending on whether the binding causes an enhancement/inhibition of the enzyme activity.



Properties of allosteric enzymes:

1. Most allosteric enzymes are **oligomeric enzymes** having several protomers (peptide subunits). **These enzymes have more than one substrate-binding site (active site) on different protomers.**
2. Allosteric sites and substrate-binding sites may occur on different protomers.
3. Allosteric sites are highly specific for specific allosteric modulators.
4. Allosteric enzymes do not follow Michaelis- Menten Kinetics.
5. The allosteric enzyme shows **cooperativity**. When enzymes contain more than one active site, the **binding of a substrate molecule to the first site may influence binding of many other substrate molecules to other active sites**. This phenomenon is called cooperativity.

6. **Cooperativity can be positive or negative**. If binding of the first substrate molecule facilitates binding of subsequent substrate molecules it is called **"positive cooperativity"**, and, if the binding of the first substrate decreases the binding of subsequent substrate molecules it is called **"negative cooperativity".**

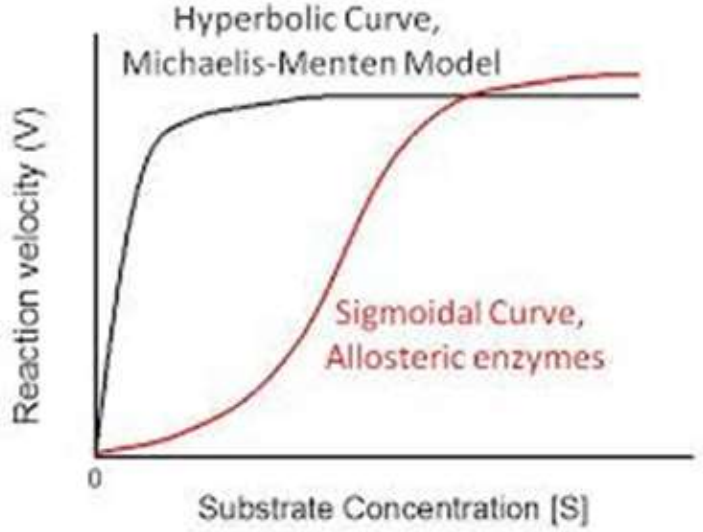
7. Allosteric effectors may be homotropic or heterotopic.
   - **Homotropic allosteric enzyme**: If the substrate and allosteric modulators are same for the allosteric enzyme. (the prefix 'homo' refers to them being the same**)**. They are usually activators of the enzyme.
   - **Heterotopic allosteric enzyme:** If the allosteric modulators are different from substrate. It can either activate or inhibit the enzyme.

Kinetics of allosteric enzymes:

Allosteric enzymes do not follow Michaelis- Menten Kinetics. This is because allosteric enzymes have multiple active sites. These multiple active sites exhibit the property of cooperativity. Instead they exhibit **sigmoidal kinetics**. Plotting of the V_0 against [S] for a cooperative enzyme, produces a characteristic sigmoidal shape. According to this curve, V_0 rises at a very low rate when [S] is low, . But at higher [S], there is a rapid and immediate increase in enzyme activity.

Hill Equation: Neither the Michaelis-Menten expression nor its derived plots can be used to evaluate cooperative kinetics. Instead of that Hill equation is used to describes sigmoid kinetics of allosteric enzymes.

$$V = \frac{V_{max}[S]^n}{(K_{0.5})^n + [S]^n}$$



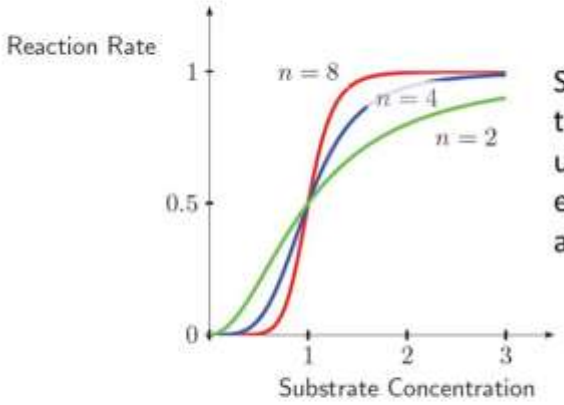
n is the **Hill coefficient**, its value is a function of the number, kind, and strength of the interactions of the multiple substrate binding sites on the enzyme.

When n = 1, Michaelis-Menten kinetic behavior is observed.

If n >1, the enzyme is said to exhibit positive cooperativity.

If n <1, the enzyme is said to exhibit negative cooperativity.

Hill Equation



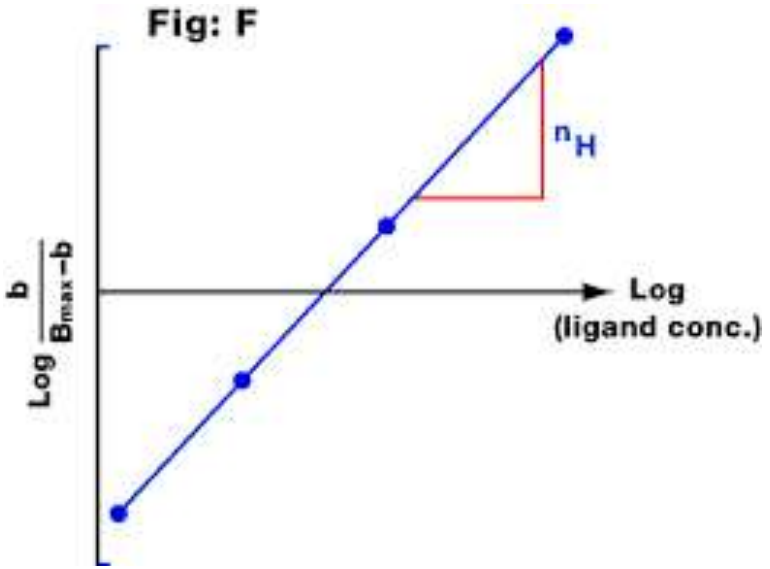
Some researchers feel that the underlying model is so unrealistic that the Hill equation should be considered an empirical result.

$$v = \frac{\text{Vmax } S^{n}}{K + S^{n}}$$ ← Hill Coefficient

The Hill equation can also be linearly transformed.

Log $(\frac{V_0}{Vmax - V_0})$ = n log [S] – log K

Plotting of log[S] against log $\frac{V_0}{Vmax - V_0}$) gives a straight line. where k' is a complex constant.



Classification of allosteric enzymes:

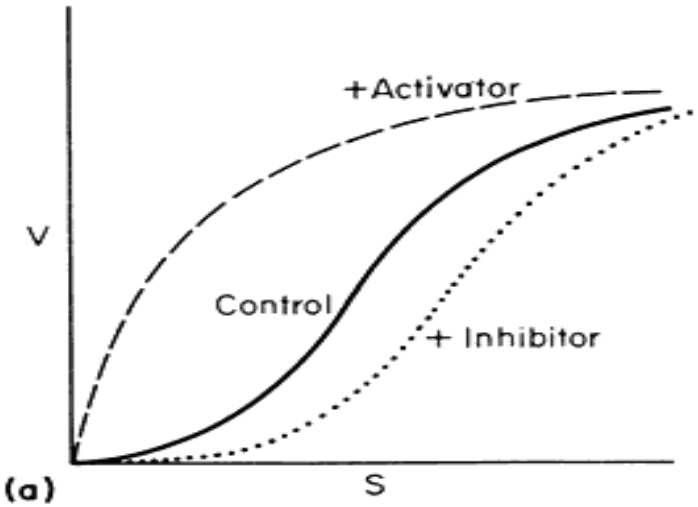
K- series:

1. Binding of **allosteric inhibitor** reduces the substrate affinity of the enzyme (enhances Km) but there is no change in Vmax. **It shifts the sigmoid curve to the right.**
2. Binding of **allosteric activator** enhances the substrate affinity of the enzyme (decreases Km) but there is no change in Vmax. **It shifts the sigmoid curve to the left.**
3. Example: Phsphofructokinase-1 is allosterically inhibited by ATP and activated by Fructose-2,6-bis phosphate.

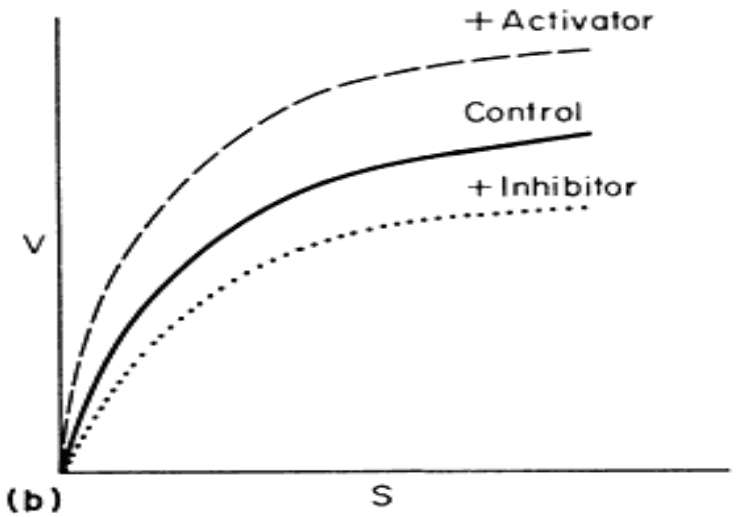
V- series:

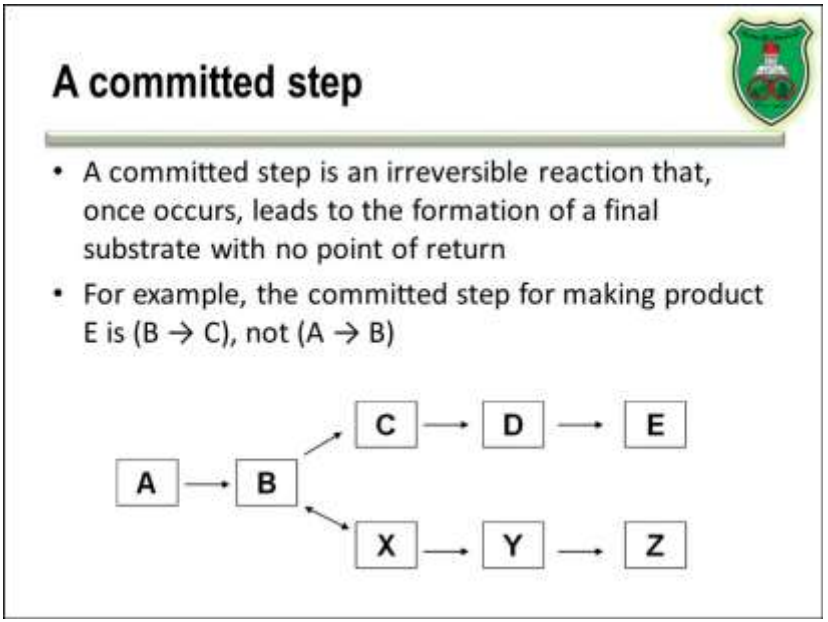
1. Binding of **allosteric inhibitor** reduces the Vo as well as Vmax. But substrate affinity of the enzyme remain unaltered. **It shifts the curve to the right.**
2. Binding of **allosteric activator** enhances the Vmax. But substrate affinity of the enzyme remain unaltered. **It shifts the curve to the left.**
3. Example- Acetyl Co-A carboxylase is allosterically inhibited by palmitoyl Co-A and activated by Citrate.

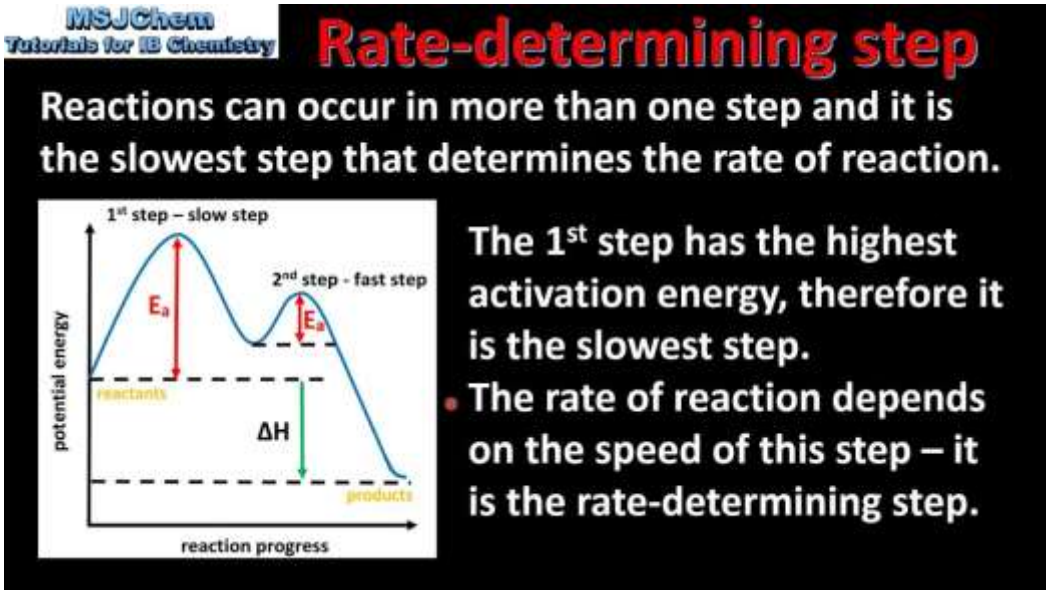
K- SERIES



V- SERIES







RATE-LIMITING ENZYMES:

1. A rate-limiting enzyme is **a key enzyme which determines the overall rate and direction of a metabolic pathway**.
2. A rate-limiting enzyme catalyzes the stage that requires the **greatest activation energy.**
3. A rate-limiting enzyme catalyzes the **slowest step** in a metabolic pathway.
4. A rate-limiting enzyme is **highly regulated.**

For example,
Glycolysis depends primarily on the activity of three key rate-limiting enzymes,

1. **hexokinases (HKs),**
2. **phosphofructokinase-1 (PFK-1), and**
3. **pyruvate kinases (PKs)**

Isoenzymes:

1. Isozymes (also known as isoenzymes) are enzymes that differ in amino acid sequence but **catalyze the same chemical reaction**. Isozymes were first described by Hunter and Markert (1957).

2. In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants) of enzymes. In many cases, they are **coded for by homologous genes** that have diverged over time.

3. They catalyze same reaction but differing in their **amino acid sequence, kinetic properties, regulatory properties, electrophoretic mobility, cofactor they use, in their subcellular distribution** and also in their parent gene loci.

4. The existence of isozymes permits the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stage.

Lactate dehydrogenase isoenzymes

Isoenzyme name	Composition	Composition	Present in	Elevated in
LDH1	(H_4)	HHHH	Heart, RBC	myocardial infarction
LDH2	(H_3M_1)	HHHM	Heart, RBC	myocardial infarction
LDH3	(H_2M_2)	HHMM	lungs and spleen	leukemia
LDH4	(H_1M_3)	HMMM	lungs and spleen	viral hepatitis
LDH5	(M_4)	MMMM	Skeletal muscle, Liver	Skeletal muscle and liver diseases

Isozymes of hexokinase

- **Isozymes** - multiple forms of hexokinase occur in mammalian tissues and yeast
- Hexokinases I, II, III are active at normal glucose concentrations (K_m values ~10^{-6} to 10^{-4}M)
- Hexokinase IV (**Glucokinase**, K_m ~10^{-2}M) is active at higher glucose levels, allows the liver to respond to large increases in blood glucose

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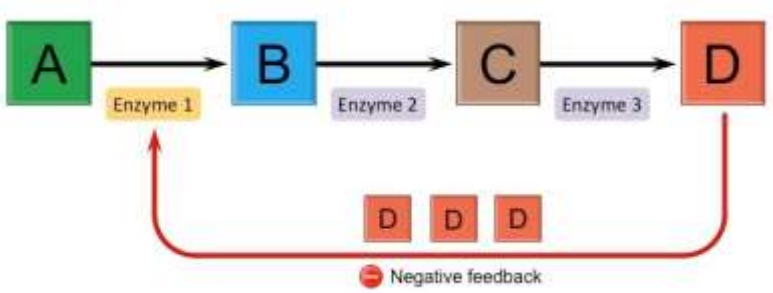
FEEDBACK INHIBITION

1. Most biochemical processes are complex and multi-step, requiring multiple enzymes to get from the starting substrate to the desired end product.
2. Feedback inhibition is the mechanism by which the **end products produced at the end of a series of reactions help to suppress the activity of the enzymes involved in the earlier steps** of the metabolic pathway.
3. The end product binds to the allosteric site of the enzyme and slows down or stops the enzyme's activity so that little or no new end product is produced. Thus it adjusts the rate of reaction and prevent their end product from building up to dangerous levels.
4. **Examples:** Accumulation of glucose-6-phosphate will lead to inhibition of hexokinase. Hexokinase is an allosteric enzyme and the product, (glucose-6-phosphate) is an allosteric inhibitor of the enzyme.



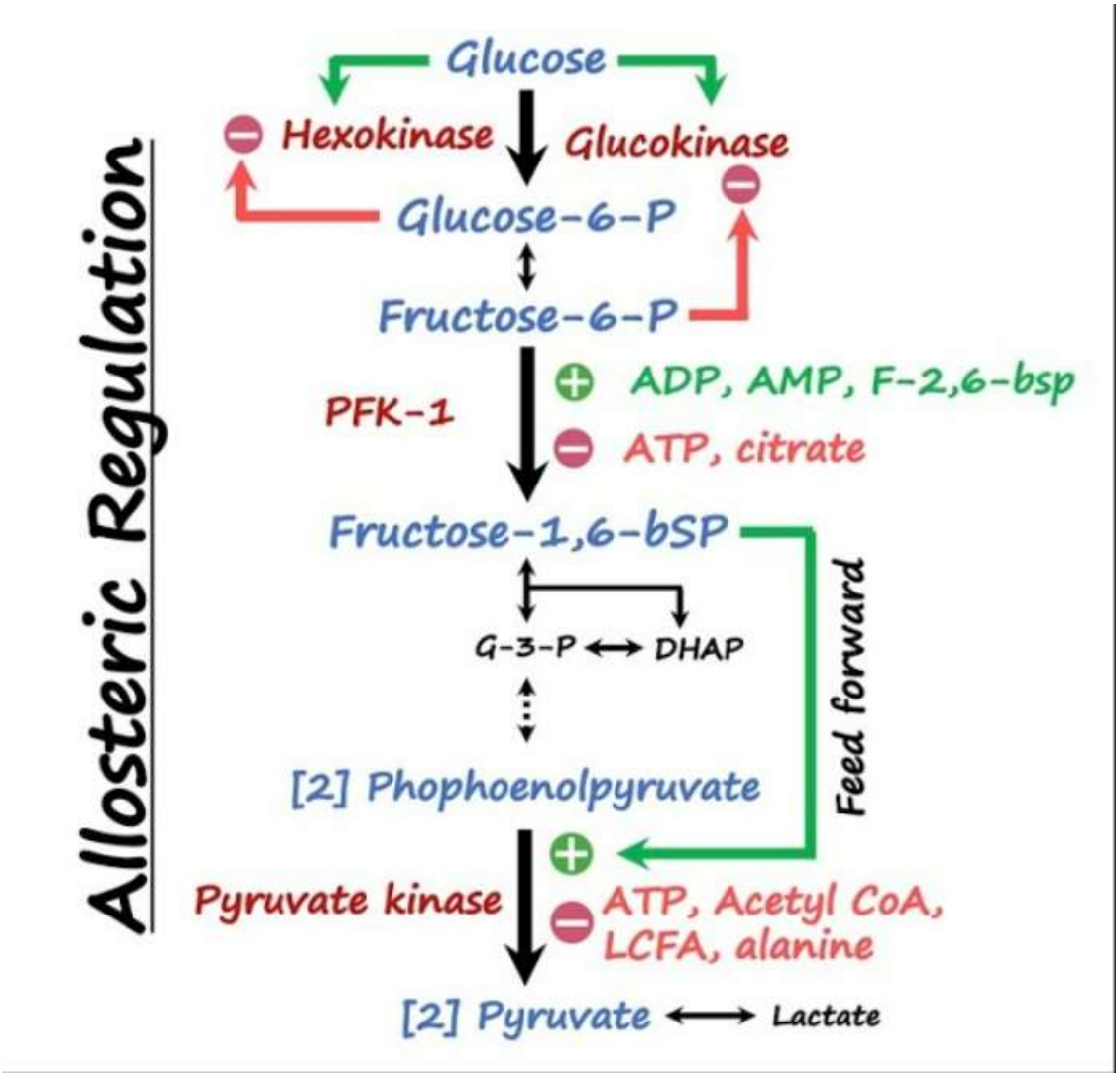
Functions of Feedback Inhibition

1. Without feedback inhibition, **raw materials and energy might be depleted** by biochemical processes when their end product is not needed. A good example of this is the production of ATP from glucose. The enzymes that produce ATP from glucose are subject to feedback inhibition by ATP. This saves glucose by preventing its unnecessary breakdown when the cell has plenty of ATP.

2. The end products of some biochemical pathways can actually be **dangerous in high concentrations**. Cholesterol is an excellent example.

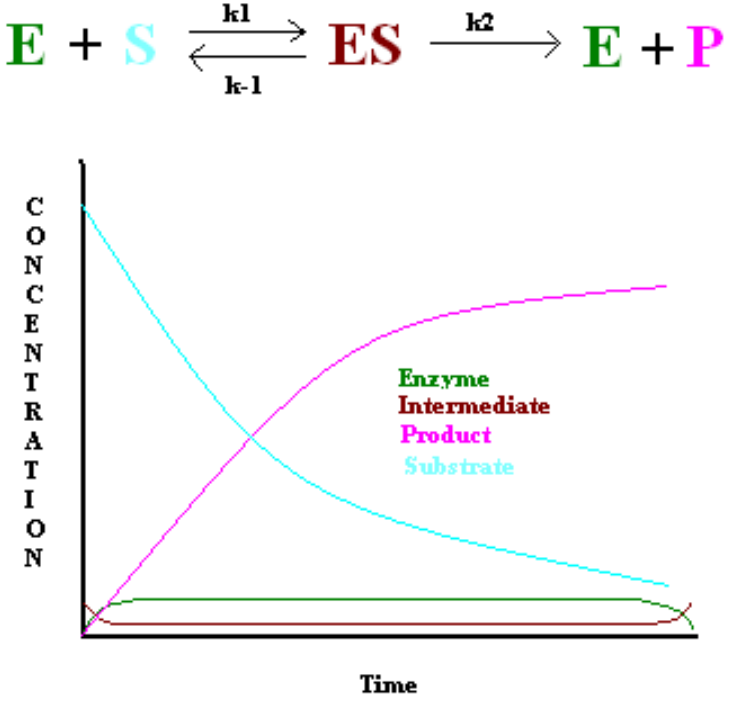
3. An essential function of life is the ability to **maintain homeostasis** in the face of changing environmental circumstances. Some chemical messengers that are involved in maintaining homeostasis are regulated through feedback regulation.

FEEDBACK INHIBITION IN GLYCOLYSIS



Steady state approximation :

In 1925, George E. Briggs and John B. S. Haldane applied the steady state approximation method to determine the rate law of the enzyme-catalyzed reaction (Figure 1).



Substrate concentrations are indicated in **blue**, product concentrations are indicated in **red**, and intermediate concentrations are indicated in **brown**. Notice that after their initial production, **the concentration of the reaction-intermediates (ES) remains *relatively constant* (slope of brown curve is approximately zero)** throughout the course of the reaction.

1. **Rate of formation of ES complex equals the rate of breakdown of ES .**
2. The steady state approximation is applies to a consecutive reaction with a slow first step and a fast second step ($k1 \ll k2$)
3. If the first step is very slow in comparison to the second step, intermediates in the reaction is consumed as quickly as it is generated. **Concentration of ES remains the same through out the duration of the reaction.**