Lecture 6

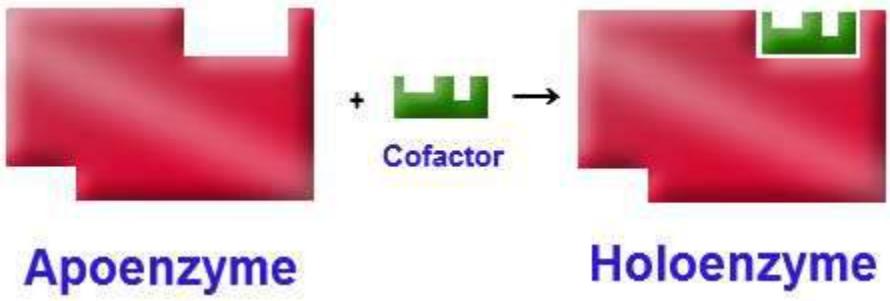
Enzymes

Enzyme Structure:

Enzymes are proteins, like the proteins the enzymes contain chains of amino acids linked together. The characteristic of an enzyme is determined by the sequence of amino acid arrangement. When the bonds between the amino acid are weak, they may be broken by conditions of high temperatures or high levels of acids. When these bonds are broken, the enzymes become nonfunctional. The enzymes that take part in the chemical reaction do not undergo permanent changes and hence they remain unchanged to the end of the reaction.

Enzymes are highly selective, they catalyze specific reactions only. Enzymes have a part of a molecule where it just has the shape where only certain kind of substrate can bind to it, this site of activity is known as the *'active site'*. The molecules that react and bind to the enzyme is known as the *'substrate'.*

Most of the enzymes consists of the protein and the non-protein part called the '*cofactor*'. The proteins in the enzymes are usually globular proteins. The protein part of the enzymes are known '*apoenzyme'*, while the non-protein part is known as the cofactor. Together the apoenzyme and cofactors are known as the '*holoenzyme'*.



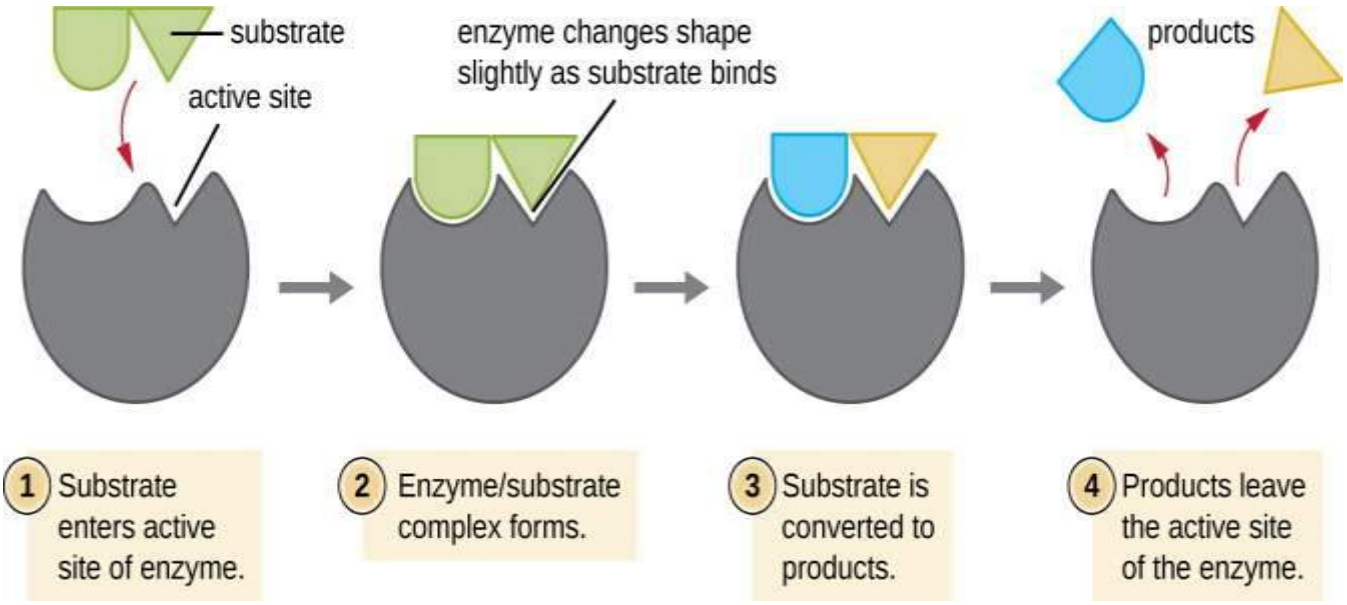
Enzymes are usually much larger than their substrates. Only a small portion of their structure (around 2–4 amino acids) is directly involved in catalysis: the catalytic site. This catalytic site is located next to one or more binding sites where residues orient the substrates. The catalytic site and binding site together comprise the enzyme's active site. The remaining majority of the enzyme structure serves to maintain the precise orientation and dynamics of the active site.

In some enzymes, no amino acids are directly involved in catalysis; instead, the enzyme contains sites to bind and orient catalytic cofactors. Enzyme structures may also contain allosteric sites where the binding of a small molecule causes a conformational change that increases or decreases activity.

A small number of RNA-based biological catalysts called ribozymes exist, which again can act alone or in complex with proteins. The most common of these is the ribosome which is a complex of protein and catalytic RNA components.

Enzymes mechanism

An enzyme attracts substrates to its active site, catalyzes the chemical reaction by which products are formed, and then allows the products to dissociate (separate from the enzyme surface). The combination formed by an enzyme and its substrates is called the enzyme–substrate complex.



When two substrates and one enzyme are involved, the complex is called a ternary complex; one substrate and one enzyme are called a binary complex. The substrates are attracted to the active site by electrostatic and hydrophobic forces, which are called non-covalent bonds because they are physical attractions and not chemical bonds.

Nomenclature:

Except for some of the originally studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in "ase". The International Union of Biochemistry (I.U.B.) initiated standards of enzyme nomenclature which recommend that enzyme names are often derived from its substrate or the chemical reaction it catalyzes.

Examples are lactase, alcohol dehydrogenase and DNA polymerase. Different enzymes that catalyze the same chemical reaction are called isozymes.

Classification:

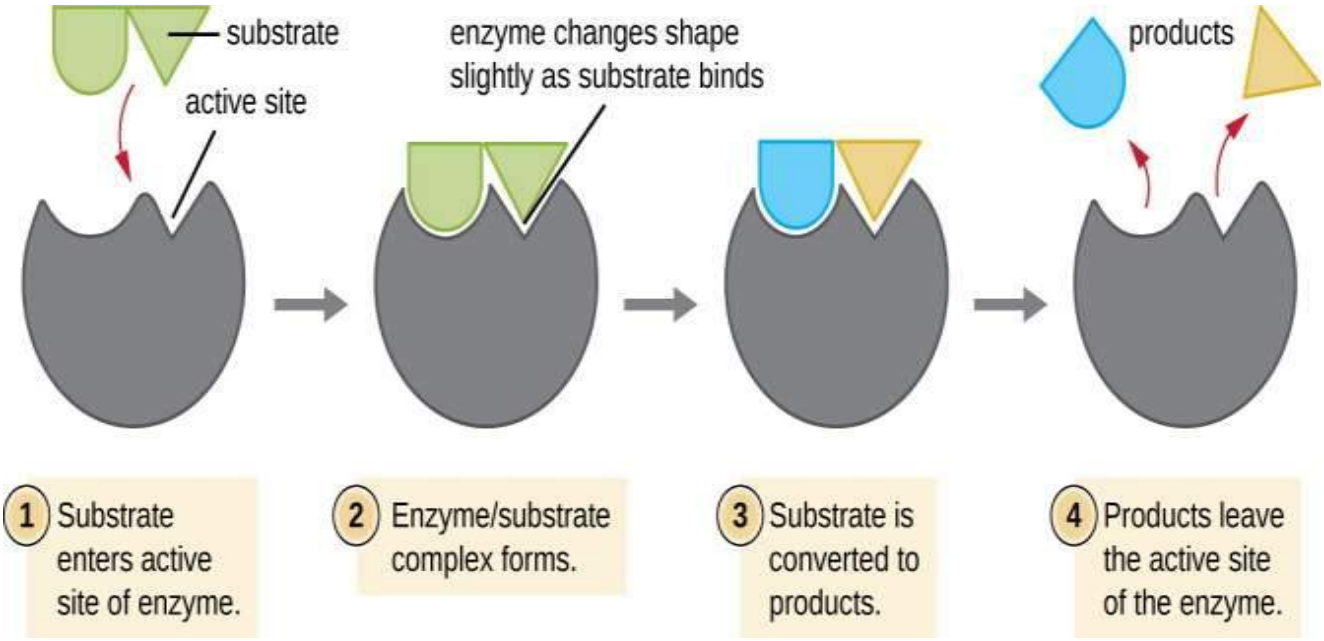
Enzymes can be classified by the kind of chemical reaction catalyzed :

1. **Addition or removal of water**
    1. **Hydrolases - these include esterase, carbohydrase, nuclease, deaminase, amidase, and protease.**
2. **Hydrases such as fumarase, enolase, aconitase and**

3. **Transfer of electrons**
    1. **Oxidase**
    2. **Dehydrogenase**
4. **Transfer of a radical**
    1. **Transglycosidase - of monosaccharide**
    2. **Transphosphorylase and phosphomutase - of a phosphate group**
    3. **Transaminase - of amino group**
    4. **Transmethylase - of a methyl group**
    5. **Transacetylase - of an acetyl group**
5. **Splitting or forming a C-C bond**
    1. **Desmolase**
6. **Changing geometry or structure of a molecule**
    1. **Isomerase**
7. **Joining two molecules through hydrolysis of pyrophosphate bond in ATP or other tri-phosphate**
    1. **Ligase**

<u>Mechanism of Catalysis :</u>

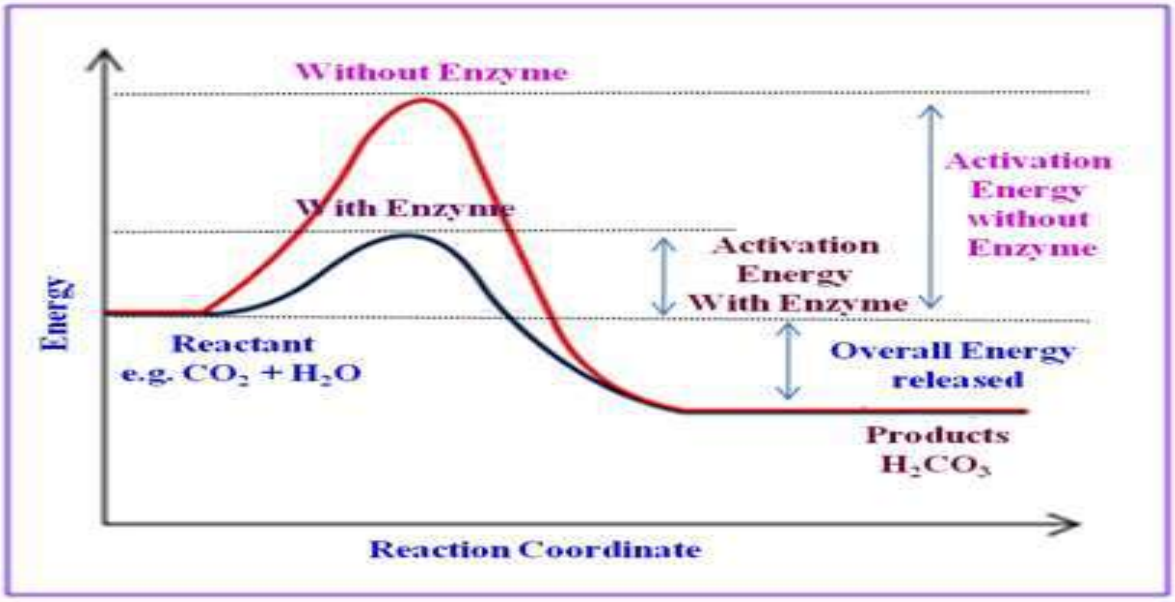
The substance used to change the speed of the reaction is called a catalyst. <u>Enzymes</u> are a type of catalysts which are responsible for increasing the rate of reaction. The <u>catalysis</u> in which enzymes act as a catalyst is called enzyme catalysis.



Enzymes are able to lower the activation energy for various biochemical reactions. They do this by binding the reactant(s), known as the substrate(s), to an active site within the enzyme. At the active site, the substrate(s) can form an activated complex at lower energy. Once the reaction completes, the product(s) leaves the active site, so the enzyme is free to catalyze more reactions.

Enzymes can accelerate reactions in several ways, all of which lower the activation energy ($\Delta G^{\ddagger}$, Gibbs free energy)

1. **By stabilizing the transition state:**
2. **By providing an alternative reaction pathway:**
3. **By destabilizing the substrate ground state:**



Thermodynamics of enzymatic reaction :

Thermodynamic is the study of energy and its transformations. It predicts the net direction of reactions, but not how fast it will occur.

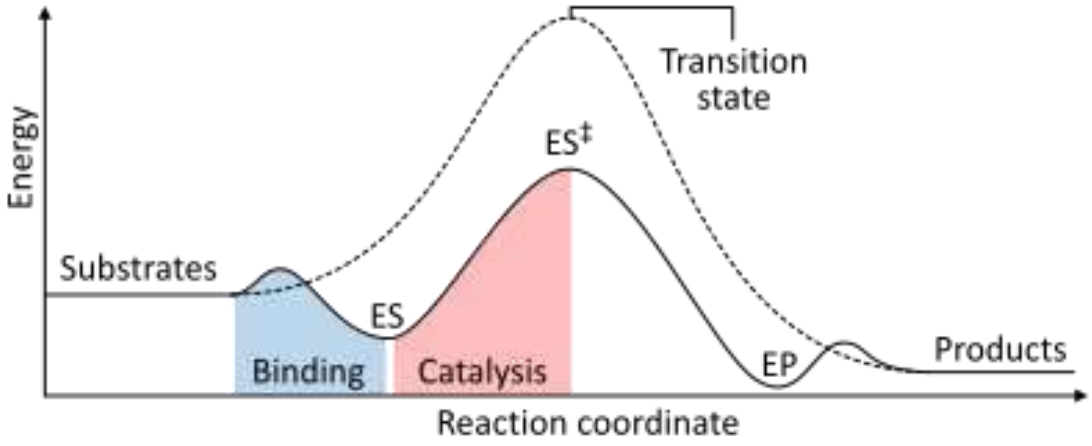
The thermodynamic quantity is the energy difference resulting from the free energy (ΔG) given off during a chemical reaction.

Thermodynamics refers to either:

(1) The energy released during a reaction, in which case ΔG will be negative and the reaction exergonic or spontaneous, or

(2) The energy consumed during a reaction, in which case ΔG will be positive and the reaction endergonic or nonspontaneous.

A thermodynamic reaction favors the products, resulting in a spontaneous reaction that occurs without the need to supply energy. This indicates that the reactions' most stable state is that of the products.



In uncatalyzed biochemical reaction (dashed line), substrates need a lot of activation energy to reach a transition state, which then decays into lower-energy products. When enzyme catalyzed the biochemical reaction (solid line), the enzyme binds the substrates (ES), then stabilizes the transition state ($ES^{‡}$) to reduce the activation energy required to produce products (EP) which are finally released.

<u>Specificity of Enzymes:</u>

One of the properties of enzymes that makes them so important is the specificity they exhibit relative to the reactions they catalyze. Specificity is the ability of an enzyme to choose exact substrate from a group of similar chemical molecules.

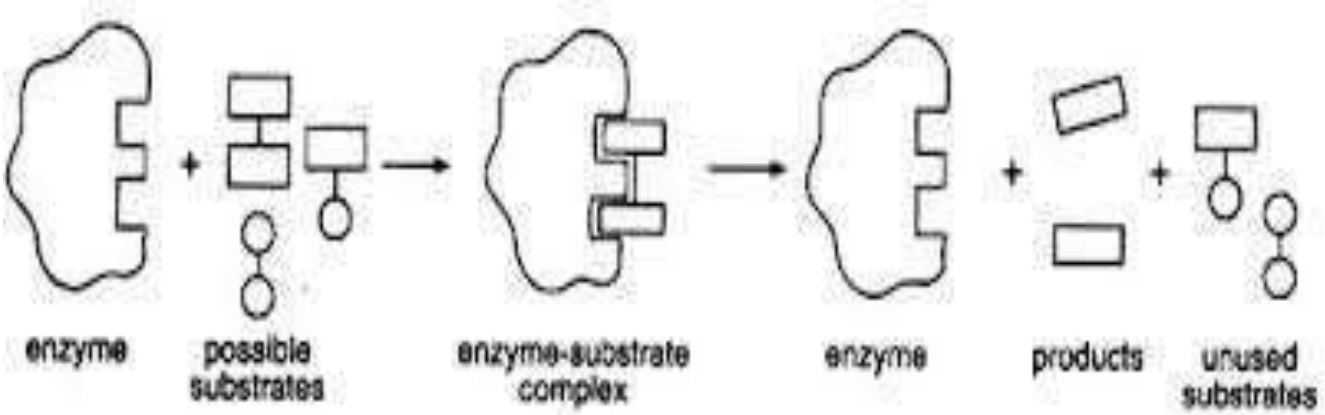


Fig. 8.19 : Enzyme specificity

In general, the specificity shown by enzyme are grouped into 6 types:

1. Absolute specificity (Substrate specificity):

The enzyme will catalyze only one reaction. It means that one and only one substrate will fit with a particular enzyme.

For example the enzyme glutamate dehydrogase will catalyze only the removal of the nitrogen group from glutamate- NOT any other amino acid. It has absolute specificity.

Urease is absolutely specific for its substrate urea (NH_2CONH_2).

2. Group specificity:

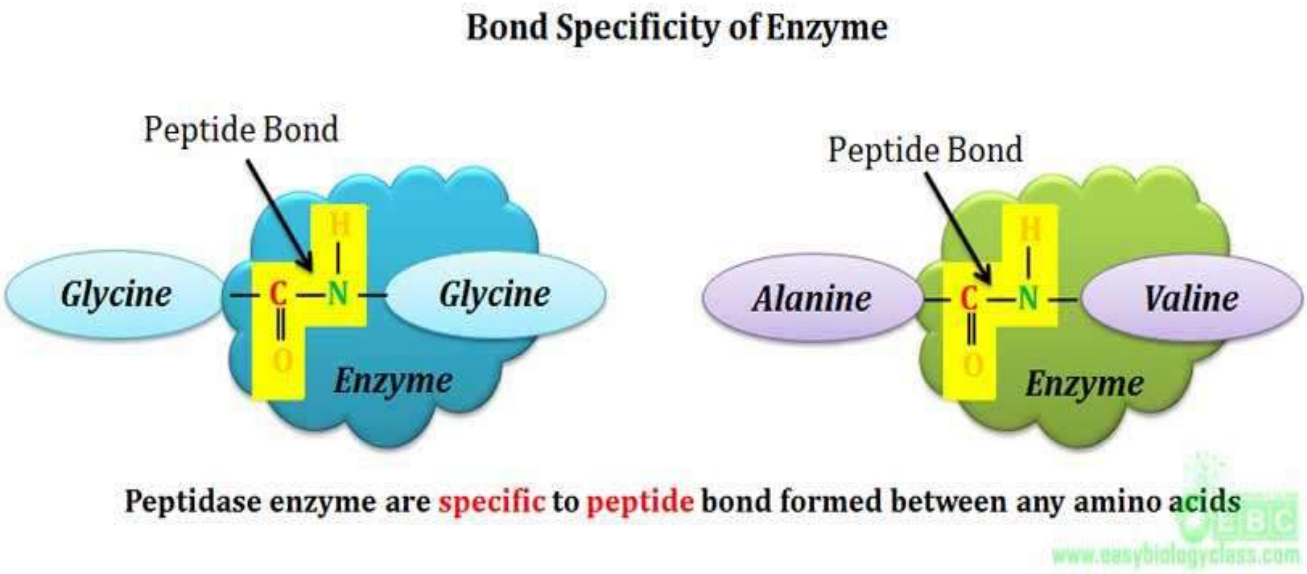
The enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.

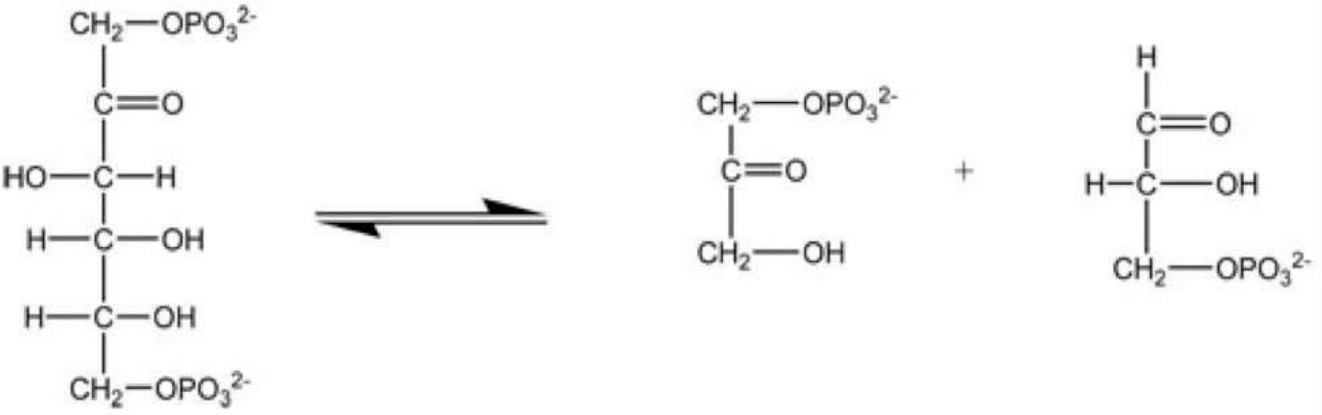
For example the enzyme sulfur oxygenase reductase catalyzes the reduction of inorganic sulfur compounds and elemental sulfur to sulfate. This enzyme has a group specificity of only sulfur compounds.

3. Bond specificity (relative specificity).

The enzyme recognizes particular chemical bond types. Figure below is a reaction that illustrates an enzyme cleaving a specific bond (peptide bond or carbon-carbon bond) of the reactant in order to create two products.

Peptidase enzyme is specific to peptide bond





4. Stereochemical specificity:

The enzyme will act on a particular steric or optical isomer or one enantiomer only.

For example the enzyme D-Lactate dehydogenase complexed with D-lactic acid.

The enzyme D-amino acid oxidase is specific for D-amino acids only, and will not catalyze the oxidation of the L-amino acid stereoisomers.

5. Geometrical specificity:

Some enzymes will work with a small range of similar substrates, for example alcohol dehydrogenase will oxidize methanol and n-propanol to aldehydes.

6. Co-factor specificity :

Co-factor specificity is between the enzyme and the co-factor and it is not between the enzyme and substrate .Certain enzymes require cofactor for their reactivity. Cofactor may be organic such as nicotinamide adenine dinucleotide (NAD+) , NADP+ , alcohol dehydrogenase, malate dehydrogenase, and lactate dehydrogenase which are called coenzyme , and if it is inorganic like Fe++ , Mg++ , and Zn++ , it is termed as cofactor.

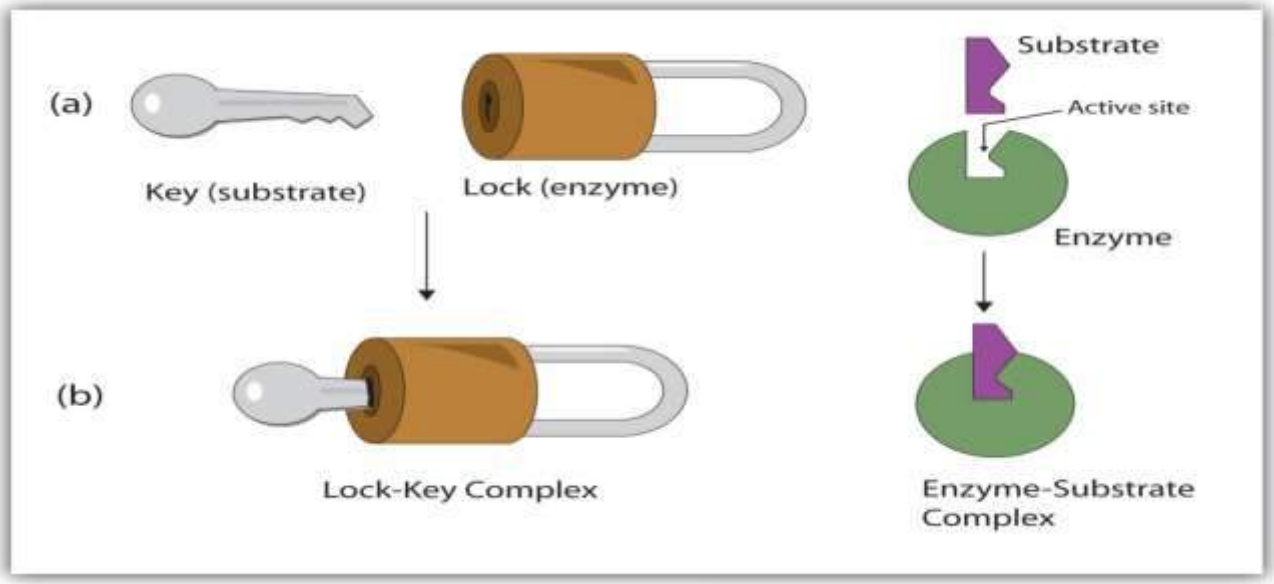
Model of enzyme mechanism:

There are two models for enzyme mechanism:

1. Lock and Key Theory:

The specific action of an enzyme with a single substrate can be explained using a Lock and Key analogy first postulated in 1894 by Emil Fischer. In this analogy, the lock is the enzyme and the key is the substrate. Only the correctly sized key (substrate) fits into the key hole (active site) of the lock (enzyme).

Smaller keys, larger keys, or incorrectly positioned teeth on keys (incorrectly shaped or sized substrate molecules) do not fit into the lock (enzyme). Only the correctly shaped key opens a particular lock. This is illustrated in graphic below:



This model explains enzyme specificity, but fails to explain the stabilization of the transition state that enzymes achieve.

2. Induced fit model :

In 1958, Daniel Koshland suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme.

This model proposes that the binding of the reactant, or substrate, to the enzyme active site results in a conformational change to the enzyme. This change stabilizes the transition state complex, and thus lowers the activation energy.

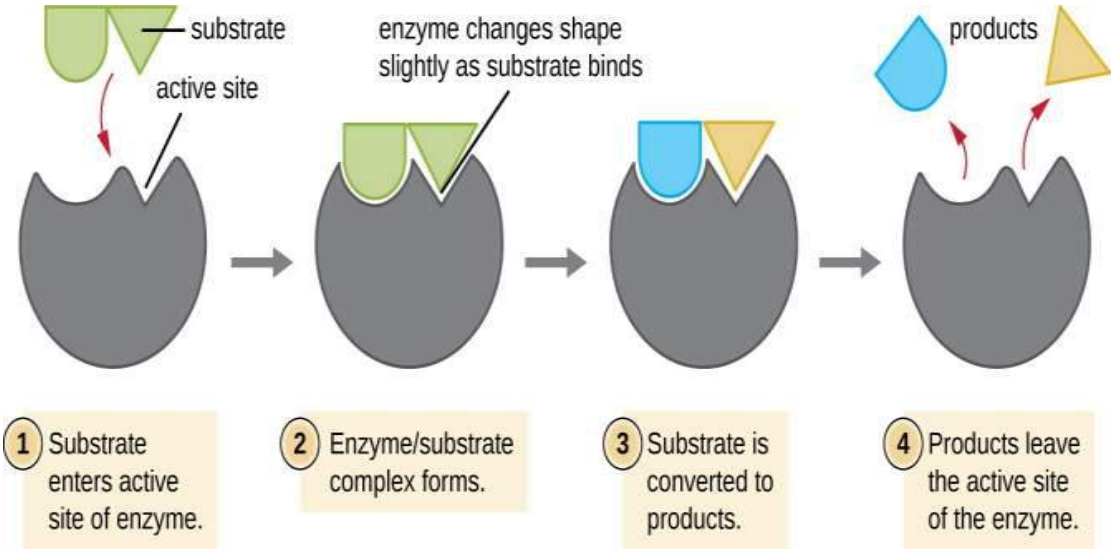
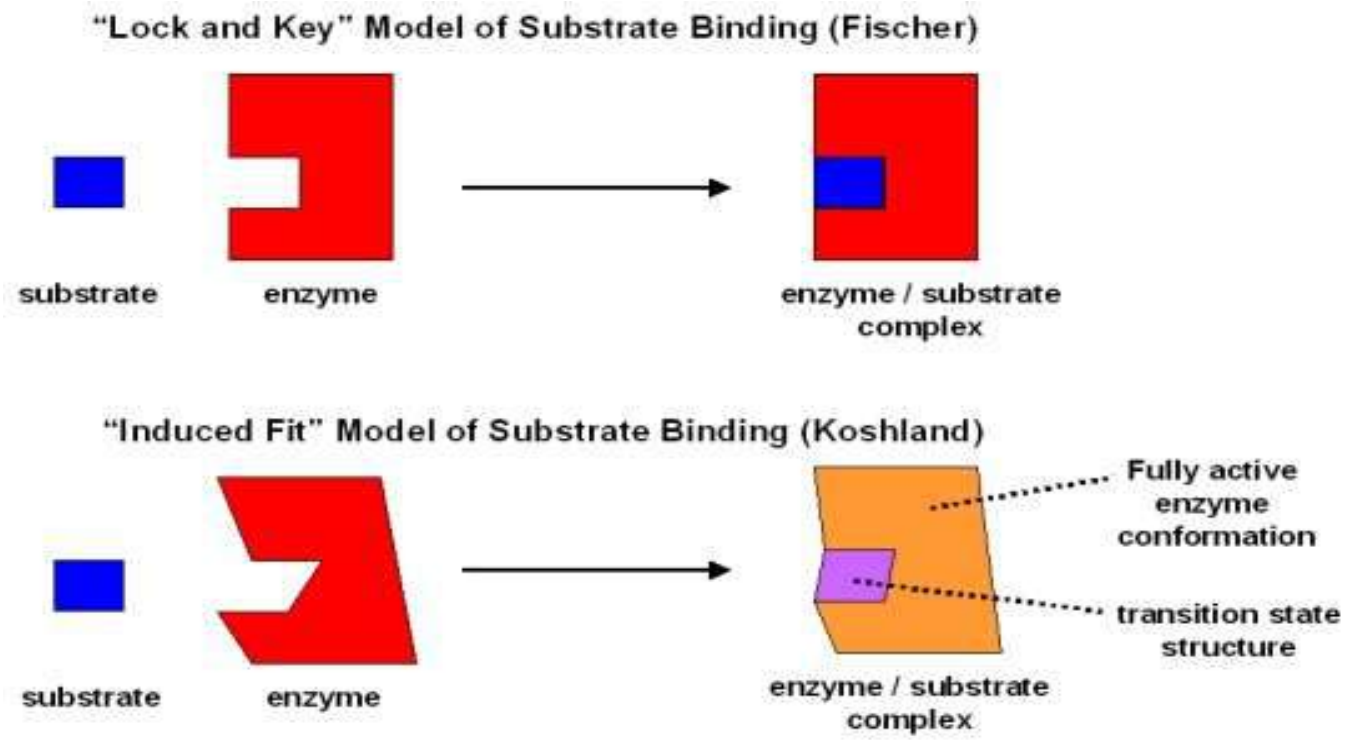


Figure below shows Lock and Key model and Induced Fit model of substrate binding to enzyme



<u>Transition state stabilization</u>:

All chemical transformations pass through an unstable structure called the transition state. By binding substrates to their active sites, enzymes stabilize the structure of the transition state.

Enzymes interact with a substrate by means of strain or distortions, moving the substrate towards the transition state. By binding substrates to their active sites, enzymes stabilize the structure of the transition state. In enzyme-catalyzed reactions, the overall activation energy of the reaction is lowered when an enzyme stabilizes a high energy transition state intermediate.

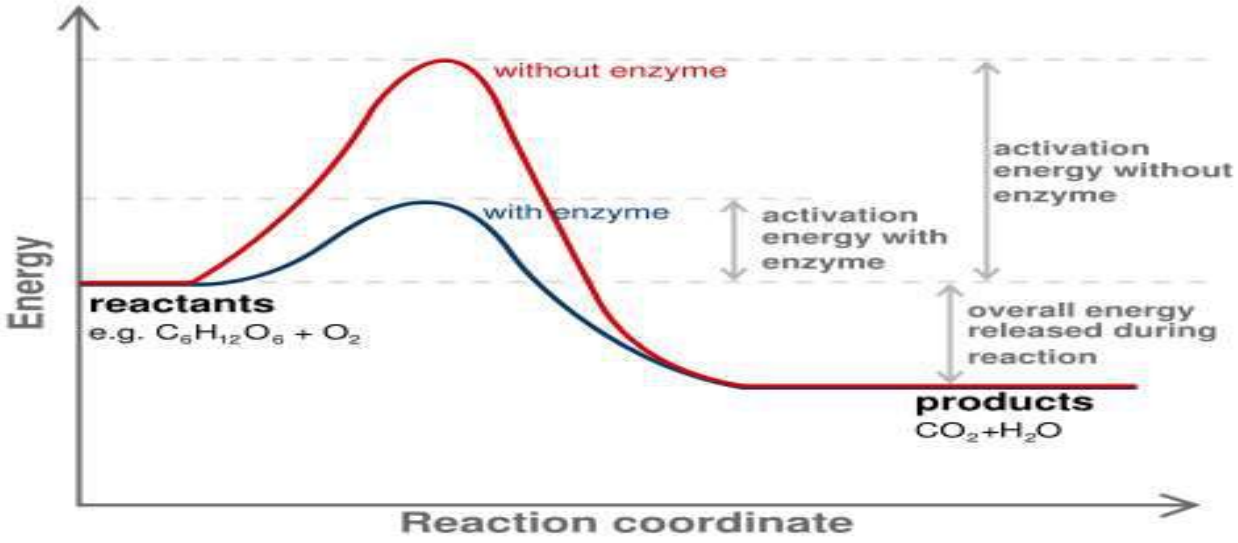
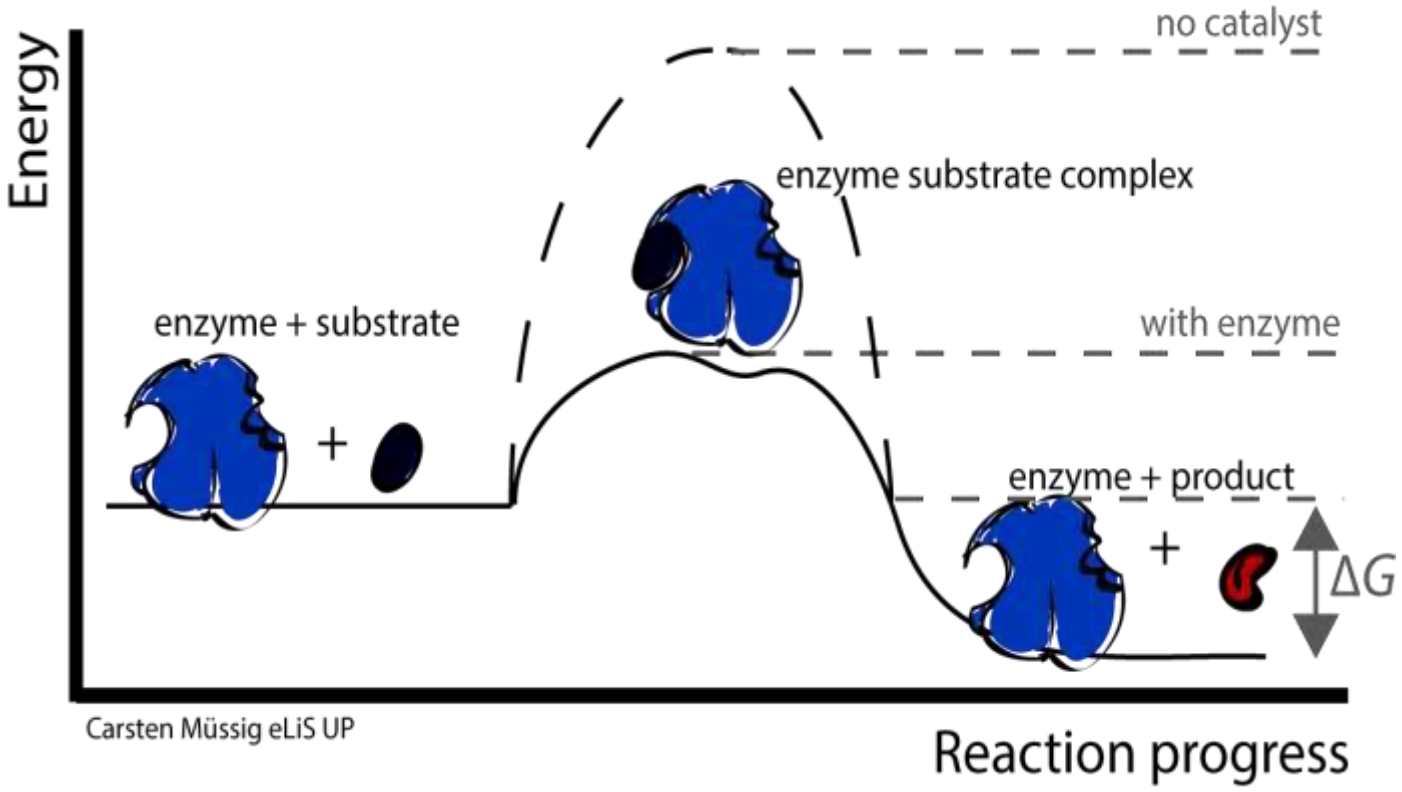
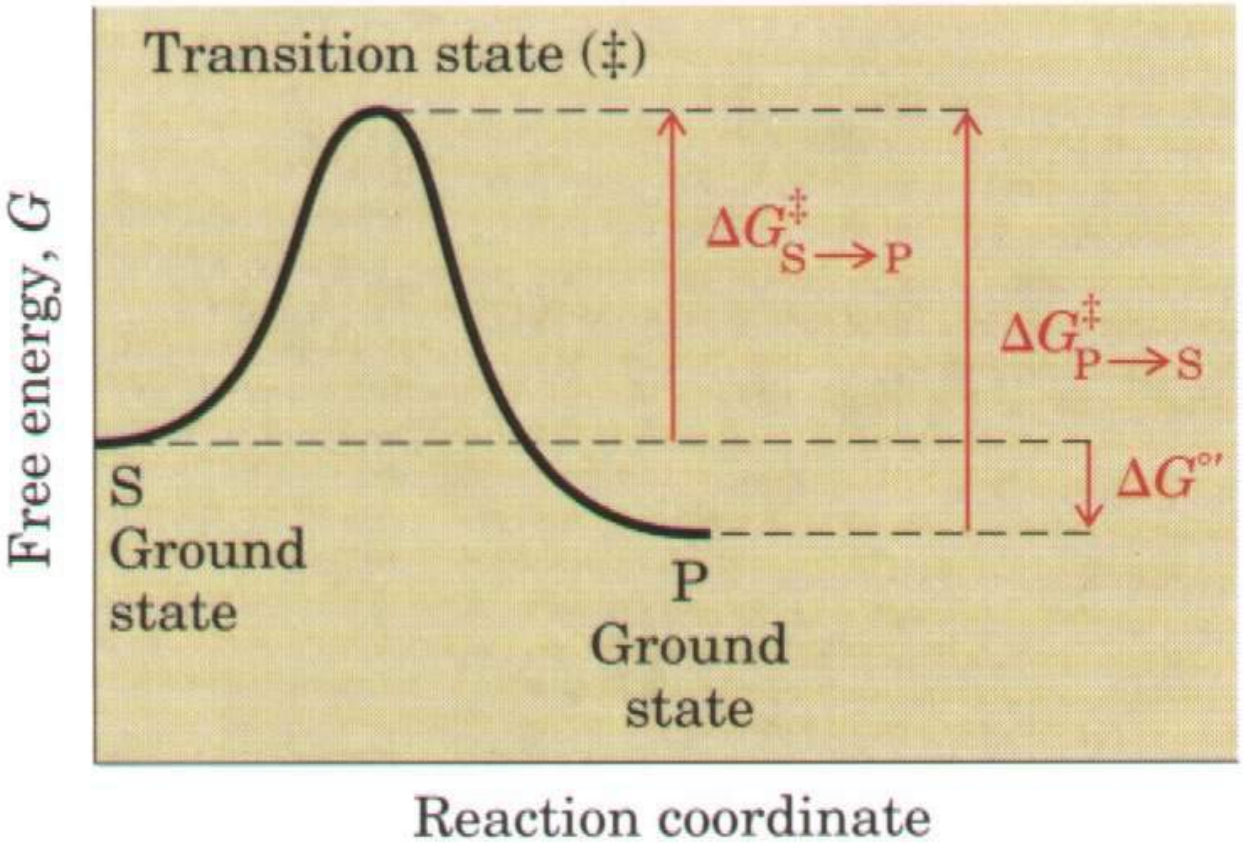


Figure :Enzymes Decrease the Activation Energy



ΔG is the difference between $\Delta \underline{G}^{\ddagger}$ of reactant and product as shown below :

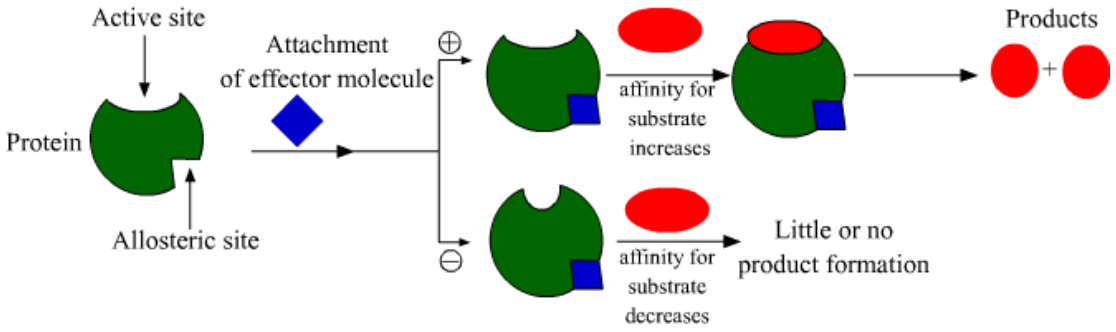

Transition state (‡)
$\Delta G^{\ddagger}_{S \rightarrow P}$
$\Delta G^{\ddagger}_{P \rightarrow S}$
$\Delta G^{\circ\prime}$
S
Ground
state
P
Ground
state
Free energy, G
Reaction coordinate

الكيمياء الحيوية / السنة الثالثة /اقسم الصيدلة

Lecture 7

Allosteric modulation

Allosteric modulation is binding of a ligand at a regulatory site other than the orthosteric (natural) (non-allosteric) site (endogenous ligand binding site). It acts by causing a conformational change in a protein, which results in a change in the binding affinity of the orthosteric (endogenous) ligand/substrate. An allosteric modulator can increase, decrease, or block enzyme catalytic activity.



<u>Biological function of Enzymes:</u>

1. They are major components in signal transduction and cell regulation, often via kinases and phosphatases ..

2. They take part in movement with the help of the protein myosin hydrolyzing ATP to generate muscle contraction .

3. ATPases in the cell membrane acts as ion pumps involved in active transport mechanism.

4. Enzymes present in the viruses are for infecting cell such as the HIV integrase.

5. Enzymes play an important role in the digestive activity of the enzymes. Enzymes such as amylases and proteases break down large molecules (starch or proteins, respectively) into smaller ones, so they can be absorbed by the intestines.

6. Various enzymes work together in order forming metabolic pathways. Example: Glycolysis.

Cofactors:

Some enzymes do not need additional components to show full activity. Others require non-protein molecules called cofactors to be bound for activity. Cofactors can be either inorganic (e.g., metal ions and iron-sulfur clusters) or organic compounds (e.g., flavin and heme). These cofactors serve many purposes; for example, metal ions can help in stabilizing nucleophilic species within the active site.

An example of an enzyme that contains a cofactor is carbonic anhydrase, with a zinc cofactor bound as part of its active site.

Organic cofactors can be either coenzymes, which are released from the enzyme's active site during the reaction, or prosthetic groups, which are tightly bound to an enzyme. Organic prosthetic groups can be covalently bound (e.g., biotin in enzymes such as pyruvate carboxylase).

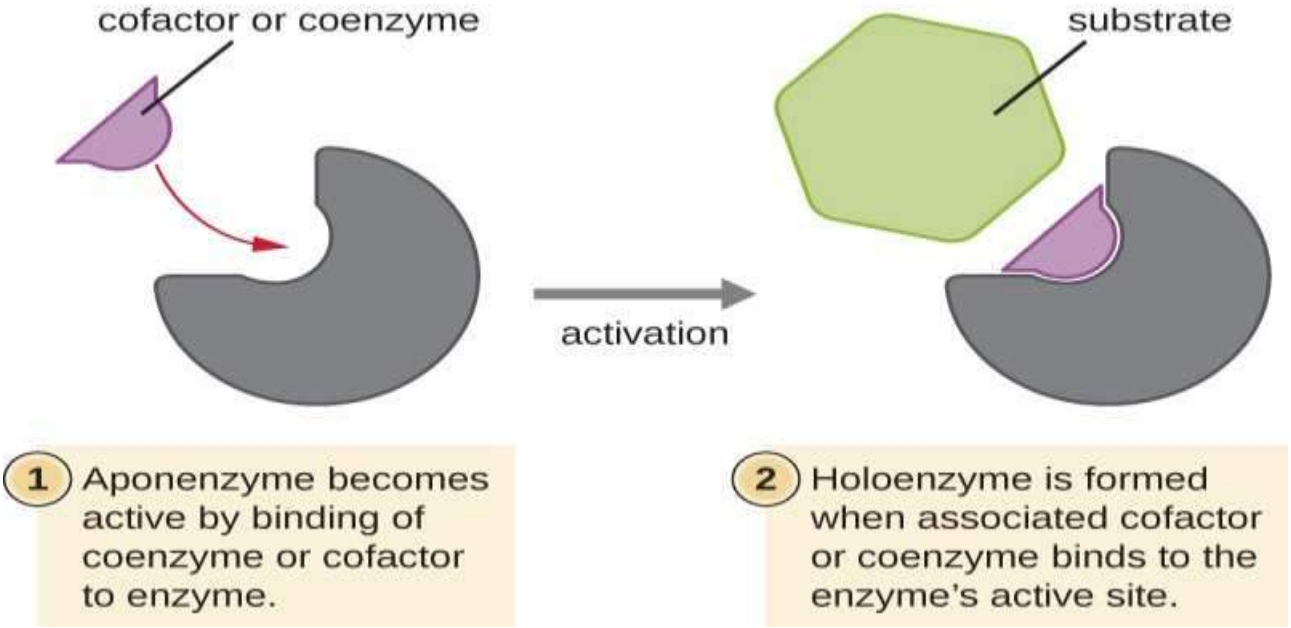
Coenzymes :

Coenzymes are small organic molecules that can be loosely or tightly bound to an enzyme. Coenzymes transport chemical groups from one enzyme to another. Examples include NADH, NADPH and adenosine triphosphate (ATP). Some coenzymes, such as flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), thiamine pyrophosphate (TPP),

and tetrahydrofolate (THF), are derived from vitamins. These coenzymes cannot be synthesized by the body.

The chemical groups carried include the hydride ion (H^-) carried by NAD or $NADP^+$, the phosphate group carried by adenosine triphosphate, the acetyl group carried by coenzyme A, formyl, methenyl or methyl groups carried by folic acid and the methyl group carried by S-adenosylmethionine.

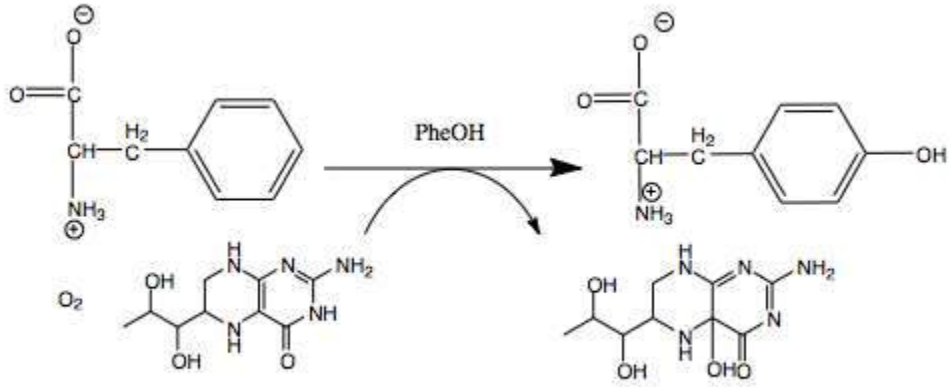
Some cofactors and coenzymes, like coenzyme A (CoA), often bind to the enzyme's active site, aiding in the chemistry of the transition of a substrate to a product .In such cases, an enzyme lacking a necessary cofactor or coenzyme is called an apoenzyme and is inactive. Conversely, an enzyme with the necessary associated cofactor or coenzyme is called a holoenzyme and is active. NADH and ATP are also both examples of commonly used coenzymes that provide high-energy ,electrons or phosphate groups, respectively, which bind to enzymes, thereby activating them.



Involvement in disease:

One example of enzyme deficiency is the most common type of phenylketonuria. Phenylalanine hydroxylase (PAH) is an enzyme that

catalyzes the hydroxylation of the aromatic side-chain of phenylalanine to generate tyrosine as shown below:



PheOH generates tyrosine from phenylalanine with concommitant hydroxylation of tetrahydrobiopterin.

Deficiency in PAH activity due to mutations in the PAH gene causes hyperphenylalaninemia (HPA), and when blood phenylalanine levels increase above 20 times the normal concentration, the metabolic disease

Another example is pseudocholinesterase deficiency, in which the body's ability to break down choline ester drugs is impaired. Oral administration of enzymes can be used to treat some functional enzyme deficiencies, such as pancreatic insufficiency, and lactose intolerance.

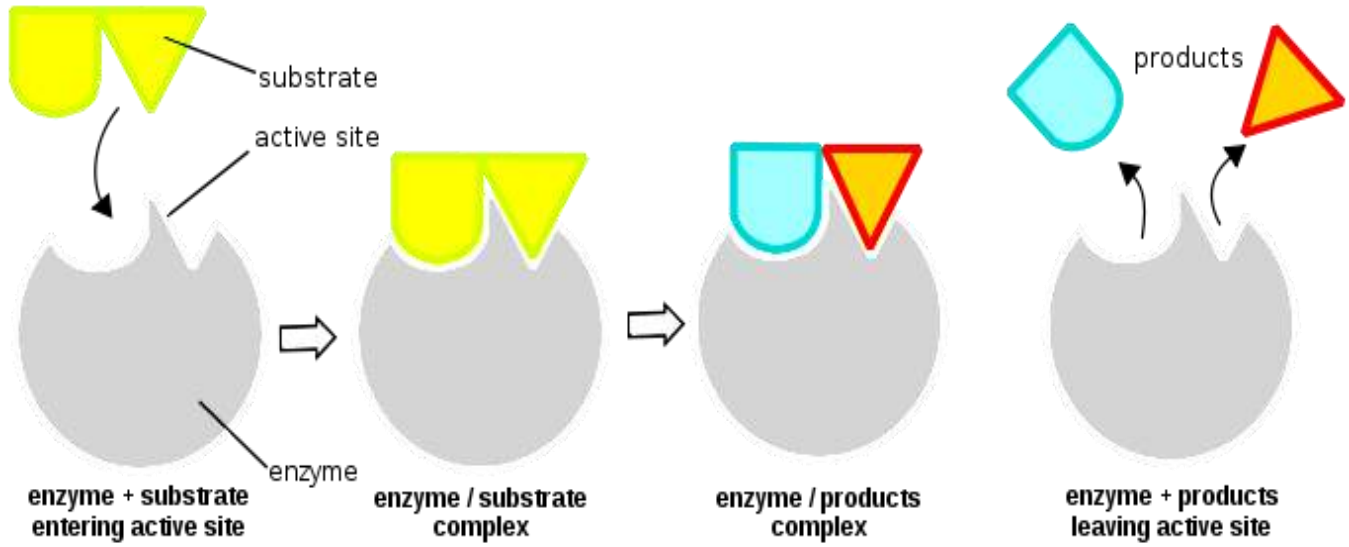
Kinetics

Enzyme kinetics is the investigation of how enzymes bind substrates and turn them into products. In 1913 Michaelis and Menten proposed a quantitative theory of enzyme kinetics, which is referred to as Michaelis–Menten Enzyme kinetics.

The major contribution of Michaelis and Menten was to think of enzyme reactions in two stages :

In the first, the substrate binds reversibly to the enzyme, forming the enzyme-substrate complex which is sometimes called the Michaelis-Menten complex .

In the second step the enzyme catalyzes the chemical step in the reaction and releases the product.



The Michaelis–Menten equation describes how the (initial) reaction rate v_0 depends on the position of the substrate-binding equilibrium and the rate constant k_2.

$$V_0 = V_{max}\left(\frac{[Substrate]}{[Substrate] + K_m}\right)$$

In this equation:

V_0 is the initial velocity of the reaction (mole/time).

V_{max} is the maximal rate of the reaction.

[Substrate] is the concentration of the substrate(molar).

K_m is the Michaelis-Menten constant which shows the concentration of the substrate when the reaction velocity is equal to one half of the maximal velocity for the reaction.

For enzyme kinetic reaction :

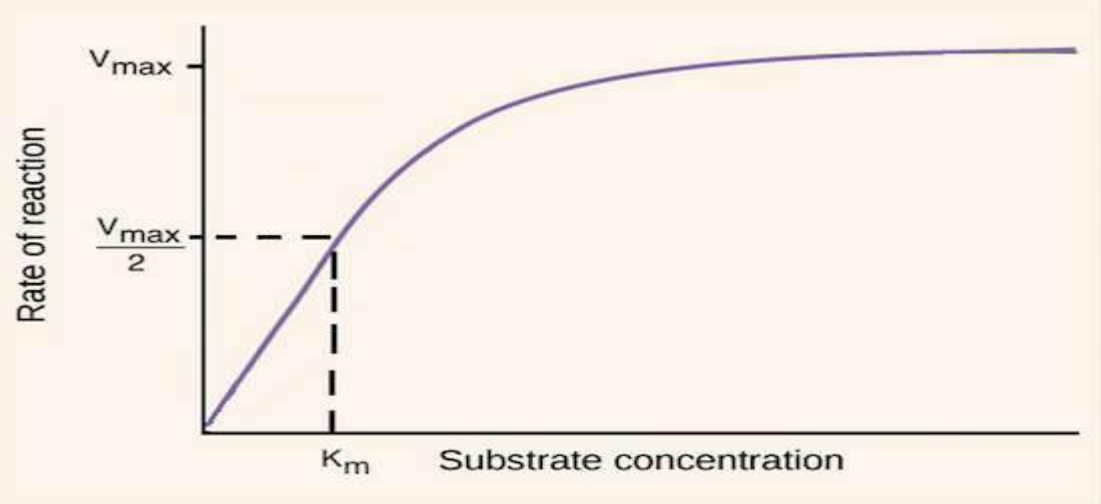
$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \xrightarrow{k_{+2}} P + E$$

K_m can be calculated by using two methods :

1)from equation below :

$$K_m = (K_{-1} + K_{+2}) / K_{+1}$$

From equation above if $K_{+1}$is increased to form ES , the concentration of complex [ES] is increased and the velocity of reaction will increase and the value of K_m decreases which indicates a large binding affinity between enzyme and substrate while a high K_m indicates that the enzyme does not bind as efficiently with the substrate and V_{max} will only be reached if the substrate concentration is high enough to saturate the enzyme.



From curve above we see that as the concentration of substrates increases at constant enzyme concentration, the active sites on the protein will be occupied as the reaction is proceeding. When all the active sites have been occupied, the reaction is complete, which means that the enzyme is at its maximum capacity and increasing the concentration of substrate will not increase the rate of <u>turnover number</u> (number of substrate molecule converted to product per enzyme molecule per second,) or K_{cat} and typically is 10^{-2} – 10^{4} s^{-1}

2) from Michaelis-Menten equation :

K_m can also be calculated from Michaelis-Menten equation:

$$V_0 = V_{max}\left(\frac{[Substrate]}{[Substrate] + K_m}\right)$$

When the velocity of reaction V_o equal 1/2 maximum velocity then by substituted this value in Michaelis-Menten equation we will get :

$$1/2\ V_{max} = V_{max}\ ([S])\ /\ K_m + [S]$$

$$2\ [S] = K_m + [S]$$

$$K_m = [S]$$

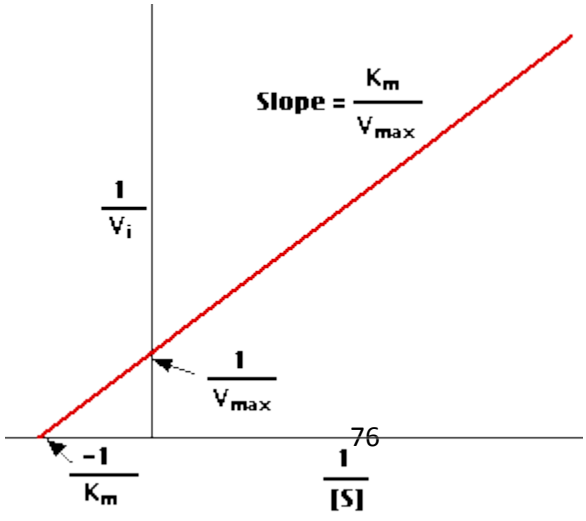
That means K_m is equal to concentration of substrate when the velocity is equal half maximum velocity

<u>Determination of V_{max} and K_m :</u>

The more precise way to determine V_{max} and K_m. is by plotting $(1/V_{max})$ against $(1/[S])$ and a straight line will produce with the equation :

$$y = mx + c$$

a *y*-intercept equivalent to $1/V_{max}$ and an *x*-intercept of the graph representing $-1/K_M$ and slop = K_M / V_{max} .



- **V_{max} is determined by the point where the line crosses the $1/V_i = 0$ axis (so the [S] is infinite).**
- **Note that the magnitude represented by the data points in this plot decrease from lower left to upper right.**
- **K_m equals V_{max} times the slope of line. This is easily determined from the intercept on the X axis.**

Q1: How will K_m affect the rate of an enzymatic reaction?

Solution :

K_m is the [S] needed to reach $1/2V_{max}$, so, a higher K_m will mean a slower rate of reaction. Then vice versa, a lower K_m indicates a faster rate of reaction.

Q2. The reaction between nicotineamide mononucleotide and ATP to form nicotineamide–adenine dinucleotide and pyrophosphate is catalyzed by the enzyme nicotinamide mononucleotide adenylyltransferase. The following table provides typical data obtained at a pH of 4.95. The substrate, S, is nicotinamide mononucleotide and the initial rate, *v*, is the μmol of nicotinamide–adenine dinucleotide formed in a 3-min reaction period.

[S] (mM)	v (μmol/sec)	[S] (mM)	v (μmol/sec)
0.138	0.148	0.560	0.324
0.220	0.171	0.766	0.390
0.291	0.234	1.460	0.493

Determine values for V_{max} and K_m.

***Answer* :**

Figure shows plot for this data and the resulting regression equation. Using the *y*-intercept, we calculate V_{max} as

$$V_{max} = 1 / y\text{−intercept} = 1 / 1.708 \text{ mol} = 0.585 \text{ mol}$$

and using the slope we find that K_m is

$$K_m = \text{slope} \times V_{max} = 0.7528\ \text{molimM} \times 0.585\ \text{mol} = 0.440\ \text{mM}$$

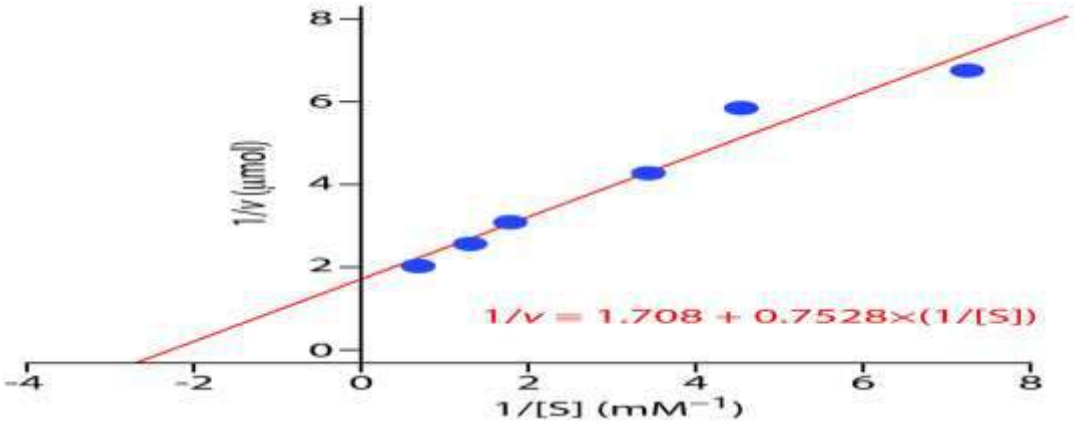


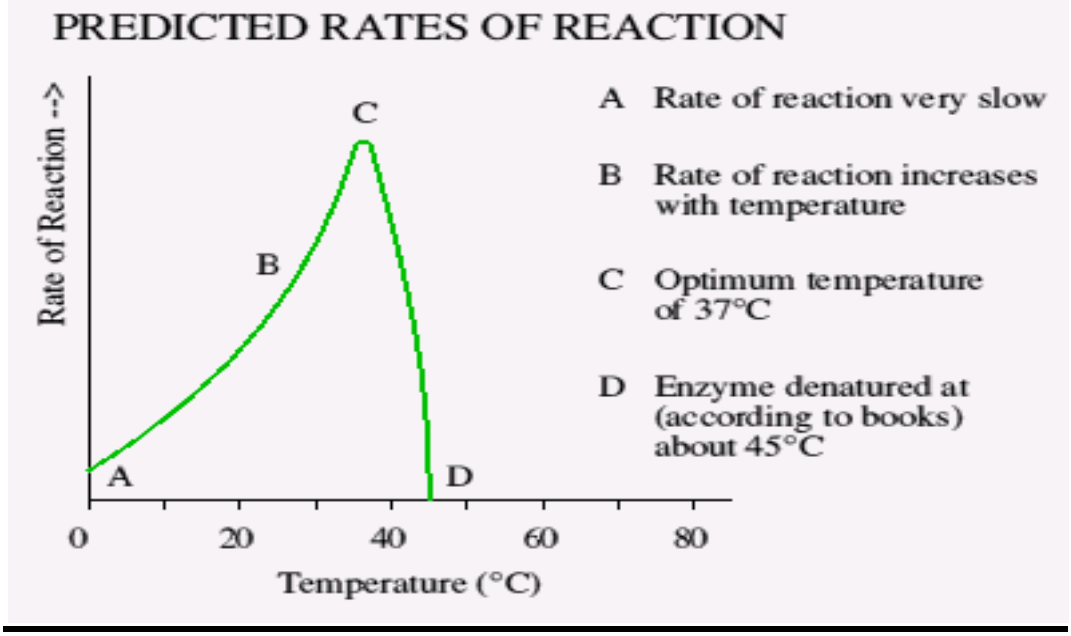
Figure: plot 1/[S] against 1/ V

Factors affecting Enzyme Activity:

Several factors affect the rate at which enzymatic reactions proceed - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.

1- Effect of temperatures on enzyme activity :

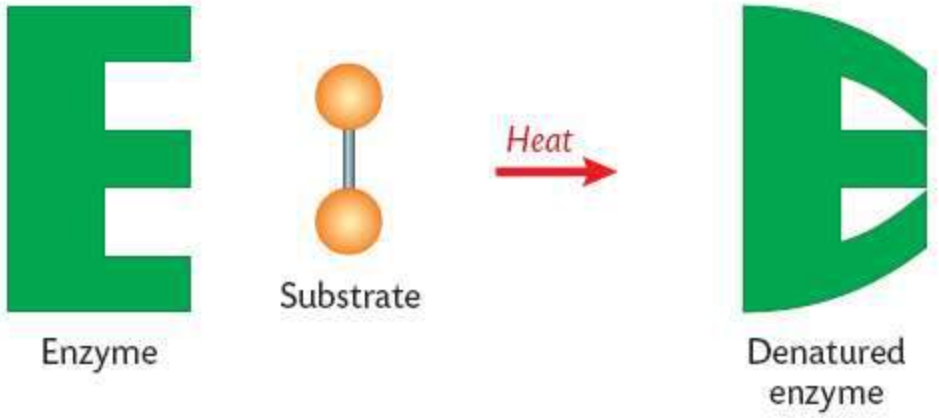
- **Low temperatures → low Kinetic Energy of enzymes and substrates. No/Very few enzyme-substrate complexes are formed so Enzymes are inactivated.**
- **Increasing the temperature will lead to the increase in kinetic energy of enzyme and substrate molecules.**
- **Enzyme and substrate molecules move with increasing speed and collide more frequently with each other.**



- **This increases the rate of enzyme-substrate complex formation This increases the rate of enzyme-substrate complex formation and product formation.**
- **As the temperature continues to increase, the rate of enzyme activity also increases until the optimal temperature is reached.**
- **Optimal temperature is the temperature at which the enzyme works best. Rate of product formation is highest. optimal temperature is usually around human body temperature (37.5 ºC) for the enzymes in human cells.**
- **Beyond Optimal Temperatures, At high temperatures (>60°C), weak bonds within the enzyme molecule are broken**
- **Enzyme loses its shape and its active site.**
- **Loss of shape leads to a loss of function. Enzyme is said to have denatured due to intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.**

- **Denaturation is the change in 3D structure of an enzyme or any other protein caused by heat or chemicals such as acids or alkali, causing it to lose its function.**

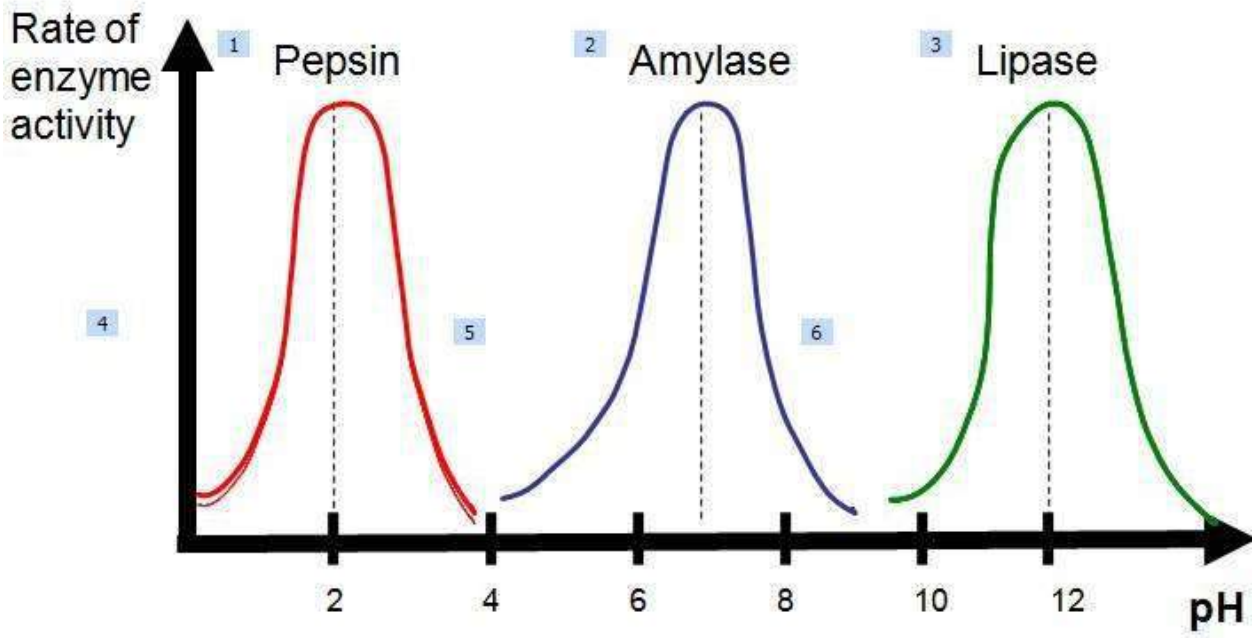
<u>Denaturation :</u>



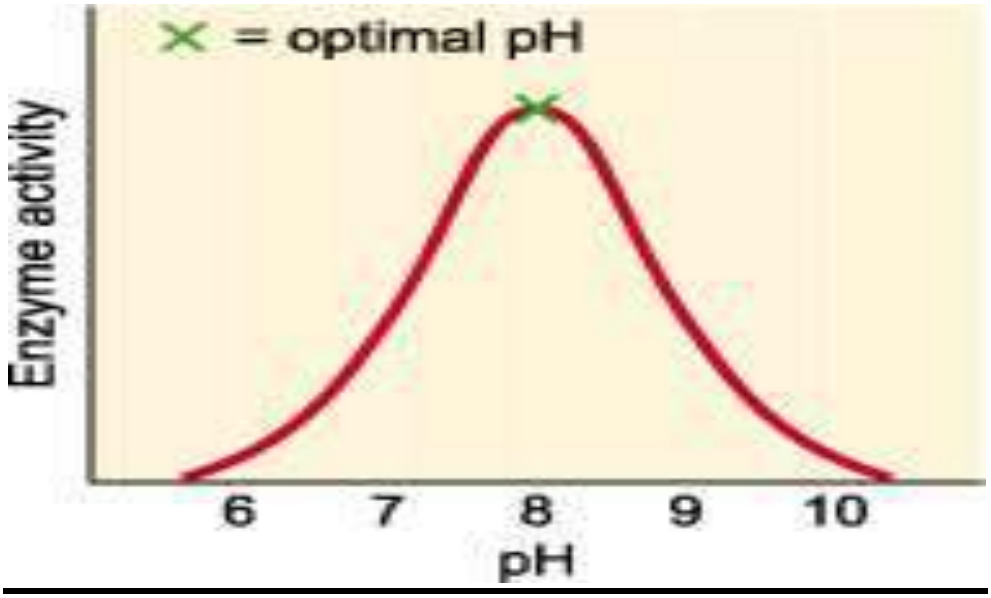
Different enzymes denature at different temperatures. Most enzymes denature at temperatures higher than 60°C. However, there are some enzymes that stay active even at high temperatures like 80°C (Enzymes in the bacteria *Thermus aquaticus*).

2- <u>Effect of pH on enzyme activity</u>

- **Enzyme works best within a narrow pH range**
- **Each enzyme works best at particular pH, known as its optimum pH level.**

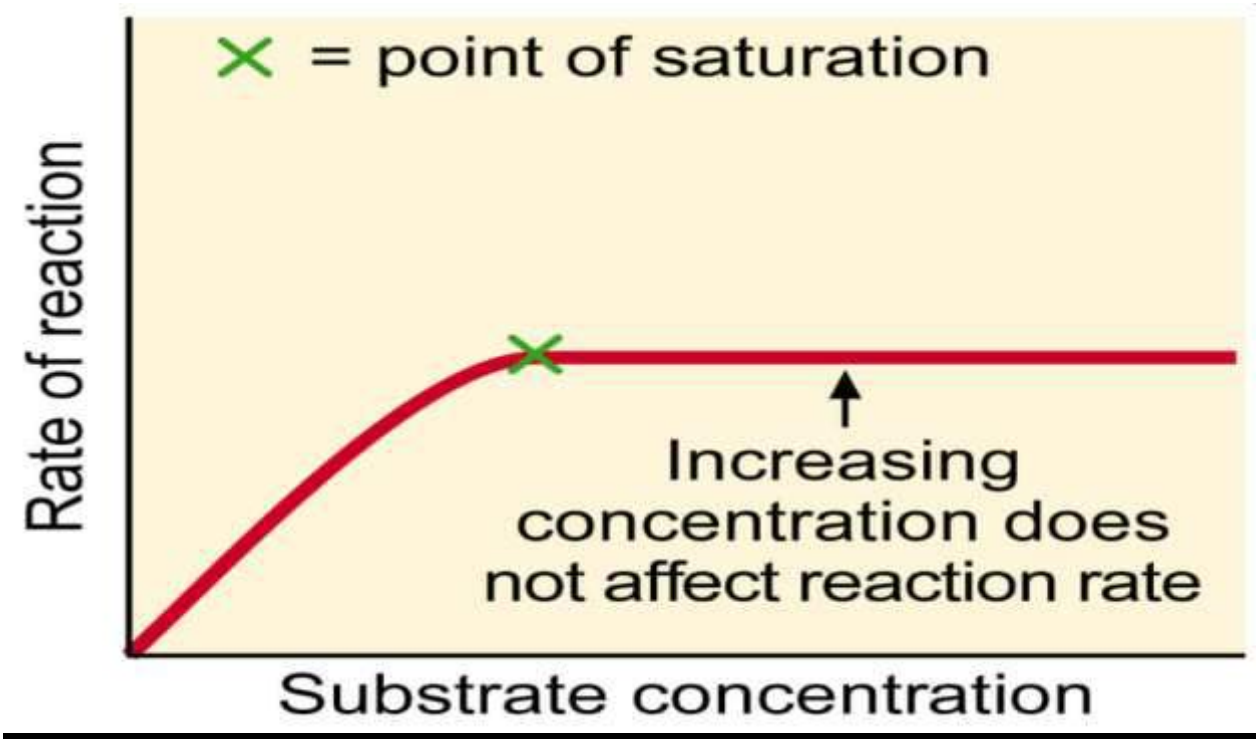


- **At extreme pH levels, enzymes lose their shape and function and become denatured because changes in pH can make and break intra- and intermolecular bonds of the enzyme and, therefore, its effectiveness.**



3-Effect of Substrate on Enzyme Activity:

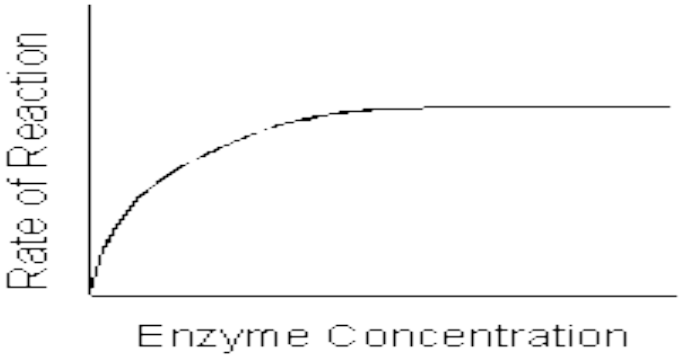
The rate of an enzyme-catalysed reaction depends on the concentrations of substrate. As the concentration of substrate is increased the rate of reaction increases. For a given enzyme concentration, the rate of reaction increases with increasing substrate concentration up to a point, above which any further increase in substrate concentration produces no significant change in reaction rate. This is because the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. The enzyme/substrate complex has to dissociate before the active sites are free to accommodate more substrate. (See graph)



4- Effect of Concentration of enzyme on Enzyme Activity:

As the enzyme concentration increases, the rate of the reaction increases linearly (directly proportionally) , because there are more enzyme molecules available to catalyse the reaction. At very high enzyme concentration the substrate concentration may become rate-limiting, so the rate stops increasing.

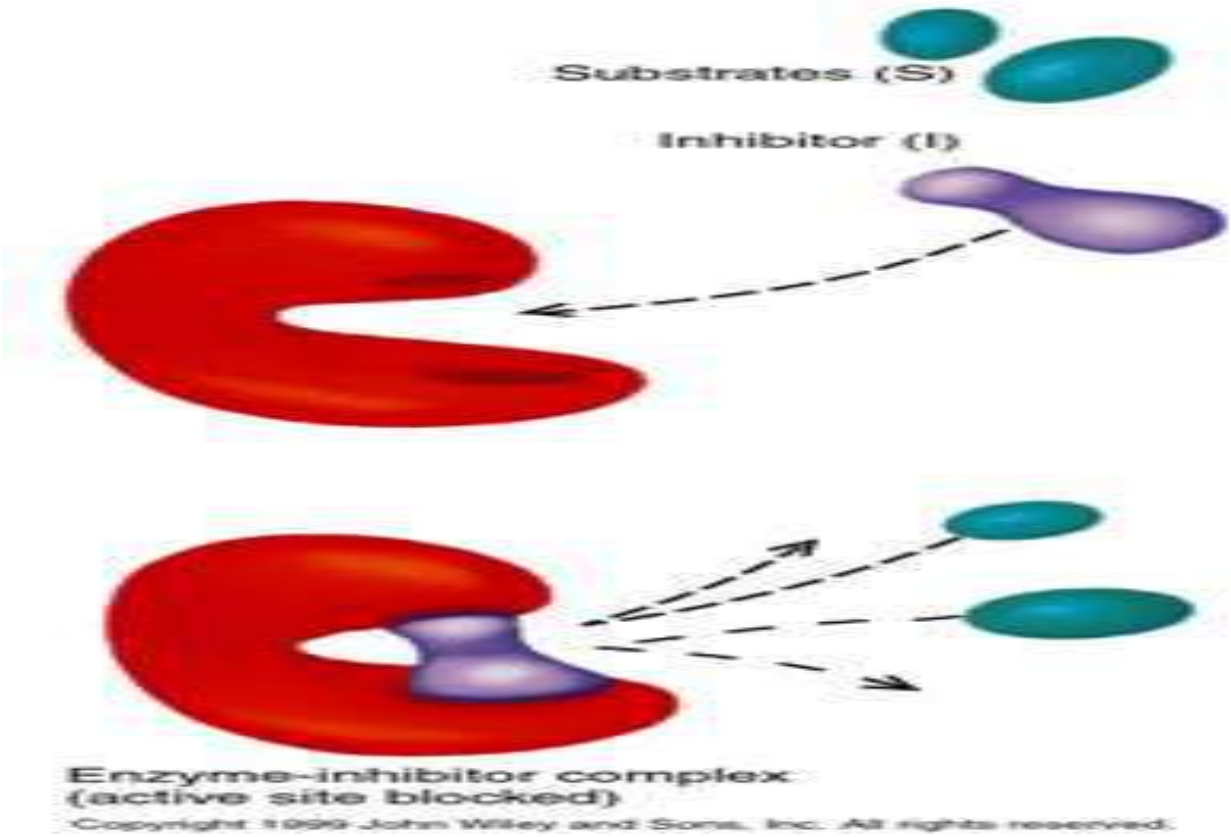
As the concentration of an enzyme increases the rate of reaction also increases, provided that the substrate is in excess.



5- Effect of Inhibitors on Enzyme Activity:

Enzyme inhibitors are molecules that interact in some way with the enzyme to prevent it from working in the normal manner. Poisons and drugs are examples of enzyme inhibitors. Inhibitors change the shape of the enzyme and make it nonuasable to a substrate , i.e. inhibit the activity of enzymes .

Inhibitors can also act as a substrate and bind to the enzyme. This prevents the enzyme from binding with its intended substrate. When this happens the enzyme is said to be denatured



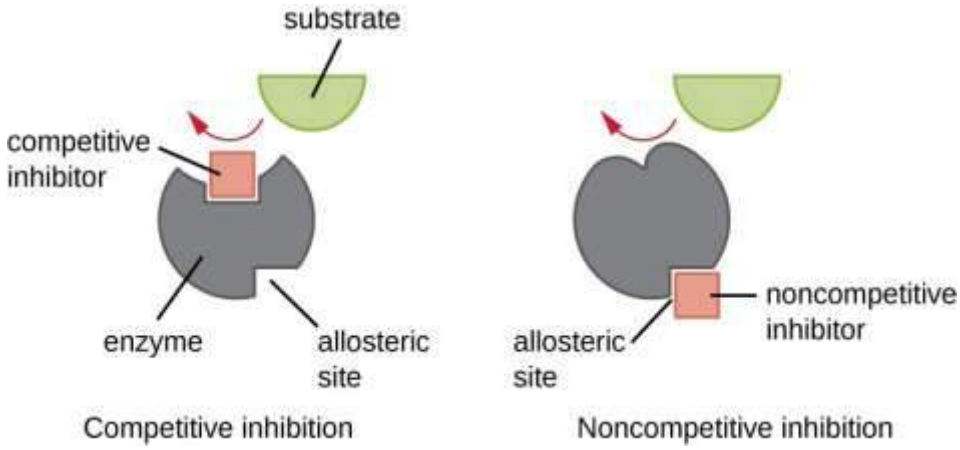
Inhibitors inhibit the activity of enzymes, reducing the rate of their reactions.

Lecture 8

Enzyme Inhibition

Type of inhibitors:
There are five types of inhibitor :
1-a competitive inhibitor:

A competitive inhibitor is a molecule similar enough to a substrate that it can compete with the substrate for binding to the active site by simply blocking the substrate from binding. For a competitive inhibitor to be effective, the inhibitor concentration needs to be approximately equal to the substrate concentration.



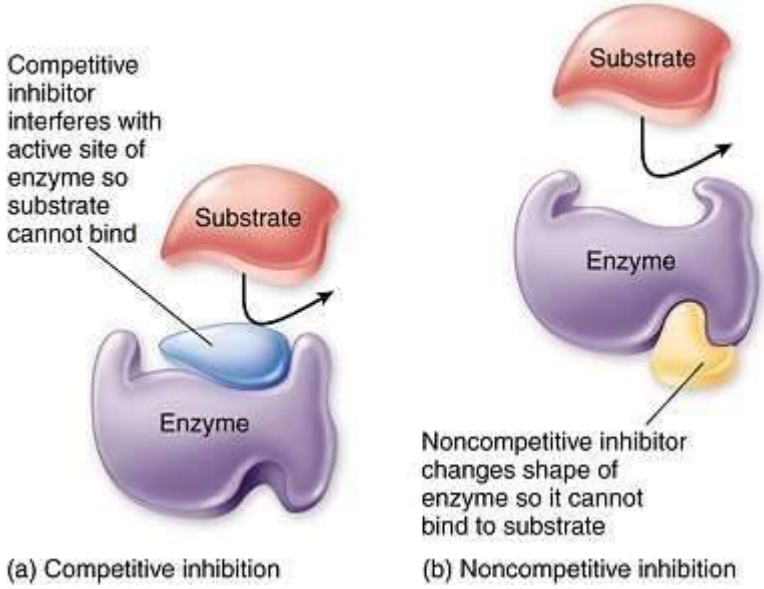
For example, the drug methotrexate (anti-cancer drug) is a competitive inhibitor of the enzyme dihydrofolate reductase, which catalyzes the reduction of dihydrofolate to tetrahydrofolate.

Sulfa drugs are another example of competitive competition. They are used to treat bacterial infections because they bind to the active site of an enzyme within the bacterial folic acid synthesis pathway. When present in a sufficient dose, a sulfa drug prevents folic acid synthesis, and bacteria are

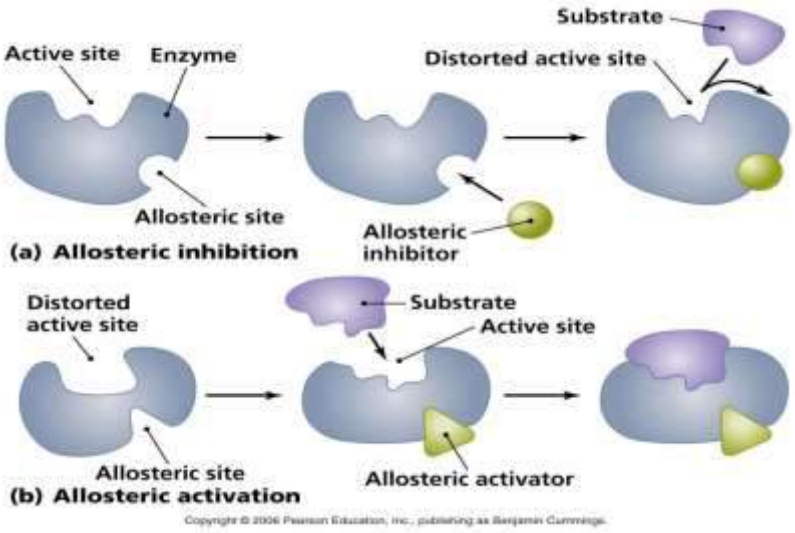
unable to grow because they cannot synthesize DNA, RNA, and proteins. Humans are unaffected because we obtain folic acid from our diets.

2- non-competitive(allosteric) inhibitor :

A noncompetitive inhibitor binds to the enzyme at an allosteric site, a location other than the active site where the substrate binds. The substrate still binds with its usual affinity. However the inhibitor reduces the catalytic efficiency of the enzyme. In contrast to competitive inhibition, non-competitive inhibition cannot be overcome with high substrate concentration.



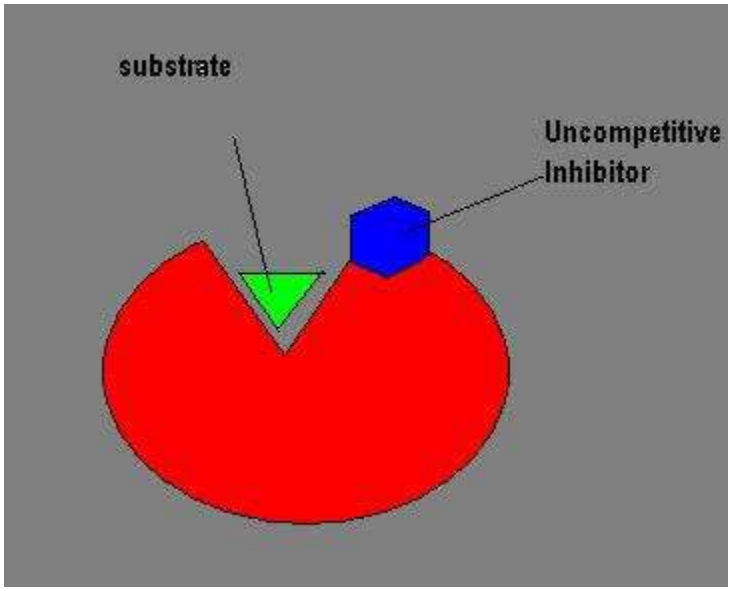
In addition to allosteric inhibitors, there are allosteric activators that bind to locations on an enzyme away from the active site, inducing a conformational change that increases the affinity of the enzyme's active site(s) for its substrate(s).



3-Uncompetitive (anti-competitive) inhibitor :

An uncompetitive inhibitor cannot bind to the free enzyme, only to the enzyme-substrate complex to stop enzyme from reacting with substrate to form product; hence, these types of inhibitors are most effective at high substrate concentration.

In the presence of the inhibitor, the enzyme-substrate complex is inactive. Uncompetitive inhibition occurs in reactions with two or more substrates or products

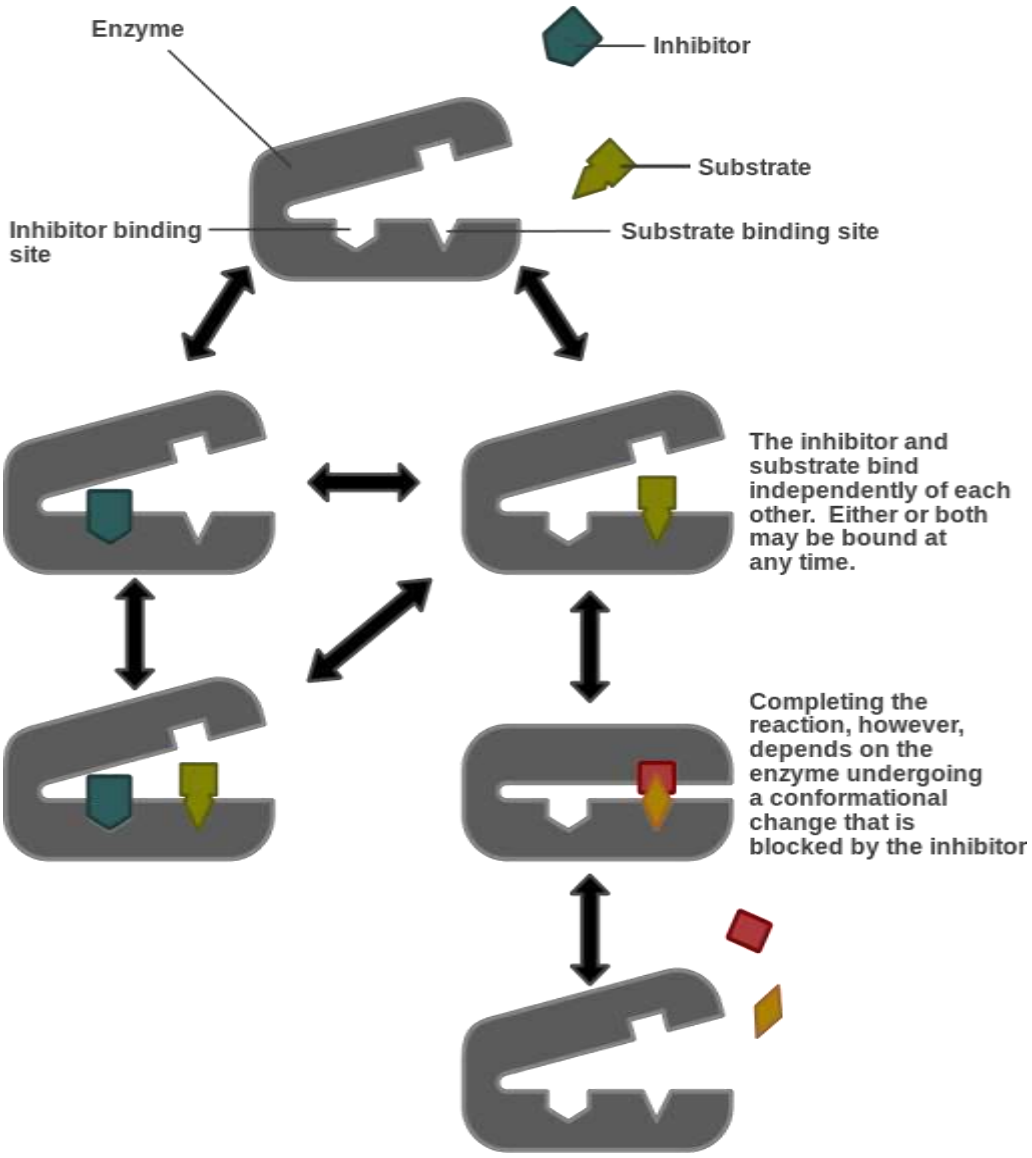


This type of inhibition is rare such as tertiary amines (R_3N) which binds to enzyme acetylcholinesterase in its various forms, but the acyl-intermediate-amine complex cannot break down into enzyme plus product.

4-Mixed – type inhibitor

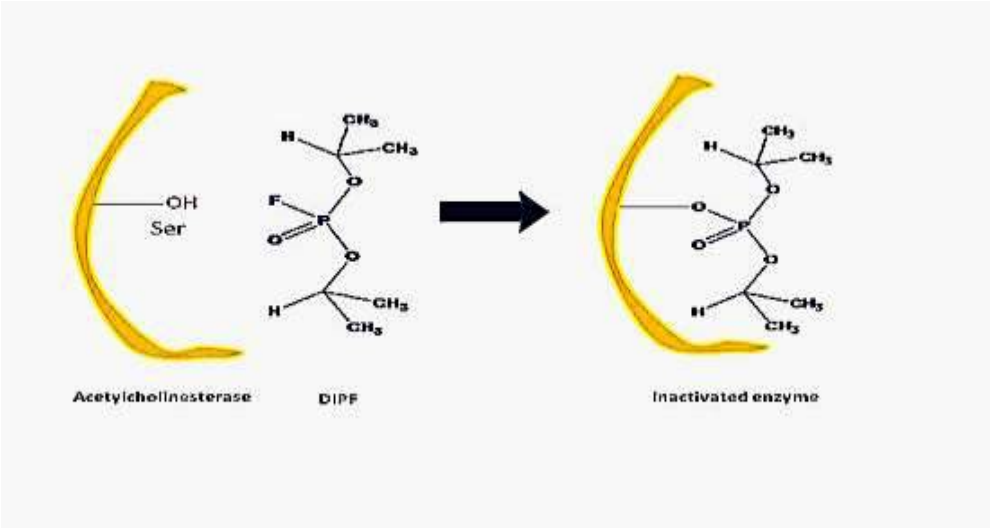
A mixed inhibitor binds to an allosteric site and the binding of the substrate and the inhibitor affect each other. The enzyme's function is reduced but not eliminated when bound to the inhibitor. This type of inhibitor does not follow the Michaelis-Menten equation.

It is called "mixed" because it can be seen as "mixture" of competitive inhibition, in which the inhibitor can only bind the enzyme if the substrate *has not* already bound, and uncompetitive inhibition, in which the inhibitor can only bind the enzyme if the substrate *has* already bound.



5- Irreversible inhibitor :

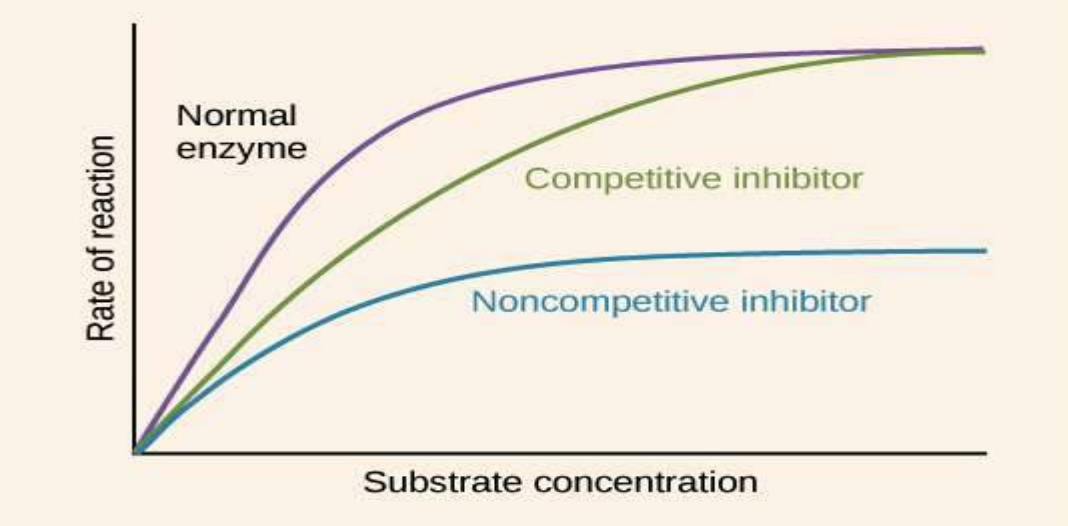
Irreversible inhibitors are covalently or non-covalently bound to the target enzyme and dissociates very slowly from the enzyme. There are three types of irreversible inhibitors: group-specific reagents, reactive substrate analogs also known as affinity labels and suicide inhibitors. Like diisopropylphosphofluoridate (DIPF)which reacts with enzyme acetylcholinesterase to form inactivated enzyme as shown below :



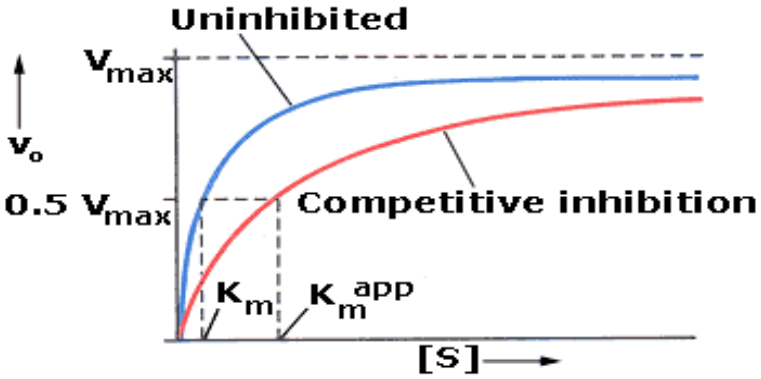
Another example of Irreversible inhibitor are Penicillin and aspirin which are common drugs that form a covalent bond to the protein and make inactivation to the enzyme.

<u>Inhibition kinetics :</u>

If a certain amount of competitive inhibitor added to enzyme chemical reaction, and a certain amount of noncompetitive inhibitor added instead. We would get results as follows:



With a competitive inhibitor, the reaction can reach its normal V_{max}, but it takes a higher concentration of substrate to get it there. In other words, V_{max}, it is unchanged, but the apparent K_m is higher.



As in competitive inhibition, inhibitor can bind to enzyme (E) not with enzyme-substrate complex(ES), hence the value of K_m(Michaelis-Menten constant) increases as the inhibitor interferes with substrate binding, but the maximum velocity (V_{max}) remained same.

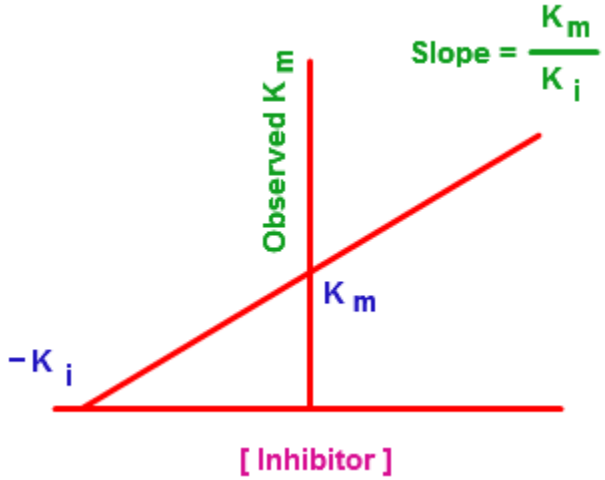
Hence the observed Km will be

$$K_{M,obs} = K_M \cdot \left[1 + \frac{[\text{Inhibitor}]}{K_i}\right]$$

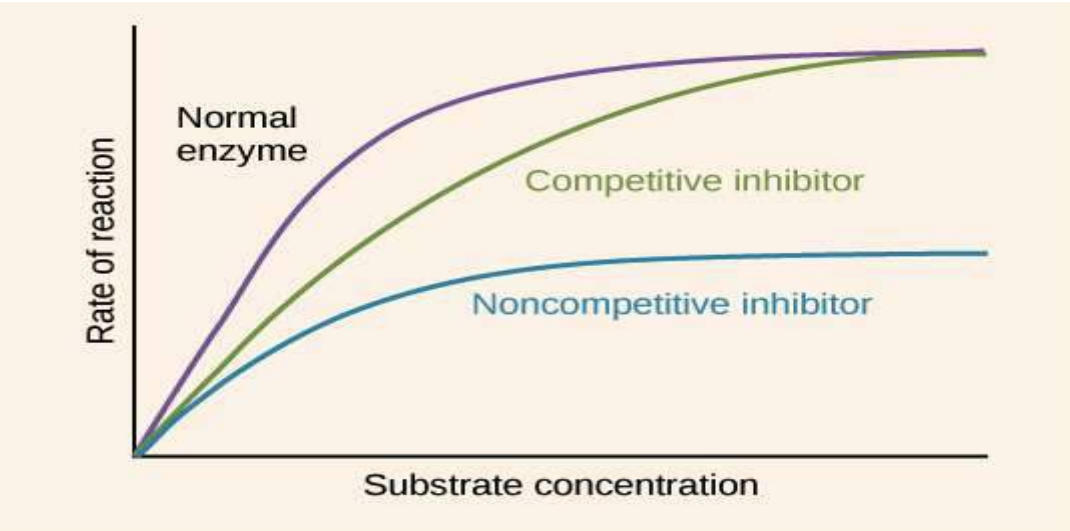
Where K_i = dissociation constant for inhibitor binding (Binding affinities

$$K_i = \frac{[\text{Inhibitor}]}{\frac{K_{M,obs}}{K_M} - 1.0}$$

The curve between the concentration of inhibitor, and observed Km will be linear with K_m intercept at Y-axis and negative K_i at X-axis.



While With a noncompetitive inhibitor, the reaction can never reach its normal V_{max}, regardless of how much substrate we add. A subset of the enzyme molecules will always be "poisoned" by the inhibitor, so the effective concentration of enzyme (which determines V_{max}) is reduced. However, the reaction reaches half of its new Vmax at the same substrate concentration, so Km is unchanged. The unchanged Km reflects that the inhibitor doesn't affect binding of enzyme to substrate, just lowers the concentration of usable enzyme.



Control of enzyme activity :

Enzymes can be regulated by other molecules that either increase or reduce their activity. Molecules that increase the activity of an enzymes are called activators, while molecule that decrease activity of an enzyme are called inhibitors.

Enzymes activities are regulated by five basic techniques.

1. Allosteric control.

Allosteric proteins have different regulatory and catalytic binding sites. Allosteric proteins are cooperative proteins, where binding of a substrate in one active site affects the activity of the rest of the binding sites. Some substrate binding will favor the protein to be in the inactive T (tense) state, while other substrate binding will favor the protein to be in the active R (relaxed) state, depending on the biological needs. Allosterically regulated enzymes do not however obey Michaelis-Menten kinetics

2. Isoenzymes.

Isoenzymes have different amino acid sequences but catalyze the same reaction as enzymes. They usually have different K_m and V_{max} values, and different regulatory techniques. The advantages of isoenzymes is that it can catalyze the same reaction under the different environments within the different organelles.. For example lactate dehydrogenase (LDH) has two isozymes that have an amino acid sequence that is 75% similar. The H isozyme is present in the heart muscle and the M isozyme is expressed in the skeletal muscle.

3. Reversible covalent modification.

An enzyme's activity can be altered by covalently attaching a different group to its active site. It blocks the natural substrate from binding to the active site. The most common forms of covalent modification are phosphorylation and dephosphorylation as well as aceylation and deacylation. Not all forms of covalent modification are readily reversible.

4.Proteolytic Activation.

Many enzymes are present in the body in their inactive forms call zymogen or proenzyme. They are not activated until a digestive enzyme cleaves it. The cleavage alters the three dimension shape of the enzyme, forming the active site in the right orientation. The zymogens become active enzymes in an irreversible reaction, typically the hydrolysis of bonds in the zymogen.

5.Control by Limiting Amount of Enzyme.

The amount of enzymes gets produced can be controlled at the transcription level.

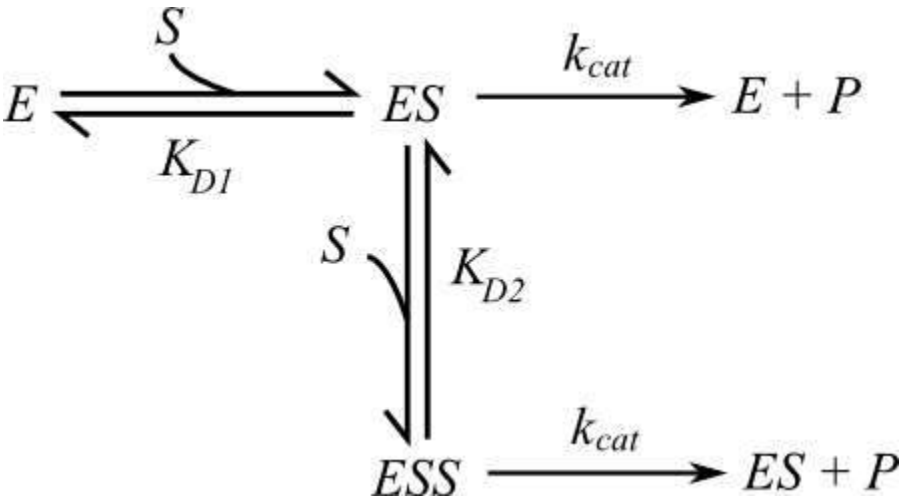
In Double Displacement (Ping Pong reaction), two compounds switch places to form new compounds. Two reactants yield two products.

<u>Multi-substrate reactions :</u>

The Michaelis –Menten model of enzyme kinetics was derived for single substrate reactions. Enzymatic reactions requiring multiple substrates and yielding multiple products are more common and yielding multiple products are more common than single-substrate reaction. In these types of reactions, the all the substrates involved are bound to the enzyme before catalysis of the reaction takes place to release the products.

We can begin with the simplest model of multiple binding:

a two-site sequential model. Here, an enzyme *E* can bind a single molecule of substrate *S* to form a singly-occupied complex *ES* with equilibrium dissociation constant K_{D1}. *ES* can either react irreversibly to form product *P* with rate k_{cat1}, or can bind a second substrate molecule *S* to form a doubly-occupied complex *ESS* with dissociation constant K_{D2}. In turn, *ESS* can irreversibly form *P* with rate k_{cat2}. This is represented schematically below:



In order to calculate the reaction velocity for this system, we need to know the relative concentrations of the active species *ES* and *ESS*. From the definitions of the two dissociation constants, we have:

$$K_{D1} = \frac{[E][S]}{[ES]} \Rightarrow [ES] = \frac{[E][S]}{K_{D1}}$$

$$K_{D2} = \frac{[ES][S]}{[ESS]} \Rightarrow [ESS] = \frac{[ES][S]}{K_{D2}} = \frac{[E][S]^2}{K_{D1}K_{D2}}$$

<u>A ternary complex mechanisms :</u>

A ternary complex is a protein complex containing three different molecules that are bound together. This is seen in multi-substrate enzyme-catalyzed reactions where two substrates and two products can be formed. The ternary complex is an intermediate between the product formation in this type of enzyme-catalyzed reactions.

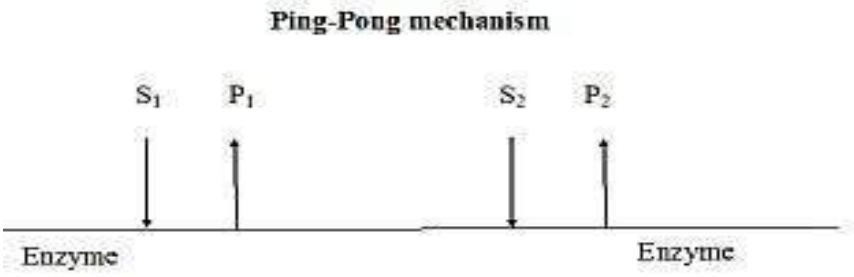
In a Ternary Complex Mechanism two substrates bind to the enzyme (substrate A and substrate B) to form a complex that is known as the EAB - Ternary Complex. The order of the substrate binding can either be in a specific sequence (ordered) or random sequence as well. An enzyme that follows the ternary complex mechanism would have a Line weaver-Burk Plot that has two lines that intersect on a(1/ substrate concentration) against (1/velocity) graph. The plot represents a linear graph of the (1/S)

and (1/V) . A specific enzyme that has a ternary-complex mechanism is DNA polymerase. DNA polymerase functions to add nucleotides to DNA.

<u>Ping Pong (double displacement) Mechanism :</u>

For this mechanism, an enzyme can be in two states. One of the states is labeled E and the other state that is also known as the intermediate and that is chemically modified is labeled E*. In this mechanism, the first substrate (substrate A) binds to enzyme turning it into E* by the transfer of a chemical group to the active site and then the substrate is released.

Once substrate A is released, substrate is able to bind to the modified Enzyme (E*) forming the unmodified Enzyme once again (regeneration). When a Line-Weaver Burk plot is graphed, two sets of parallel will be formed opposite of the Ternary Complex Mechanism.



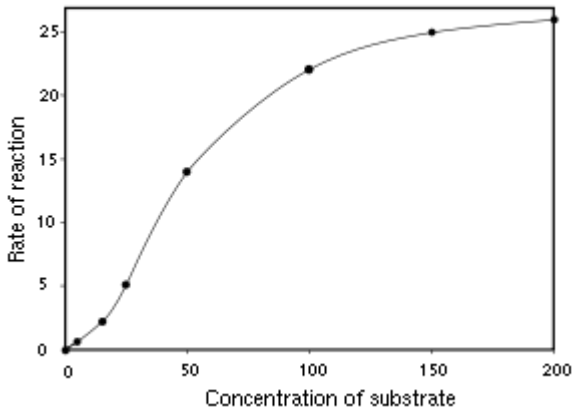
Substrate S_1 binds to the enzyme, followed by release of product P_1. Subsequently new substrate S_2 binds to the enzyme, a product P_2 leaves the complex.

Specific enzymes that follow this mechanism include oxidoreductases and serine proteases. Some of the serine proteases include the digestive enzymes of chymotrypsin and trypsin. For the example of the chymotrypsin, an acyl-enzyme is formed after the breakdown of the tetrahedral intermediate, which is formed after the nucleophilic attack of Ser to the carbonyl forming the intermediate. Once the intermediate breaks down, the acyl-enzyme is formed which acts as the modified Enzyme (E*). The acyl-enzyme however breaks down later into the intermediate complex as the amine group of the acyl-enzyme (E*) leaves and hydrogen functions as a nucleophile to attack the carbonyl forming the tetrahedral intermediate once again.

Non-Michaelis–Menten kinetics :

Some enzymes produce a sigmoid *v* by [S] plot, which often indicates cooperative binding of substrate to the active site. This means that the binding of one substrate molecule affects the binding of subsequent substrate molecules. This behavior is most common in multimeric enzymes with several interacting active sites.

Here, the mechanism of cooperation is similar to that of hemoglobin, with binding of substrate to one active site altering the affinity of the other active sites for substrate molecules. Positive cooperativity occurs when binding of the first substrate molecule *increases* the affinity of the other active sites for substrate. Negative cooperativity occurs when binding of the first substrate *decreases* the affinity of the enzyme for other substrate molecules.



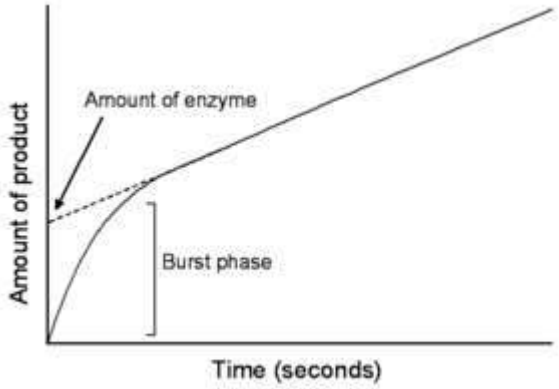
Saturation curve for an enzyme reaction showing sigmoid kinetics.

Bacterial aspartate transcarbamoylase and phosphofructokinase, which show positive cooperativity while allosteric enzymes include mammalian tyrosyl tRNA-synthetase, which shows negative cooperativity,

Positive cooperativity makes enzymes much more sensitive to [S] and their activities can show large changes over a narrow range of substrate concentration. Conversely, negative cooperativity makes enzymes insensitive to small changes in [S].

Pre-steady-state kinetics :

In the first moment after an enzyme is mixed with substrate, no product has been formed and no intermediates exist. The study of the next few milliseconds of the reaction is called pre-steady-state kinetics. Pre-steady-state kinetics is therefore concerned with the formation and consumption of enzyme–substrate intermediates (such as ES or E*) until their steady-state concentrations are reached.



Pre-steady state progress curve,
Showing the burst phase of an enzyme reaction.

This approach was first applied to the hydrolysis reaction catalyzed by chymotrypsin. Often, the detection of an intermediate is a vital piece of evidence in investigations of what mechanism an enzyme follows. For example, in the ping–pong mechanisms that are shown above, rapid kinetic measurements can follow the release of product P and measure the formation of the modified enzyme intermediate E*. In the case of chymotrypsin, this intermediate is formed by the attack of the substrate by the nucleophilic serine in the active site and the formation of the acyl-enzyme intermediate.

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Lecture 9

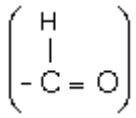
carbohydrate

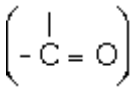
Carbohydrates: Chemistry and classification:

Definition of Carbohydrates :

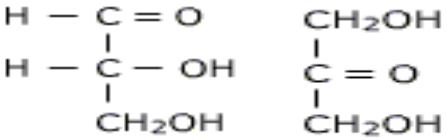
Carbohydrates are polyhydroxy aldehydes ($HOCH_2(CHOH)_nCHO$) or polyhydroxy ketones($HOCH_2(CHOH)_{n-1}$ $(C=O)$ CH_2OH)

hydroxy group O-H

aldehyde group 

keto group 

example :



Chemical formula of carbohydrate :

The formulas of many carbohydrates can be written as carbon hydrates, $C_n(H_2O)_n$.

Biomedical Importance of Carbohydrates

1. The carbohydrates are a source of energy for the body e.g. glucose and glycogen in our body and starch in plants.
2. Components of other molecules like glycolipids, and glycoproteins (attached to proteins or lipids on cell surface membrane).

3. Degradation products act as "promoters" or 'catalysts'.

4. Certain carbohydrate derivatives are used as drugs like cardiac glycosides/antibiotics.

5. One of three essential components of DNA and RNA.

6. Intermediate in respiration (e.g. glyceraldehydes).

7• Degradation products utilized for synthesis of other substances such as fatty acids, cholesterol, amino acid, etc.
8. Lactose is a sugar of milk in mammary gland.

9. Ribose is present in coenzyme e.g. ATP,FAD,NAD and in RNA.

10. Deficiency of enzymes of metabolic pathway of carbohydrates causes disease e.g. Glycogen storage disease, lactose intolerance, hyperglycosurea causes diabetes

Classification of Carbohydrates:

Carbohydrates are classified into three types

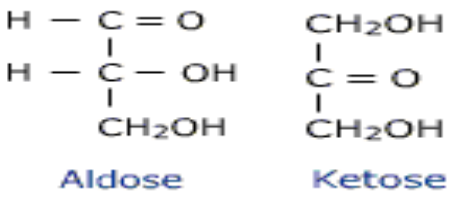
Monosaccharides - Disaccharides – Polysaccharides

Structure of monosaccharides and sterochemistry :

There are two structures for monosaccharides :

1.Open chain structure :

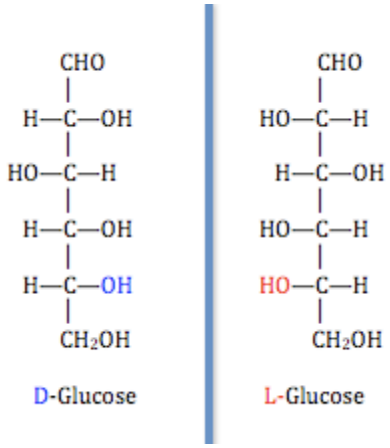
Monosaccharides are the simplest form of carbohydrates may be subclassify as aldoses or ketoses.



The sugar is an aldose if it contains an aldehyde functional group and ketose if it contains a ketone functional group. Monosaccharides may be further classified based on the number of carbon atoms in the backbone, which can be designated with the prefixes tri-(3), tetr-(4), pent-(5), hex-(6), hept-(7), etc. in the name of the sugar.

Open chain structure of monosaccharides are often represented by a Fischer Projection

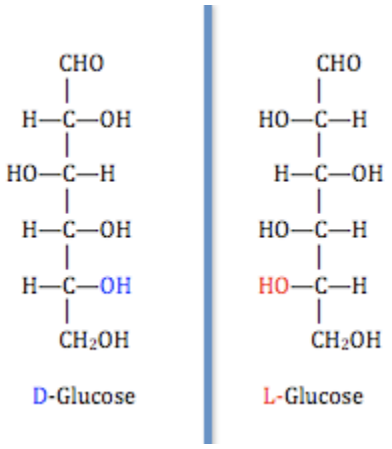
On the Fischer projection, if the farthest hydroxyl(-OH) group is on the right, then it is classified as D sugar, if the hydroxyl group is on the left, then it is a L sugar.we have another isomers d- and l-isomer which represent enantiomer which rotate the plan polarized light into right or lift respectively .It sometime give (+) or (-) sign .



There are three type of isomers in open chain structure:

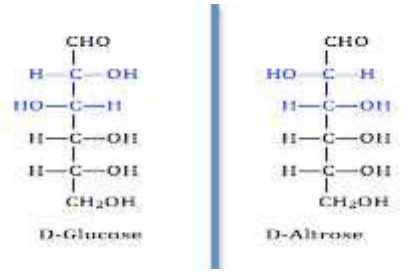
1. Enantiomers:

Two carbohydrates are said to be enantiomers if they are nonsuperimposable mirror images of one another. An example of an enantiomer is the D and L isomers of glucose, as shown in figure below :



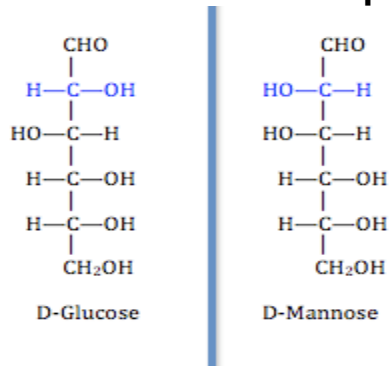
2. Diastereoisomers :

Carbohydrates are classified as diastereomers if their chiral carbons are connected to the exactly the same substrates but connected at differing configurations (R or S). Unlike an enantiomer, diastereomers are NOT object and mirror image. An example of two carbohydrates that are diastereoisomers are D-Glucose and D-Altrose as seen in the figure below :



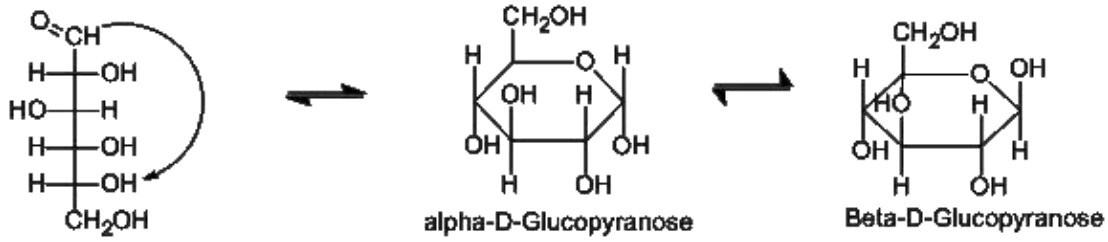
3. Epimers

Epimers are two diastereomers (optical isomers) that differ only at one stereocenter (configuration about one chiral atom). As shown in the figure below, D-Glucose and D-Mannose are an example of an epimer.



2.Cyclic structure

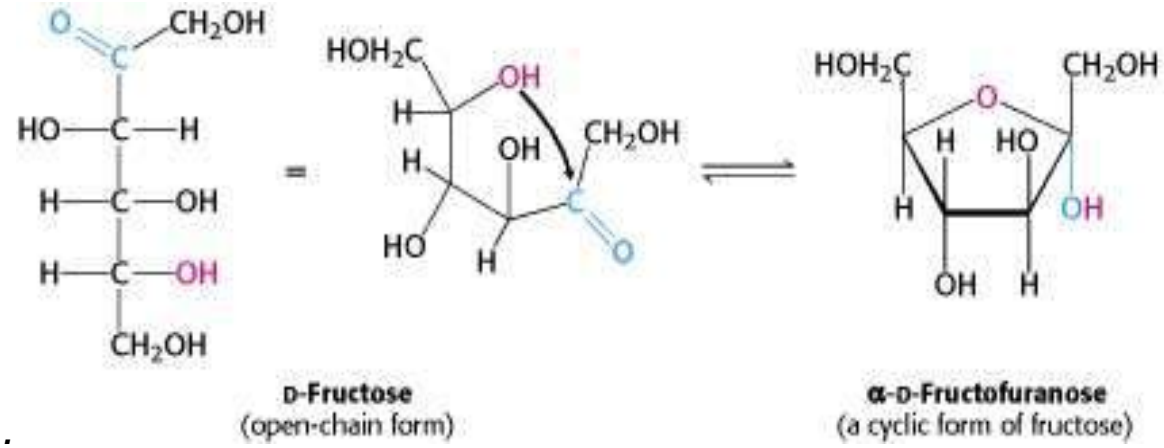
This structure is formed due to condensation between hydroxyl group at C_5 and aldehyde group at C_1 to give hemiacetal which is called pyranose.



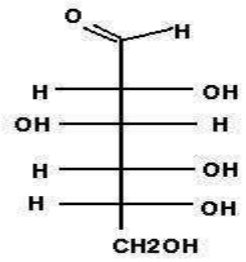
Two isomers called alpha (α) and beta (β) –anomer is formed which is diastereomers that differ at the chiral carbon formed on ring closure.

C_1 in cyclic structure is called anomer-carbon atom which is the more reactive carbon due to joining into two electronegative oxygen atoms .

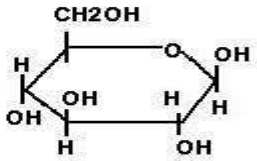
The C-2 keto group in the open-chain form of a ketohexose, such as fructose, can form an *intramolecular hemiketal* by reacting with either the C-6 hydroxyl group to form a six-membered cyclic hemiketal or the C-5 hydroxyl group to form a five-membered cyclic hemiketal. The five-membered ring is called a *furanose* because of its similarity to *furan.*



So we have two projections , Fischer and Haworth progection



Fischer projection



Haworth Projection

D- Glucose

Classification of Monosaccharides :

Monosaccharides can be classified according to the number of carbon atoms in a molecule into four types as shown in following table :

Number of Carbon Atoms	General Name	Examples
3	Triose	Glyceraldehyde (glycerose), dihydroxyacetone.
4	Tetrose	Erythrose
5	Pentose	Ribose, deoxyribose, arabinose, xylose, ribulose.
6	Hexose	Glucose, fructose, galactose, mannose

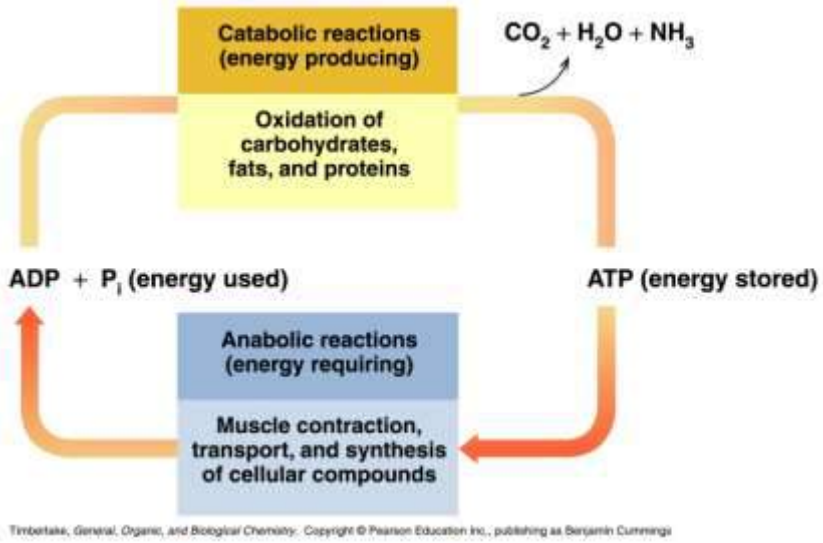
Metabolism of carbohydrates

Metabolism :

Metabolism is all chemical reactions occurring in cell . Metabolism involves two reactions :

1.Catabolic reactions that break down large, complex molecules to provide energy and smaller molecules.

2.Anabolic reactions that use ATP energy to build larger molecules as shown in diagram below



Metabolic Pathways for Carbohydrates:

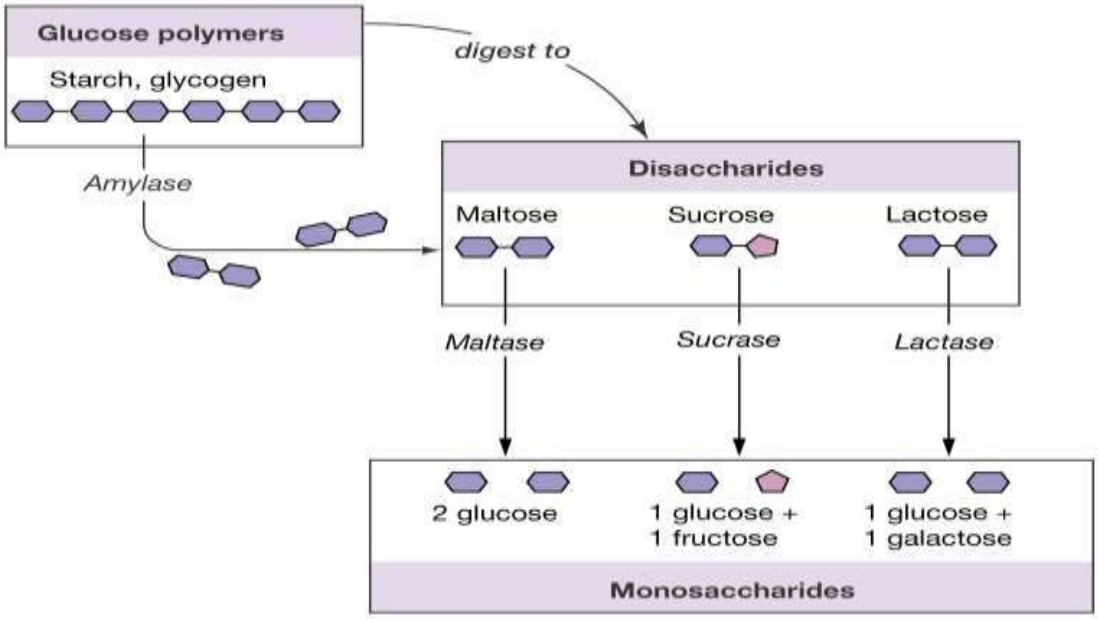
There are five stages of Metabolic Pathways for Carbohydrates

1-Digestion of Carbohydrates 2- Glycolysis 3- Kreb's cycle

4- Glycogenesis 5- Glycogenolysis

1-Digestion of Carbohydrates:

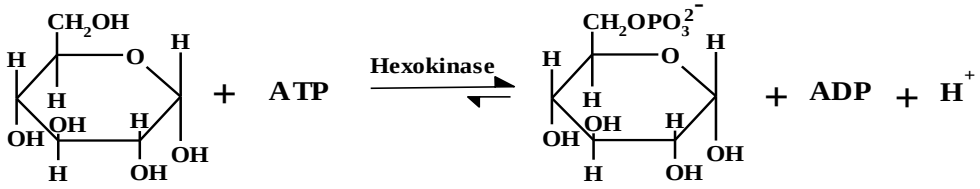
Carbohydrate metabolism begins with digestion of polysaccharides into disaccharides which is converted into monosaccharides which are absorbed into the blood stream as shown below :



2-Glycolysis:

The breakdown of glucose to provide energy begins with glycolysis. Glucose is converted into two, three-carbon molecules of pyruvate through a series of ten different reactions.

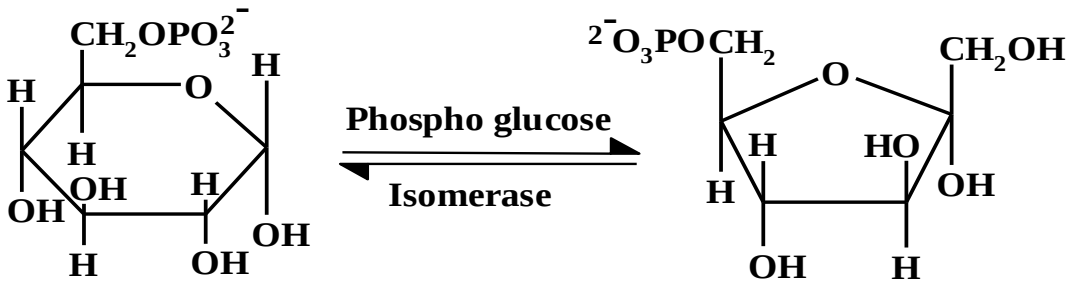
1- Glucose is converted to glucose-6-phosphate using ATP and by the help of hexokinase in the presence of Mg^{2+} or Mn^{2+} ion .In this reaction one molecule of ATP is consumed.



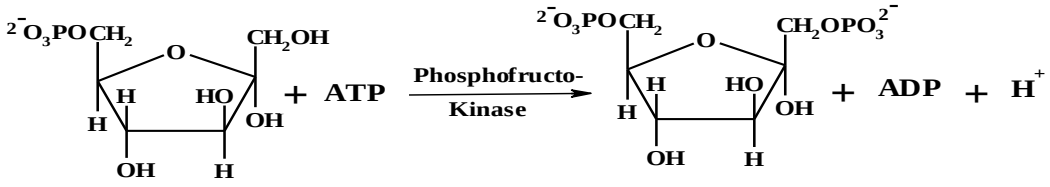
Glucose **Glucose 6- Phosphate**

Hexokinase is inhibited by high levels of glucose-6-phosphate, which prevents the phosphorylation of glucose so it is one of point of regulate of glycolysis of glucose.

2- Glucose 6-phosphate is converted into fructose 6-phosphate by the help of Phosphoglucose Isomerase



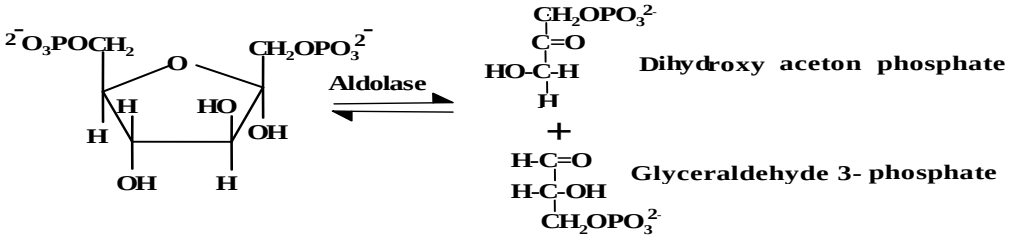
3- Fructose 6-phosphate is converted into fructose 1,6-diphosphate by the help of Phosphofructo Kinase. In this reaction another molecule of ATP is consumed.



Fructose 6- Phosphate **Fructose 1,6- diphosphate**

Phosphofructokinase, an allosteric enzyme, is inhibited by high levels of ATP and activated by high levels of ADP and AMP.

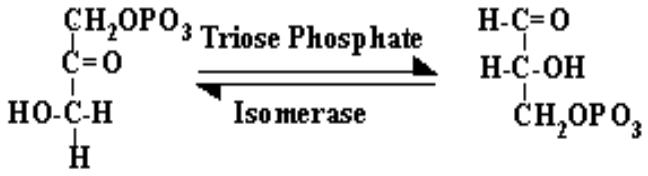
4-Fission of fructose 1,6-diphosphate (compound contains 6 carbon atoms) into dihydroxy acetone phosphate (compound contains 3 carbon atoms) and glyceraldehyde 3-phosphate (compound contains 3 carbon atoms) by the help of aldolase .



Fructose 1,6 diphosphate

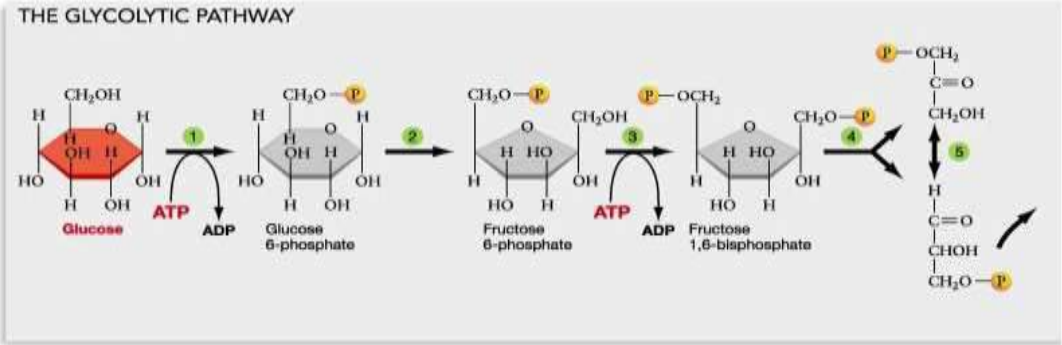
5- dihydroxy acetone phosphate is converted into glyceraldehydes 3-phosphate by the help of triose Phosphate Isomerase .This reaction is reversible which means the same enzyme can catalyzes the reaction in both directions.

The net gain of five reactions (1 → 5) glycolysis one glucose molecule and formation two glyceraldehydes 3-phosphate molecules and consume two ATP molecule These reactions are energy consumed and the concentration of glyceraldehydes 3-phosphate effected on the direction of reaction, if it is low the reaction is shifted to right .



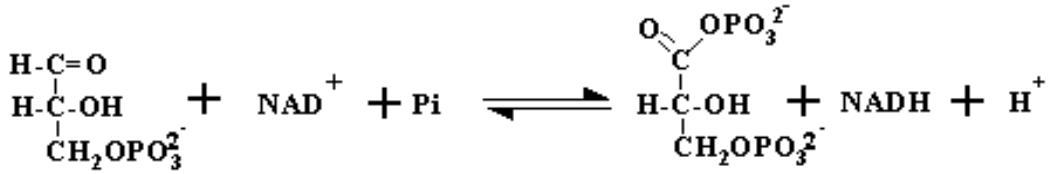
Dihydroxyaceton Phosphate **Glyceraldehyde 3- Phosphate**

Reactions from 1 to 5 are shown below:



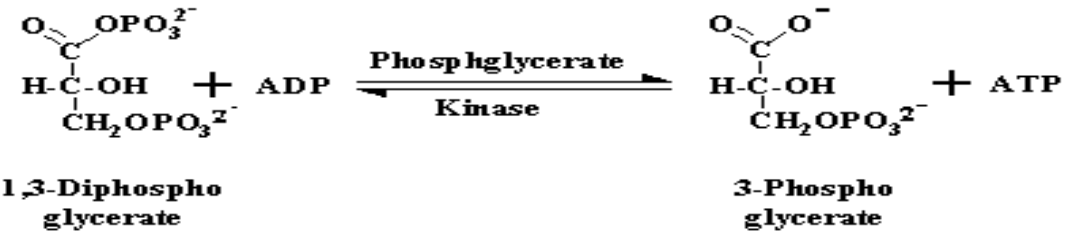
6-two glyceraldehyde 3-phosphate molecules is converted into 1,3-diphosphoglycerate (compound of high energy)by the help of glyceraldehydes 3-phosphate dehydrogenate and in the presence of coenzyme nicotinamide adenosine dinucleotide (NAD), and electron transfers from glyceraldehyde 3-phosphate to NAD^+ and this reaction endothermic reaction .

During the sixth reaction, glyceraldehyde 3-phosphate is oxidized to 1,3 - diphosphoglycerate while reducing nicotinamide adenosine dinucleotide (NAD) to NADH



Glyceraldehyde 3- phosphate **1,3-Diphospho glycerate**

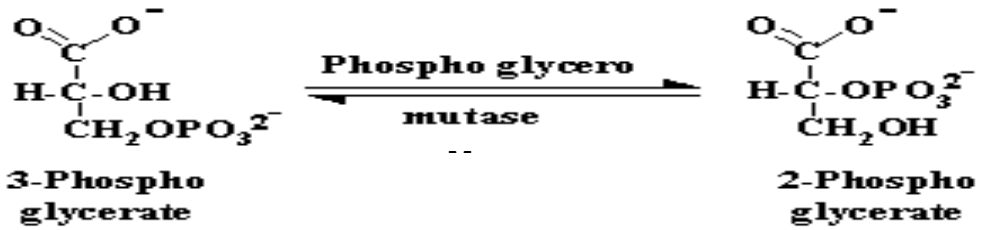
7-two molecules of 1,3 –diphosphoglycerate is converted into two molecules of 3-phosphoglycerate and formation of 2 ATP from 2 ADP by the help of phosphoglycerate kinase.



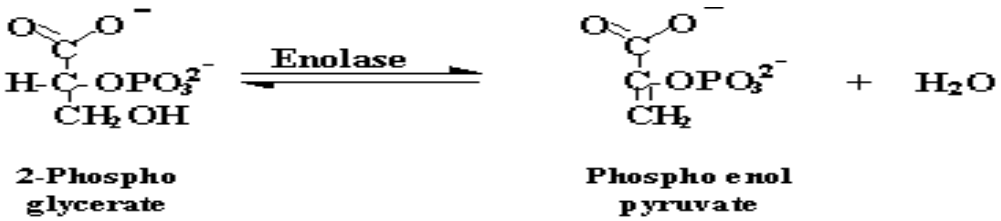
This type of phosphorylation is called substrate –level phosphorylation

that means phosphate group is transferred to ADP without transfer of electron differ from oxidative phosphorylation.

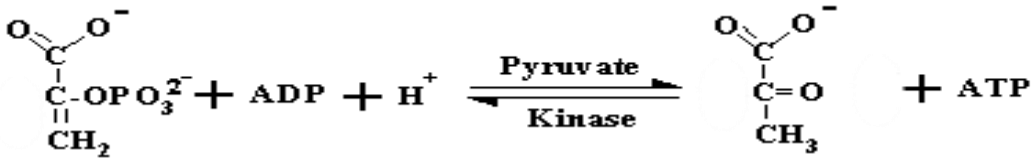
8- two molecules of 3-phosphoglycerate is converted to two molecules of 2-phosphoglycerate by transferring phosphate group from position 3 to position 2 by the help of Phospho glycero mutase and in the presence of Mg^{2+} .



9-formation of two molecules of phosphoenolpyruvate (compound rich in energy) from two molecules of 2-phosphoglycerate by the help of Enolase and in the presence of Mg^{2+} .



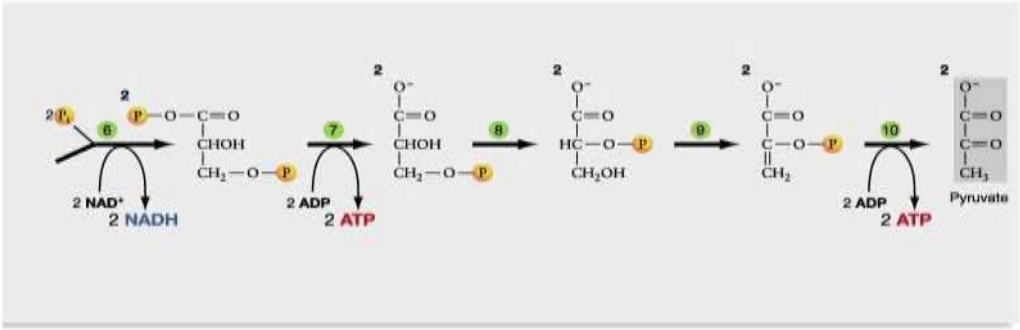
10-Formation of two molecules of pyruvate and ATP from two molecules of phosphoenolpyruvate and ADP by the help of pyruvate kinase and in the presence of K^{+}.



Phospho enol pyruvate **Pyruvate**

Pyruvate kinase, another allosteric enzyme is inhibited by high levels of ATP or acetyl CoA.

Reactions from 6 to 10 are shown below :



So in glycolysis,

- **Two ATP add phosphate to glucose and fructose-6-phosphate.**
- **Four ATP are formed in energy-generation by direct transfers of phosphate groups to four ADP.**
- **There is a net gain of 2 ATP and 2 NADH.**

$C_6H_{12}O_6$ + 2ADP + 2Pi + $2NAD^+$ ⟶

Glucose

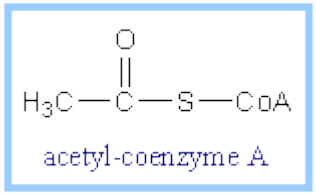
$2C_3H_3O_3^-$ + 2ATP + 2NADH + $4H^+$

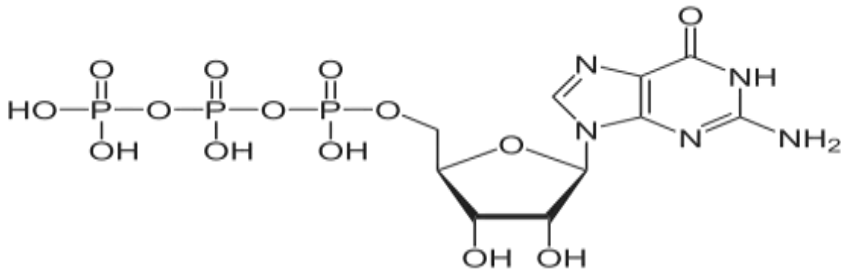
Pyruvate

Lecture 10

3-Kreb's cycle

Kreb's cycle– also known as the tricarboxylic acid (TCA) cycle or citric acid cycle – is a series of chemical reactions used by all aerobic organisms to generate energy through the oxidation of acetyl-CoA derived from carbohydrates, fats and proteins into carbon dioxide and chemical energy in the form of guanosine triphosphate (GTP).





In addition, the cycle provides precursors of certain amino acids as well as the reducing agent NADH that is used in other biochemical reactions and nictotinamide adenine dinucleotide (NAD^+)