# E-content for Program: M.Sc. Zoology (2nd semester)
# Core Course (CC- 7): Biochemistry

**Name of Faculty: Dr. Richa Rani, *M.Tech.*, *Ph.D.***
**Assistant Professor, Post Graduate Department of Zoology,**
**Patna University, Patna-800005, Bihar**
**Email: richarani.pu@gmail.com**
**Contact: 6206460541**

## Unit - IV: Enzyme Biochemistry

## INTRODUCTION

### Enzymes:

The term 'enzyme' was coined by Friedrich Wilhelm Kuhne in 1878. Enzymes, the catalysts of biological systems, are remarkable molecular devices that determine the patterns of chemical transformations. They are also involved in the transformation of one form of energy into another. For instance-in cellular respiration, which takes place in mitochondria, the free energy contained in small molecules derived from food is converted first into the free energy of an ion gradient and then into a different currency—the free energy of adenosine triphosphate (ATP).

### Characteristics of enzymes:

- Nearly all known enzymes are protein in nature except ribozyme, which is a RNA molecule.
- Enzymes catalyze the biochemical reactions in living cells.
- Enzymes speed up the rate of chemical reactions, but the properties of the reaction--whether it can take place at all and the degree to which the enzyme accelerates the reaction--depend on energy differences between reactants and products.
- Enzymes accelerate reactions by facilitating the formation of the transition state. They catalyze reactions by stabilizing transition states, the highest-energy species in reaction pathways.
- Enzymes speed up the rate of biochemical reaction by lowering the energy of activation.
- Enzyme does not change the reaction equilibrium, but only decrease the time it takes to reach the equilibrium.
- Enzymes are not consumed and the chemical nature of an enzyme is not altered by entering a biochemical reaction.
- Major factors that affect the reaction rate of enzymes are enzyme concentration, substrate concentration, pH and temperature.

### Enzyme related important terms:

1. **Substrate:** The reactant in the biochemical reaction is called substrate.

2. **Active site and its common features:**

All enzymes molecules contain a special cleft or region in its structure for binding with its substrate known as 'active site' or 'catalytic site' or 'substrate site' of an enzyme.

- The active site is a three-dimensional cleft, or crevice, occupying a very small portion of the enzyme molecule, formed by groups that come from different parts of the amino acid sequence.
- The active site takes up a small part of the total volume of an enzyme.
- Active sites are unique microenvironments. Active site in the enzyme molecules usually excludes water, unless it is a reactant. It contains amino acids such as aspartic acid, glutamic acid, lysine, serine etc. The side chain groups such as --COOH, --NH2, --CH2OH etc., serve as catalytic groups in the active site.
- Substrates are bound to enzymes by multiple weak attractions such as electrostatic interactions, hydrogen bonds, and van der Waals forces.
- The specificity of binding depends on the precisely defined arrangement of atoms in an active site.

3. **Specificity:**

- Enzyme catalyzed reaction are highly specific ie. a particular enzyme catalyzes particular type of biochemical reaction.
- Four types of enzyme specificity have been recognized:

  **i) Absolute specificity:** Enzyme acts on only one type of substrate. For example, catalase acts on $H_2O_2$ only. Similarly, Urease acts only on urea to produce ammonia and carbon dioxide.

  **ii) Group specificity:** Enzyme acts on group of structurally related substrates. For example, Lactic dehydrogenase (LDH) catalyzes the interconversion of pyruvic and lactic acid and also a number of other structurally-related compounds. Alcohol dehydrogenase brings dehydrogenation of many alcohols such as methanol, ethanol etc. Similarly, hexokinase phosphorylates many hexose sugars including, glucose, galactose, mannose etc.

  **iii) Optical specificity:** A particular enzyme will react with only one of the two optical isomers. For example, arginase acts only on L-arginine and not on its D-isomer. Similarly, D-amino acid oxidase oxidizes the D-amino acids only. Although, the enzymes exhibit optical specificity, some enzymes, however, interconvert the two optical isomers of a compound; For example, alanine racemase catalyzes the interconversion between L- and D-alanine.

  **iv) Geometrical specificity:** Some enzymes exhibit specificity towards the cis and trans forms. For instance, fumarase catalyzes the interconversion of fumaric and malic acids. It does not react with maleic acid which is the cis isomer of fumaric acid or with D-malic acid.

4. **Catalytic efficiency or Turnover number:**

- The catalytic efficiency of an enzyme is expressed in turnover number or molecular activity.
- It is defined as the number of substrate molecules converted into product on a single enzyme molecule per unit time, when the enzyme is fully saturated with substrate. It is expressed as $k_{cat}$ (units of reciprocal time). It is given by $k_{cat} = V_{max}/[E_t]$.

5. **Cofactor and Apoenzyme:**

- A cofactor is a non-protein chemical compound that is bound (either tightly or loosely) to an enzyme and is required for catalysis.
- Cofactors can be subdivided into two groups: (1) metals and (2) small organic molecules called **coenzymes**. Often derived from vitamins, coenzymes can be either tightly or loosely bound to the enzyme.
- Tightly bound coenzymes are called **prosthetic groups**.
- Loosely associated coenzymes are more like cosubstrates because, like substrates and products, they bind to the enzyme and are released from it.
- Some enzymes are composed of a protein moiety (**apoenzyme**) and a non-protein moiety (**cofactor or coenzyme**).
- An enzyme without its cofactor is referred to as an apoenzyme; the complete, catalytically active enzyme is called a holoenzyme.

**Apoenzyme** (**inactive**) + **cofactor** = **holoenzyme** (**active**)

6. **Activation Energy:**

- All the chemical reactions in a biological system have an energy barrier that must be crossed by the reactant molecules in order to convert itself into the product. The required amount of energy needed to break this energy barrier or to start a reaction is called the activation energy.
- Enzymes lower the activation energy of a reaction not by changing the reaction's free energy ($\Delta G$), but by reducing the activation energy required to reach the transition state.
- If energy of activation is higher, rate of reaction is slower and if it is lower, the rate of reaction is faster.

## 4.1. Enzyme: Classification and nomenclature

### *4.1.1 Nomenclature:*

Initially, enzymes were named by suffixing "*ase*" after the name of substrate or according to the:

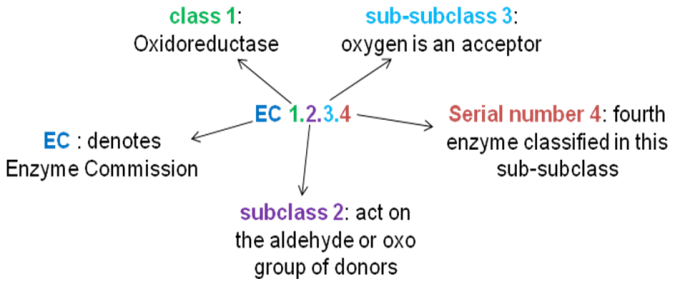
- substrate catalyzed: for ex- lactase (acting upon lactose), maltase (acting upon maltose), sucrase (acting upon sucrose) etc.
- type of reaction catalyzed: for ex- hydrolases (catalyzing hydrolysis), phosphorylases (phosphorylation) etc.
- substrate utilized and type of reaction catalyzed: for ex- succinic dehydrogenase (catalyzes the dehydrogenation of substrate succinic acid)
- substrate synthesized: for ex- Fumarse (forms fumarate from malate)

✓ To bring some consistency to the classification of enzymes, in 1964 the International Union of Biochemistry established an Enzyme Commission to develop a nomenclature for enzymes.

✓ According to this system, enzymes are identified by a unique name and a code number, that reflect type of reaction catalyzed and substrates involved.

✓ Enzyme Commission (EC) number consists of four elements, separated by periods. The first digit identifies the class of reaction catalyzed. The second digit (the subclass) says about the type of compound or group involved. The third digit, the sub-subclass, specifies the type of reaction involved or the substrates on which the group acts. The fourth digit is a serial number that is used to identify the individual enzyme within a sub-subclass.

**Example 1: Nomenclature EC 1.2.3.4**; EC: denotes Enzyme commission, the digits indicate that the enzyme is an oxidoreductase (class 1), that it acts on the aldehyde or oxo group of donors (subclass 2), that oxygen is an acceptor (sub-subclass 3) and that it was the fourth enzyme classified in this sub-subclass (serial number 4).




**Example 2: Nomenclature EC 2.7.1.1** represents class 2 (a transferase), sub-class 7 (transfer of phosphate), sub-subclass 1 (an alcohol group as phosphate acceptor). The last digit indicates the enzyme hexokinase or ATP: D-hexose-6-phosphotransferase, which catalyzes the transfer of phosphate from ATP to the hydroxyl group on carbon 6 of glucose.

### *4.1.2 Classification:*

Enzymes are classified into six major classes (based on the type of reaction catalyzed) and a seventh class, the translocases, added in 2018 (Tipton, K. Translocases (EC 7): A new EC Class. Enzyme Nomenclature News, August 2018)). These are summarized in Table 1.

**Table 1: Enzyme classification**

| Class | Name (Enzyme Code) | Reaction catalyzed | Function | Example |
|---|---|---|---|---|
| 1 | Oxidoreductases (EC 1) | $A_{red} + B_{ox} = A_{ox} + B_{red}$ | catalyze oxidation reduction reactions | Alcohol dehydrogenase, Acetyl-CoA dehydrogenase, Cytochrome oxidase, Catalase |
| 2 | Transferases (EC 2) | A-B + C = A + B-C | catalyze transfer or exchange of certain functional groups | Choline acetyltransferase, Phosphorylase, Hexokinase |
| 3 | Hydrolases (EC 3) | A-B + $H_2O$ = A-H + B-OH | catalyze hydrolysis of their substrate by adding constituents of water across the bond they split | Lipase, Beta-galactosidase, Arginase, Trypsin. Pepsin |
| 4 | Lyases or Desmolases (EC 4) | A=B + X-Y = A-Y + B-X | catalyze group elimination reactions to form double bonds | Aldolase, Fumarase, Histidase |
| 5 | Isomerases (EC 5) | AB = BA | catalyze geometric or structural changes or isomerizations (bond rearrangements) | Alanine racemase, Cis-trans isomerases, Retinine isomerase, Glucosephosphate isomerase |
| 6 | Ligases or Synthetases (EC 6) | A + B + ATP = A-B + ADP + $P_i$ (or AMP + $PP_i$) | catalyze the synthesis of two molecular substrates into one molecular compound coupled with hydrolysis of a diphosphate bond in ATP or a similar triphosphate. | Acetyl-CoA synthetase, Glutamine synthetase, Acetyl-CoA carboxylase |
| 7 | Translocases (EC 7) | AX + B* = A + X + *B (side 1) (side 2) | catalyze the movement of ions or molecules across membranes or their separation within membranes (transfer from 'side 1' to 'side 2') | Translocase of the outer membrane (TOM) and Translocase of the inner membrane (TIM) of the mitochondrion |

## 4.2 Mechanism of enzyme action

***4.2.1. The formation of an enzyme–substrate complex is the first step in enzymatic catalysis.***

Formation of Enzyme-substrate complex is described by two models:

I. **Lock and Key model:**

- In the 1890s the German chemist Emil Fischer (1852–1919) proposed **Lock and Key model.** In '**Lock and Key'** hypothesis, active site has a rigid shape i.e. the shape of active site of enzyme is complementary to the shape of substrate molecules.
- Enzymes can catalyze only substrates which fit perfectly on the active site of the enzyme and results in the formation of an enzyme substrate complex.
- Once the product is formed, they no longer fit into the active site and escape into surrounding medium.
- This model does not work for all enzymes.

II. **Induced fit model:**

- In 1959, Koshland suggested a modification to the '**Lock and Key'** hypothesis which is known as '**Induced fit'** hypothesis.
- Koshland presumed that the active site is flexible, but structurally not complementary and suggested that the shape of the enzyme, active site and substrate is modified as the substrate interacts with the enzyme.
- In this model, there is a greater range of substrate specificity and model is applicable to a wider range of enzymes.

**Evidence for the existence of an enzyme–substrate complex** At a constant concentration of enzyme, the reaction rate increases with increasing substrate concentration until a maximal velocity is reached. *The fact that an enzyme-catalyzed reaction has a maximal velocity suggests the formation of a discrete ES complex.*

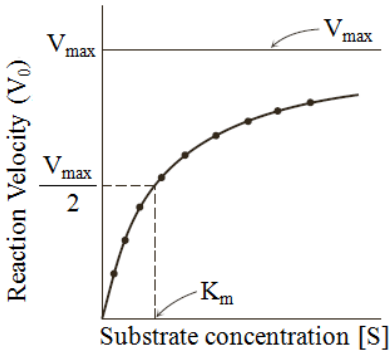



**Figure 1:** Reaction velocity versus substrate concentration in an enzyme catalyzed reaction.

***4.2.2. The binding energy between enzyme and substrate is important for catalysis.***

Enzymes increase reaction rates by decreasing the activation energy, but where does the energy to lower the activation energy come from?. It comes from the free energy released by the interactions between complementary enzyme and its substrate, known as binding energy. Binding energy is maximum when the enzyme facilitates the formation of the transition state.

However, the transition state is very unstable collapsing to the accumulation of either substrate or product, which is determined by the energy difference between the substrate and the product i.e., ΔG of the reaction (Figure 2).

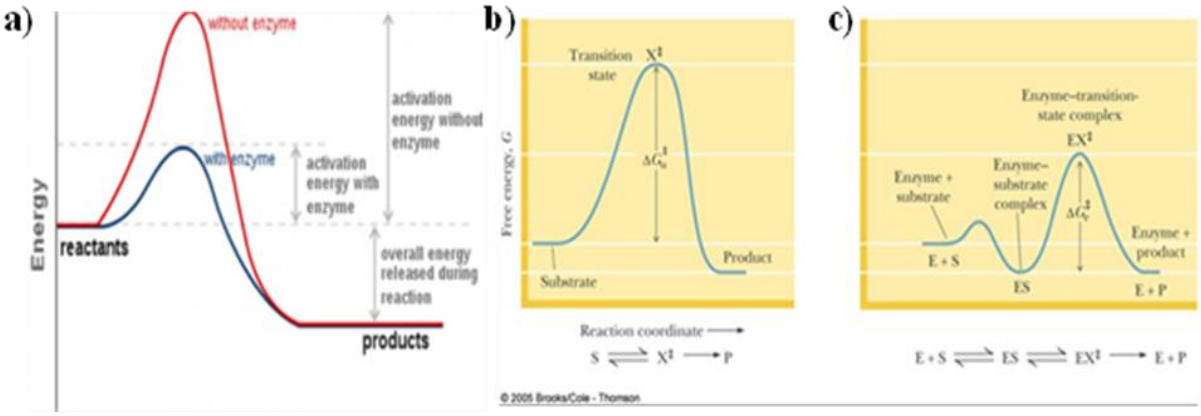



**Figure 2: a)** Enzymes catalyze reactions by lowering the activation energy. Free energy of activation for **b)** the uncatalyzed reaction, ΔGu is larger than that of **c)** the enzyme catalyzed reaction, ΔGe.

### 4.3 Kinetics of enzyme catalyzed reaction

The study of the rates of chemical reactions is called kinetics, and the study of the rates of enzyme-catalyzed reactions is called enzyme kinetics.

In 1913, the Leonor Michaelis and Maud L. Menten assumed that enzyme and substrate are in thermodynamic equilibrium with the enzyme-substrate complex, and proposed a mechanism to explain the dependence of the initial rate ($V_0$) of enzyme-catalyzed reactions on concentration.

In the beginning of the reaction, the enzyme may be present in lower quantities, whereas [S] may be in excess. The initial rate (or initial velocity), designated $V_0$ can then be explored as a function of [S]. At [S] << [E], $V_0$ increases almost linearly with an increase in [S]. At At [S] >> [E], $V_0$ increases by smaller and smaller amounts in response to increases in [S]. Finally, a point is reached beyond which increases in $V_0$ are vanishingly small as [S] increases. This plateau-like $V_0$ region is close to the maximum velocity, $V_{max}$.

At any given point, the enzyme exists in two forms, the free or uncombined form E and the combined form ES. At low [S], most of the enzyme is in the uncombined form E and the equilibrium of Equation 1 is pushed toward formation of more ES as [S] increases. The maximum initial rate of the catalyzed reaction ($V_{max}$) is observed when virtually all the enzyme is present as the ES complex and [E] is vanishingly small.

When the enzyme is first mixed with a large excess of substrate, there is an initial period, the pre-steady state, during which the concentration of ES builds up. The reaction quickly achieves a steady state in which [ES] (and the concentrations of any other intermediates) remains approximately constant over time. The concept of a steady state was introduced by G. E. Briggs and Haldane in 1925. The measured $V_0$ generally reflects the steady state and the analysis of the

initial rate is referred to as steady-state kinetics. $V_0$ is determined by the breakdown of ES to form product, which is determined by [ES]. The initial rate of product formation, $V_0$ is given by

$$V_0 = (dP/dt)_0 = k_2[ES] \qquad \text{Equation 1}$$

Considering [Et] as the total enzyme concentration (the sum of free and substrate-bound enzyme), free or unbound enzyme [E] can be represented by

$$[E] = [E_t] - [ES]$$

The idea that the combination of an enzyme with its substrate molecule to form an ES complex is a necessary step was expanded into a general theory of enzyme action, by Michaelis and Menten. They postulated that the enzyme first combines reversibly with its substrate to form an enzyme-substrate complex in a relatively fast reversible step:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \qquad \text{Equation 2}$$

The ES complex then breaks down in a slower second step to yield the free enzyme and the reaction product P:

$$ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P \qquad \text{Equation 3}$$

Early in the reaction, the concentration of the product, [P] is negligible, and we assume that the reverse reaction, P → S (described by $k_{-2}$), can be ignored. So, overall reaction reduces to

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P \qquad \text{Equation 4}$$

In 1924, George Briggs and John Haldane suggested the steady-state assumption. In a steady state, the concentrations of intermediates, i.e. [ES] remain the same even if the concentrations of reactants and products are changing. This steady state is reached when the rates of formation and breakdown of the ES complex are equal.

$$\text{Rate of formation of ES} = k_1[E][S] = k_1([E_t] - [ES])[S] \qquad \text{Equation 5}$$

$$\text{Rate of breakdown of ES} = (k_{-1} + k_2)[ES] \qquad \text{Equation 6}$$

Under steady-state assumption,

$$k_1([E_t] - [ES])[S] = (k_{-1} + k_2)[ES] \qquad \text{Equation 7}$$

Rearranging Equation 7, we get

$$k_1[E_t][S] - k_1[ES][S] = (k_{-1} + k_2)[ES]$$
$$k_1[E_t][S] = (k_1[S] + k_{-1} + k_2)[ES]$$

$$k_1[E_t][S] = (k_1[S] + k_{-1} + k_2)[ES] \qquad \text{Equation 8}$$

Solving for [ES], we obtain

$$[ES] = \frac{k_1[E_t][S]}{k_1[S] + k_{-1} + k_2} \qquad \text{Equation 9}$$

Dividing Equation 9 by $k_1$, we get

$$[ES] = \frac{[E_t][S]}{[S] + (k_{-1} + k_2)/k_1} \qquad \text{Equation 10}$$

The term $(k_{-1} + k_2)/k_1$ is defined as the Michaelis constant, Km. When $k_2$ is rate-limiting, $k_2 << k_{-1}$ and $K_m$ reduces to $k_{-1}/k_1$, which is known as the **dissociation constant**, $\mathbf{K_d}$, of the ES complex.

Therefore, Equation 10 can be written as

$$[ES] = \frac{[E_t][S]}{K_m + [S]} \qquad \text{Equation 11}$$

Substituting the right side of Equation 11 for [ES] in Equation 1, we get

$$V_0 = \frac{k_2[E_t][S]}{K_m + [S]} \qquad \text{Equation 12}$$

Because the maximum velocity occurs when the enzyme is saturated (that is, with $[ES]=[E_t]$), $V_{max}$ can be defined as $k_2[E_t]$. Substituting this in Equation 12 gives:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \qquad \text{Equation 13}$$

This is known as the Michaelis-Menten equation, the rate equation for a one-substrate enzyme-catalyzed reaction. It tells about the quantitative relationship between the initial velocity $V_0$, the maximum velocity $V_{max}$, and the initial substrate concentration [S], all related through the Michaelis constant $K_m$ ($K_m$ has units of molar concentration).

When $V_0$ is exactly one-half $V_{max}$, then

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]} \qquad \text{Equation 14}$$

$$\frac{1}{2} = \frac{[S]}{K_m + [S]} \qquad \text{Equation 15}$$

Solving for $K_m$,

$$K_m + [S] = 2[S] \text{ or, } K_m = [S], \text{ when } V_0 = V_{max}/2 \qquad \text{Equation 16}$$

Thus, $K_m$ is equivalent to the substrate concentration at which $V_0$ is one-half $V_{max}$.

### *4.3.1 Transformations of the Michaelis–Menten Kinetics*

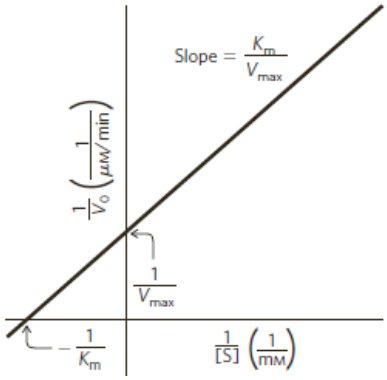
**1. Lineweaver–Burk or double-reciprocal plot**

It was suggested by H. Lineweaver and Dean Burk, obtained from Equation 13 by employing the double-reciprocal plot of $1/V_0$ versus $1/[S]$.

By taking the reciprocal of both sides of the Michaelis-Menten, Equation 13 gives

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \qquad \text{Equation 17}$$

**Figure 3:**
**A double-reciprocal or Lineweaver–Burk plot**




A plot of $1/V_0$ versus $1/[S]$, yields a straight line with a slope of $K_m/V_{max}$ and a y-intercept of $1/V_{max}$ and x-axis intercept of $-1/K_m$.

**2. Eadie–Hofstee plot (Woolf-Eadie-Augustinsson-Hofstee or Eadie- Augustinsson plot)**

It was obtained by multiplying both sides of Equation 17 by $V_0V_{max}$, i.e.

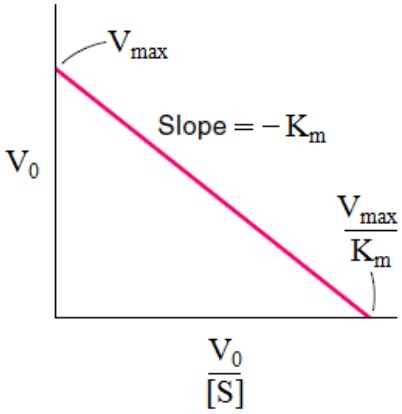
$$V_{max} = \frac{K_m V_0}{[S]} + V_0$$

Rearrangement gives,

$$V_0 = V_{max} - \frac{K_m V_0}{[S]} \qquad \text{Equation 18}$$

A plot of $V_0$ versus $V_0/[S]$, gives a straight line with slope equal to $-K_m$ and intercepts $V_{max}$ on the $V_0$ axis and $V_{max}/K_m$ on the $V_0/[S]$ axis.

**Figure 4: Eadie–Hofstee plot**



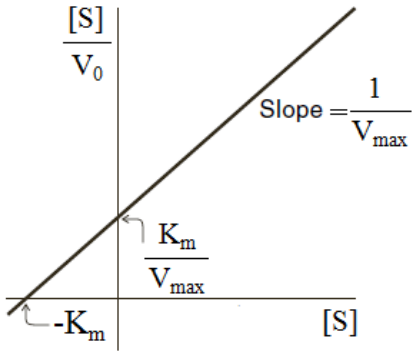
**3. Hanes-Woolf (Langmuir) plot**

It was obtained by multiplying both sides of Equation 17 by [S], i.e.

$$\frac{[S]}{V_0} = \frac{[S]}{V_{max}} + \frac{K_m}{V_{max}} \qquad \text{Equation 19}$$

A plot of $[S]/V_0$ versus [S], gives a straight line with slope equal to $1/V_{max}$ and intercepts $K_m/V_{max}$ on the $[S]/V_0$ axis and $-K_m$ on the [S] axis.

**Figure 5: Hanes-Woolf plot**

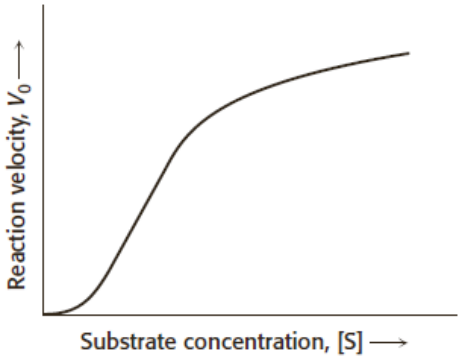



**4.3.2 Allosteric enzymes do not obey Michaelis–Menten kinetics**

An important group of enzymes that do not obey Michaelis–Menten kinetics are the allosteric enzymes. These enzymes consist of multiple subunits and multiple active sites. Allosteric enzymes often display sigmoidal plots of the reaction velocity $V_0$ versus substrate concentration [S], rather than the hyperbolic plots predicted by the Michaelis–Menten equation.

In allosteric enzymes, the binding of substrate to one active site can alter the properties of other active sites in the same enzyme molecule. A possible outcome of this interaction between subunits is that the binding of substrate becomes cooperative; that is, the binding of substrate to one active site facilitates the binding of substrate to the other active sites. In addition, the activity of an allosteric enzyme may be altered by regulatory molecules that are reversibly bound to specific sites other than the catalytic sites.

**Figure 6: Kinetics for an allosteric enzyme**



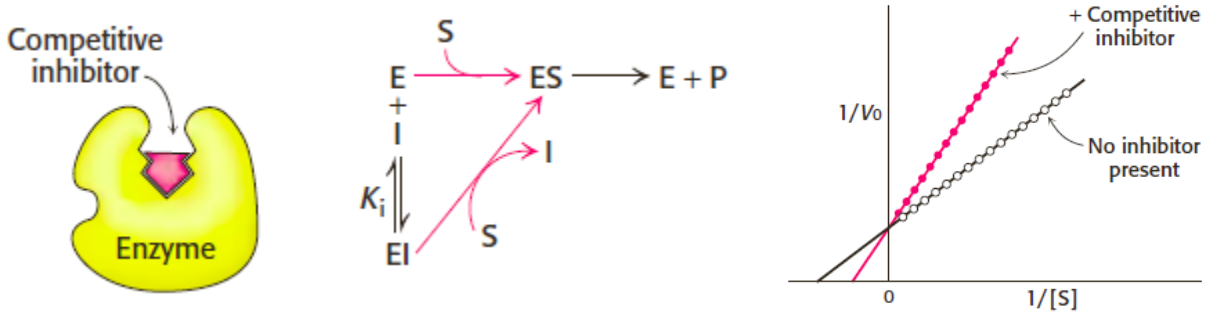
### 4.3.3 Enzyme inhibition

1) **Irreversible inhibition:** It is characterized by very slow dissociation of inhibitor from its target enzyme, as it is tightly bound to the enzyme, either covalently or noncovalently. For ex- Penicillin acts by covalently modifying the enzyme transpeptidase, thereby preventing the synthesis of bacterial cell walls and thus killing the bacteria. Aspirin acts by covalently modifying the enzyme cyclooxygenase, reducing the synthesis of signaling molecules in inflammation.

2) **Reversible inhibition**: In contrast to irreversible inhibition, it is characterized by a rapid dissociation of the enzyme–inhibitor complex. It may be of three types:

**1. Competitive inhibition:**

Inhibitor binds at the active site and thus prevents the substrate from binding. Competitive inhibition can be relieved by increasing the substrate concentration. Drugs such as ibuprofen are competitive inhibitors of enzymes that participate in signaling pathways in the inflammatory response.




The equation that describes the double-reciprocal plot for a competitive inhibitor is

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)\left(\frac{1}{[S]}\right) \qquad \text{Equation 20}$$

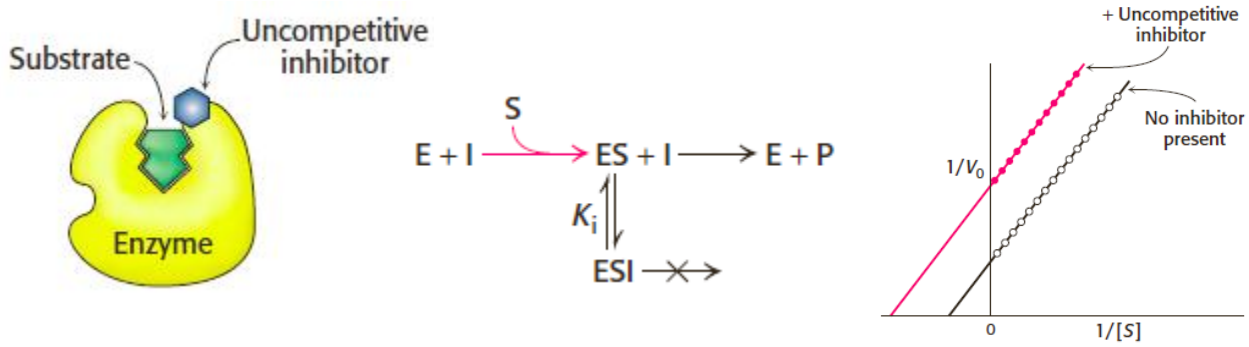
The effect of a competitive inhibitor is to increase the apparent value of $K_m$, meaning that more substrate is needed to obtain the same reaction rate. This new value of $K_m$, called $K^{app}_m$ , is numerically equal to

$$K^{app}_m = K_m(1 + [I]/K_i)$$

Where, [I] is the concentration of inhibitor and $K_i$ is the dissociation constant for the enzyme–inhibitor complex. The intercept is unchanged because a competitive inhibitor does not alter $V_{max}$. The increase in the slope of the $1/V_0$ versus 1/[S] plot indicates the strength of binding of a competitive inhibitor.

**2. Uncompetitive inhibition:**

Inhibitor binds only to the enzyme–substrate complex. Uncompetitive inhibition cannot be overcome by the addition of more substrate. The herbicide glyphosate, also known as Roundup, is an uncompetitive inhibitor of an enzyme in the biosynthetic pathway for aromatic amino acids.



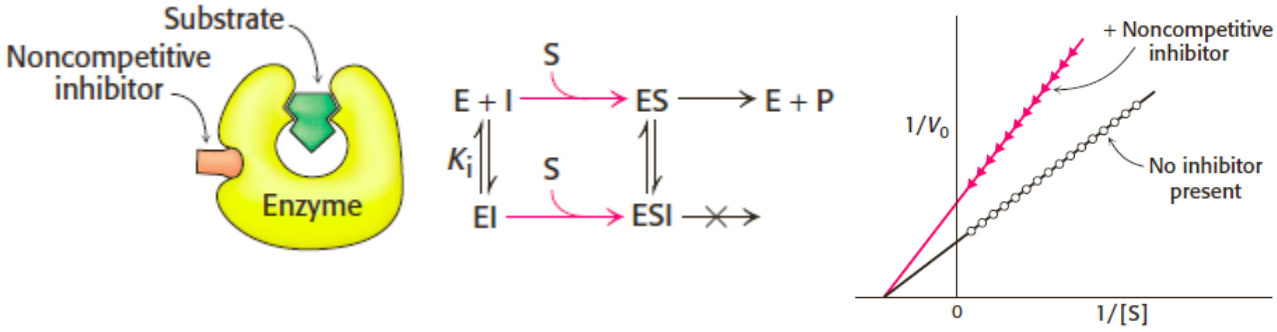

The equation that describes the double-reciprocal plot for an uncompetitive inhibitor is

$$\frac{1}{V_0} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right) \qquad \text{Equation 21}$$

The slope of the line, $K_m/V_{max}$, is the same as that for the uninhibited enzyme, but the intercept on the y-axis will be increased by $1 + [I]/K_i$. Consequently, the lines in double-reciprocal plots will be parallel.

**3. Noncompetitive inhibition:**

Inhibitor does not prevent the substrate from binding. In noncompetitive inhibition, substrate can still bind to the enzyme–inhibitor complex. However, the enzyme–inhibitor–substrate complex does not proceed to form product. Noncompetitive inhibition, like uncompetitive inhibition, cannot be overcome by increasing the substrate concentration. In noncompetitive inhibition, the inhibitor decreases the turnover number. Deoxycycline, an antibiotic, functions at low concentrations as a noncompetitive inhibitor of a proteolytic enzyme (collagenase). It is used to treat periodontal disease.



The value of $V_{max}$ is decreased to a new value called $V^{app}_{max}$, whereas the value of $K_m$ is unchanged. The maximal velocity in the presence of a pure noncompetitive inhibitor, $V^{app}_{max}$, is given by

$$V^{app}_{max} = V_{max}/(1+ [I]/K_i)$$

Thus, $V_{max}$ is lowered although $K_m$ remains unchanged.

**Table 2: Summary of Lineweaver-Burk plot for enzyme inhibition**

| Competitive inhibition | Uncompetitive inhibition | Noncompetitive inhibition |
|---|---|---|
| $K_m$ increased<br>$V_{max}$ unaffected | $K_m$ decreased<br>$V_{max}$ decreased | $K_m$ unaffected<br>$V_{max}$ decreased |

**4.4 Non-genetic Regulation of enzyme activity:**

**(a) Feedback inhibition:** An enzyme regulation process in which the final product of a metabolic pathway inhibits that pathway upstream. The end product of the pathway binds to the regulatory enzyme at the start of the pathway and decreases its activity. It prevents a cell from wasting energy as well as chemical resources by synthesizing more product than is needed.

**(b) Allosteric inhibition:** Allosteric regulation occurs when an inhibitor binds to an enzyme at an allosteric site and affects its function by permanently changing the shape of the enzyme or the active site.
Allosteric regulation may either inhibit or stimulate an enzyme's activity by

1. **Negative Allosterism** - Inhibitor binding sites alters the shape of the active site of the enzyme making it to an inactive configuration.

2. **Positive Allosterism** - Activator binding sites that alters the shape of inactive site of enzyme to an active configuration.

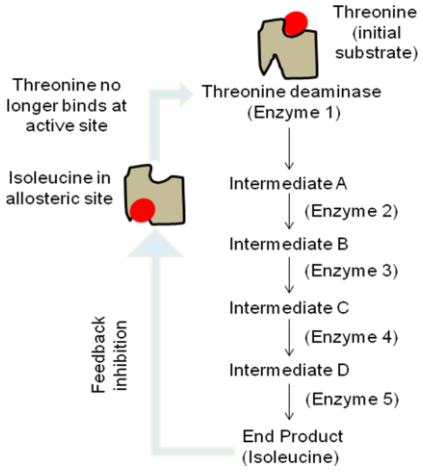



**Figure 7: Allosteric inhibition**

## 4.5 Free radicals, Antioxidants and detoxification

**4.5.1 Free radicals:** This are highly reactive and unstable molecules mainly formed within mitochondria, and associated with alternation of cellular metabolic process, through damage to various cell components such as proteins, DNA, RNA etc.

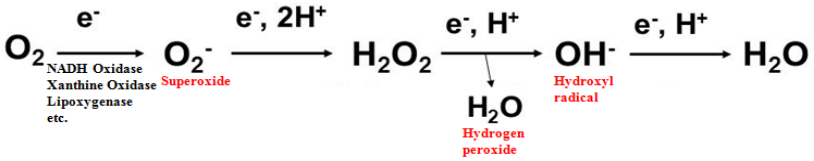
**Sources of free radicals:**

About 1-4% of oxygen taken up during cellular metabolism in the body is converted into free radicals.
There are two main sources of free radicals:

1. **Exogenous:** Ionizing and ultraviolet radiation, pollution, chemicals and diets including fat enriched processed food.
2. **Endogenous:** Several cellular processes such as metabolism, respiration, phagocytosis and inflammation

**Formation of free radicals:**

Oxygen in the body splits into single atom with unpaired electrons in the presence of different enzymes. Below is the reaction showing formation of free radicals through oxygen metabolism:




**Different types of free radicals:**

1. Superoxide, $O_2^-$
2. Hydrogen peroxide, $H_2O_2$
3. Hydroxyl radical, $OH^-$
4. Singlet oxygen, $^1O_2$
5. Hydroperoxy radical, $HOO^-$
6. Lipid peroxide radical, $ROO^-$
7. Nitric oxide, $NO^-$
8. Peroxynitrite, ONOO-

**Properties of free radicals:**

Most of the free radicals are derivatives of oxygen (Reactive Oxygen Species, ROS) in biological systems, but nitrogen derived free radicals (Reactive Nitrogen Species, RNS) are also produced through reactive metabolites or intermediates.

**Main features of the free radicals:**

1. Highly reactive
2. Very short half-life
3. Generate new radicals by chain reactions
4. Cause damage to biomolecules, cells and tissues

**Harmful effects of free radicals**:

1. Different forms of free radical cause oxidation of sulfhydryl groups and modification of amino acid, ultimately lead to fragmentation and conformational changes of protein.
2. Free radicals cause lipid peroxidation (oxidative degradation of lipid).
3. Glycation increases the susceptibility of proteins to the attack by free radicals.
4. Free radicals cause DNA strand breaks, fragmentation of bases that lead to cytotoxicity and mutations.
5. Free radicals are associated with cause and causal of different diseases such as cardiovascular diseases, cancers, inflammatory diseases, respiratory diseases etc.

### 4.5.2 Antioxidants

The substances that significantly delay or reduce the oxidation of the substrate, and lower the effect of oxidants through binding together with harmful molecules, and ultimately reduce the oxidant destructive power. Antioxidants are also known as the scavengers of free radicals and help in damage repair by different ways i.e. prevent the transfer of electron from O2 to organic molecules, stabilize free radicals and terminate free radical reactions.

**Types of antioxidants:**

- **Plasma/nutrient antioxidants:** Such antioxidants are supplemented through food such as β-carotene, α-tocopherol, ascorbic acid (Vitamin C) etc. Some of them are derived during metabolic processes (metabolic antioxidants), for instance uric acid, ceruloplasmin, bilirubin, transferrin, ferritin, albumin and glutathione.
- **Cell membrane antioxidants**: These include α-tocopherol (Vitamin E).
- **Intracellular/enzymatic antioxidants:** These include mainly enzymes such as glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase etc.

**Sources of antioxidants:**

Meats enriched with Coenzyme Q, Selenium enriched sea foods, whole grains, green tea, grape seeds, onions, walnuts, pomegranates etc.

### 4.5.3 Detoxification:

This includes physiological and psychological processes through which the body identifies, neutralize, and eliminate harmful substances such as toxins, toxic metabolites, habits and patterns. Generally, these toxins are grouped under following general categories: antinutrients,

internal metabolic toxins, heavy metals, chemicals, allergens, infectious organisms, social, emotional, and spiritual challenges.

Diet-derived products or cellular metabolites are eliminated through liver. Detoxification process is carried out with help of antioxidants and detoxifying enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase. Liver and kidney are the main organs involved in detoxification and facilitation of xenobiotics (foreign drugs or chemicals) excretion. Cytochrome P450 and associated molecules are crucial components of the microsomal monooxygenase system, which is involved in metabolizing potentially toxic compounds, such as drugs and bilirubin.

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