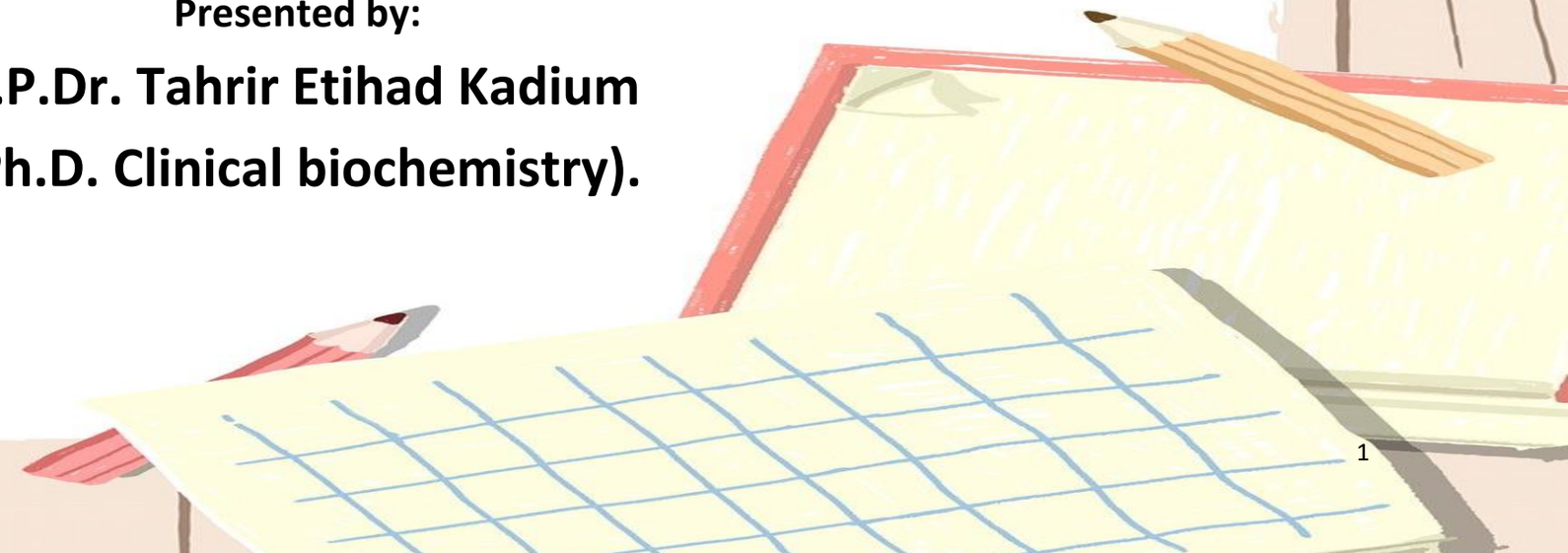
Biochemistry Module For Medical Students
Lecture 7 : Enzymes II
Factors Affecting Enzyme Activity & Enzyme Regulation.

Presented by:

A.P.Dr. Tahrir Etihad Kadium

(Ph.D. Clinical biochemistry).



Learning Objectives:

The main objectives of this lecture are to understand:

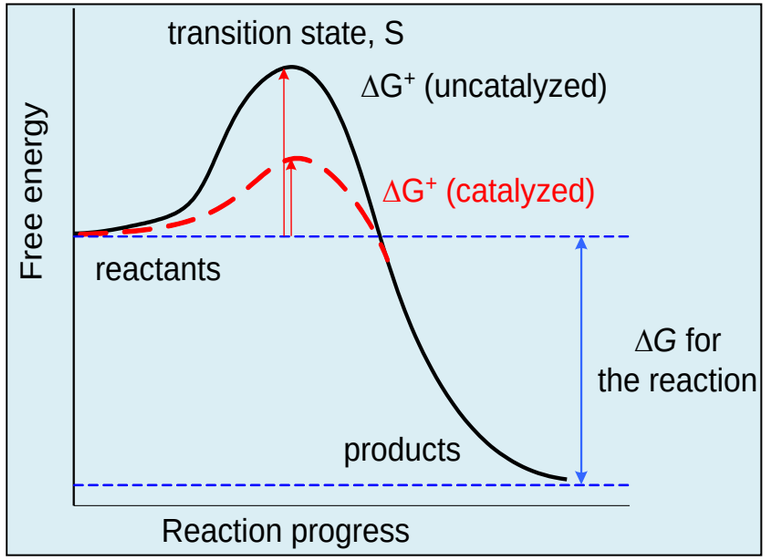
- **Outline the mechanism of enzymatic action.**
- **Describe the factors influencing enzyme activity.**
- **Define the Michaelis-Menten equation and its significance.**
- **Outline enzyme regulation.**
- **Describe Inhibition and list the types of inhibition.**
- **Learn examples of inhibition in biological system as Clinically used drugs.**

How Enzymes Work:

The mechanism of enzyme action can be viewed from two different sides.

A. **Energy changes occurring during the reaction.**

B. **Chemistry of the active site**



The enzymes provide an alternate, energetically favorable reaction pathway different from the uncatalyzed reaction.

A. Energy changes occurring during the reaction:

Most of chemical reactions have energy barrier, which separating the reactants and the products.

This barrier, called the (free energy of activation), is the energy difference between that of the reactants and a high-energy intermediate (transition state) that occurs during the formation of product.

For example, (Figure) shows the changes in energy during the conversion of a molecule of reactant A to product B as it proceeds through the transition state (high-energy intermediate), T*:

A ⟷ T* ⟷ B

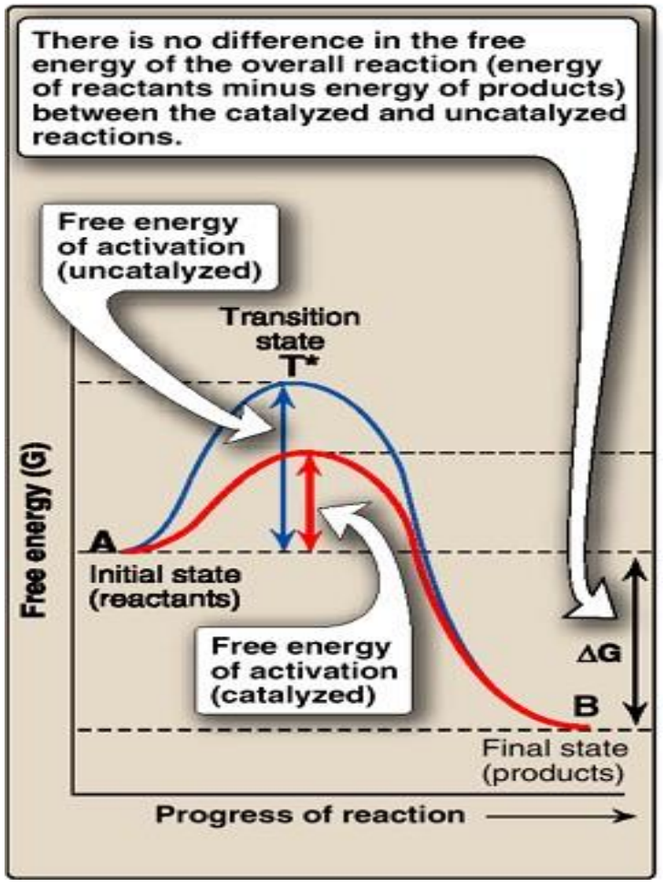
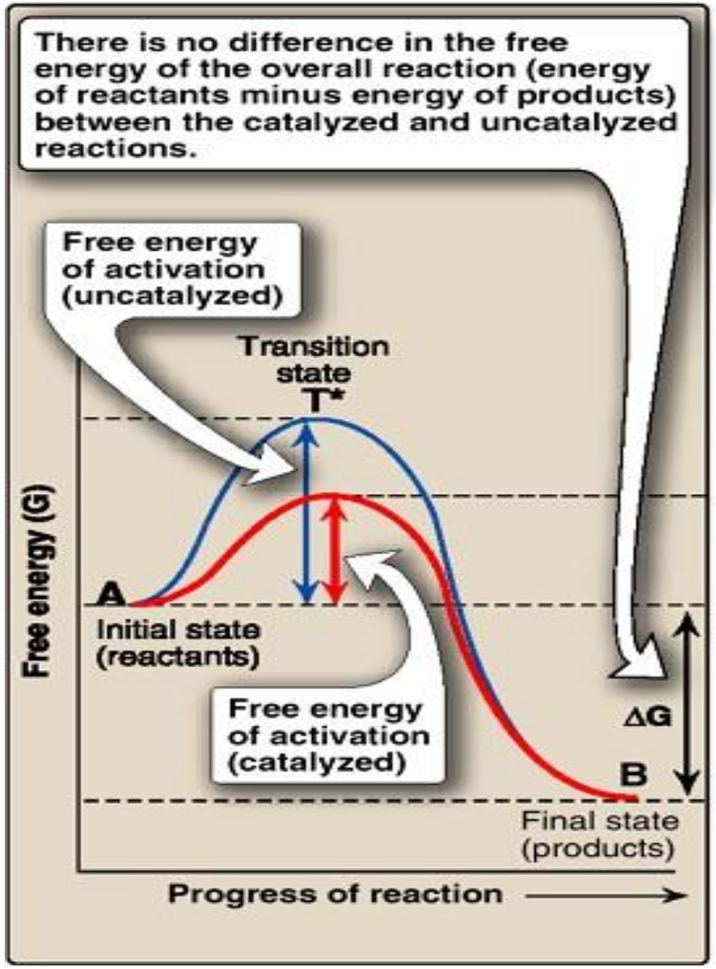


Figure :the effect of enzyme on the activation energy of the reaction.

1. Free energy of activation:

Because of the high free energy of activation, the rates of uncatalyzed chemical reactions are often slow.

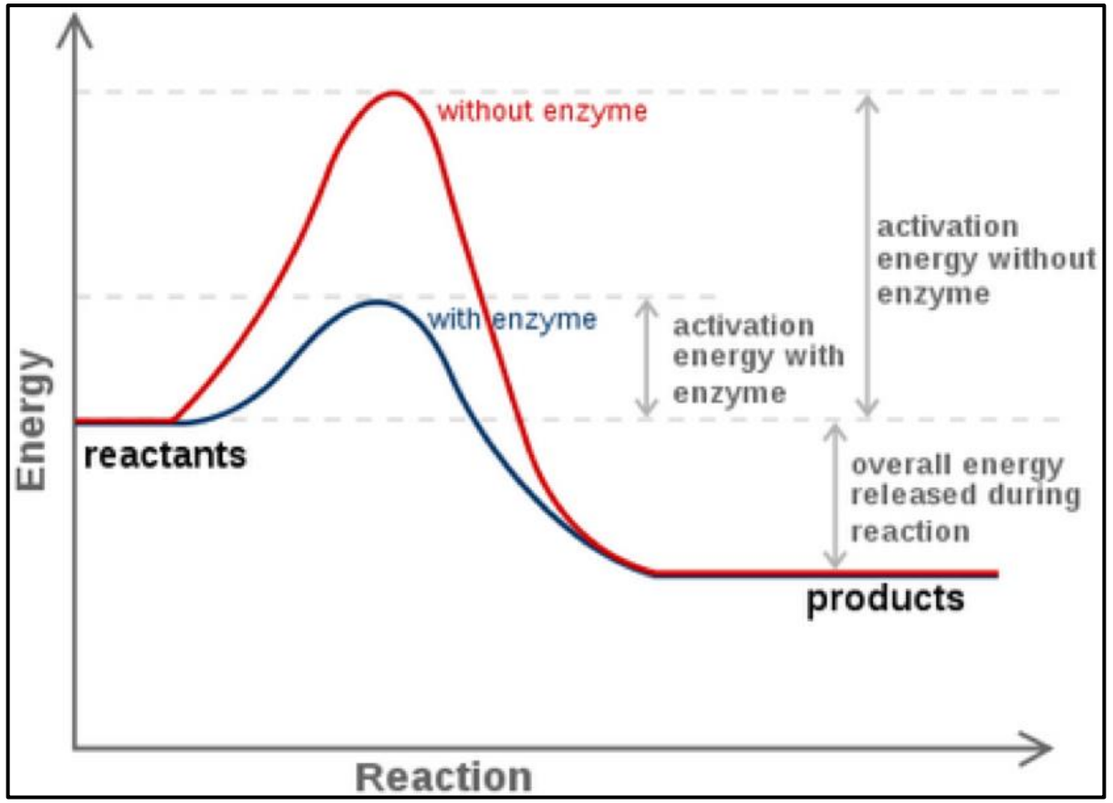


2. Rate of reaction:

- **For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state. The absence of an enzyme, only a small proportion of molecules may possess enough energy to achieve the transition state between reactant and product.**
- **The rate of reaction is determined by the number of such energized molecules.**
- **General, the lower the free energy of activation, the more molecules have sufficient energy to pass through the transition state, and, thus, the faster the rate of the reaction.**

3. Alternate reaction pathway:

An enzyme allows a reaction to proceed rapidly under conditions existing in the cell by providing an alternate reaction pathway with a **lower free energy of activation**.



Factors Affecting Reaction Velocity:

The factors that influence the reaction velocity of enzymes that give us clues as how enzymes function in the living cell (that is *in vivo*) are:

A. Substrate concentration

B. Temperature

C. pH

A. Substrate concentration:

The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per time unit. velocity is usually expressed as (µmol) of product formed per minute.

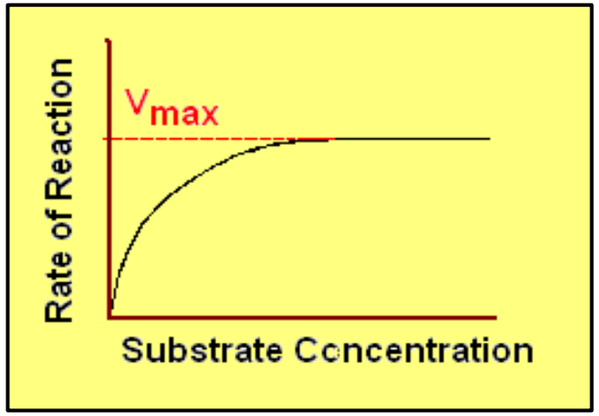


Figure :the effect of substrate concentration on the reaction velocity

The rate of an enzyme-catalyzed reaction increases with increasing substrate concentration until a maximal velocity (V max), is reached (Figure).

The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present.

B. Temperature:

1. Increase of velocity with temperature:

The reaction velocity increases with temperature until a peak velocity is reached (optimum temperature) (Figure). This increase is the result of the increased number of molecules having sufficient energy to pass over the energy barrier and form the products of the reaction.

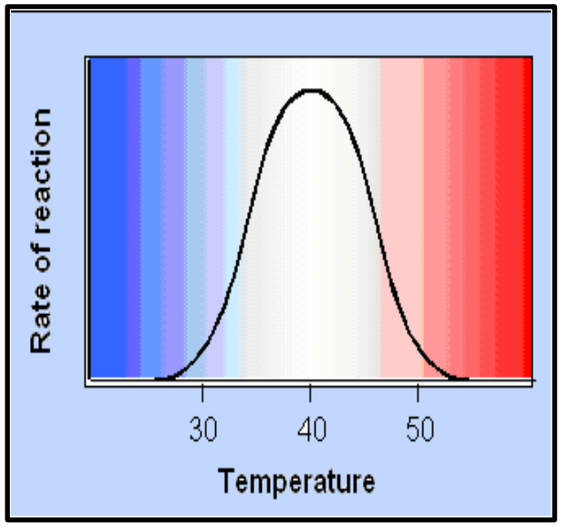


Figure : the effect of temperature on the rate of reaction

2. Decrease of velocity with higher temperature:

Further elevation of the temperature results in a decrease in reaction velocity as a result of temperature-induced denaturation of the enzyme.

- **NOTE: the optimum temperature for most human enzymes is between 35-40 °C. Human enzymes start to denature at temperature above 40 °C.**

C. pH:

1.Effect of pH on the ionization of the active site:

Catalytic activity may require that an amino group of the enzyme be in the protonated form (NH_3^+).

At alkaline pH this group is deprotonated, and the rate of the reaction, therefore, declines.

2. Effect of pH on enzyme denaturation:

Extremes of pH can also lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid side chains.

3. The pH optimum varies for different enzymes:

The pH at which maximal enzyme activity is achieved (optimum pH) is different for different enzymes, For example, *pepsin*, a digestive enzyme in the stomach, is maximally active at pH=2, whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment (Figure).

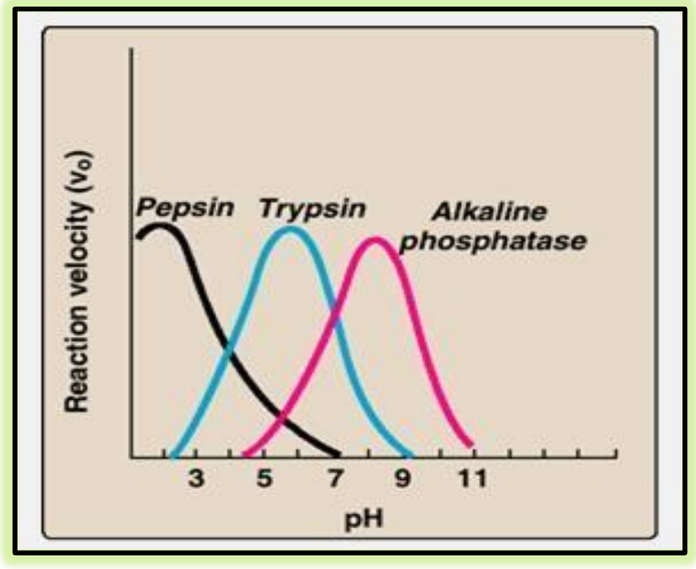


Figure : effect of pH on rate of reaction.

MICHAELIS-MENTEN MODEL & EFFECTS OF SUBSTRATE CONCENTRATION

- Michaelis-Menten Model:

"According to this model the enzyme reversibly combines with substrate to form an ES complex that subsequently yields product, regenerating the free enzyme."

where:

- S is the substrate
- E is the enzyme
- ES-is the enzyme substrate complex
- P is the product
- K1,K-1 and K2 are rate constants

Michaelis-Menten equation:

A. Reaction model:

Michaelis and Menten are two scientists proposed a simple model. In this model, the enzyme reversibly combines with its substrate to form an ES complex that subsequently breaks down to product, regenerating the free enzyme.

B. Michaelis and Menten Equation:

The Michaelis-Mentenequation describes how reaction velocity varies with substrate concentration.

$$Vo = \frac{V_{max}[S]}{Km + [S]}$$

V0 = Initial velocity (moles/times)

[S] = substrate concentration (molar)

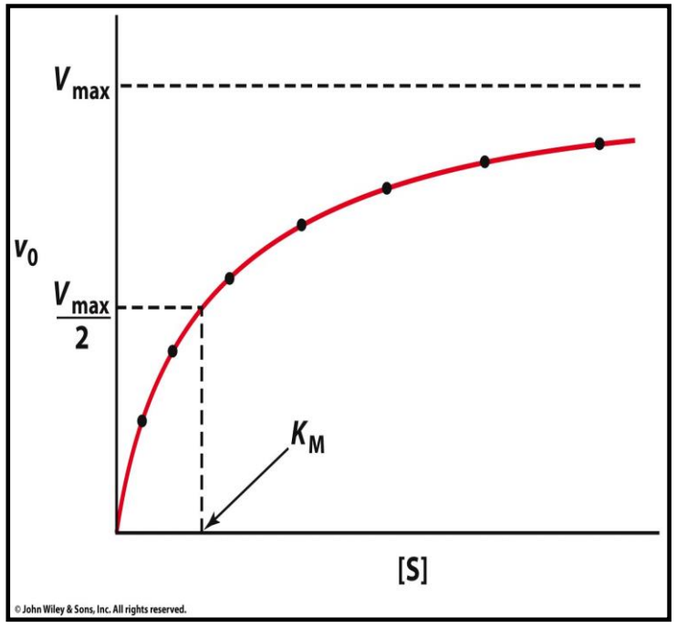
V_{max}= maximum velocity

K_m = substrate concentration at half V_{max}

Important conclusions about Michaelis-Menten kinetics:

Km- the Michaelis constant: is characteristic of an enzyme and its particular substrate, and reflects the affinity (attraction) of the enzyme for that substrate.

Km: is numerically equal to the substrate concentration at which the reaction velocity is equal to ½ Vmax. Km does not vary with the concentration of enzyme.

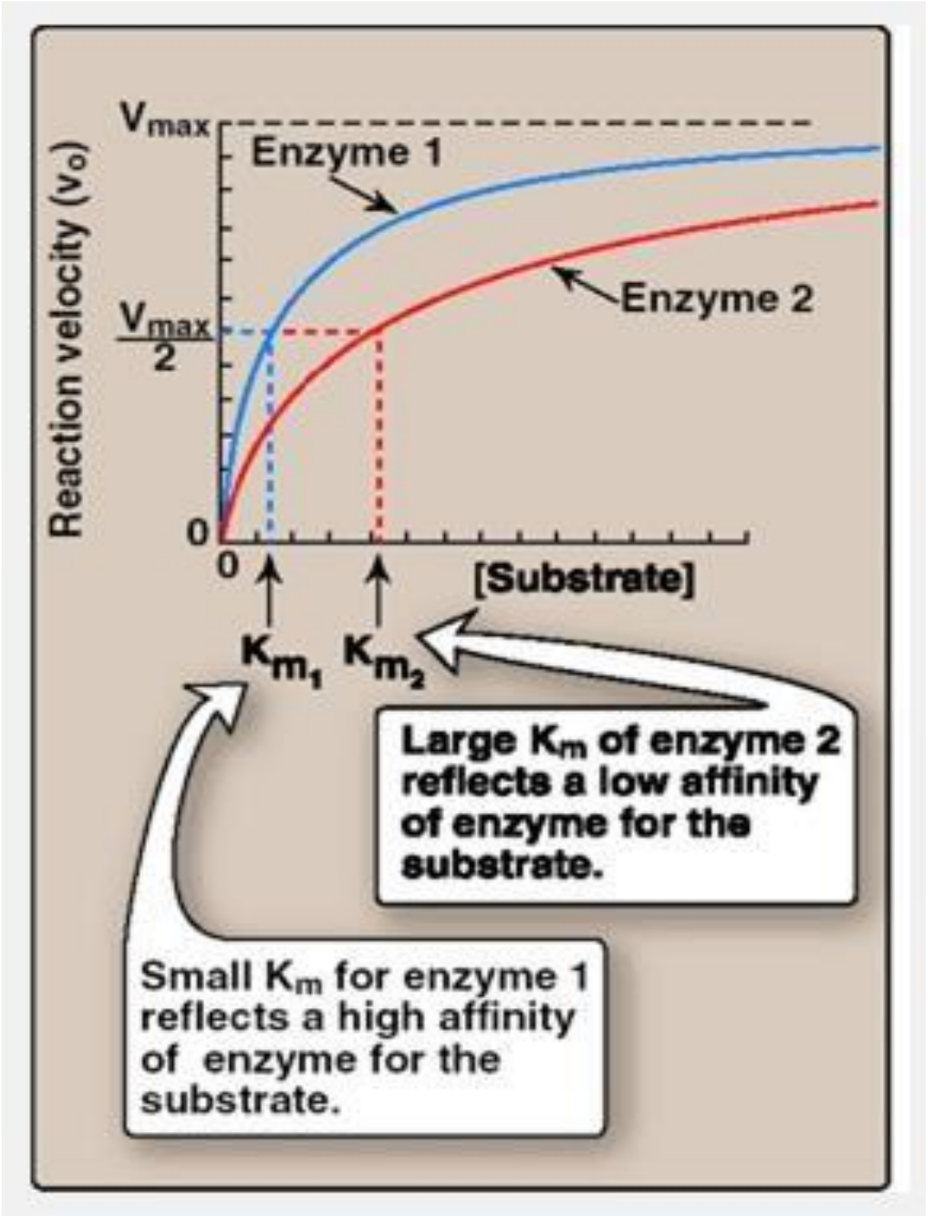


a. <u>Small Km:</u>

A numerically small (low) Km reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme (Figure).

b. <u>Large Km:</u>

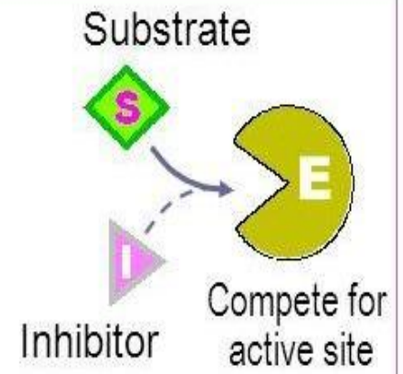
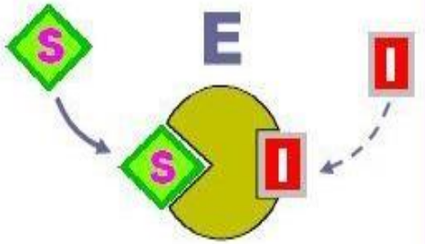
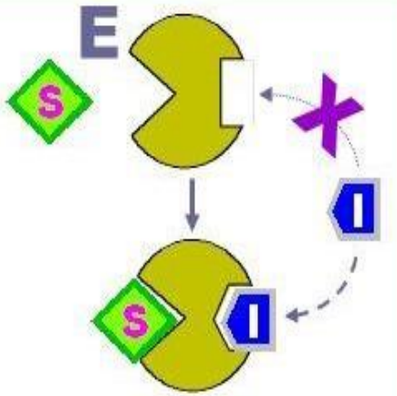
A numerically large (high) Km reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme.



Inhibition of Enzyme activity:

- **Inhibitors are certain molecules that can decrease the catalytic rate of an enzyme-catalyzed reaction (diminish the velocity).**
- **The process is called the enzyme inhibition.**
- **Inhibitors can be normal body metabolites and foreign substances (drugs and toxins).**
- **Enzyme inhibition may be reversible or irreversible.**

Enzyme Inhibition (Mechanism)

	Competitive	Non-competitive	Uncompetitive
Cartoon Guide			
Equation and Description	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\Downarrow\Uparrow$ EI	$E + S \rightleftarrows ES \rightarrow E + P$ $+ \quad +$ $I \quad I$ $\Downarrow\Uparrow \quad \downarrow\uparrow$ $EI + S \rightarrow EIS$	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\Downarrow\Uparrow$ EIS
	[*I*] binds to free [E] only, and competes with [S]; increasing [S] overcomes Inhibition by [*I*].	[*I*] binds to free [E] or [ES] complex; Increasing [S] can not overcome [*I*] inhibition.	[*I*] binds to [ES] complex only, increasing [S] favors the inhibition by [*I*].

Reversible inhibition:

- **Inhibitors are bound to enzymes non-covalently, and can dissociate from the enzyme and recovery of enzyme activity happen.**

Irreversible inhibition:

- **Occurs when an inhibited enzyme does not regain activity on dilution of the enzyme-inhibitor complex.**
- **Irreversible inhibitors generally result in the destruction or modification of an essential amino acid required for enzyme activity, this is due to some type of covalent link between enzyme and inhibitor.**
- **Binding can cause a partial loss or complete loss of the enzymatic activity.**

- **Example: penicillin and related antibiotics bind covalently to a peptidase involved in cell wall synthesis in bacteria.**

<u>The most commonly encountered types of reversible inhibition are</u>

A. Competitive inhibitors and

B. Un-competitive inhibitors

C.Mixed inhibitors:

A.Competitive inhibition:

- **The competitive inhibitor competes with substrate for the active site of an enzyme, while the inhibitor (I) occupies the active site, it prevents binding of (S) to the enzymes.**
- **Competitive inhibitors resemble (look like) the substrate and combine with the enzyme to form an EI complex, but without leading to catalysis, this combination will reduce the efficiency of the enzyme, figure.**
- **Because the inhibitor binds reversibly to the enzyme, the competition can be favoring the substrate by adding more substrate. When [S] far exceeds [I], the probability that an inhibitor molecule will bind to the enzyme is minimized and the reaction exhibits a normal Vmax.**

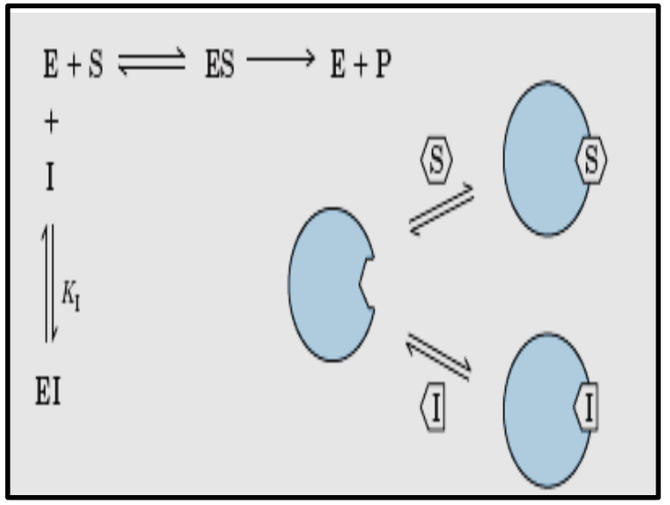


Figure :Competitive inhibition.

Statin drugs as examples of competitive inhibitors:

This group of anti-hyperlipidemic agents competitively inhibits the first committed step in cholesterol synthesis. This reaction is catalyzed by enzyme hydroxymethylglutaryl CoA (HMG CoA) reductase.

Statin drugs compete effectively to inhibit HMG CoA reductase. By doing so, they inhibit cholesterol synthesis, thereby lowering plasma cholesterol levels.

B. Uncompetitive inhibition:

Uncompetitive inhibition occurs when the inhibitor and substrate bind at different sites on the enzyme (inhibitor does not resemble structurally to substrate. The uncompetitive inhibitor can bind to the ES complex, thereby preventing the reaction from occurring.

C. Mixed inhibitors (classically was known as non- competitive):

Bind at a separate site, but may bind to either free Enzyme or ES complex.

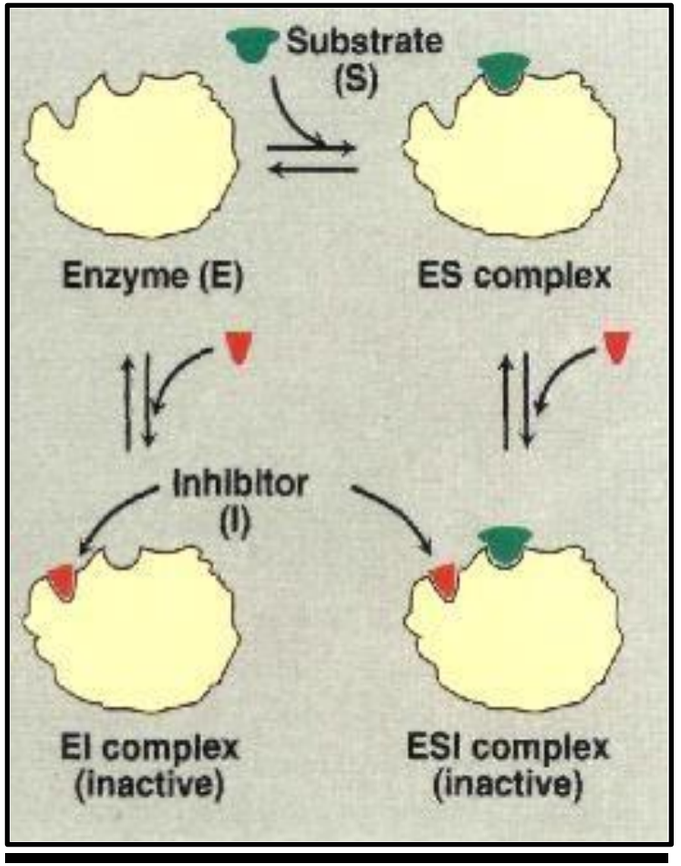


Figure : Non-competitive inhibition.

Irreversible Inhibition:

- **The irreversible inhibitors are those that bind covalently with or destroy a functional group on an enzyme that is essential for the enzyme's activity, or those that form a particularly stable noncovalent association.**

- **A special class of irreversible inhibitors is the suicide inactivators.**

- **These compounds are relatively unreactive until they bind to the active site of a specific enzyme.**

- **A suicide inactivator undergoes the first few chemical steps of the normal enzymatic reaction, but instead of being transformed into the normal product, the inactivator is converted to a very reactive compound that combines irreversibly with the enzyme.**

Examples of Suicide inhibitors :

Aspirin commonly used drug for pain relieve. Anti-inflammatory action of aspirin is also based on the suicide inhibition . Aspirin acetylate a serine residue in the active center of cyclooxygenase thus inhibiting the prostaglandins (PG) synthesis and inflammation.

Enzyme inhibitors as drugs:

- **Most drugs act as enzyme inhibitors. For example, the β-lactam antibiotics, such as penicillin and amoxicillin, act by inhibiting enzymes involved in bacterial cell wall synthesis.**

Plasma enzymes levels as diagnostic tools in disease states

- **Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma. The activities of many of these enzymes are routinely determined for diagnostic purposes in diseases of the heart, liver, skeletal muscle, and other tissues.**

- **The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Therefore, determining the degree of elevation of a particular enzyme activity in the plasma is often useful in evaluating the prognosis for the patient.**

- **For example: Liver Enzymes: alanine aminotransferase (ALT) & Aspartate a aminotransferase (AST). Elevated levels of ALT in plasma signals possible damage to hepatic tissue.**

Summary:

- **Enzymes are protein catalysts that increase the velocity of a chemical reaction by lowering the energy of the transition state. Enzymes are not consumed during the reaction they catalyze.**
- **Enzyme molecules contain a special pocket or cleft called the active site. The active site contains amino acid side chains that participate in substrate binding and catalysis.**
- **The active site binds the substrate, forming an enzyme–substrate (ES) complex. Binding is thought to cause a conformational change in the enzyme (induced fit) that allows catalysis. ES is converted to enzyme-product (EP), which subsequently dissociates to enzyme and product.**
- **An enzyme allows a reaction to proceed rapidly under conditions prevailing in the cell by providing an alternate reaction pathway with a lower free energy of activation.**

- **Most enzymes show Michaelis-Menten kinetics, and a plot of the initial reaction velocity (v_o) against substrate concentration[S].**
- **Any substance that can diminish the velocity of such enzyme-catalyzed reactions is called an inhibitor.**
- **The most common types of reversible inhibition are competitive and uncompetitive.**
- **Enzymes have important diagnostic and therapeutic value in medicine.**

Evaluation and Assessments:

After reading you lecture try to answer the following questions:

1) **How the enzyme work?**
2) **What are the factors that affects velocity reaction?**
3) **Explain Enzyme inhibition and types of inhibition?**
4) **What are suicidal inhibiters?**
5) **What in Enzyme Km ?**
6) **Clinical significance of enzymes as diagnostic tools in disease?**