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PAPER – VI – PHYTOCHEMISTRY

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Unit – II

Enzymology - Classification and nomenclature of enzymes; physico-chemical properties of enzymes; cofactors and coenzymes; isozymes; kinetics of enzyme action; significance of Km; Mechanism of enzyme action (Lock and key hypothesis and Induced fit model) factors affecting enzyme activity, Allosteric modification and feedback regulation.

Vitamins: Classification and Structure – Water soluble (B complex and Ascorbic acid) and fat soluble (A, D, E and K), Deficiency symptoms and Sources of vitamin.

Definition of Enzymes:

Enzymes are biocatalysts of proteinous nature, which accelerate the rate of biochemical reactions but do not affect the nature of final product. Like catalyst the enzymes regulate the speed and specificity of reaction without being used up but unlike catalysts enzymes are produced by the living cells only. Like catalysts enzymes also influence the rate of biochemical reaction so that they can take place at a relatively low temperature. Thus the enzymes are known to lower the activation energy. In many cases enzymes initiate the biological reaction. Louis Pasteur was the first to recognize the importance of enzymes while studying the fermentation process and denoted it as –ferment‖-an integral part of living cells.

It was Edward Buchner who in 1897 extracted the enzyme from yeast cells, responsible for fermentation of sugar to alcohol. In 1926, James B. Sumner isolated and crystallized urease and also postulated that all enzymes are proteins. Enzymes are the large globular proteins with molecular weight ranging from 13,000 to millions Dalton. The catalytic efficiency of an enzyme depends upon its three dimensional conformation. Moreover, these biocatalysts are highly specific for the reaction as well as other conditions e.g. each enzyme has its own optimum pH, temperature, etc. for maximum performance. Some enzymes require an additional moiety known as cofactors for their biological activity.

Classification and nomenclature of enzymes

The International Union of Biochemistry and Molecular Biology is entrusted with designating names to enzymes in addition to assigning a number in order to identify them.

Apart from a few originally studied enzymes such as rennin, pepsin and trypsin, almost all the enzyme names end in –ase‖. As per the standards, focal points of nomenclature of enzymes are both the type of reaction catalyzed and the substrate acted upon.

Most commonly, enzymes are names to provide data on the function as opposed to the structure of the enzyme. However, there are 3 significant features of nomenclature process of enzymes, which are:

- Suffix -*ase* recognizes a substance as that of an enzyme
    - Suffix -in is observed in the name of first enzymes learnt as pepsin, chymotrypsin, trypsin
- Prefix is identified by the type of reaction the enzyme catalyzes
    - Enzyme hydrolase : catalyzes a hydrolysis reaction
    - Enzyme oxidase : catalyzes an oxidation reaction
- In addition to the type of reaction, the identity of the substrate is taken into consideration
    - Glucose oxidase – catalysis of glucose oxidation
    - Lactate dehydrogenase – catalysis of eliminating hydrogen from lactate ion
- Some times, the reaction type is given instead of substrate‖
    - Lactase – hydrolysis of lactose is catalyzed
    - Urease – hydrolysis of urea is catalyzed

Example of Naming:

As per the standard International Union of Biochemistry, name of enzyme comprises 2 parts –

1. Name of the substrate for the enzyme

2. Type of reaction catalyzed by the enzyme. The second part therefore, ends with ‑ase‖ suffix

Example – Lactate dehydrogenase

Conventions of Naming – EC Numbers

The nomenclature developed by the International Union of Biochemistry and Molecular Biology has something called EC numbers where each enzyme is preceded by EC. The first number in this series classifies this enzyme on the basis of its mechanism.

EC Numbers

There are six groups of classification of enzymes as per the reaction that is being catalyzed. Therefore, all enzymes are designated as ‑EC number‖. This classification does not consider protein structure, amino acid sequence or even the chemical mechanism.

EC number is a 4 digit number for instance – a.b.c.d. Here ‑a‖ is class, ‑b‖ is subclass, ‑c‖ is sub-subclass and ‑d‖ is the sub-sub-subclass. The ‑b‖ and ‑c‖ part of the EC number describes the reaction, ‑d‖ differentiates between different enzymes with similar function on the basis of the actual substrate in the reaction.

Example – EC number of Alcohol: NAD+ oxidoreductase is 1.1.1.1

Six Classes of Enzymes – Enzyme Classification

- **EC 1. Oxidoreductases**
- **EC 2. Transferases**
- **EC 3. Hydrolases**
- **EC 4. Lyases**
- **EC 5. Isomerases**
- **EC 6. Ligases**

Classification of Enzymes:

In older times enzymes were classified into two broad categories:

(i) Hydrolysing:

Catalysing hydrolysis of larger molecules into smaller ones, e.g., carbohydrates or amylases, proteases, lipases, esterases, phosphorylases, amidases. Digestive enzymes are hydrolysing in nature. They are often grouped into three types— proteolytic, amylolytic and lipolytic,

(ii) Desmolysing:

Catalysing reactions other than hyrolysis, e.g., aldolases, dehydrogenases, oxidases, peroxidases, catalases, carboxylases, etc. The modem system of enzyme classification was introduced by International Union of Biochemistry (IUB) in 1961. It groups enzymes into the following six categories.

a. Oxidoreductases:

They take part in oxidation and reduction reactions or transfer of electrons.

$$\text{S reduced} + \text{S}' \text{ oxidised} \xrightarrow{\text{oxido reductase}} \text{S oxidised} + \text{S}' \text{ reduced}$$

Oxidoreductases are of three types— oxidases, dehydrogenases and reductases, e.g., cytochrome oxidase (oxidises cytochrome), succinate dehydrogenase, nitrate reductase.

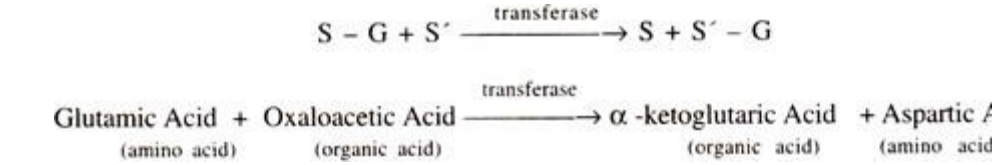
$$2AH_2 + O_2 \xrightarrow{\text{oxidase}} 2A + 2H_2O$$

$$AH_2 \xrightarrow{\text{dehydrogenase}} A + 2(H)$$

$$A + 2(H) \xrightarrow{\text{reductase}} AH_2$$

b. Transferases:

They transfer a group from one molecule to another e.g., glutamate- pyruvate transaminase (transfers amino group from glutamate to pyruvate during synthesis of alanine). The chemical group transfer does not occur in the Free State.

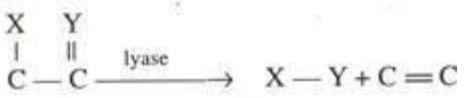


c. Hydrolases:

They catalyse hydrolysis of bonds like ester, ether, peptide, glycosidic, C-C, C halide, P—N, etc. which are formed by dehydration condensation. Hydrolases break up large molecules into smaller ones with the help of hydrogen and hydroxyl groups of water molecules. The phenomenon is called hydrolysis. Digestive enzymes belong to this group, e.g., amylase (hydrolysis of starch), sucrase, lactase.

d. Lyases:

The enzymes cause cleavage, removal of groups without hydrolysis, addition of groups to double bonds or removal of a group producing double bond, e.g., histidine decarboxylase (breaks histidine to histamine and CO_2), aldolase (fructose-1, 6-diphosphate to dihydroxy acetone phosphate and glyceraldehyde phosphate).



Fructose 1, 6-diphosphate – aldolase → Dihydroxy acetone phosphate + Glyceraldehyde phosphate.

e. Isomerases:

The enzymes cause rearrangement of molecular structure to effect isomeric changes. They are of three types, isomerases (aldose to ketose group or vice-versa like glucose 6-phosphate to fructose 6-phosphate), epimerases (change in position of one constituent or carbon group like xylulose phosphate to ribulose phosphate) and mutases (shifting the position of side group like glucose-6-phosphate to glucose-1- phosphate).

$$\text{Glucose 6-phosphate} \xrightarrow{\text{isomerase}} \text{Fructose 6-phosphate}$$

$$\text{Glucose 6-phosphate} \xrightarrow{\text{mutase}} \text{Glucose 1-phosphate}$$

f. Ligases (Synthetizes):

The enzymes catalyse bonding of two chemicals with the help of energy obtained from ATP resulting in formation of such bonds as C-O, C-S, C-N and P-O, e.g., pyruvate carboxylase. It combines pyruvic acid with CO_2 to produce oxaloacetic acid.

$$\text{Pyruvic acid} + CO_2 + ATP + H_2O \xrightleftharpoons{\text{Pyruvate Carboxylase}} \text{Oxaloacetic acid} + ADP + Pi$$

Table 2. IUB classification of enzymes

Major Class (Type of reaction catalyzed)	Common exmaples	Kind of reaction	Specific Example
1. ***Oxidoreductases*** (*Transfer of electrons*)	Oxidases Reductases Dehydrogenase	$A^{+3} + B^{+2} \rightarrow A^{+2} + B^{+3}$	Alcohol + NAD ↓ *Alcohol dehydrogenase* Aldehyde + $NADH_2$
2. ***Transferases*** (*Transfer of functional groups*)	Transaminase Transketolase Transaldolase	$A-X+B \rightarrow A+B-X$	Glucose + ATP ↓ *Glukokinase or hexokinase* Glucose-6-Phosphate + ADP
3. ***Hydrolases*** (*Hydrolysis Reactions*)	Amylases Lipases Proteases Nucleases	$A{-}B+H_2O \rightarrow A{-}OH + B{-}H$	Sucrose ↓ *Sucrase* Glucose + Fructose
4. **Lyases or *Desmolases*** (*Group elimination to form double bonds without hydrolysis*)	Aldolase Decarboxylase Fumarase Citrate synthase	$\underset{X}{A}-\underset{Y}{B} \rightarrow A=B+X-Y$	Histidine ↓ *Histidine decarboxylase* Histidine + CO_2
5. ***Isomerases*** (*Transfer of Groups within a molecule*)	Isomerase Mutase Epimerase	$\underset{Y}{A}-\underset{X}{B} \rightarrow \underset{X}{A}-\underset{Y}{B}$	Glucose – 6-Phosphate ↓ *Isomerase* Fructose-6-Phosphate
6. ***Ligases or Synthetases*** (*Bond formation couples with ATP hydrolysis*)	Synthetases Carboxylases	$A+B+ATP \rightarrow$ $A-B+ADP+Pi$	Pyruvate + CO_2 + ATP ↓ *Pyruvate carboxylase* Oxaloacetate + ADP + Pi

Properties Of Enzymes Can Be Classified Into:

1. Physical properties
2. Chemical Properties
3. General properties

Physical Properties of Enzymes

- Physically enzymes behave as colloids or as substance of high molecular weight.
- Enzymes are destroyed or inactivated at temperature below the boiling point of water.
- At 60 degrees Celsius most enzymes in liquid medium are inactivated.
- Dried enzymes extract can endure temperature 100 degree Celsius to 120 degrees Celsius or even higher. Thus enzymes are thermos-labile.
- There is always a specific temperature of optimum activity of every enzyme, which usually ranges from 25 degrees Celsius to 45 degrees Celsius. Enzymatic action is highest at 37 degrees Celsius and enzymes become inactive when temperature rises above 60 degrees Celsius.

Chemical Properties Of Enzymes

- Catalytic properties: Enzymes are biological catalyst. The small quantity of enzymes catalyses the larger quantities of substances. It means, enzymes have high capability to convert giant quantities of substrate into product. Enzymes increase the rate of reaction and remain unaffected by the reaction which they catalyse.
- Specificity of enzyme: Enzymes are highly specific in nature, i.e., a particular enzyme can catalyse a particular reaction. For example, Enzyme sucrase can catalyse only hydrolysis of sucrose.

General Properties Of Enzymes

- Enzymes initiate and accelerate the rate of biochemical reaction.

- The activity of enzymes depends upon the acidity of medium (pH specific). Each catalyst is most active at a specific pH. For example, pH 2 for pepsin, pH 8.5 for trypsin. Most intracellular enzymes function at near neutral pH.
- Enzymes can accelerate the reaction in either direction.
- All enzymes possess active sites which participate in the biochemical reactions.
- Enzymes are very unstable compounds mostly soluble in water, dilute glycerol, NaCl and dilute alcohol.
- Enzymes act actively at optimum temperature.
- All enzymes are protein in nature but all proteins may not be an enzyme.
- Enzymes lower the energy of activation of the substance molecule so the biochemical reaction can take place at normal body temperature which is 37 degrees Celsius.

Chemical Nature of Enzymes

All enzymes are proteins, however all proteins are not enzymes. However, there are some conjugated enzymes with a non-protein moiety attached to the protein part of enzyme, which is called Apo enzyme. The non-protein part is known as co factor. If the co factor is of inorganic nature like potassium calcium, magnesium, manganese it is known as prosthetic group. Prosthetic group is generally tightly bound to the protein part of enzyme and it is difficult to separate it with simple method like diffusion. The enzyme with prosthetic group and Apo enzyme is called holoenzyme.

If co factor attached to an enzyme protein is organic moiety like NADP, NAD, FAD, etc , it is called coenzyme. A co enzyme is generally loosely bound to Apo enzyme and can easily be separated than prosthetic group. Co enzymes are heat resistant also.

Difference between Apo enzyme and co-factor:

- Apo enzyme is protein part of enzyme hence a macromolecule whereas co factor is the non protein part of enzyme hence a micro molecule.

- Apo enzyme is an enzyme which acts only in presence of a co-factor whereas co-factor may be metal ion or a complex organic compound makes an Apo enzyme functional.
- Apo enzyme is thermo-labile whereas co-factor is thermostable.
- Apo enzyme conducts enzymatic activities whereas co-factor moulds the enzyme or co enzyme or carries the groups removed from the substrate.
- Apo enzyme is specific for an enzyme system whereas co-factor work with many enzymes.

The Most Important Properties Of An Enzyme Are:

1. Catalytic Property
2. Specificity
3. Reversibility
4. Sensitiveness to heat and temperature and pH

Catalytic Property:

Enzymes have extra-ordinary catalytic power. They are active in very small quantities. A small amount of enzyme is enough to convert a large quantity of substrate. The enzymes remain unchanged after the reaction. The turnover number of enzymes ranges from 0.5 to 600000. Turn over number is the number of substrate molecules converted by one molecule of enzymes per second when its active site is saturated with substrate.

Specificity:

Enzymes are very specific in their action. Particular enzymes act on particular substrates only. Enzymes are also specific to a particular type of reaction. In some rare cases, the specificity may not be too strong. Enzymes show different types of specificity as follows:

1. Bond Specificity: It is also called as relative specificity. Here the enzymes are specific for a bond. eg; peptidase is specific or peptide bond, lipase is specific for ester bond in a lipid.

2. Group Specificity: It is also called structural specificity. Here the enzymes are specific for a group. eg; pepsin hydrolyse the peptide bonds in with the amino group belongs to aromatic amino acids.

3. Substrate Specificity: It is also called absolute specificity. Here the enzyme acts only on a particular substrate. eg; arginase acts only on arginine; carbonic anhydrase acts only on carbonic acid.

4. Optical Specificity: It is also called stereo-specificity. This is the highest specificity shown by an enzyme. Here the enzymes are specific not only to the substrate but also to its optical configuration. e.g. L amino acid oxidase acts only L-amino acids, not on D-amino acids. Similarly, the alpha-amylase act only on alpha-14 glycosidic linkage of starch and glycogen. It is not able to hydrolyse the beta-14 glycosidic linkage of cellulose.

5. Co-factor Specificity: This shows that enzymes are not only specific to the substrate but also specific to its co-factors.

6. Geometric Specificity: Here the specificity is very less. Some enzymes will work with a small range of similar substrates having similar structural geometry. e.g. alcohol dehydrogenase can oxidise methanol and n-propanol to aldehydes.

Reversibility:

Most of the enzymes catalysed reactions are reversible. The reversibility of the reaction depends upon the requirements of the cell. In some cases, there are separate enzymes for forward and reverse reaction. Some enzyme-catalysed reactions are not reversible.

Sensitivity To Heat, Temperature And pH:

Enzymes are very sensitive to heat and temperature. They are thermolabile. The maximum activity of Associate in Nursing protein is at traditional temperature. The correct temperature for the utmost activity is termed optimum temperature. Enzymes will

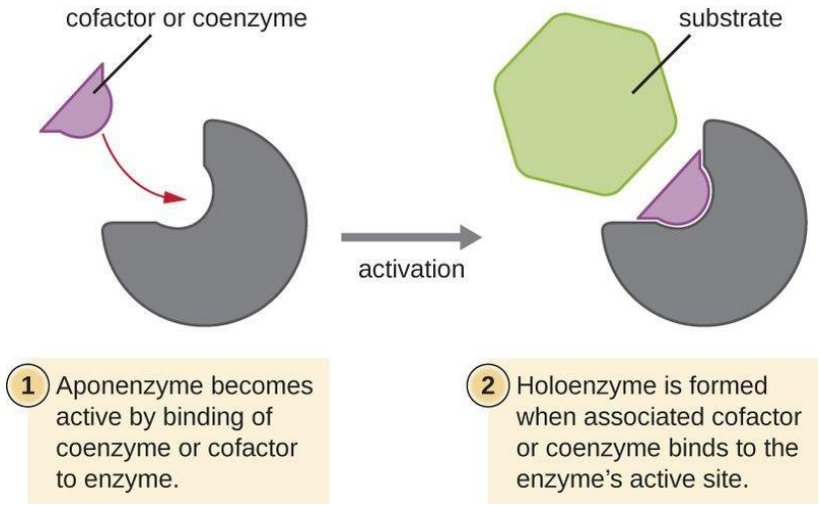
be inactive at very low temperatures; this is the reason for preserving food and vegetables in the refrigerator. The enzymatic activity increases with the increase in temperature up to a certain level. At higher temperature (60-70 degree Celsius), the enzyme is destroyed or denatured. Do you know an enzymes active at very high temperature? It is Taq-Polymerase used in PCR reactions. The optimum temperature for it is 75 to 80 degrees Celsius.

The optimum pH of most endo-enzyme is pH 7.0 (neutral pH). However, digestive enzymes can function at different pH. For example, salivary amylase act best at pH 6.8, pepsin act best at pH2 etc. Any fluctuation in pH scale from the optimum causes ionization of R-groups of amino acids that decrease the protein activity. Sometime a change in pH causes the reverse reaction, e.g. at pH 7.0 phosphorylase break down starch into glucose 1- phosphate while at pH5 the reverse reaction occurs.

Coenzymes:

Many reactions of substrates are catalyzed by enzymes only in the presence of a specific non-protein organic molecule called the coenzyme. Coenzymes combine with the apoenzyme (the protein part) to form holoenzyme. The coenzymes are also regarded as co-substrates.

Coenzymes are heat-stable, dialyzable non-protein organic molecules and the prosthetic groups of enzymes.



- **Coenzymes** are nonprotein organic molecules that bind loosely to an enzyme. Many (not all) are vitamins or are derived from vitamins. Many coenzymes contain adenosine monophosphate (AMP). Coenzymes may be described as either cosubstrates or prosthetic groups.
- **Cofactors** are inorganic species or at least nonprotein compounds that aid enzyme function by increasing the rate of catalysis. Typically, cofactors are metal ions. Some metallic elements have no nutritional value, but several trace elements function as cofactors in biochemical reactions, including iron, copper, zinc, magnesium, cobalt, and molybdenum. Some trace elements that appear to be important for nutrition do not appear to act as cofactors, including chromium, iodine, and calcium.
- **Cosubstrates** are coenzymes that bind tightly to a protein, yet will be released and bind again at some point.
- **Prosthetic groups** are enzyme partner molecules that bind tightly or covalently to the enzyme (remember, coenzymes bind loosely). While cosubstrates bind temporarily, prosthetic groups permanently bond with a protein. Prosthetic groups help proteins bind other molecules, act as structural elements, and act as charge carriers. An example of a prosthetic group is heme in hemoglobin, myoglobin, and cytochrome. The iron (Fe) found at the center of the heme prosthetic group allows it to bind and release oxygen in the lung and tissues, respectively. Vitamins are also examples of prosthetic groups.
- **Isozymes** (also known as **isoenzymes** or more generally as **multiple forms of enzymes**) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters (e.g. different K_M values), or different regulatory properties.

Characteristics of Enzymes:

All enzymes are proteins, but a functional enzyme has different components and these components are named differently, viz.,

Holoenzyme:

A conjugated protein and functional enzyme.

Apoenzyme:

Polypeptide segment of the enzyme, which is catalytically inactive.

Coenzyme:

The non-protein organic moiety, which can frequently be separated from the apoenzyme.

Prosthetic group:

If a substance is firmly (covalently) attached to the protein part of the enzyme, it is referred to as a prosthetic group. It is the non-protein portion of any conjugated protein. So coenzyme is a specific example of prosthetic group.

Activator:

There are many metalloprotein enzymes in which the metal ion (e.g. Mg^{++}, Mn^{++}, and Zn^{++}) is bonded either to the apoenzyme or to the coenzyme. The metal is usually designated as activator. They form a co-ordination complex between the enzyme and the substrate, and activate the substrate by prompting electronic shifts.

Pro-enzyme or Zymogens:

They are simple protein enzymes, which are secreted, in an inactive form.

Activation:

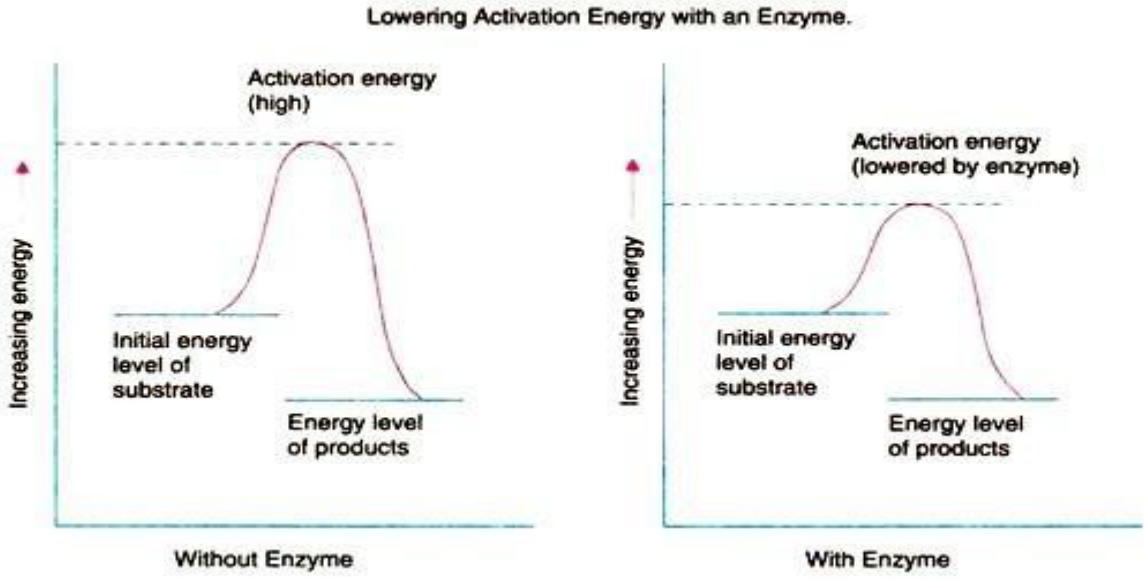
It is the process in which an inactive protein (pro-enzyme or zymogens) is transformed into an active enzyme.

Mechanism of Enzyme Action:

Enzyme is active in catalytic action of biochemical reaction. They act on substrate and forms a complex after interactions with the enzyme is called active center. The enzyme and substrate forms a complex at the active centre. This binding action makes both enzyme and substrate stable. The interaction between substrate and enzyme may be either ionic bonds and hydrogen bonds or Van der Waal forces. The active sites of enzyme have some special groups such as NH_2 COOH, -SH etc. which bind the substrate though above bonds to form a transitional (intermediate) compound called enzyme-substrate complex (ES). This reaction is exergonic and releases some energy which raises energy level of the substrate molecule.

Thus, activating the substrate molecule and the phenomenon is known as activation energy or energy of activation as shown below

$$\underset{\text{(Enzyme)}}{E} + \underset{\text{(Substrate)}}{S} \longleftrightarrow E.S \text{ Complex}$$



Types of Mechanisms of Enzymes:

There are two types of mechanisms involved to explain substrate-enzyme complex formation; lock and key theory (template model), and induced-fit theory.

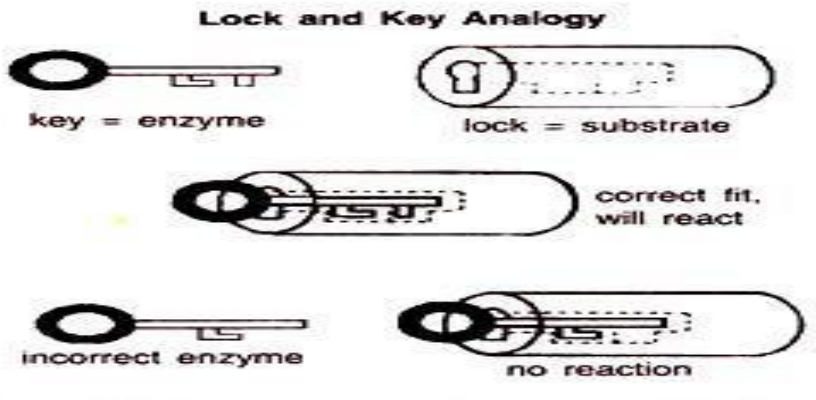
(i) Lock and Key Theory:

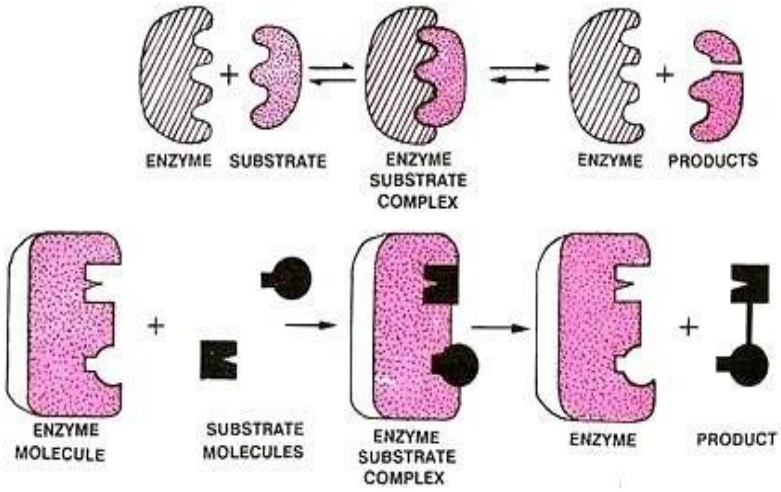
Emil Fischer (1894) explained the specific action of an enzyme with a single substrate using a theory of Lock and Key analog . According to this theory, reaction of sub-state and enzyme is analogous to lock and key.

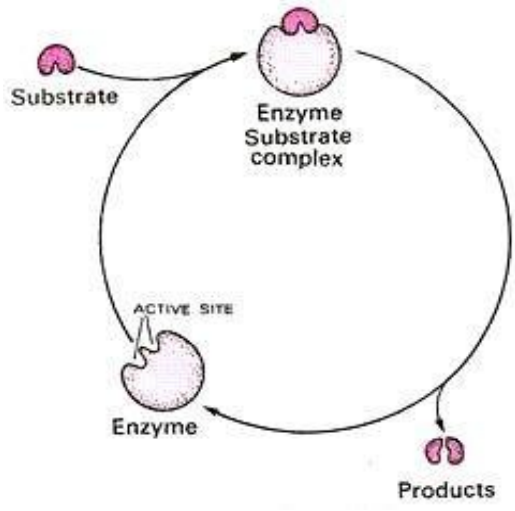
Enzyme is analogous to key, where the geometrical configuration of socket is fixed. Similarly substrate has also got fixed geometrical configuration like that of key. A particular lock can be opened or closed by a particular key. According to the particular substrate can be found at active site of particular enzyme forming substrate-enzyme complex.

Enzyme-substrate complex remains in tight fitting and active sites of enzymes are complementary to substrate molecules. Subsequently, enzyme-substrate complexes result in the transformation of substrate into the product formation due to activity of reaction sites. Since product has lower free energy, it is released. Enzymes are fixed to receive another molecule of substrate and thus enzyme activity continues. In this analogy, the lock is the substrate and the key is the enzyme. 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 as shown below







Enzyme + Substrate ⇋ Enzyme – Substrate Complex

Enzyme – Substrate Complex ⇋ Enzyme – Products Complex

Enzyme – Products Complex ⇋ Enzyme + Products

Thus the chemical reactants do not cause any alteration in the composition or physiology of the enzyme. The same enzyme molecule can be used again and again. Hence, enzymes are required in very small concentrations.

(ii) Induced Fit Theory:

It is modification of lock and key hypothesis which was proposed by Koshland in 1959. According to this theory the active site of the enzyme contains two groups, buttressing and catalytic. The buttressing group is meant for supporting the substrate. The catalytic group is able to weaken the bonds of reactants by electrophilic and nucleophilic forces. The two groups are

normally at a distance. As soon as the substrate comes in contact with the buttressing group, the active site of the enzyme undergoes conformational changes so as to bring the catalytic group opposite the substrate bonds to be broken. Catalytic group helps in bringing about chemical reaction. The substrate is converted into product. The product is unable to hold on the buttressing site due to change in its structure and bonds. Buttressing group reverts to its original position. The product is released.

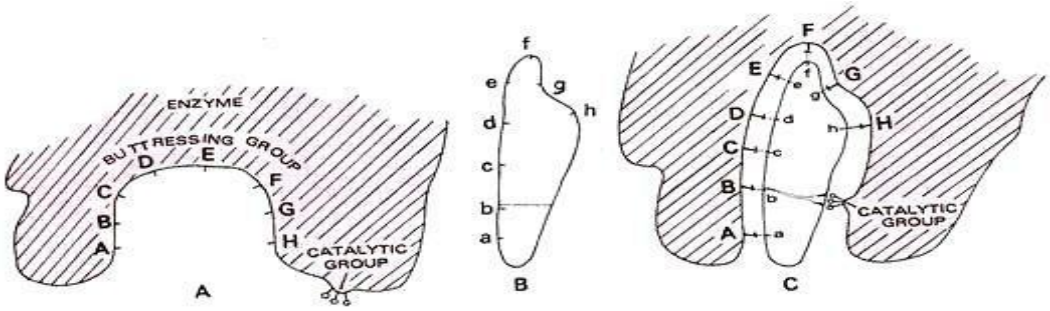
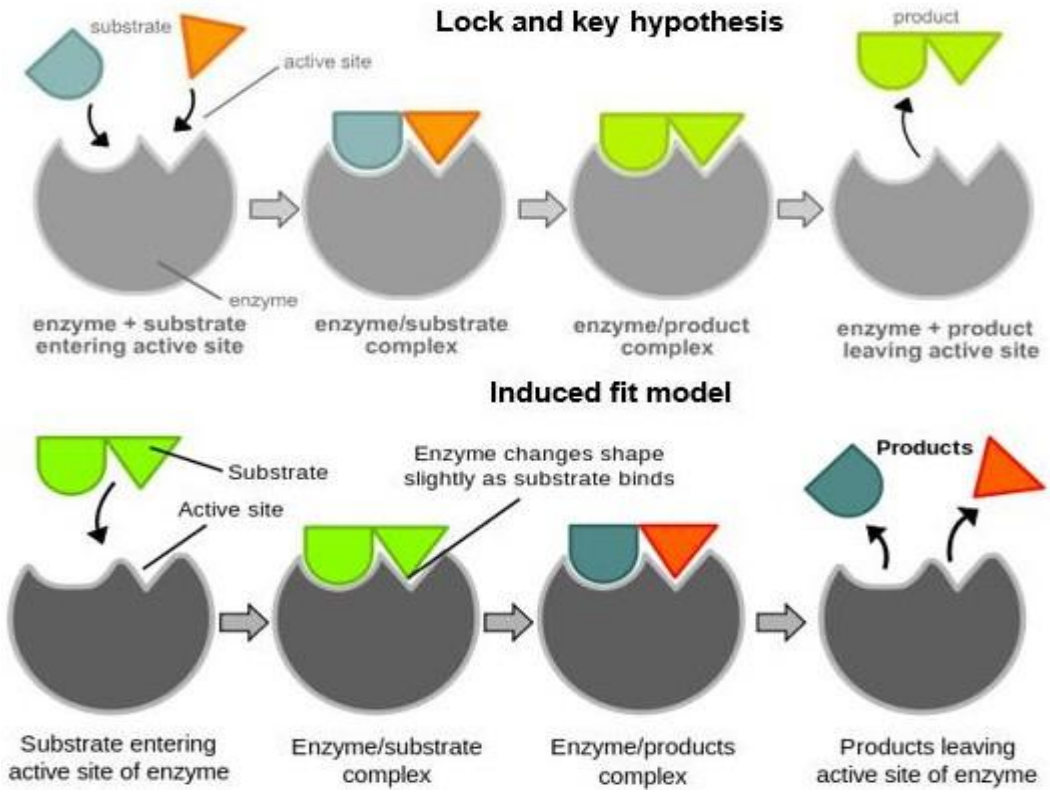


Fig. 9.35. Induced-fit theory of enzyme action. A, active site of enzyme. B, substrate molecule. C, enzyme-substrate complex with conformational changes so as to bring the catalytic group against the substrate bonds to be broken.



Enzyme Kinetics:

A number of approaches are now available to study the mechanism of enzyme action including knowledge of complete 3-D structure, site directed mutagenesis and protein engineering, still central approach is to determine the rate of reaction and how it is affected by different experimental parameters-more precisely- ‗The enzyme kinetics'.

Enzyme kinetics follows the principles of general chemical reaction kinetics; however, show a distinctive feature of saturating (Fig. 12.13). At lower substrate concentration, the initial reaction velocity is proportional to substrate concentration (1st order reaction). Further increase in substrate concentration does not affect the reaction rate and the latter became constant (zero order reaction).

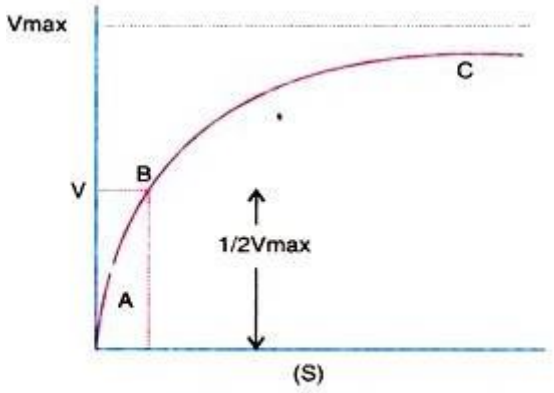


Fig. 12.13: Kinetics of enzyme – substrate relationship (Michaelis-Menten hypothesis).

In hypothetical one substrate reaction Michaelis-Menten theory, enzymes first combine with substrate to from enzyme-substrate (ES) complex.

This ES complex then breaks in second step to release free enzyme and product as:

$$E + S \underset{}{\overset{Ka}{\rightleftharpoons}} ES \qquad (1)$$

$$ES \overset{Kb}{\rightleftharpoons} E + P \qquad (2)$$

According to above equations, initial velocity of complete reaction equals the breakdown of enzyme substrate complex. Hence,

$$Vo = Kb (ES) \quad (3)$$

Where, Vo = initial velocity,

(ES) = concentration of enzyme substrate complex

However, neither of the two parameters can be determined directly, and an alternative expression of Vo is required. This can be done by considering 2″‖ order rate equation for formation of ES from E and S.

$$d(ES)/dt = Ka(E)(S) = Ka[ET] - (ES)](S) \quad (4)$$

Where, Ka= second order rate constant

(ET) = total enzyme concentration

(ES) = concentration of enzyme substrate complex

Since, starting of reaction is being considered, formation of (ES) by reaction (2) may be neglected because in the beginning for reaction in forward direction, when (S) is high and (P) is zero.

Rate equation for degradation of ES can be expressed as sum of two reactions-first reaction yielding the product and second reaction yielding E + S. Then,

$$-d(ES)/dt = Ka(ES) + Kb(ES) \quad (5)$$

However, in the steady state, when rate of formation of ES is equal to its breakdown, then equation 4 = 5

Thus:

$$(ES)/dt = -d(ES)/dt \quad (6)$$

Or, Ka [ET]-[ES] (S) = Ka (ES) + Kb (ES)

Or, [S] [(ET)-(ES)]/(ES) = Ka + Kb/Ka = Km (7)

(Ka+Kb/Ka), i.e. expressed as Km is known as Michaehs-Menten constant. Rearranging the equation 7 gives,

(ES) = (ET) (S)/Km + (S) (8)

The value of (ES), when expressed in equation:

Vo = Kb (ES)

Vo = Kb (ET) (S)/ Km + (S) (9)

When substrate concentration is high, all enzymes in system are present as ES complex.

Hence enzyme will be saturated, and reach the maximum velocity (V max), given as:

Vmax = Kb (ET)

Putting this value in equation 9, we get

Vo = Vmax (S)/Km + (S) (10)

This is Michaelis-Menten equation i.e. the rate equation for a one substrate enzyme catalyzed reaction considering special case when initial reaction rate is exactly half of Vmax, then by equation 10

Vmax/2 = Vmax (S)/Km + (s)

Or, Km + (S) = 2 (S)

Or, Km = (S)

Thus Michaelis-Menten constant is equal to substrate concentration at which initial reaction velocity is half of maximum velocity. K_m or the Michaelis-Menten constant is defined as the substrate concentration (expressed in moles/lit) to produce half-maximum velocity in an enzyme catalysed reaction.

It indicates that half of the enzyme molecules (i.e. 50%) are bound with the substrate molecules when the substrate concentration equals the K_m value.

K_m value is a constant and a characteristic feature of a given enzyme. It is a representative for measuring the strength of ES complex. A low K_m value indicates a strong affinity between enzyme and substrate, whereas a high K_m value reflects a weak affinity between them. For majority of enzymes, the K_m values are in the range of 10^{-5} to 10^{-2} moles.

Factors affecting enzyme activity

1. Concentration of Enzyme:

As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases (Fig. 66.1). In fact, this property of enzyme is made use in determining the activities of serum enzymes for diagnosis of diseases.

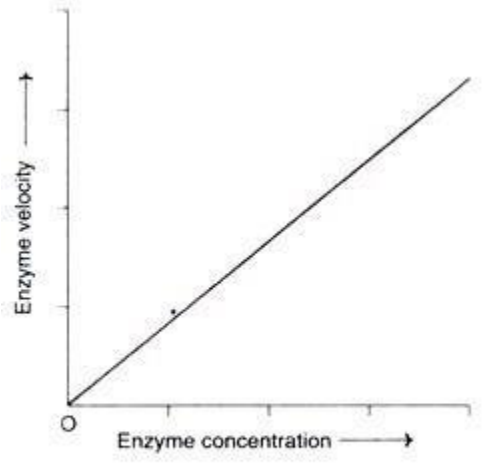


Fig. 66.1 : Effect of enzyme concentration on enzyme velocity.

2. Concentration of Substrate:

Increase in the substrate concentration gradually increases the velocity of enzyme reaction within the limited range of substrate levels. A rectangular hyperbola is obtained when velocity is plotted against the substrate concentration (Fig. 66.2). Three distinct phases of the reaction are observed in the graph

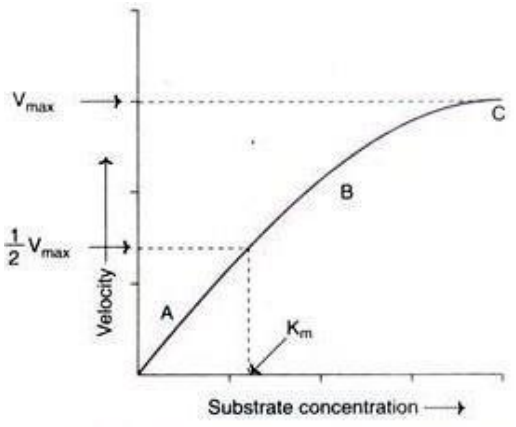


***Fig. 66.2** : Effect of substrate concentration on enzyme velocity (A–linear; B–curve; C–almost unchanged).*

3. Effect of Temperature:

Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A bell-shaped curve is usually observed (Fig. 66.4).

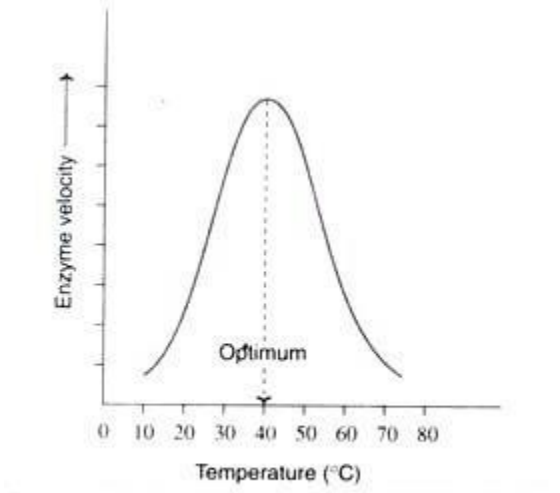


***Fig. 66.4** : Effect of temperature on enzyme velocity.*

The optimum temperature for most of the enzymes is between 40°C-45°C. However, a few enzymes (e.g. venom phosphokinases, muscle adenylate kinase) are active even at 100°C. In general, when the enzymes are exposed to a temperature above 50°C, denaturation leading to derangement in the native (tertiary) structure of the protein and active site are seen. Majority of the enzymes become inactive at higher temperature (above 70°C).

4. Effect of pH:

Increase in the hydrogen ion concentration (pH) considerably influences the enzyme activity and a bell-shaped curve is normally obtained (Fig. 66.5). Each enzyme has an optimum pH at which the velocity is maximum.

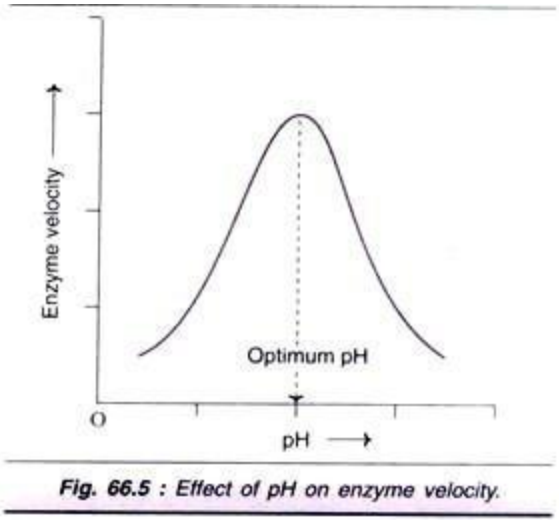


Fig. 66.5 : Effect of pH on enzyme velocity.

Most of the enzymes of higher organisms show optimum activity around neutral pH (6-8). There are, however, many exceptions like pepsin (1-2), acid phosphatase (4-5) and alkaline phosphatase (10-11) for optimum pH.

5. Effect of Product Concentration:

The accumulation of reaction products generally decreases the enzyme velocity. For certain‘ enzymes, the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the enzyme activity. In the living system, this type of inhibition is generally prevented by a quick removal of products formed.

6. Effect of Activators:

Some of the enzymes require certain inorganic metallic cations like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Na^{+}, K^{+} etc. for their optimum activity. Rarely, anions are also needed for enzyme activity e.g. chloride ion (Cl^{-}) for amylase.

Allosteric modification

Sometimes it has been found that when a series of reactions is catalysed by a number of enzymes in sequence, the accumulation of the final end-product may cause inhibition in the activity of the first enzyme of the series. This inhibition due to a compound (final end product) which is totally

different in structure from the substrate of the enzyme is called as allosteric inhibition or feedback inhibition and such an enzyme is called as allosteric enzyme.

This type of inhibition takes place due to the presence of allosteric site (Greek allo = _other‘; stereos = _space‘ or _site‘) on the surface of the allosteric enzyme away from the active site. The final end-product molecule fits in the allosteric site and in some way brings about a change in shape of the enzyme so that the active site of the enzyme becomes unfit for making complex with its substrate. The allosteric inhibition is reversible. When the concentration of the final end product in the cell falls, it leaves the allosteric site, and the activity of the allosteric enzyme is re-stored.

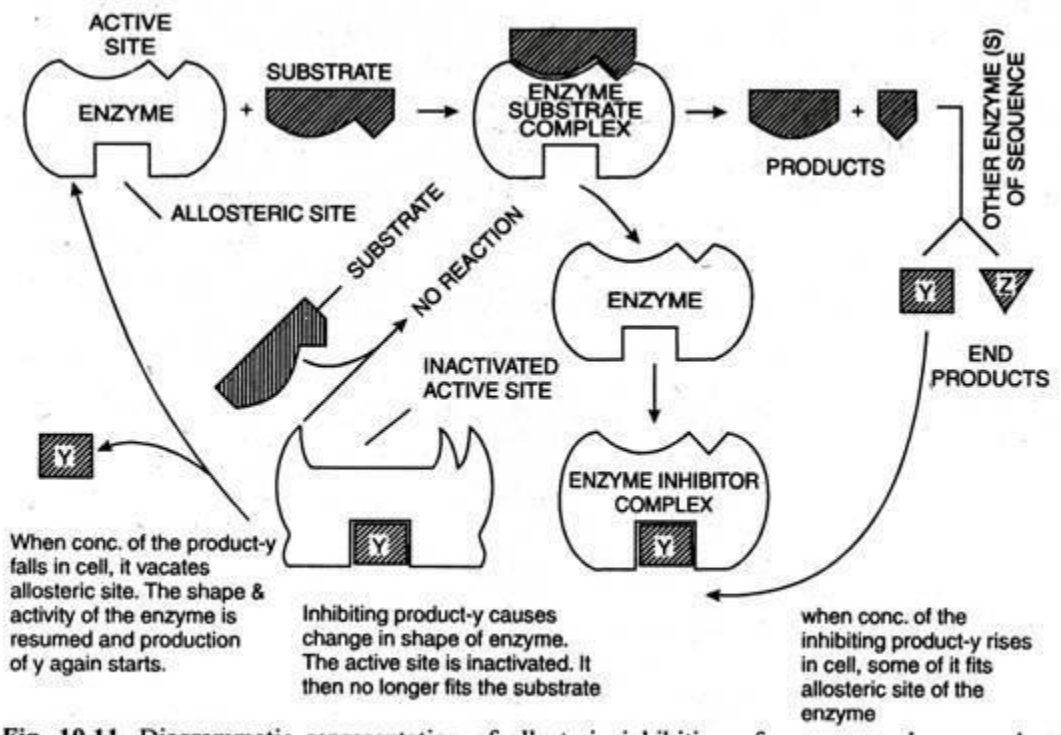


Fig. 10.11. Diagrammatic representation of allosteric inhibition of an enzyme by a product of the reaction sequence.

Allosteric inhibition is shown diagrammatically in Fig. 10.11.

One of the classical and first discovered examples of allosteric inhibition is furnished by the bacterial enzyme system of E. coli which catalyses the conversion of L-Threonine into L-Isoleucine involving 5 different enzymes in sequence viz., 1. Threonine dehydratase 2.

Acetolactate synthase 3. Ketoacid reductoisomerase 4. Dihydroxy acid dehydratase and 5. Transaminase (See Fig. 10.12).

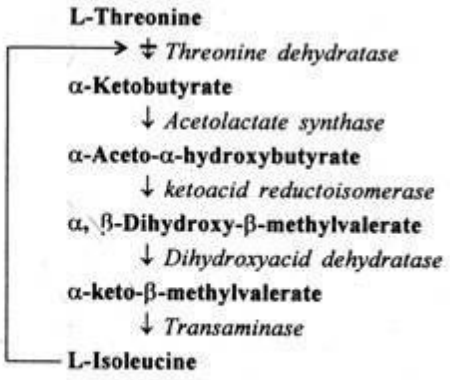


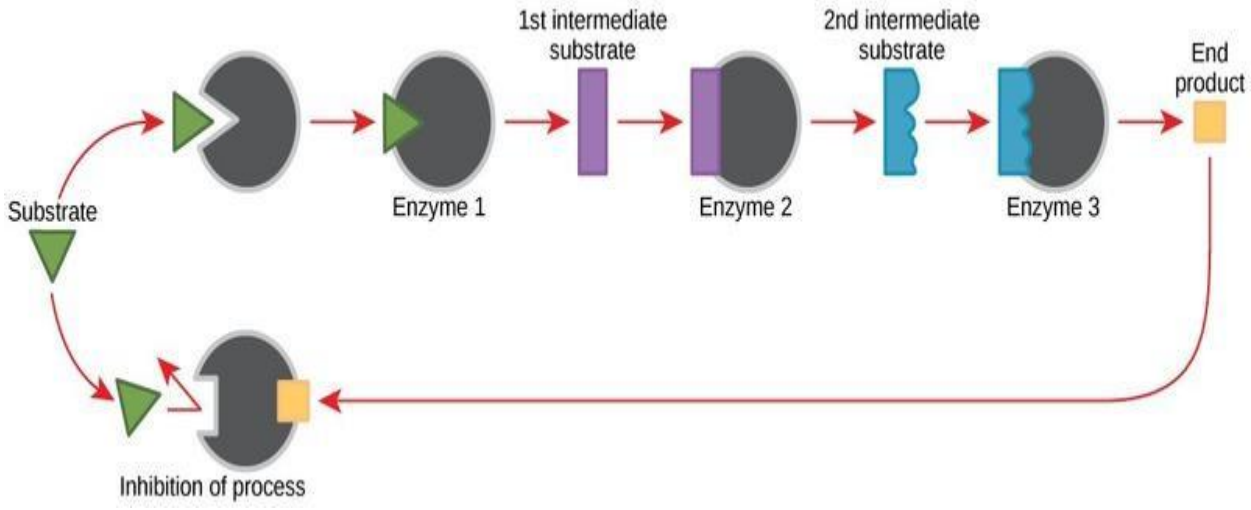
Fig. 10.12. Schematic representation of biosynthesis of Isoleucine from Threonine and allosteric inhibition of *Threonine dehydratase* by Isoleucine in *E. coli*.

In this sequence, only the first enzyme i.e., threonine dehydratase is inhibited by Isoleucine which is the end-product of this sequence. The activity of this enzyme is neither inhibited by any other intermediate of the sequence, nor is any other enzyme of this sequence inhibited by Isoleucine. The inhibition of the first enzyme threonie dehydratase is reversible. When the concentration of isoleucine in the cells increases the activity of this enzyme is decreased so that production of isoleucine falls. But, when isoleucine concentration decreases, the activity of threonine dehydratase increases and the production of isoleucine in the cells is restored‘.

Feedback inhibition (=End product inhibition):

Feedback inhibition means the inhibition of an enzyme activity in a metabolic pathway by an end product of that pathway.

In a metabolic pathway (series of enzyme catalyzed reactions) when the biosynthesis of end products reaches a sufficiency high level then each end product act as an inhibitor or negative modulator and binds to the allosteric site of the first enzyme of the pathway to stop its catalytic activity. Thus, the first irreversible step called committed step of every metabolic pathway is subjected to the control of feedback inhibition.



VITAMINS

Vitamins are chemical compounds that are required in small amounts with our regular diet in order to carry out certain biological functions and for the maintenance of our growth.

Classification of Vitamins

Vitamins are generally classified as water-soluble vitamins and fat-soluble vitamins.

1. Fat-Soluble Vitamins

- Vitamin A, D, E and K are fat-soluble. These are stored in adipose tissues and hence are called fat-soluble vitamins. These vitamins are absorbed more easily by the body in the presence of dietary fat.

2. Water-Soluble Vitamins

- Vitamins in B-group and vitamin C are water-soluble and There are nine water-soluble vitamins. They are not stored in the body. Any leftover water-soluble vitamins leave the body through the urine. Although, the body keeps a small reserve of these vitamins, they have to be taken on a regular basis to prevent shortage in the body. Vitamin B12 is the only water-soluble vitamin that can be stored in the liver for many years.

There are 13 essential vitamins. This means that these vitamins are required for the body to work properly. They are:

- Vitamin A
- Vitamin C
- Vitamin D
- Vitamin E
- Vitamin K
- Vitamin B1 (thiamine)
- Vitamin B2 (riboflavin)
- Vitamin B3 (niacin)
- Pantothenic acid (B5)
- Biotin (B7)
- Vitamin B6
- Vitamin B12 (cyanocobalamin)
- Folate (folic acid and B9)

Vitamins are grouped into two categories:

- Fat-soluble vitamins are stored in the body's fatty tissue. The four fat-soluble vitamins are vitamins A, D, E, and K. These vitamins are absorbed more easily by the body in the presence of dietary fat.
- There are nine water-soluble vitamins. They are not stored in the body. Any leftover water-soluble vitamins leave the body through the urine. Although, the body keeps a small reserve of these vitamins, they have to be taken on a regular basis to prevent shortage in the body. Vitamin B12 is the only water-soluble vitamin that can be stored in the liver for many years.

Some ―vitamin-like factors‖ are also needed by the body such as:

- Choline
- Carnitine

Function

Each of the vitamins listed below has an important job in the body. A vitamin deficiency occurs when you do not get enough of a certain vitamin. Vitamin deficiency can cause health problems.

Not eating enough fruits, vegetables, beans, lentils, whole grains and fortified dairy foods may increase your risk for health problems, including heart disease, cancer, and poor bone health (osteoporosis).

- Vitamin A helps form and maintain healthy teeth, bones, soft tissue, mucous membranes, and skin.
- Vitamin B6 is also called pyridoxine. Vitamin B6 helps form red blood cells and maintain brain function. This vitamin also plays an important role in the proteins that are part of many chemical reactions in the body. The more protein you eat the more pyridoxine your body requires.
- Vitamin B12, like the other B vitamins, is important for metabolism. It also helps form red blood cells and maintain the central nervous system.
- Vitamin C, also called ascorbic acid, is an antioxidant that promotes healthy teeth and gums. It helps the body absorb iron and maintain healthy tissue. It is also essential for wound healing.
- Vitamin D is also known as the "sunshine vitamin," since it is made by the body after being in the sun. Ten to 15 minutes of sunshine 3 times a week is enough to produce the body's requirement of vitamin D for most people at most latitudes. People who do not live in sunny places may not make enough vitamin D. It is very hard to get enough vitamin D from food sources alone. Vitamin D helps the body absorb calcium. You need calcium for the normal development and maintenance of healthy teeth and bones. It also helps maintain proper blood levels of calcium and phosphorus.
- Vitamin E is an antioxidant also known as tocopherol. It helps the body form red blood cells and use vitamin K.
- Vitamin K is needed because without it, blood would not stick together (coagulate). Some studies suggest that it is important for bone health.
- Biotin is essential for the metabolism of proteins and carbohydrates, and in the production of hormones and cholesterol.
- Niacin is a B vitamin that helps maintain healthy skin and nerves. It also has cholesterol-lowering effects at higher doses.
- Folate works with vitamin B12 to help form red blood cells. It is needed for the production of DNA, which controls tissue growth and cell function. Any woman who is pregnant should be sure to get enough folate. Low levels of folate are linked to birth defects such as spina bifida. Many foods are now fortified with folic acid.
- Pantothenic acid is essential for the metabolism of food. It also plays a role in the production of hormones and cholesterol.

- Riboflavin (vitamin B2) works with the other B vitamins. It is important for body growth and the production of red blood cells.
- Thiamine (vitamin B1) helps the body cells change carbohydrates into energy. Getting enough carbohydrates is very important during pregnancy and breastfeeding. It is also essential for heart function and healthy nerve cells.

- Choline helps in normal functioning of the brain and nervous system. Lack of choline can cause swelling in liver.
- Carnitine helps the body to change fatty acids into energy.

Vitamin A:

Clinical name:

Axerophthol, antixeropthalmic or antinyctalopic vitamin.

Chemical name:

Chemically, vitamin A ($C_{20}H_{29}OH$) is an unsaturated alcohol called retinol. Vitamin A exists in two isomeric forms: vitamin A (retinol) is a trans-isomer and occurs naturally, whereas vitamin A_2 (dehydroretinal or retional2) is a cisisomer and has 30 – 40% of vitamin A actively.

Sources:

Plant sources like green vegetables, fruits, and cereals supply pro vitamin A (β-carotene) in diet. Animal sources of vitamin A are liver, milk, butter, egg yolk etc. Liver of fresh water fish contain A_2.

Functions:

(i) Retinol (vitamin A aldehyde) combines with lysine residues of opsin protein to form rhodopsin pigments of rod cells of retina, so it is essential for night vision,

(ii) Retinoic acid (vitamin A acid) has some anticancer effects,

(iii) Vitamin A maintains the integrity of epithelial cells; permeability of cell membranes as well as the membranes of organelles,

(iv) In young animals vitamin A causes growth, formation of bones and teeth.

Deficiency Symptoms:

(i) Vitamin A deficiency causes the defective night vision called night blindness (nyctalopia or Henerolopia).

(ii) In children deficiency of vitamin A_2 causes xerophthalmia (drying of conjunctiva) and keratomalacia ulceration and softening of cornea) that may lead to complete blindness,

(iii) Toad‘s skin is another detectable early symptom of vitamin A where the skin becomes dry and rough particularly in the lateral part of forearms and sides of thigh.

Vitamin D:

Clinical name:

Clinically, Vitamin D is called antirachitic vitamin because it possesses the property of curing or preventing ticket.

Chemical name:

Chemically Vitamin D is a steroid which is related to calcium metabolism. Hence it is called calciferol. Vitamin D exists in 2 forms i.e. Vitamin D_2 (ergocalciferol) and Vitamin D_3 (cholecalciferol). They are synthesized from the provitamins by the action of UV-rays of sunlight. Because of it, vitamin D is called sunshine vitamin. The provitamin D_2 (ergosterol) occurs in plants and the provitamin D_3 (7-dehydrocholesterol) occurs in animals. The term vitamin D_1 is no longer used.

Sources:

Vit. D_3 obtained from fish liver oil, egg, milk, butter, ghee etc.

Functions:

(i) Vitamin D_3 is also considered as a prohormone which gives rise to a hormone colcitriol (1, 2, 5 Dihydroxy cholecalciferol) by various metabolic changes. Calcitriol has a role in calcium and phosphate metabolism,

(ii) Vitamin D activates the transcription of mRNA for calcium binding protein,

(iii) It helps in the growth and development of bone and teeth,

(iv) Increase the excretion of phosphate.

Deficiency symptoms:

Vitamin D deficiency leads to rickets in children and osteomalacia in adult. Rickets is characterized by bowlegs, knock knees, bending of ribs leading to pigeon‘s breast, enlargement of ankles, knees, wrists, elbow etc. In osteomalacia the bones become weak and fragile instead of being soft due to decalcification of bones.

Vitamin E:

Clinical name:

It is called antisterility vitamin or fertility vitamin because of its requirement in proper functioning of reproductive system.

Chemical name:

Tocopherol (tokes = child birth; phero = to bear, ol = alcohol)

Source:

Vegetable oil, leafy vegetables, milk, cheese, egg, meat etc.

Function:

(i) It acts as an antioxidant and prevents oxidation of vitamin A, K, essential fatty acids.

(ii) It keeps the skin glowing by reducing keratinization. Hence, it is also called as beauty vitamin,

(iii) It helps in the normal functioning of skeletal muscles, gonads and renal tubules.

Deficiency symptoms:

(i) Causes sterility and miscarriage,

(ii) Causes muscular weakness and dystrophy (degeneration.)

Vitamin K:

Clinical name:

It is called antihemorrhagic vitamin or vitamin for blood clotting or coagulation vitamin.

Chemical name:

Chemically vitamin K is a naphthoquinone derivative. Naturally it occurs in two forms, i.e. vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinone or farnoquinone). The vitamin K_3 (mendione) is a synthetic product.

Source:

Green leafy vegetables, soybean, carrots, potatoes, milk, fish and meats etc.

Function:

(i) Act as co-enzyme Q and participate in oxidative phosphorylation in ETC.,

(ii) Acts as a co-factor of carboxylase,

(iii) Required for fat absorption.

Deficiency symptoms:

(i) Delay in blood clotting,

(ii) Cause hemorrhagic disease of newborn,

(iii) Defective functioning of liver.

Vitamin C:

Clinical name:

It is called antiscorbutic acid because it prevents scurvy. It is also known as anti- rabies or and cancer vitamin.

Chemical name:

Chemically it exists in L-ascorbic acid and L-dehydroascorbic acid (ascorbone). The L-ascorbic acid is ay-lactone (with internal esterification) that is synthesized in plants and most animals except primates and guinea pigs.

Source:

Citrus fruits (lemons, oranges), grapes, apple, papaya, guava, vegetables etc.

Functions:

(i) It is required for absorption of iron,

(ii) It keeps gums and capillary walls healthy.

(iii) It gives resistance against cold and viruses. Hence, it is often called as anti-viral vitamin,

(iv)It is necessary for wound healing,

(v) It acts as co-enzyme for hydroxylation and oxidation -reduction reactions. Thus, it helps in metabolism of amino acids, collagen synthesis etc.

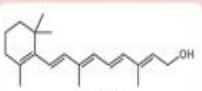
Deficiency symptoms: It causes scurvy (Sailor's disease) characterized by bleeding gums, loosening of teeth, fragile capillaries, failure in wound healing, anemia, general weakness etc.

S. No.	Vitamin	Deficiency diseases
1)	Thiamine (B_1)	Beriberi
2)	Riboflavin (B_2)	Glossitis
3)	Niacin (B_3)	Pellagra
4)	Pyridoxine (B_6)	Anaemia
5)	Cyanocobalamine (B_{12})	Pernicious anaemia
6)	Folic acid (B_9)	Anaemia
7)	Pantothenic acid	Burning feet
8)	Biotin	Nerves disorders
9)	Ascorbic acid (Vitamin C)	Scurvy
10)	Retinol (Vit. A)	Eye and Skin diseases – Night blindness, Xerophthalmia, Rupture of cornea, Scale formation on skin
11)	Calciferol (Vit. D)	Rickets, fragile bones
12)	Tocoferol (Vit. E)	Fertility disorders – Sterility in males, Abortions in females
13)	Phylloquinone (Vit. K)	Blood clotting

THE CHEMICAL STRUCTURES OF VITAMINS

Vitamins are the essential nutrients that our body needs in small amounts. More specifically, an organic compound is defined as a vitamin when it is required by an organism, but not synthesised by that organism in the required amounts (or at all). There are thirteen recognised vitamins.

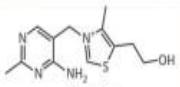
VITAMIN A



RETINOL
active form in mammalian tissues

Important for eyesight. Also strengthens immune system and keeps skin and linings of parts of the body healthy.

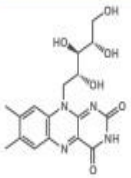
VITAMIN B1



THIAMIN
can also occur in pyrophosphate ester form

Used to keep nerves & muscle tissue healthy. Also important for processing of carbohydrates and some proteins.

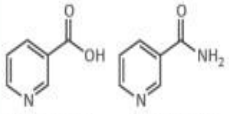
VITAMIN B2



RIBOFLAVIN
excess turns urine bright yellow

Important for body growth, red blood cell production, and keeping the eyes healthy. Also helps processing of carbohydrates.

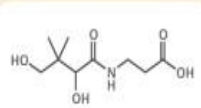
VITAMIN B3



NICOTINIC ACID **NICOTINEAMIDE**
niacin is collective name for these compounds

Helps with digestion and digestive system health. Also helps with the processing of carbohydrates.

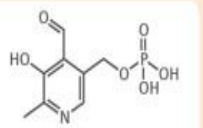
VITAMIN B5



PANTOTHENIC ACID
can also occur in pyrophosphate ester

Important for manufacturing red blood cells and maintaining a healthy digestive system. Also helps process carbohydrates.

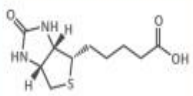
VITAMIN B6



PYRIDOXAL PHOSPHATE
active form in mammalian tissues

Helps make some brain chemicals; needed for normal brain function. Also helps make red blood cells and immune system cells.

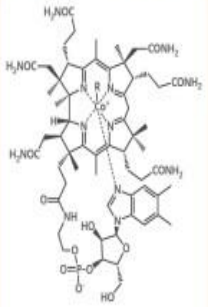
VITAMIN B7



BIOTIN
produced by intestinal bacteria

Needed for metabolism of various compounds. Often recommended for strengthening hair, but evidence is variable.

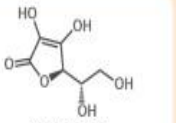
VITAMIN B12



COBALAMIN
usually contains CN as the R group

Important for the nervous system, for making red blood cells, and helps in the production of DNA and RNA.

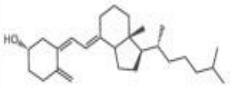
VITAMIN C



ASCORBIC ACID
deficiency can cause scurvy

Important for a healthy immune system; helps produce collagen, used to make skin and other tissues. Also helps wound healing.

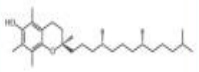
VITAMIN D



CHOLECALCIFEROL
natural form; different form used in supplements

Important for bone health and maintaining the immune system function. May also have a preventative role in cancers.

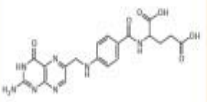
VITAMIN E



ALPHA-TOCOPHEROL
group includes tocopherols & tocotrienols

An antioxidant that helps prevent damage to cells and may have a preventative role in cancer. Also helps make red blood cells.

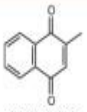
VITAMIN B9



FOLIC ACID
found as tetrahydrofolate in food

Important for brain function & mental health. Aids production of DNA & RNA. Important when tissues are growing quickly.

VITAMIN K



MENADIONE
all K vitamins are menadione or derivatives

Helps blood clot properly, & plays a key role in bone health. Newborns recieve vitamin K injections to prevent bleeding.

Key

Vitamins can be divided broadly into two classes.

WATER SOLUBLE VITAMINS
These vitamins are not stored in the body. As such, generally, they are required more frequently than the fat soluble vitamins.

FAT SOLUBLE VITAMINS
These vitamins are stored in the liver and fatty tissues until required. As such, they can be harmful if too much is taken in.