Introduction

Biochemical reactions in living cells are catalyzed by **enzymes.** They are central to every biochemical process. They catalyze the hundreds of stepwise reactions of metabolism, conserve and transform chemical energy, and make biological macromolecules from simple precursors.

Enzymes (from the Greek ***enzymos***, "**leavened**") are the chemical catalysts of biological systems. Enzymes have extraordinary catalytic power, often far greater than that of synthetic or inorganic catalysts. They have ***a high degree of specificity*** for their substrates and they accelerate chemical reactions tremendously. They function in aqueous solutions under very mild conditions of temperature and pH, unlike many catalysts used in organic chemistry.

1.Definition

Enzymes are mostly **proteins** that accelerate the reaction rates. They are vital for life and execute diverse functions in the body such as: aiding the digestion and assist metabolic processes. An enzyme is formed by stringing together about 100 to 1000 amino acids in a specific and unique order. This chain of amino acids folds into a unique shape. Most enzymes are three-dimensional globular protein in both tertiary and quaternary structure. Some Special RNA species also act as enzymes and called Ribozymes e.g. hammerhead ribozyme.

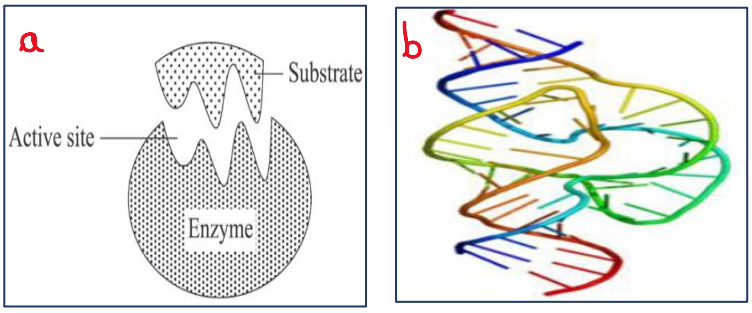


Figure 1: a. Schematic depiction of enzyme - substrate complex, **b.** Hammerhead enzyme

From a structural standpoint, there are two main categories of enzymes:

- **Apoenzyme**: The enzyme without its **non-protein moiety** is termed as apoenzyme and it is **inactive**.
- **Holoenzyme**: is an **active** enzyme with its **non-protein component**.

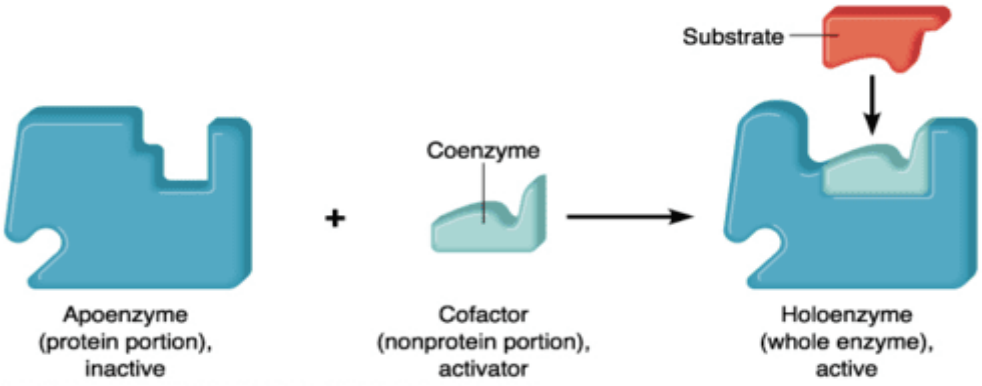


Figure 2: Apoenzyme and holoenzyme

Important Terms to Understand Biochemical Nature and Activity of Enzymes:

Cofactor:

A cofactor is a non-protein chemical compound that is bound (either tightly or loosely) to an enzyme and is required for catalysis.

Types of Cofactors:

• **Coenzymes:** The non-protein component, loosely bound to apoenzyme by non-covalent bond. ***Examples:*** vitamins or compound derived from vitamins.

• **Prosthetic groups:** The non-protein component, tightly bound to the apoenzyme by covalent bonds is called a Prosthetic group.

Example: Mg^{2+}

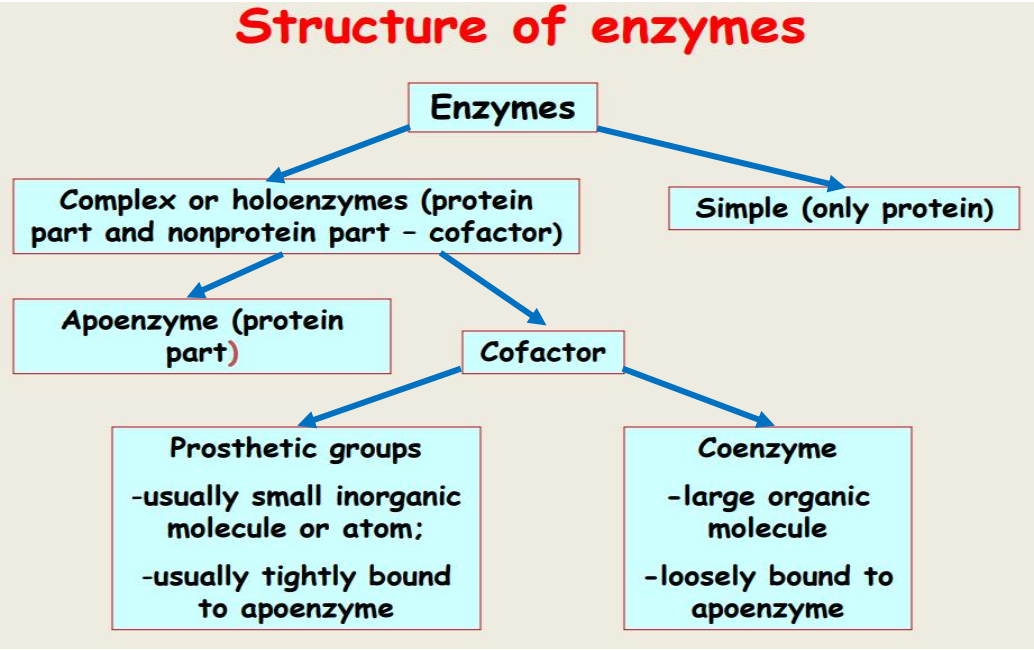
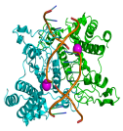


Figure 3: Enzyme structure



2. Nomenclature and classification of Enzyme

Enzyme nomenclature refers to two major systems:

- Common Names (Traditional naming)
- Systematic Names (Based on the reaction mechanism and substrate)

2.1. Common names

✓ Common names are the traditional, familiar names given to enzymes based on their function or the type of substrate they act upon.

✓ In most cases, common names end with the suffix **"-ase"**, indicating that the term refers to an enzyme. However, there are some exceptions.

Examples:

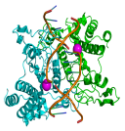
- **Cellulase:** An enzyme that breaks down cellulose (found in plant cell walls).
- **Pepsin:** A protease that does not follow the standard "-ase" suffix but is widely recognized.
- **Oxidase:** Enzymes that catalyze oxidation reactions, often involving oxygen as an electron acceptor.

2.2. Systematic Names

The systematic name of an enzyme is more formal and provides detailed information about the enzyme's substrate, the reaction it catalyzes, and its mechanism of action. Systematic names follow a structured approach and are based on **the EC number** (**E**nzyme **C**ommission **number**) as defined by the International Union of Biochemistry and Molecular Biology (**IUBMB**)Enzyme Nomenclature.

The EC number consists of four digits (**EC N-X Y Z**) that classifies an enzyme according to the following scheme:

- First digit **N**: Class (the type of reaction the enzyme catalyzes)
- Second digit **X**: Subclass (the type of bond or group involved in the reaction)
- Third digit **Y**: Sub-subclass (specific reaction or substrate)
- Fourth digit **Z**: Enzyme identifier (a unique identifier for the enzyme)



Examples of Systematic Names:

Hexokinase (EC 2.7.1.1)

- Class 2: Transferases (enzymes that transfer functional groups)
- Subclass 7: Transferring a phosphate group (phosphotransferase)
- Sub-subclass 1: Specific for the substrate glucose (glucose as the acceptor of phosphate)
- Enzyme identifier number 1: Hexokinase (the enzyme that catalyzes the phosphorylation of glucose)

Trypsin (EC 3.4.21.4)

- Class 3: of hydrolases.
- Subclass 4: means that it is a peptidase (the cleaved substrate is of peptide nature).
- Sub-subclass 21: characteristic of a catalytic serine endopeptidase.
- Enzyme identifier number 4: designates the enzyme in question.

There are six main classes of enzymes:

1.Oxidoreductases: Catalyze oxidation-reduction reactions (e.g., dehydrogenases, oxidases).

2.Transferases: Transfer functional groups (e.g., kinases, transaminases).

3.Hydrolases: Catalyze hydrolysis reactions (e.g., proteases, lipases).

4.Lyases: Catalyze the breaking of chemical bonds without water (e.g., decarboxylases, aldolases).

5.Isomerases: Catalyze isomerization reactions (e.g., racemases, epimerases).

6.Ligases: Catalyze the formation of bonds using energy from ATP (e.g., synthetases, ligases).

Table 1: Enzyme classes and the types of reaction they catalyse[a]

Class number	Class name	Reaction schema
1	Oxidoreductases	$AH_2 + B^+ = A + BH + H^+$ or $AH_2 + B = A + BH_2$
2	Transferases	$AX + B = A + BX$
3	Hydrolases	$A - B + H_2O = AH + BOH$
4	Lyases	$A = B + X - Y = \begin{matrix} A & - & B \\ \vert & & \vert \\ X & & Y \end{matrix}$
5	Isomerases	$A = B$
6	Ligases	$A + B + NTP = A - B + NDP + P$ or $A + B + NTP = A - B + NMP + PP$

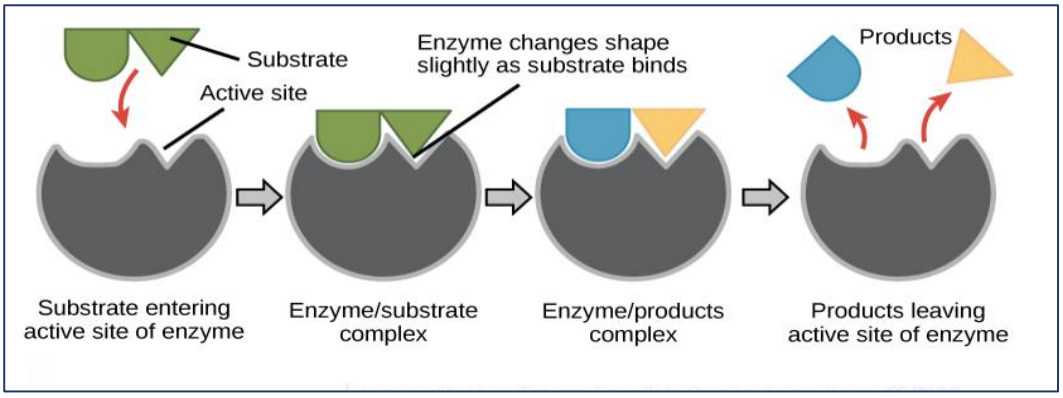
[a]Adapted with permission from McDonald AG & Tipton KF (2014) © John Wiley & Co Ltd.

3. Mechanism of Action

An **enzyme** is a protein molecule that can act as a biological catalyst. The molecules that enzymes act upon are called **substrates**. Different molecules that are created by the action of an enzyme upon a particular substrate are called **products**. Enzymes catalyze biochemical reactions by lowering its activation energy.

An enzymatic reaction contains the following main steps:

1. The binding of the substrate to the enzyme's **active site**;
2. Formation of the Enzyme-Substrate Complex (**ES Complex**);
3. Substrate transformed into products;
4. Product release and enzyme regeneration.



Substrate + Enzyme → [ES Complex] → Transition State →Product + Enzyme

Figure 4: Enzymatic reaction steps

4.Active Site

- The active site of an enzyme is the region where specific substrates bind to the enzyme, catalyzing the chemical reaction.
- The active site is a small area, a cavity or hole on the surface of the enzyme. The active site consists of 10– 15 amino acid residues brought together by folding from different parts of the primary structure of the protein.
- The active site is typically composed of:
  - **Binding Site:** part of the active site is responsible for stereospecific binding of the substrate. It is designed to specifically recognize and bind the substrate molecules with high affinity.

- **Catalytic Site:** transforming part of the active site, occurs next to the binding site, carrying out the catalysis.

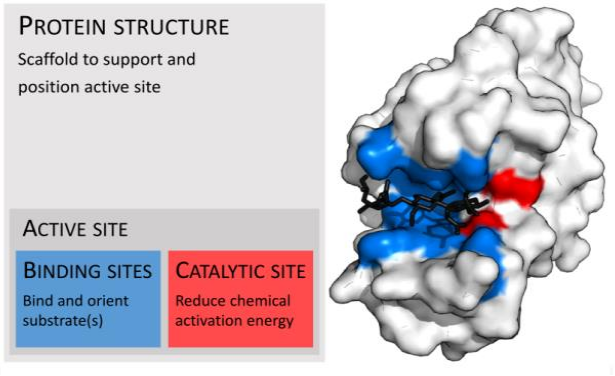


Figure 5: Active site of the enzyme

5.Enzyme kinetics and types of representation

- Enzyme kinetics is the study of the rate of biochemical reaction catalysed by an enzyme. It helps us to understand enzymatic efficiency and to determine the concentration of an enzyme in a solution or biological sample.
- The reaction rate of an enzyme is determined by the change in the amount of substrate or product per unit time.
- The concentration of substrate [S] is a key factor affecting the rate of a reaction catalyzed by an enzyme.
- In 1913, Michaelis and Menten developed a mechanism to explain how the initial rate of enzyme catalyzed reactions depends on the concentration.

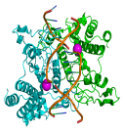
Enzymatic reaction

The general enzymatic reaction can be written as:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

Where:

- **E** is the enzyme.
- **S** is the substrate.
- **ES** is the enzyme-substrate complex.
- **P** is the product.
- **-k1** is the formation constant of the ES complex;
- **-k2** is the dissociation constant of the ES complex;
- **-k3** is the formation constant of P.



According to the reaction, the rate of disappearance of the substrate S (- ds/dt) is equal to the rate of formation of the reaction products (dp/dt): **V=-d[S]/dt = d[P]/dt**

- **The rate of formation of ES** at any time t (within the initial period when the product concentration is negligible) = k1[E][S].
- **The rate of breakdown of ES** at this time = k_2[ES] + k_3[ES], since ES can break down to form products or reform reactants.
- **Using the steady state assumption:** k_2 [ES] + k_3 [ES] = [ES] (k_2 + k_3)
- Separating the constants from the variables:

  [E][S]/ [ES] = (k2 + K3)/ k1 = km

- The final Michaelis-Menten equation is as given below:

$$v = \frac{V_{max}[S]}{[S] + K_M}$$

Where:

-**V_{max}** = The maximum velocity achieved by the system, at maximum (saturating) substrate concentrations.

- **K_M** (the Michaelis constant) = substrate concentration at which the reaction velocity is 50% of the Vmax. Its unit is mM. It is also the concentration at which concentration of substrate is half the maximal velocity is observed.

- **[S]** = concentration of the substrate S,

- The curve V=f([S]) is a hyperbolic curve that approaches a limiting value, the maximum speed or Vmax.

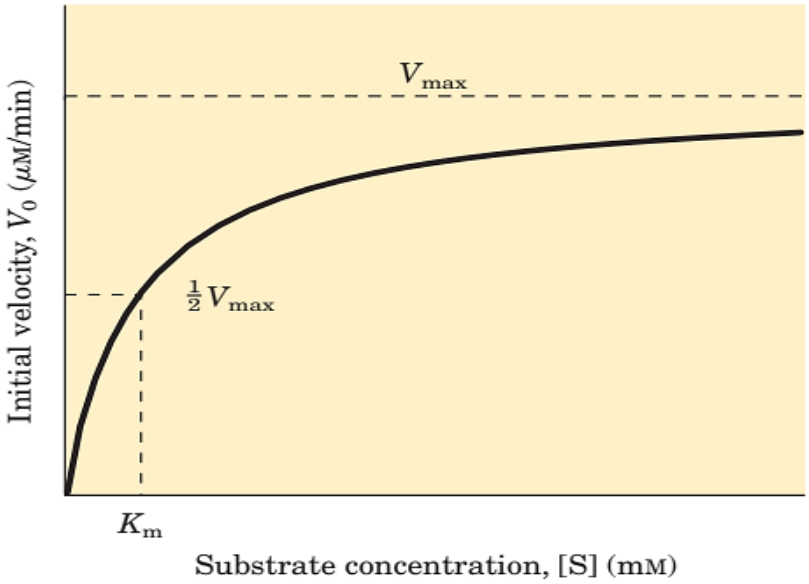


Figure 6: Graphical determination for V_{max} and K_M.

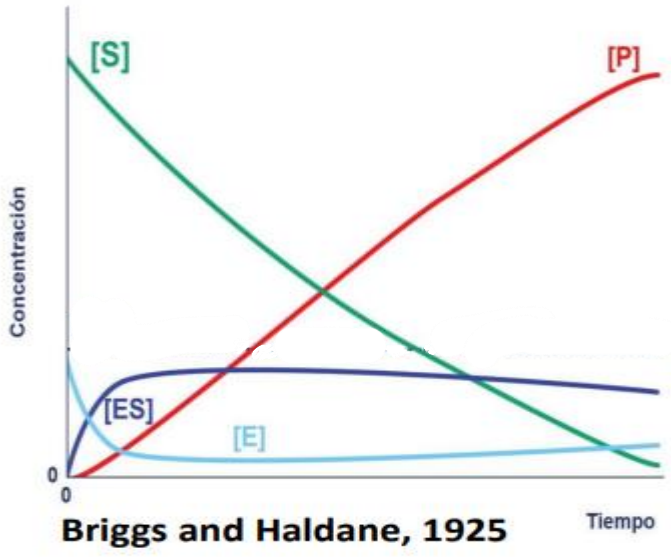


Figure 7: Enzymatic reaction progress

- To simplify the graphical representation of the Michaelis-Menten equation, the hyperbola is transformed into a straight line.
- Equation is the equation for a straight line: $y = ax + b$, where $y = 1/v_0$ and $x = 1/[S]$.

$$Vo = \frac{Vmax\,[S]}{Km + [S]}$$ --- Michaelis-Menten equation

Lineweaver-Burk Equation $$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

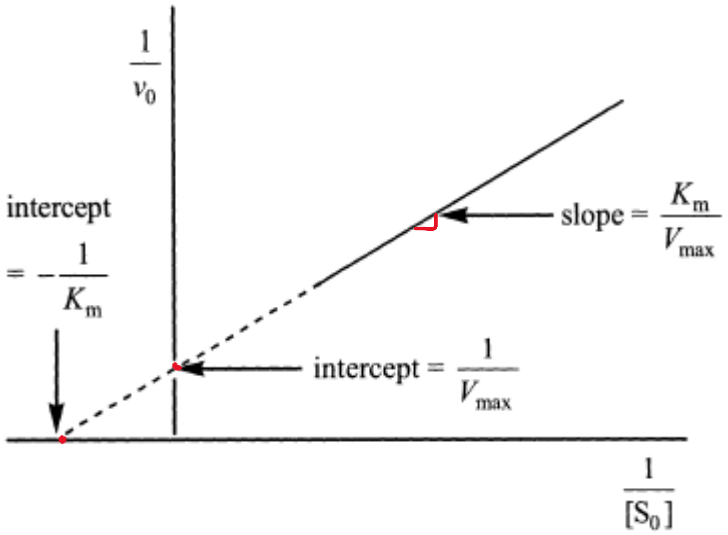
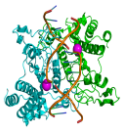


Figure 8: The Lineweaver-Burk plot.



6.Enzyme inhibition

An enzyme catalyzed reaction can be hindered or reduced by a number of substances. Some others like urea are known as denaturants, being non-specific in their mode of action. But if any compound act in a fairly specific way in inhibiting the catalysis of a particular enzyme they are called **inhibitors.** The loss in activity can either be of two types:

- **Reversible**, where the activity can be restored by the removal of the inhibiting compound. It's temporary.
- **Irreversible**, where the loss of activity cannot be recovered within the stipulated time of interest. It is permanent. Irreversible inhibition behaves as time dependent loss of enzyme concentration with lowered Vmax or incomplete in activation with time dependent change in both Km and Vmax.

Heavy metal ions like mercury, cause irreversible inhibition, which bind strongly to the amino acid backbone termed as "suicide inhibition".

By definition Inhibitors are a class of compounds which decrease or reduce the rate of an enzyme-catalyzed reaction. There are a variety of compounds like drugs, antibiotics and metabolites which can act as inhibitors.

Enzyme activity is usually regulated by the phenomenon called **feedback mechanism** where the end product is responsible for inhibiting the enzyme's activity. In fact, the amount of products formed is controlled by enzyme inhibition.

6.1. Reversible inhibitions

In this type of inhibition, the hindrance is temporary and thus noncovalent interactions like hydrogen bonds, ionic bonds or hydrophobic bonds form between inhibitors and the enzyme.

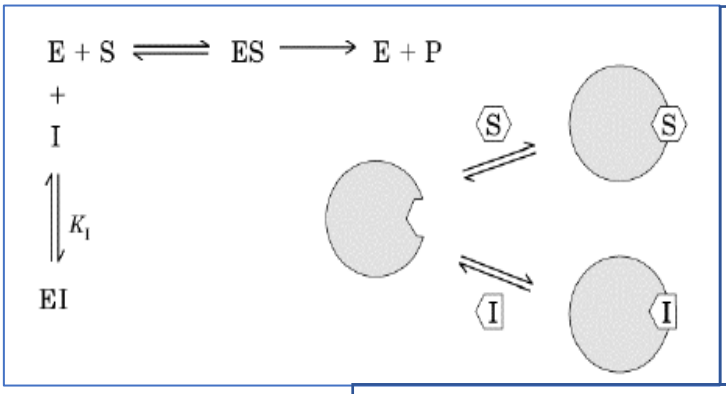
the inhibitors can easily remove or exchange by dilution or dialysis. After removing the inhibitor, enzyme can be fully restored in reversible inhibition. Equilibrium is established between free inhibitor and enzyme-inhibitor [EI] complex.

Reversible inhibition can be of three types

- Competitive inhibition;
- Non-competitive inhibition, and;
- Uncompetitive inhibition.

Competitive inhibition: **Competitive inhibitors** often resemble the substrates whose reactions they inhibit, and because of this structural similarity they may compete for the same binding site on the enzyme. Competitive inhibition can be reversed by increasing the concentration of the substrate.

Example: Malonate is a competitive inhibitor of enzyme succinate dehydrogenase, and competes with succinate.



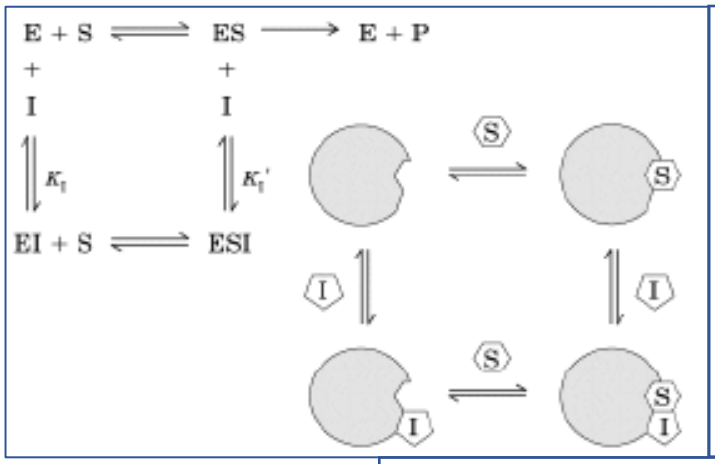
The Lineweaver-Burk equation in the presence of a competitive inhibitor will be :

$$\frac{1}{v_0} = \frac{K'_m}{V_{max}} \cdot \frac{1}{[S_0]} + \frac{1}{V_{max}}$$

Figure 9 : Competitive inhibition

Noncompetitive inhibition: A **non-competitive inhibitor** can combine with an enzyme molecule to produce a ***dead-end complex***, regardless of whether **a substrate molecule is bound or not.**

A **non-competitive inhibitor** is one which reacts with enzyme-substrate or [ES] complex. It does not affect the binding of the substrate, but slows down the reaction rate for formation of the enzyme-product [EP] complex. The only factor on which the extent of hindrance or inhibition depends is the inhibitor concentration.



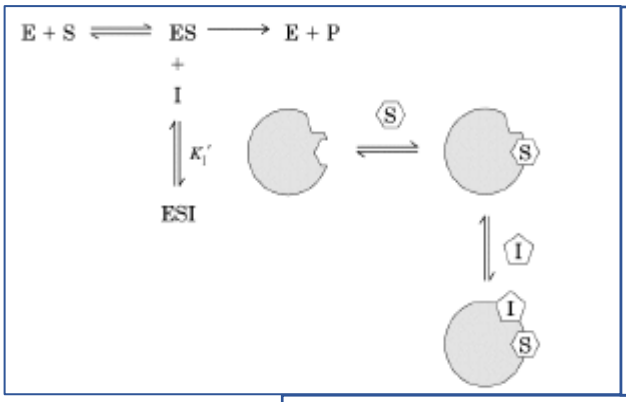
The Lineweaver-Burk equation in the presence of a competitive inhibitor will be :

$$\frac{1}{v_0} = \frac{K_m}{V'_{max}} \cdot \frac{1}{[S_0]} + \frac{1}{V_{max}}$$

Figure 11 : Noncompetitive inhibition

Uncompetitive Inhibition: It's an anti-competitive inhibition; where **the inhibitor** binds only to the substrate-enzyme complex. This type of inhibition works best in case of high concentration of the substrate. The substrate and the uncompetitive inhibitor do not resemble each other.

Example: -Lithium and phosphoinositide cycle.

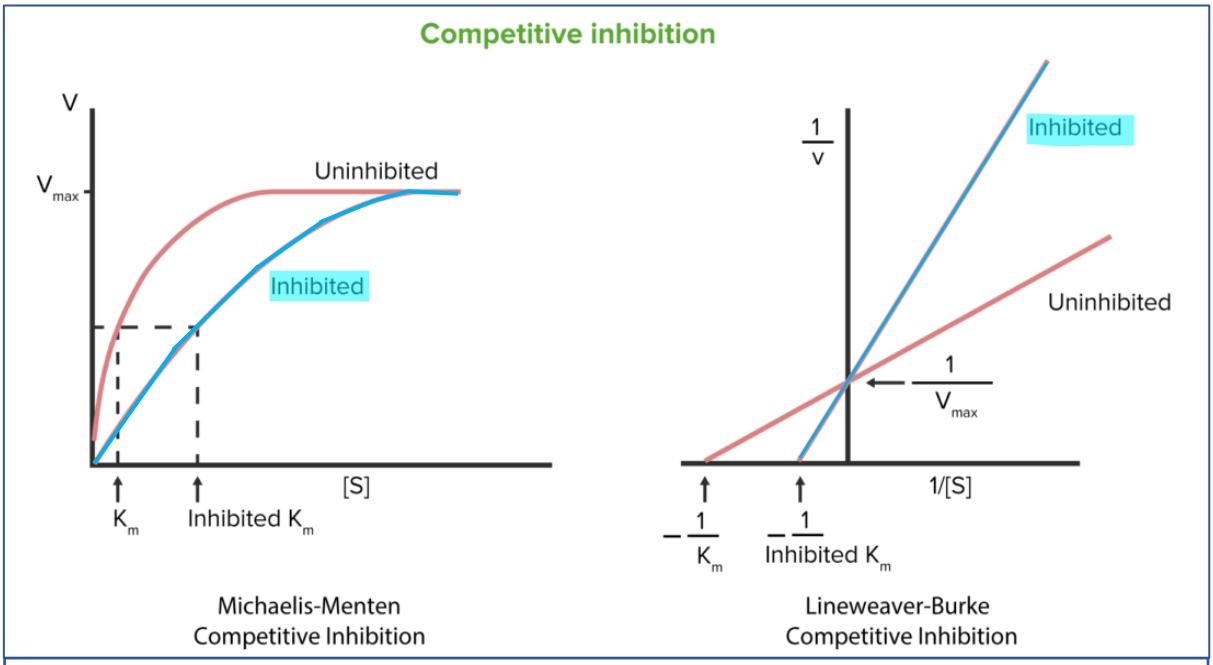


The Lineweaver-Burk equation in the presence of a competitive inhibitor will be:

$$\frac{1}{v_0} = \frac{K'_m}{V'_{max}} \cdot \frac{1}{[S_0]} + \frac{1}{V_{max}}$$

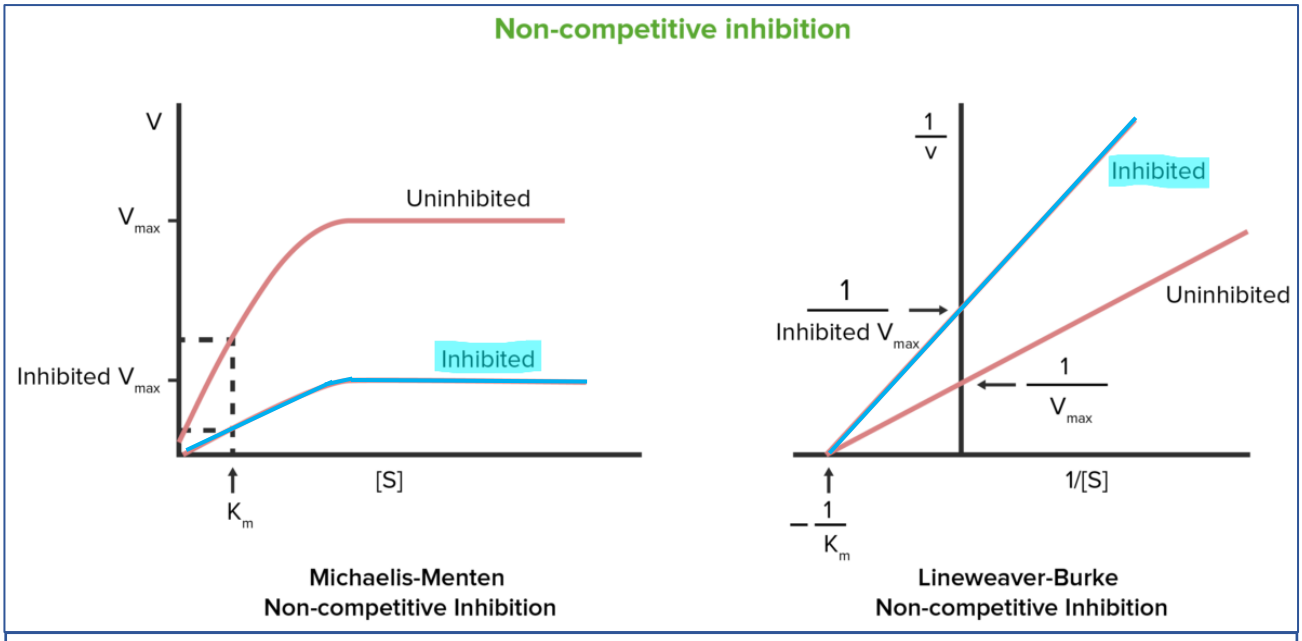
Figure 11 : Uncompetitive inhibition

❖ kinetics of reversible inhibition types



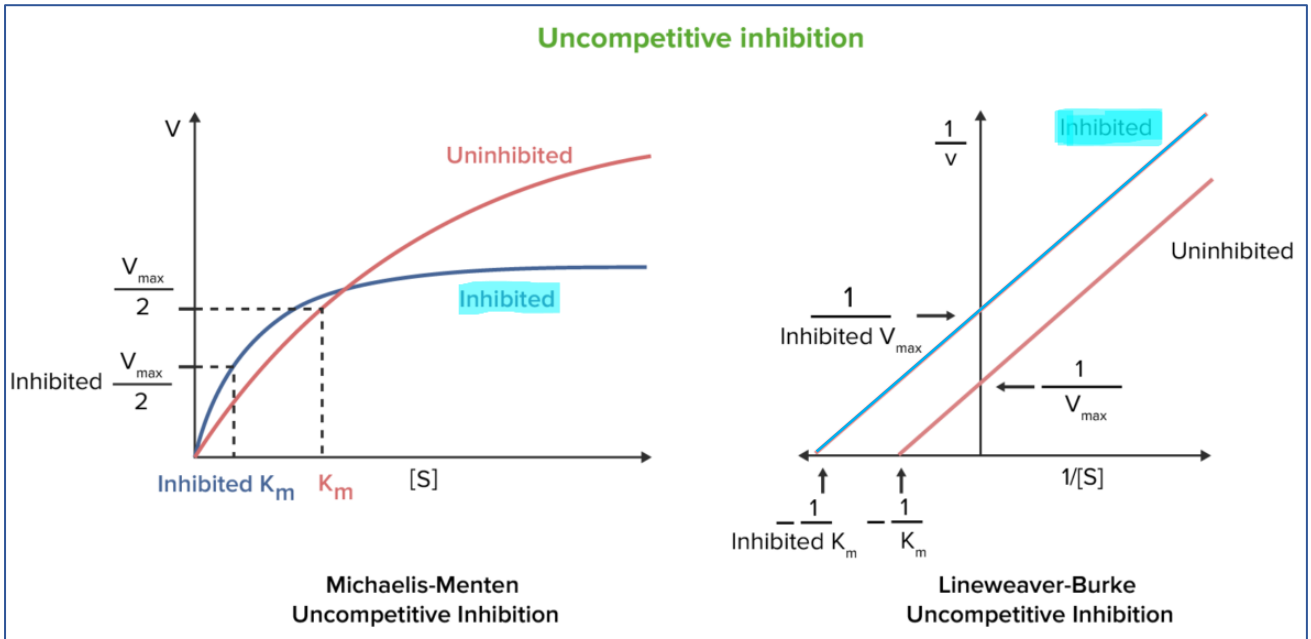
- ***According to its kinetics :***

Vmax of the reaction is **unchanged,** while **km increase** (Vmax = Vmax'; Km' > Km)



- ***According to its kinetics :***

Vmax decrease but **km** will remain the same (Vmax' < Vmax; Km' = Km)

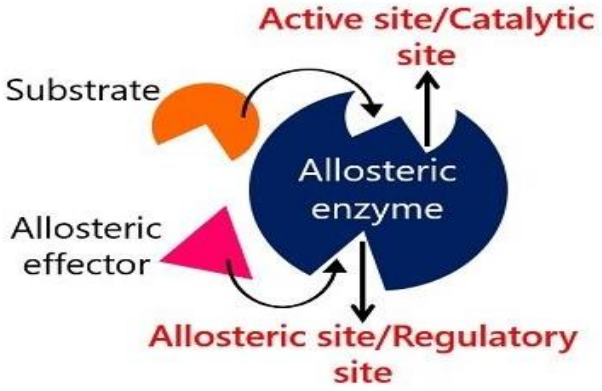


- ***According to its kinetics :***

Vmax and **Km** decrease (Vmax' < Vmax; Km' < Km)

7.Allosteric phenomenon

An **allosteric enzyme** possesses at least 2 spatially distinct binding sites on the protein molecules the active or the catalytic site and the regulator or **the allosteric site**. The metabolic regulator molecule binds at the allosteric site and produces a change in the conformational structure of the enzyme, so that the geometrical relationship of the amino acid residues in the catalytic site is modified. Consequently, the enzyme activity either increases (activation) or decreases (inhibition).



However, the term **allosteric inhibition** is usually reserved for the situation where the inhibitor, rather than forming a dead-end complex with the enzyme, influences conformational changes which may alter the binding characteristics of the enzyme for the substrate or the subsequent reaction characteristics (or both).

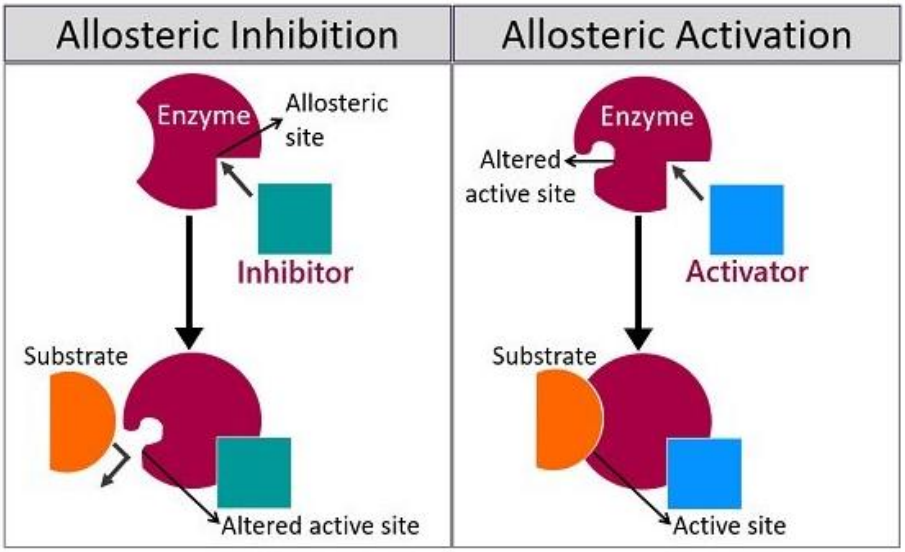


Figure13: Allosteric regulation