BIO CATALYST
Enzyme: Study of Kinetics and Mechanism

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Abstract

This chapter deals with the classification, structure binding, kinetics and properties of enzymes along with the classification and properties of coenzymes. Enzyme plays a pivotal role in any physiological process occurring in plants and animals including human. The specific nature of enzyme is the property of greater interest. The kinetics of enzyme catalysis including the behaviour of enzyme by variation of its concentration, substrate concentration and product formation in different time intervals are incorporated in the chapter. The rate law expression of enzyme catalyzed reaction is derived which helps in better understanding of enzymatic action. The chapter includes various examples of enzymatic action with examples and site of their production described in various diagrammatic representations and tables. The enzymes, as can be concluded from the chapter, is a valuable part of life supporting physiological actions.

Keywords – EC numbers, classifications, turnover number, specificity, lock and key hypothesis, induced fit theory, Michaelis constant, coenzyme.

The word Enzyme (a Greek word en – in zyme – leaven i.e., present in yeast) was coined by Kuhne in 1878. Buchner, in 1896 extracted a substance capable to carry out fermentation from the cell of a yeast which was later known as zymase enzyme. In the year 1926, Professor J.B. Sumner was capable to isolate, from jack beans, by means of acetone, the crystalline form of enzyme urease. Enzymes are proteinaceous compounds that increases the rate of biological reactions occurring in living organisms and are also often extracted from the living cell to catalyse some important reactions for commercial purposes.

Enzymes which are active outside the living cells are called exoenzymes, e.g., enzymes occurring in digestive juices, lysozyme of tears while the enzymes which are active inside the living cells are called endozymes, e.g., endo amylase that break large amylose molecule to dextrin, enzymes participating in Krebs cycle, enzymes of glycolysis, and so on.
In the latter half of nineteenth and early half of twentieth century several successful attempts were made to extract, characterise and commercially exploit several enzymes. However, it was only possible to crystallise enzymes and establish its catalytic behaviour associated with proteins between 1920 and 1930. This established the belief that enzymes are proteins acquiring the property of catalysis. It was during 1980s biologists observed some ribonucleic acid (RNA) molecules known to be ribozymes also have catalytic effects in gene expression. Simultaneously the bio chemists successfully developed the technology to generate the antibodies called abzymes having significant catalytic role in industries and therapeutics.

I Enzyme Classification

The EC Numbers

As per the reaction, six groups of enzymes are categorized. The enzymes are classified according to the "EC numbers". However, the classification is not applicable for protein structure, sequence of amino acid or the mechanism involved in chemical reactions.

EC number involves a four-digit number for instance – a.b.c.d, where "a" is called class, "b" is classified as subclass, "c" is termed as sub-subclass and "d" is known as the sub-sub-subclass. The digits "b" and "c" explain the bio-chemical reaction, "d" is said differentiates, different enzymes having same type of property based on the given substrate in the reaction. As for example, the EC number of Alcohol: NAD+ oxidoreductase is mentioned as 1.1.1.1

Enzyme Classification: The Six Classes of Enzymes

Class 1. Oxidoreductases

These enzymes catalyze the process that involves the transfer of oxygen atoms or hydrogen atoms or electrons from one of the substrates to another substrate. They are hence, called oxidases, reductases or dehydrogenases. For this purpose, an electron donor or an acceptor is said to be required for completion of the reactions as these reactions involve reduction and oxidation.

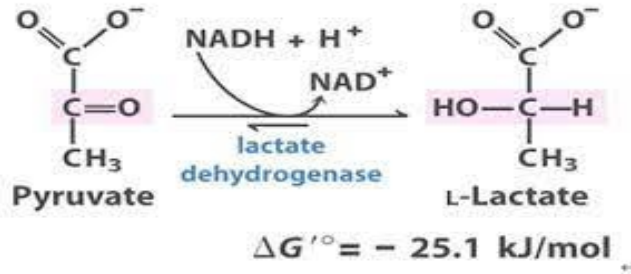

Fig. 1- Reduction of pyruvate to form L- lactate

Class 2. Transferases

These catalysts are used to transfer a group from one substrate to another. However, the atom cannot be hydrogen.

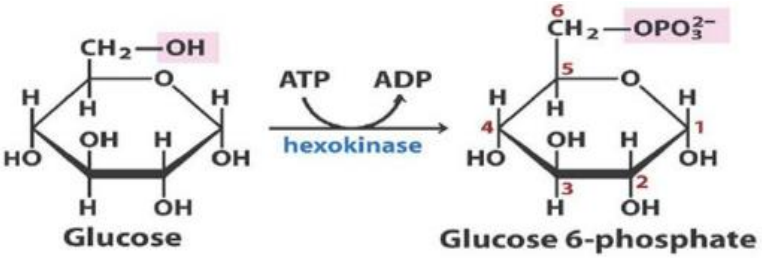

Fig. 2- Formation of glucose- 6- phosphate from glucose

Class 3. Hydrolases

These catalysts are used in the hydrolysis reactions. The examples of these enzymes include esterases, lipases, nitrilases, proteases.

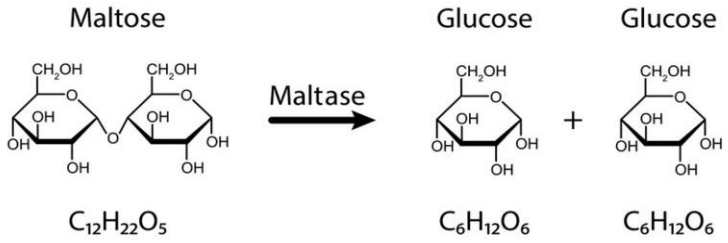

Fig. 3 – Enzymatic hydrolysis of maltose

Class 4. Lyases

These enzymes catalyze the removal of functional groups from the substrates without any hydrolysis reaction. The reaction involves either introduction of a double bond in the product or addition reaction. These enzymes such as aldolases and decarboxylases are responsible for elimination and addition reactions.

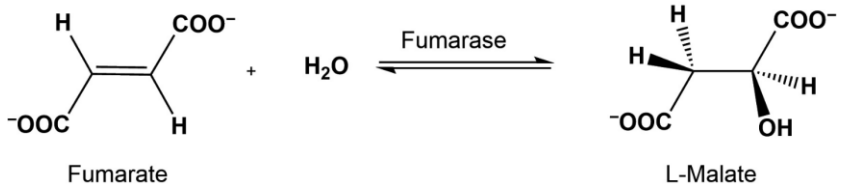

Fig. 4 – Enzymatic conversion of fumarate to malate

Class 5. Isomerases

These enzymes are involved in the catalysis of isomerization reactions resulting in cis-trans isomerization and racemization.

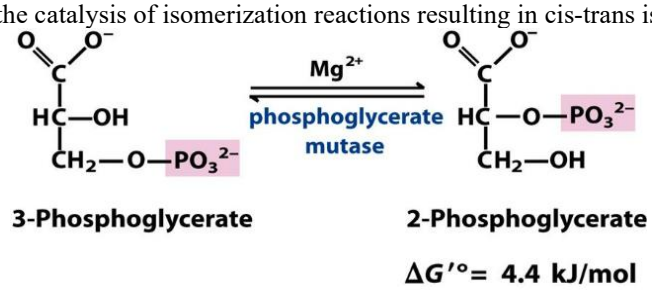

Fig. 5 – Isomerization by enzymatic action

Class 6. Ligases

These enzymes are responsible for introducing new bonds, along with involvement of Adenosine triphosphate.

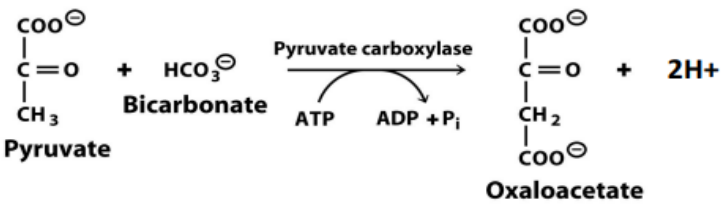

Fig. 6 – Conversion of pyruvate to oxaloacetate by formation of new bond

Enzyme Classification: Secondary Classes of Oxidoreductases

Hydrogen or electron donor	Oxidoreductases: second EC digit
Alcohol (CHOH)	1
Aldehyde or ketone (C = O)	2
-CH-CH-	3
Primary amine ($CHNH_2$ or $CHCH_3^+$)	4
Secondary amine (CHNH)	5
NADH or NADPH (when another redox catalyst in the acceptor)	6

Table 1 – Secondary classes of Oxidoreductases

Enzyme Classifications: Tertiary Classes of Oxidoreductases in the EC System

Hydrogen or electron acceptor	Oxidoreductases: third EC digit
NAD^+ or $NADP^+$	1
Fe^{3+} (e.g., cytochromes)	2
O_2	3
Other	4

Table 2- Tertiary classes of oxidoreductases

Enzymes as catalysts

The catalytic activity of an enzyme is represented by a constant k_{cat}, which is called turnover rate or frequency number. The constant k_{cat} is the amount of substrate molecules that converts to product per unit time. To consider an example, one molecule of an enzyme carbonic anhydrase results in catalysing the conversion of around 6 lakh molecules of carbon dioxide (CO_2) and water (H_2O), producing bicarbonate (HCO_3^-), every second.

Enzyme	Turnover number (per second)
Lysozyme	0.5
Tryptophan synthetase	2
DNA polymerase I	15
Chymotrypsin	100
Lactate dehydrogenase	1000
Acetylcholinesterase	25,000
3-Ketosteroid isomerase	280,000
Carbonic anhydrase	600,0000

Table 3 – Turnover number

Specificity of Enzymes

The enzymes are highly specific in their role to increase the rate of biochemical reactions. A single enzyme can catalyze only a single step or reaction of any physiological process or at the maximum a group of similar type of substrate molecules can undergo reaction catalyzed by any single enzyme i.e., some of the enzymes show group specificity e.g., an enzyme called alkaline phosphatase removes a phosphate group from a variety of biomolecules.

There are also some enzymes which show absolute specificity e.g., glucose oxidase, an enzyme, shows total absolute specificity for β-D-glucose but does not catalyse any other reaction of any other monosaccharides.

Chemical Specificity of Enzymes

Types of Specificity	Action	Examples
Low or Bond Specificity	Acts on any compound with a specific type of bond	Amylase and Lipase
Moderate Specificity	Acts on chemicals not only on specific bonds but also on specific structure surrounding it.	Pepsin, trypsin and chymotrypsin
Absolute Specificity	Acts only one substrate	Uricase, Lactase & Maltase
Optical Specificity	Acts only on stereo-specific structure	L-amino acid oxidase and D-aminoacids oxidase
Dual Specificity (Two substrates)	Acts on Two separate substrates	Xanthin oxidase acts on hypoxanthine and xanthin
Dual Specificity (One substrate but two reactions)	One enzyme act on one substrate by two separate reactions	Isocitrate dehydrogenase acts on isocitrate by oxidation and decarboxylation.

Table 4 – Chemical specificity of enzymes

Classification of enzymes on the basis of chemical nature

(i) Simple Enzymes:

Some enzymes act as simple protein which upon hydrolysis yield amino acids. These also include digestive enzymes as well.

Types of Digestion	Enzyme	pH Level	Produced in	Site of Release
Lipid (fat)	Lipase	Basic	Small Intestine	Small Intestine
Protein	Pepsin	Acidic	Gastric Glands	Stomach
	Peptidase	Basic	Small Intestine	Small Intestine
	Trypsin	Basic	Pancreas	Small Intestine
Carbohydrate	Maltase	Basic	Small Intestine	Small Intestine
	Pancreatic Amylase	Basic	Pancreas	Small Intestine
	Salivary amylase	Neutral	Salivary Glands	Mouth
Nucleic Acid	Nucleosidase	Basic	Small Intestine	Small Intestine
	Nuclease	Basic	Small Intestine	Small Intestine

Table 5 – List of simple enzymes and their characteristics

(ii) Conjugate Enzymes:

These enzymes contain two constituents – a protein part known as apoenzyme (e.g., flavoprotein) while another part consist of a non-protein called cofactor. Together they are called holoenzyme. Some examples of conjugate enzymes are transferases, oxidoreductases, ligases, and isomerases.

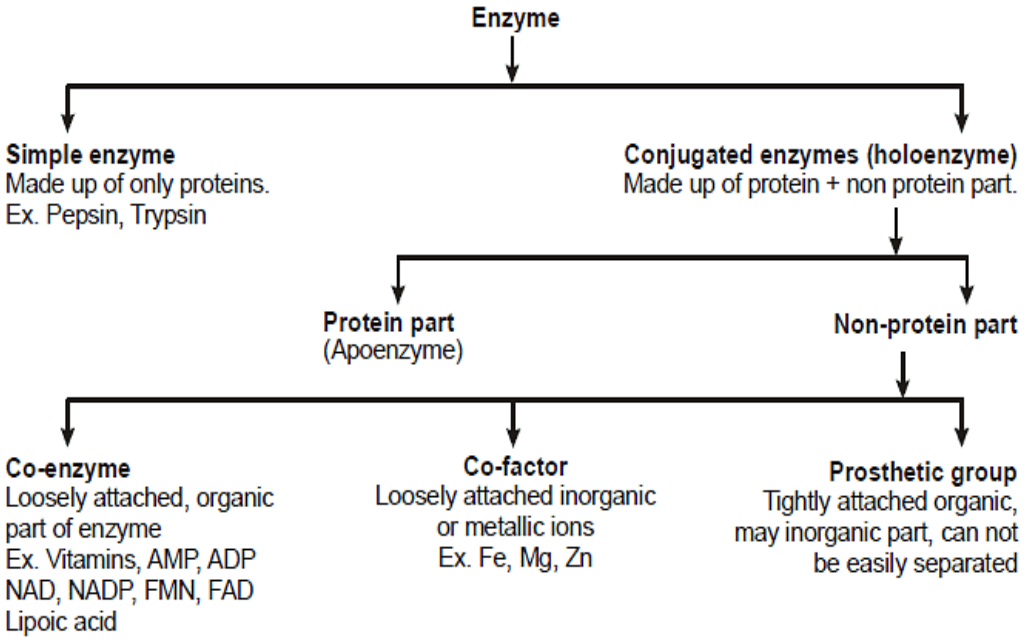

Fig. 7 – Flowchart of enzyme classification

The enzymes are functional only when apoenzyme and cofactor exist together. The cofactor can be bivalent cations of metals like Ca, Mg, Zn, Co etc or can be non-protein organic compound called coenzyme such as flavin, haem etc or can be a prosthetic group such as pyridoxal sulphate, flavin mononucleotide (FMN), biotin etc.

(iii) Metallo-enzymes:

The metal cofactors containing both monovalent metal cation of Na, K etc and divalent metal cations of Mg, Mn, Cu etc can be involved in enzymatic reactions. These cations can either be loosely held by the enzyme or can go into the composition of molecule itself. Some of the examples are carboxypeptidase (which acts as hydrolytic enzyme containing Zn coordinated to many amino acid residues of proteins), catalase (used for the decomposition of H_2O_2 containing iron – porphyrin complexes), haemoglobin (also contains iron – porphyrin complexes), chlorophyll (contains magnesium- porphyrin complexes)

(iv) Isoenzymes (Isozymes):

Sometimes same species, tissue or even same cell contains many enzymes in multiple forms which can catalyze the same reaction but have different amino acid composition possessing different physicochemical properties. These enzymes are called isoenzyme or isozyme. As for example glycolytic enzyme, hexokinase which exist as four isoenzymes in various tissues, lactate dehydrogenase (LDH) which carry out anaerobic glucose metabolism, contains two isoenzymes in human out of which one is present in heart while the other is present in skeletal muscles.

PROPERTIES OF ISOZYMES

1. **Different metabolic patterns in different organs.**

The isozymes have different properties for glycogen phosphorylase present in the skeletal muscle and liver which indicates different behaviour of breakdown of glycogen in the given two tissues.

2. **Different locations and metabolic roles for isozymes in the same cell.**

In mitochondrion and cytosol, the isozymes isocitrate dehydrogenase can cited as example.

3. **Different stages of development in embryonic or foetal tissues and in adult tissues.**

LDH isozyme is different as the liver organ develops in adult form from the child phase. Few enzymes of glucose catabolism present in malignant cells of especially responsible for cancer is present only in foetal isozymes.

4. **Different responses of isozymes to allosteric modulators.**

Hexokinase IV (glucokinase) enzyme of liver and those of other tissues differ in their inhibition sensitivity by glucose 6-phosphate.

Lactate dehydrogenase (LDH), enzymes that are responsible for the reduction of pyruvate to lactate are present in different forms as described below.

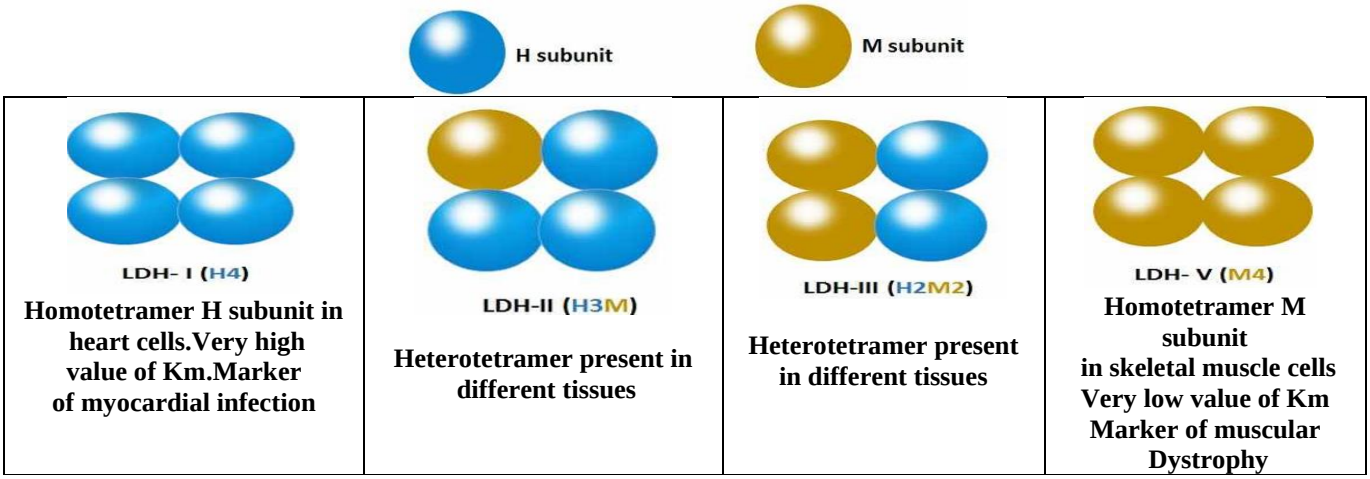

Fig. 8 – Classification of Tetramers

II Structure of Enzyme and its Binding to Substrate

1. Lock and Key Hypothesis:

The enzymes containing amino acids are basically globular proteins ranging in molecular weight corresponding to less than 100 to even more than 2,000 amino acid units. The amino acids are arranged in one or even more polypeptide chains that can exist as bent and folded forms constituting a particular three-dimensional structure which incorporate smaller area called active sites where the substrate actually binds. These active sites contain fewer number (usually less than 10) of the integral amino acids. The shape and charge present on these active sites permit them to bind a single type of biomolecule which makes the enzyme exceedingly specific in its behaviour as was first proposed by a German chemist Emil Fischer in the year 1894. This is called “Lock and Key” theory.

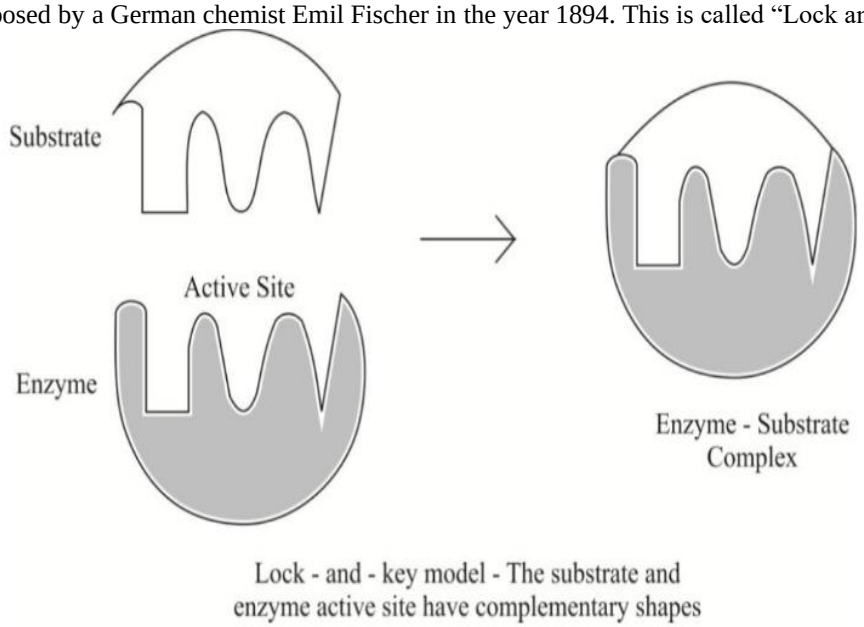

Fig. 9 – Diagrammatic representation of Lock and Key hypothesis

The rest of the protein molecule is responsible to stabilize the active site providing suitable atmosphere for the site to interact with the substrates. Hence the active site does not separate out from the rest of the protein without losing its catalytic behaviour.

2. Induced-Fit Theory:

Due to close approach of substrate or product, the active site of enzymes gets induced by this and undergoes a modification in its conformation. This results in better blend of enzyme and substrate.

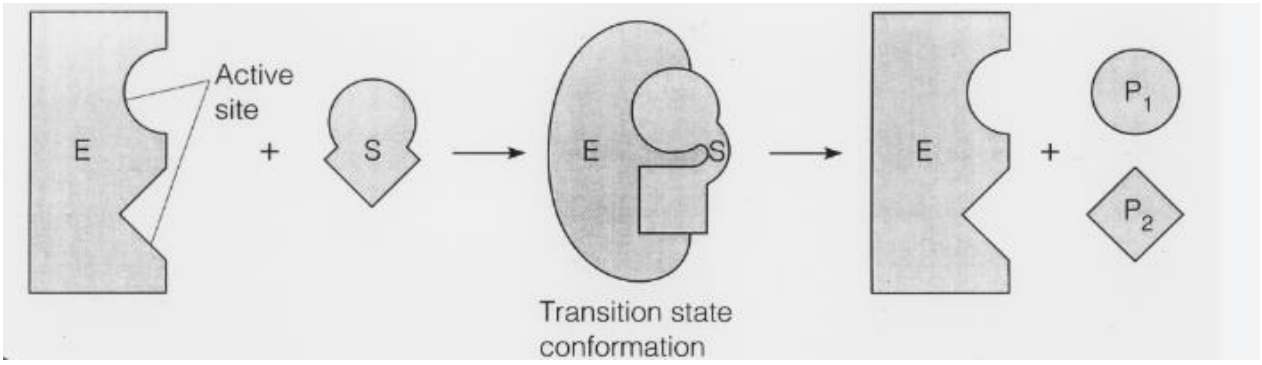

Fig. 10 – Diagrammatic representation of Induced Fit theory

This is known as the induced-fit theory. The structure of the substrate gets changed during this process in a large number of cases thus forming a functional enzyme- substrate complex.

III Kinetics aspect of the Enzyme

The study of aspects which explain and evaluate the extent of enzyme-catalysed reactions is termed as enzyme kinetics. The theory of kinetics is important to assess and understand this concept of the participation of enzymes in metabolic activity as well as biotechnology.

There are two methods of measurement of enzyme activity.

1. Discontinuous method
2. Continuous method

In discontinuous method we mix enzyme and substrate together and measure the quantity of product yielded over a period of time intervals. It's an easier and quicker method generally used either when we have little knowledge about the system and we just make a primary investigation or when we have greater knowledge about the system and we are confident about the time interval we take.

In continuous method we usually note the rate of a reaction catalyzed by the enzyme. We can also evaluate the speed of reaction by noting down the rate of disappearance of biomolecule in different time intervals. In the kinetics of enzyme catalyzed reaction, we often use a non-natural substrate like chromogen which results in brightly coloured products. This makes us to easily follow the reaction by using colorimeter or spectrophotometer. The example of continuous assays (measurements) is as follows:

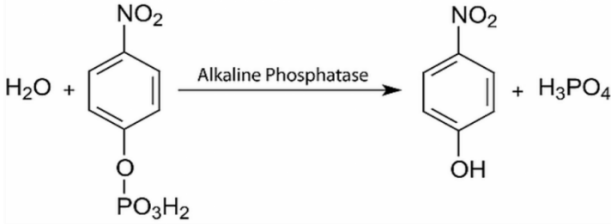

Fig. 11 – Example of Continuous assays

The enzymatic action is powerfully influenced by pH and so we have to establish the pH at a precise value and keep it persistent throughout the experiment. Thus, we use a buffer solution for this purpose. We mix the chromogen with buffer solution and then introduce the enzyme. The observations are noted in spectrophotometer which shows that initially the rate of reaction is fast but after sometimes it may decrease as shown below:

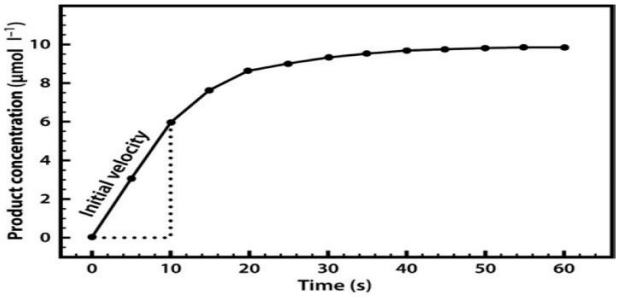

Fig. 12 - Formation of Product in an Enzyme-Catalyzed Reaction, Plotted Against Time.

Upon increasing the enzyme concentration by two times the rate of reaction gets doubled while upon decreasing the concentration of enzyme by 50% the rate is observed to be half of the initial value. Hence a linear relationship exists between rate of reaction and concentration of the enzyme as observed in the following curve:

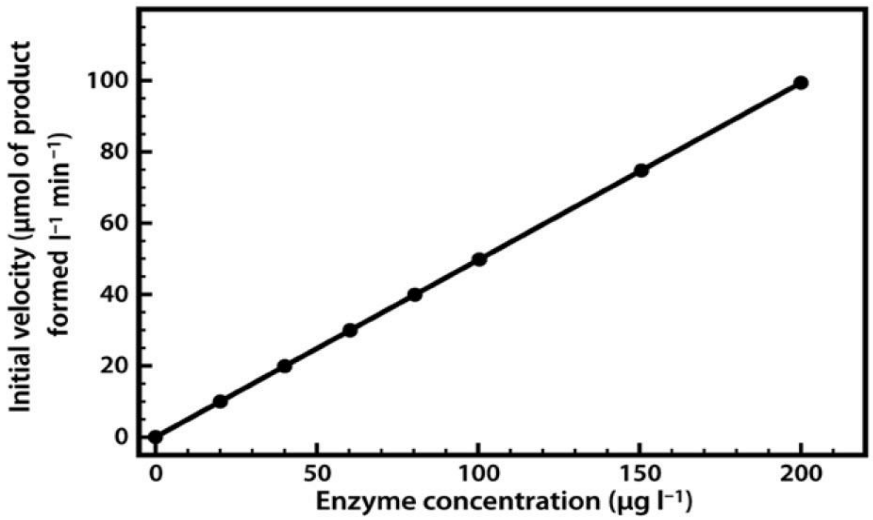

Fig. 13 - Relationship Between Enzyme Concentration and the Rate of an Enzyme-Catalyzed Reaction.

Keeping the concentration of enzyme constant if the concentration of substrate the rate is observed to increase considerably during initial period of time. Further increase in the concentration of substrate results in decline of the rate of the reaction, until a stage is reached where the substrate concentration has virtually negligible effect on the rate. Here the enzyme is supposed to approach its saturation with respect to substrate, and acquires its maximal velocity (V_{max}), a theoretical limit that will not be actually achieved in any experiment as observed in the graph given below:

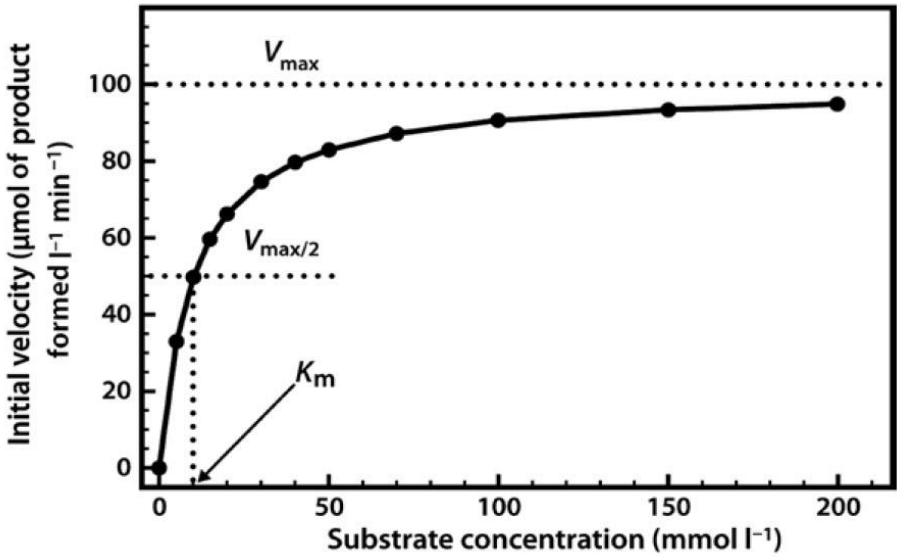

Fig. 14 - Relation Between Substrate Concentration and Rate of an Enzyme-Catalyzed Reaction.

Mathematically the equation is as follows:

$$y = \frac{a \times x}{x + b}$$

The two constants ***a*** and ***b*** describe a hyperbolic curve, where the constant ***a*** is V_{max}, constant ***b*** is the value on x-axis which gives half of the maximal value of y. This is known as the ***Michaelis constant (K_m)*.**

The ***Michaelis–Menten equation***, is mathematically defined as:

$$\text{Initial rate of reaction } (v_0) = \frac{V_{max} \times \text{Substrate concentration}}{\text{Substrate concentration} + K_m}$$

The above relation is derived mathematically by giving the mechanism of enzyme catalyzed reaction as follows:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Where k_1 is the rate constant for forward reaction between enzyme (E) and substrate (S) to form enzyme - substrate complex (ES), k_{-1} is the rate of backward reaction in the equilibrium condition where ES yields back E and S and k_2 is the rate of formation of product (P) producing back the enzyme (E) in same concentration as E was initially consumed.

This derivation requires two important assumptions:

(i) considering the initial velocity of the reaction ***(v_0)***, when the concentration of product is negligibly small (i.e. [S] ≫ [P]), where we can ignore the possibility of any product yielding back the substrate

(ii) the concentration of substrate is much larger than the concentration of enzyme (i.e. [S] ≫ [E]).

The derivation can be written as:

$$v_0 = \frac{d[P]}{dt} = k_2 \times [ES]$$

Since ES is formed as intermediate, its concentration unknown. Also, the rate law expression must be expressed in terms of concentration of reactant. Hence, we have to express [ES] in terms of concentration of E and S.

Applying steady-state approximation method:

The rate of accumulation of ES remains constant when the concentration of ES approaches its maximum value.

Rate of formation of ES complex = Rate of disappearance of ES complex

$$\text{Rate of ES complex formation} = k_1[E][S]$$

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

Applying steady state:

$$k_1[E][S] = k_{-1} + k_2[ES]$$

hence:

$$[ES] = \frac{k_1[E][S]}{k_{-1} + k_2}$$

The Michaelis constant ***K_m*** is hence as follows:

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

Simplifying [ES] equation we get:

$$[ES] = K_m [E][S]$$

As [S] ≫ [E], hence the concentration of unreacted substrate [S] is almost equal to the total concentration of substrate and the concentration of unreacted enzyme [E] is equal to the difference in ***total enzyme concentration*** **$[E]_T$** and that combined with substrate [ES]. The equation can now be written as:

$$[ES] = \frac{[E]_T[S]}{[S] + K_m}$$

Introducing [ES] into the initial velocity equation we get:

$$v_o = k_2[E]_T \frac{[S]}{[S] + K_m}$$

Here $k_2[E]_T$ represents V_{max}, the maximal velocity. Thus, Michaelis and Menten gave their final equation as:

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

The enzymes catalysing the same reaction, but derived from different organisms, can have widely different ***K_m*** values. An enzyme with multiple substrates can have different ***K_m*** values for each substrate.

Typical Range of Values of the Michaelis Constant

Enzyme	K_m (mmol l^{-1})
Pepsin	0.3
Tyrosyl-tRNA synthetase	0.9
Ribonuclease	8
Chymotrypsin	15
Carbonic anhydrase	26

Table 6 – Km values of enzymes

Lower the ***K_m*** value smaller is the amount of enzyme required for substrate to achieve saturation value reaching the maximum rate at relatively lower concentration of substrate. Higher value of Km shows the necessity for high concentration of substrate to achieve maximum rate of reaction. Thus, ***K_m*** is a measure of the interaction of any enzyme towards its substrate.

The significance of *K_m* value:

Lower ***K_m*** value as compared to the concentration of substrate indicates that the enzyme saturates with substrate, and reaction occurs at a constant rate.

Higher ***K_m*** value as compared to the concentration of substrate indicates that the enzyme will not get saturated along with substrate due to which the rate of reaction will change along with the concentration of substrate as a result the rate of formation of product will vary with the available concentration of substrate.

In case that an enzyme catalyzes different substrates, the substrate having lowest ***K_m*** value is often the enzyme's 'accepted' substrate.

In case of two different enzymes (with similar ***V_{max}***) compete for the same substrate in different metabolic reactions, then the pathway with the lower ***K_m*** value of enzyme is considered as the 'favoured pathway'.

Phosphofructokinase (PFK) is responsible to catalyse the first step of glycolytic pathway, producing energy as ATP for the cell and glucose-1-phosphate uridylyl transferase (GUT) is responsible to synthesize glycogen. Both of them use hexose

monophosphates as substrates. But, K_m of PFK is lower than GUT due to which at lower concentration of cellular hexose phosphate, PFK is dynamic and GUT is remains sedentary but when hexose phosphate concentration is higher, both the pathways become active.

Very often when high concentration of substrate is not taken then it is not possible to guess K_m values from the graph of velocity vs substrate concentration because the concentration of substrate is not enough to estimate maximal velocity, due to which we cannot evaluate half-maximal velocity and hence K_m. To solve our purpose, we can use Lineweaver–Burk plot (often called the double-reciprocal plot) which is a linear curve where the graph produced can easily be extrapolated, to calculate the values of V_{max} and K_m as follows:

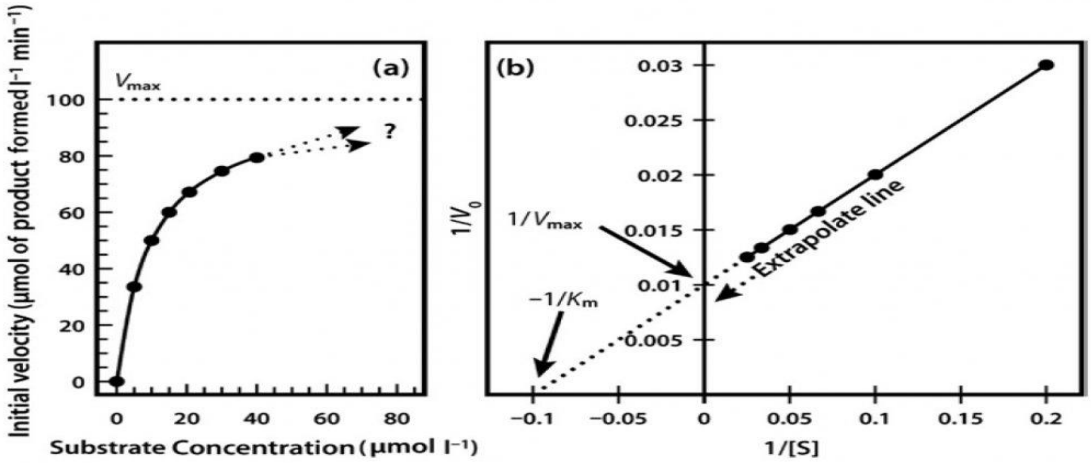

Fig. 15 - Kinetics of Menton: (i) Direct plot of data (ii) Lineweaver-Burk plot of the same kinetic data.

A limitation of Lineweaver–Burk plot is the impact on measurements taken at very low concentration of substrate. These concentrations may lead to error as it is difficult to make solution of multiple dilutions This can affect reaction rates as they are slow. Often, such points when converted on the Lineweaver–Burk plot have a noteworthy influence on the line of best fit assessed from the data, and hence the extrapolated values of V_{max} and K_m can show fault in calculation. This can be studied by observing following two graphs:

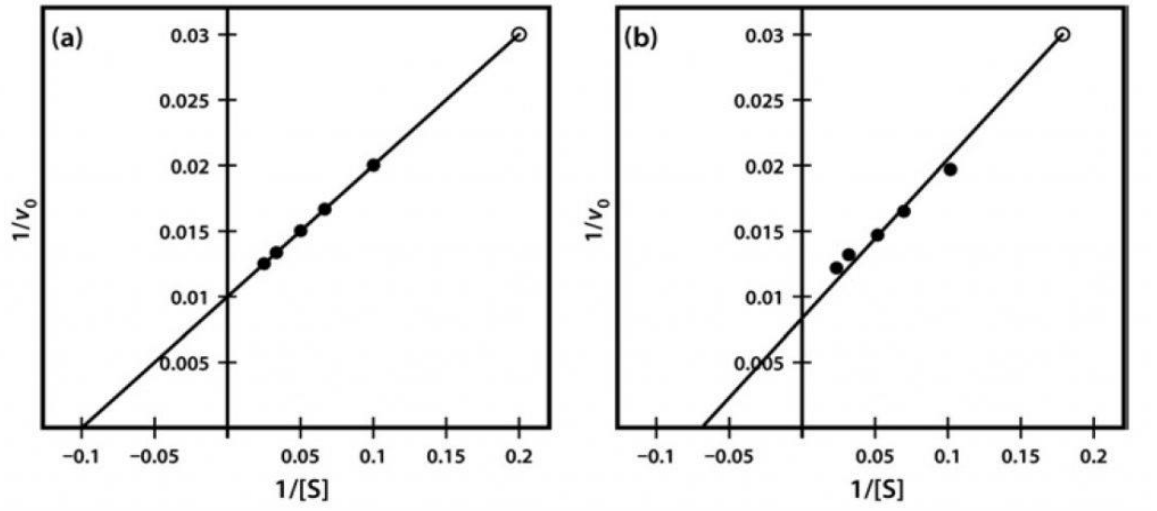

Fig. 16 - Lineweaver-Burk Plot of similar kinetic data, which differ only in a single data point

IV Properties of Enzymes:

1. Enzymes are bio catalysts.

2. Reversibility:

Enzymes controlled reactions can theoretically said to be reversible. However, the reversibility of reaction actually depends upon factors like requirement of energy, presence of reactant, concentration of final products and pH of reaction medium. The reaction can also be irreversible as in case of decarboxylation and hydrolysis reactions.

The thermodynamics of the reactions control the forward and backward shift of any reaction. The pathways of respiration and photosynthesis best describe this thermodynamic effect. The enzymes responsible for glycolysis and pentose phosphate pathway dissimilate glucose while there are certain enzymes which act in the opposite direction in photosynthesis yielding glucose from carbon dioxide gas and water.

3. Heat sensitivity:

The enzymes are affected by temperature and are thermolabile. Generally, enzymes have optimum effect between 25°-35°C. They become unproductive at freezing temperatures and undergo denaturation around 50°-55° C. However, thermal algae and bacteria show exceptional behaviour in which enzymes remain effective even at 80°C. Enzymes present in seeds and spores do not undergo denaturation even at 60°-70°C.

4. pH-sensitive:

Enzyme catalyses the reaction at a specific value of pH, e.g., sucrase is active at pH 4-5, pepsin at pH 2, trypsin is effective at pH 8.5. Any change in pH of reaction mixture makes the enzymes unproductive.

5. Specificity of actions:

The substrate specificity of enzymes can be classified as group specificity and stereo-specificity.

The group specificity of enzymes can be described when they function on a number of substrates of same homologous series. For example, hexokinase is an enzyme which transfer phosphate group from ATP to a minimum of 23 hexoses or their derivatives such as glucose, mannose, fructose, and glucosamine. Some group specific enzymes show absolute group specificity, by catalyzing only a single compound but not its homologues. Manno kinase, glucokinase and fructokinase are responsible in the reactions involving phosphorylation reaction of mannose, glucose and fructose respectively.

Enzymes can also be stereo-specific exhibiting both optical and geometric isomerism.

(i) optically specific enzyme catalyze either dextro (d) or laevo (l) isomer of the biomolecule e.g., d. amino acid oxidase can oxidise only d. amino-acids and l. amino acid oxidases can oxidise only l. amino acids.

(ii) geometrical specificity can be studied by the effect of enzyme on the cis and trans isomers e.g., fumaric hydratase can catalyze only the fumaric acid, the trans-isomer and not the cis-isomer maleic acid.

6. Enzyme inhibition:

Such molecules that result in the decrease of rate of an enzyme-catalyzed reaction are called inhibitors and the phenomenon is described as enzyme-inhibition. A diagrammatic representation is shown below:

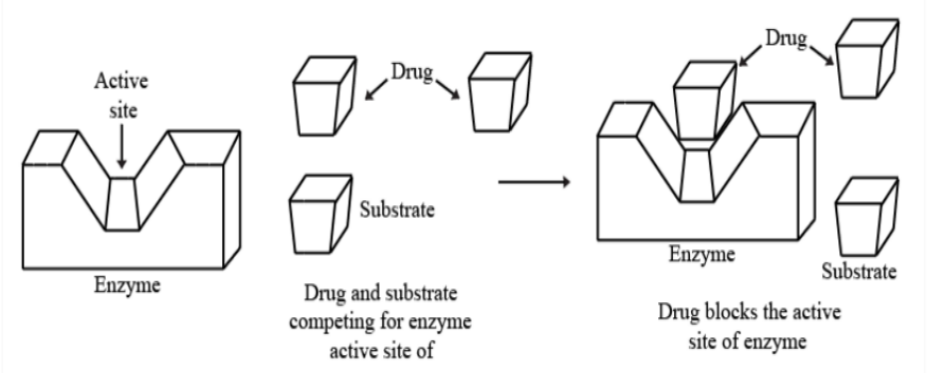

Fig. 17 – Mechanism of Enzyme Inhibition

Types of enzyme inhibitors:

I. On the basis of specific nature:

1. **Co-enzyme inhibitor:** These inhibitors result in inhibition of only the coenzymes e.g., cyanide hydrazine, hydroxyl amine inhibits the co- enzyme pyridoxal phosphate.

2. **Ion-cofactor inhibitor:** These are responsible for inhibiting the cofactors which are part of holoenzyme e.g., fluoride chelated Mg^{2+} ion of the enzyme enolase.

3. **Prosthetic group inhibitor:** They are responsible for the inhibition of the prosthetic group part of any holoenzyme e.g., the cyanide results in the inhibition of haem component of cytochrome oxidase.

4. **Apoenzyme inhibitor:** These results in inhibition of apoenzymes e.g., antibiotics.

5. **Physiological modulator:** The example of this type of inhibitor is Adenosine inhibited generation of superoxide ion by the neutrophils which are stimulated with N-formyl methionyl leucyl phenylalanine (FMLP), concanavalin A (Con A), calcium ionophore A23187.

II. On the basis of its origin:

1. **Natural enzyme inhibitor:** Natural enzyme inhibitors are those which have grown to defend a plant or animal against predators. The examples of these inhibitors are alkaloids, sesquiterpene, saponins, polysaccharides, flavonoids, dietary fibres, ferulic acid, tannins, limonene, and oleuropeins.

2. **Artificial enzyme inhibitor (synthetic):** These are competitive inhibitor drug usage is extensive. Examples include tetrahydrofolate: (anticancer drug), para-aminobenzoic acid: antibiotic etc.

III. On the basis of reversible or irreversible inhibition

1. **Reversible inhibition:** In this type of enzyme inhibition the activity of an enzyme is recovered after the inhibitors are removed.

Types of reversible inhibition:

(i) Competitive inhibition

Competitive inhibitors are substrate equivalent which fix to a particular substrate that gets attached to the site of enzyme i.e., the active site due to which there occurs competition between the inhibitor and the substrate to get attached with the given enzyme. This inhibition can be overcome by the increase in the concentration of substrate. In this case Vmax remains same but there occurs increase in the value of K_m. In this case the inhibitor gets initially attached with the enzyme but when the concentration of substrate increases the inhibitor gets released from the enzyme resulting in the binding of the substrate to the enzymes due to which the value of V_{max} remains same but the value of K_m increases. Example: conversion of succinate to fumarate by succinate dehydrogenase

(ii) Non-competitive inhibition:

In this type of inhibition, there is no competition between the given substrate and the inhibitor because the inhibitor gets attached with the enzyme leaving the substrate binding site. Since the binding site of substrate and inhibitor to enzyme is not same so, the inhibitor does not affect the affinity of enzyme towards substrate indicating the failure of inhibitor to overcome the increase in the concentration of substrate. Here the V_{max} decreases but Km remains same. It can be thus inferred that the attachment of substrate and inhibitor is equal. The inhibitor usually results in the change in the conformation of enzyme after binding due to which the substrate fails to get attached with the enzyme. This explains the decrease in the value of $V_{max.}$ Example: Heavy metal poisoning. Hg, Pb etc. results in the distortion of the -SH group present at allosteric site in the enzyme. Doxycycline is an example of non-competitive inhibitor of proteinase enzyme of bacteria. The removal of non-competitive inhibitor is possible by pH treatment or by hydrolysis. However, in the case of metal poisoning, chelator can be effectively used.

(iii) Uncompetitive inhibitor:

The uncompetitive inhibitor is observed in the reactions involving multiple substrates. It is one of the rare types of inhibition which is similar to non-competitive but this gets attached with only ES-complex. Here the substrate first binds to the enzyme yielding the ES-complex and the binding site for inhibitor forms at allosteric site resulting in the binding of the inhibitor. This results in the distortion of the active as well as the allosteric site of enzyme due to which there occurs inhibition of the catalysis. In this case, V_{max} as well as K_m decreases. Examples: lactate dehydrogenase gets inhibited by oxalate; alkaline phosphatase gets inhibited by l-phenylalanine.

(iv) Mixed inhibition:

Mixed inhibition can be observed in the multi-substrate reactions. It is described as the blend of competitive and the non-competitive inhibition. The mixed inhibitor results in the binding of both the active site and the allosteric site. This results in decrease of V_{max} and increase in the value of K_m. The decrease in V_{max} is due to non-competitive binding of inhibitor to the allosteric site resulting in the distortion of the enzyme. K_m increases as inhibitor can get attached with the active site competing with the substrate. This type of inhibition cannot be removed by increasing the concentration of the substrate. Examples: Ketoconazole is an example of mixed inhibitor that binds to 5 –**α** reductase enzyme, Palladium ion is mixed inhibitor of oxidoreductase enzyme.

V Coenzymes:

Coenzymes are compounds with similarity in behaviour as enzymes because they increase the rate of bio-chemical reactions, however unlike the enzymes, they are not proteins.

Definition:

Coenzymes can be defined as a particular type of cofactor which is an organic compound of non- proteinaceous nature, acting as a carrier molecule acting in conjunction with a particular enzyme.

The cofactor that gets firmly attached with apoenzyme is labelled as a prosthetic group; and the cofactor of organic nature which gets attached with the enzyme protein only when reaction occurs, is known as a coenzyme.

In biological process inside the cell hydrogen atoms or electrons on certain occasion, are removed from one compound and transported to another. In such process a specific enzyme catalyzes the elimination reaction, however, a specific coenzyme must be available there to support this transfer. The coenzyme momentarily attaches with the eliminated group and deliver it to an acceptor.

Chemistry of Coenzymes:

Coenzymes are mostly chemically derived from the nucleotides with the nitrogen base portion of it being substituted by some other chemical unit, such as a derivative of a specific vitamin.

The physiology of cell involves the following important coenzymes:

1. Flavin derivatives or Flavin nucleotides (FMN and FAD)

Among the two byproducts of riboflavin (vitamin B_2), flavin mononucleotide (FMN) and flavin adenine nucleotide (FAD) one of them is used as a cofactor by most of the respiratory enzymes.

Structure:

Riboflavin contains a ribose sugar, and a flavin part. Flavin has a complex three ring structure. Flavin mononucleotide (FMN) contains a phosphate group attached to riboflavin and is also called riboflavin monophosphate. If FMN joins to Adenosine mono phosphate (AMP), then a dinucleotide called flavin adenine dinucleotide (FAD) is obtained.

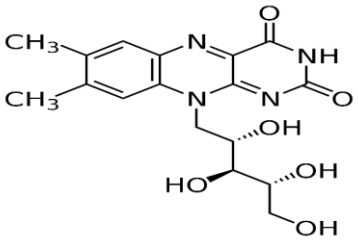

Fig. 18 – Structure of Riboflavin

Functions:

FMN or FAD gets attached itself with any apoenzyme which is known as flavoprotein (FP) which is responsible to catalyze the elimination of hydride ion (H^-) and hydrogen ion i.e., proton (H^+) from any metabolite.

$FAD + MH_2 \longrightarrow FADH_2 + M$

$FMN + MH_2 \longrightarrow FMNH_2 + M$

Here MH_2, is described as a substrate, $FADH_2$, is said to be the reduced form of FAD, and $FMNH_2$ is inferred as the reduced form of FMN. One of the important sources of hydrogen can be the reduced pyridine nucleotide.

$H^+ + NADH + FAD \longrightarrow NAD^+ + FADH_2$

When the flavoproteins gets reduced, they transfer their electrons to the cytochromes.

2. Pyridine derivatives or Pyridine nucleotides (NAD and NADP).

The first nucleotide coenzyme to be discovered was co-zymase, or diphosphopyridine nucleotide.

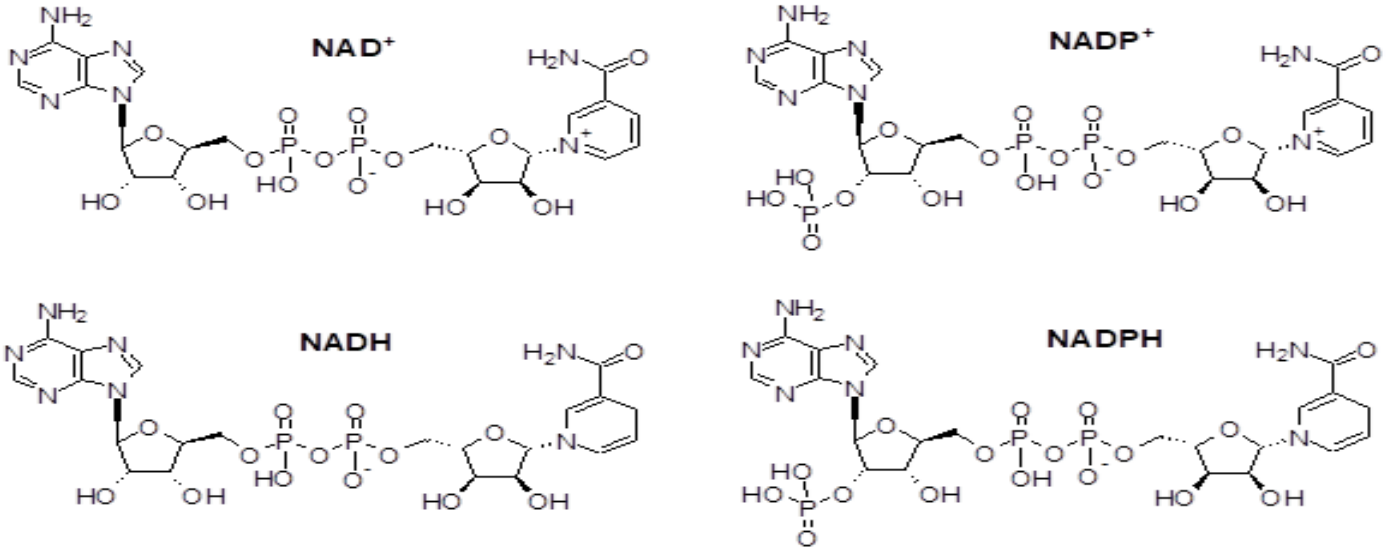

Fig. 19 – Structure of pyridine derivatives

NAD is involved in cellular respiration while NADP is involved in photosynthesis**.**

NAD	NADP
A coenzyme involved in redox reaction in the cellular respiration	A coenzyme involved in redox reactions inside the cell during photosynthesis
Does not contain phosphate group	Contains phosphate group at the 2' position of ribose ring which contains adenine moiety
Reduced form is NADH	Reduced form is NADPH
Oxidized form is NAD^+	Oxidized form is $NADP^+$
Oxidized form is more abundantly present in the cell	Reduced form is more abundantly present inside the cell
NAD^+ is mostly used as oxidizing agent	NADPH is mostly used as reducing agent
Used in glycolysis, Kreb's cycle, fatty acids and sterol synthesis	Used in Calvin cycle, pentose phosphate pathway, lipid synthesis, chain elongation in fatty acid and synthesis of cholesterol

Table 7 – Comparative study of NAD and NADP

3. Coenzyme A

Coenzyme A is synthesized naturally from the pantothenate (vitamin B5), which is present in some of the foods like meat, vegetables, cereal grains, legumes, eggs, and milk.

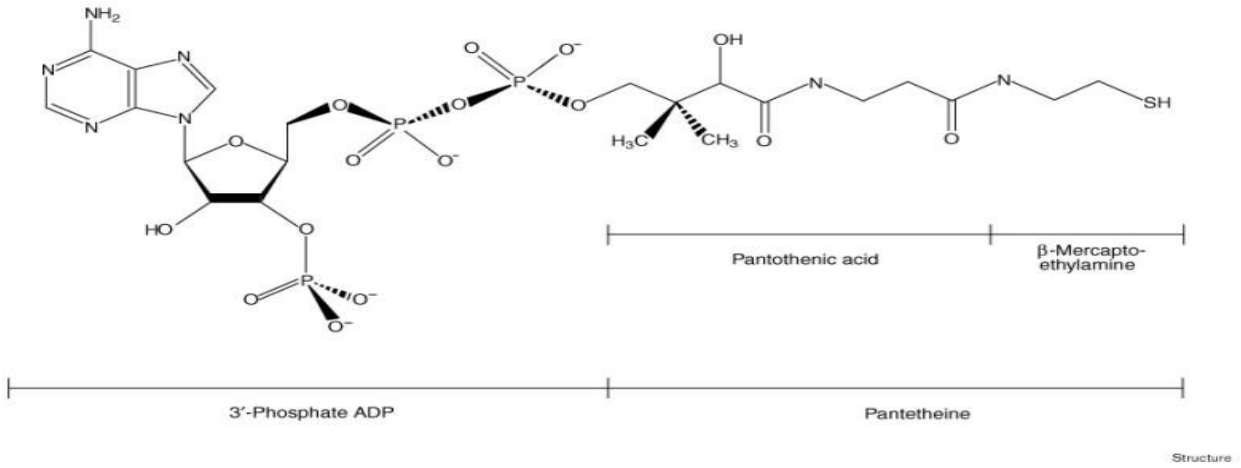

Fig. 20 – Structure of Coenzyme A

It plays a very important role in the preparation and oxidation reaction of the fatty acids, pyruvate oxidation occurring in the citric acid cycle, encoding genome sequenced and so on. It affects both anabolic and catabolic reactions. Its derivative Acetyl- CoA assists post - translational and allosteric regulations of enzymes pyruvate dehydrogenase and carboxylase in order to sustain and support preparation and degradation of pyruvate.

4. Coenzyme Q

In mitochondria, a quinone known as ubiquinone or Coenzyme Q. They are also found in microsome and cell nuclei, etc.

The coenzyme Q comprises of a quinone with a side chain having varying length according to the source of the mitochondria. In total 10 isoprenoids units in side chain known as coenzyme Q_{10} is present in the quinone present in the animal tissues.

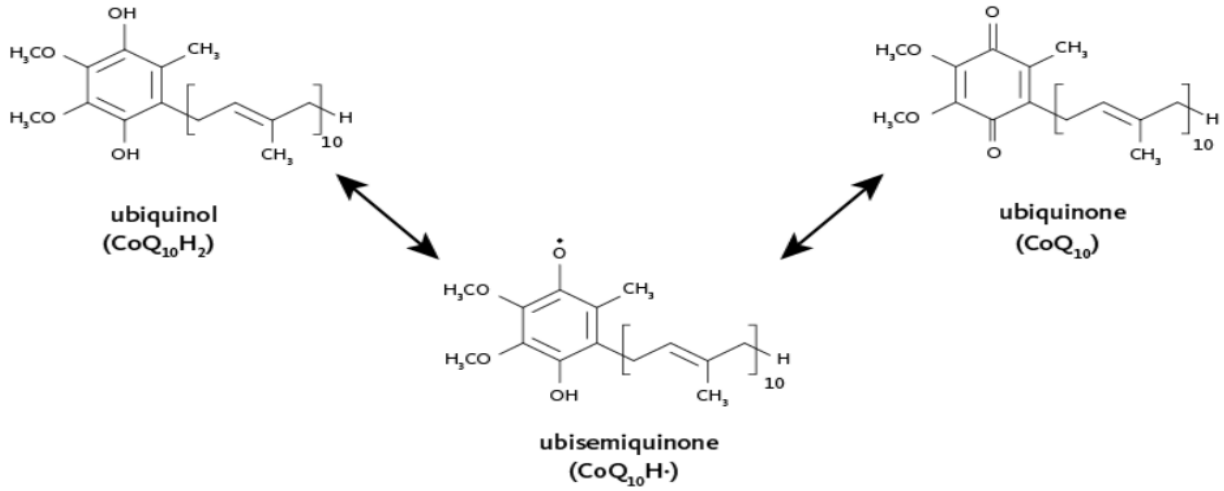

Fig. 21 – structures of Coenzymes Q

The coenzyme Q act as constituent of the electron transport chain functioning in the mitochondria. It plays the role of an supplementary hydrogen carrier between the flavin coenzymes (FAD and FMN) and the cytochromes.

5. Cytochromes

Cytochromes are the electron transfer proteins containing different haem groups that primarily generate ATP via an electron transport system within the mitochondria, endoplasmic reticulum and bacterial redox chains.

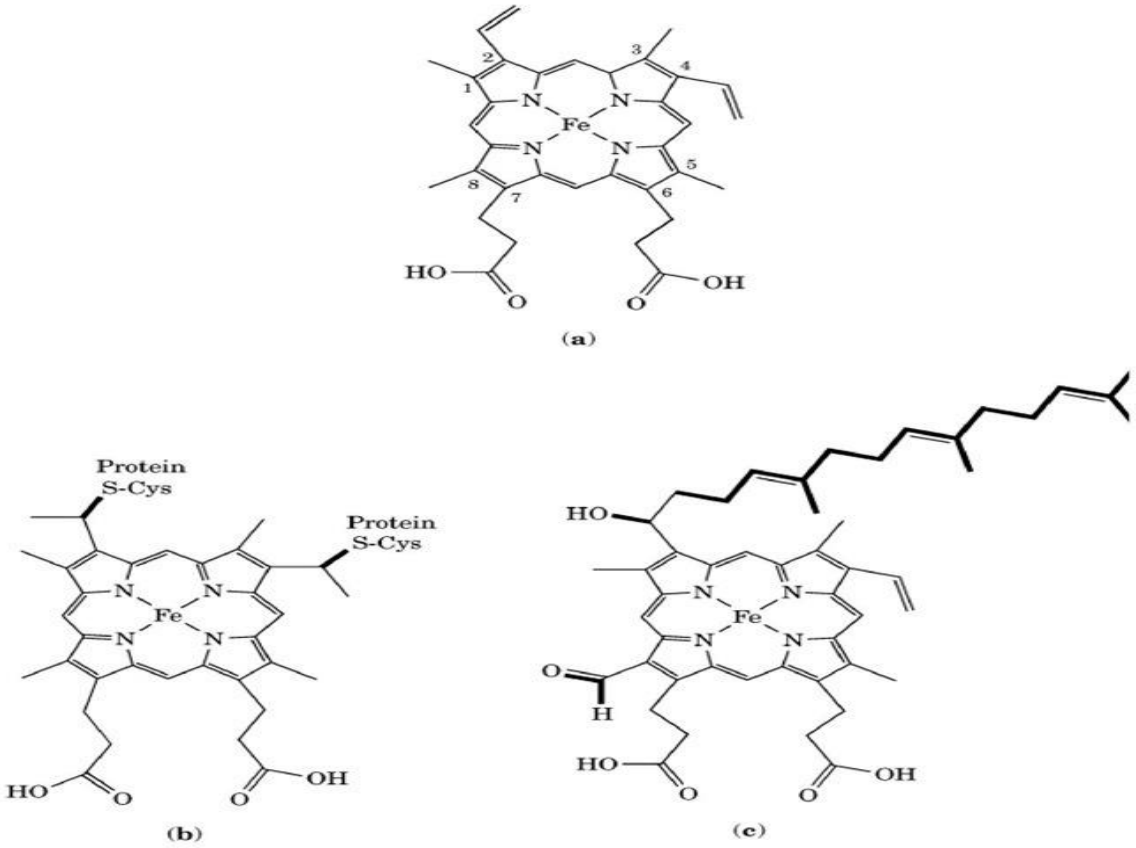

Fig. 22 – Structures of Cytochromes

The physiological activity in all the cytochromes is reversible redox reaction of iron between ferrous and ferric cationic forms. The cytochromes a and b and some type of cytochrome c form the vital proteins of the inner membrane of mitochondria while the cytochrome c is said to be a soluble protein associated with the outer surface of the mitochondrial membrane by electrostatic force of attraction.

The following is a scheme showing the electron transport system.

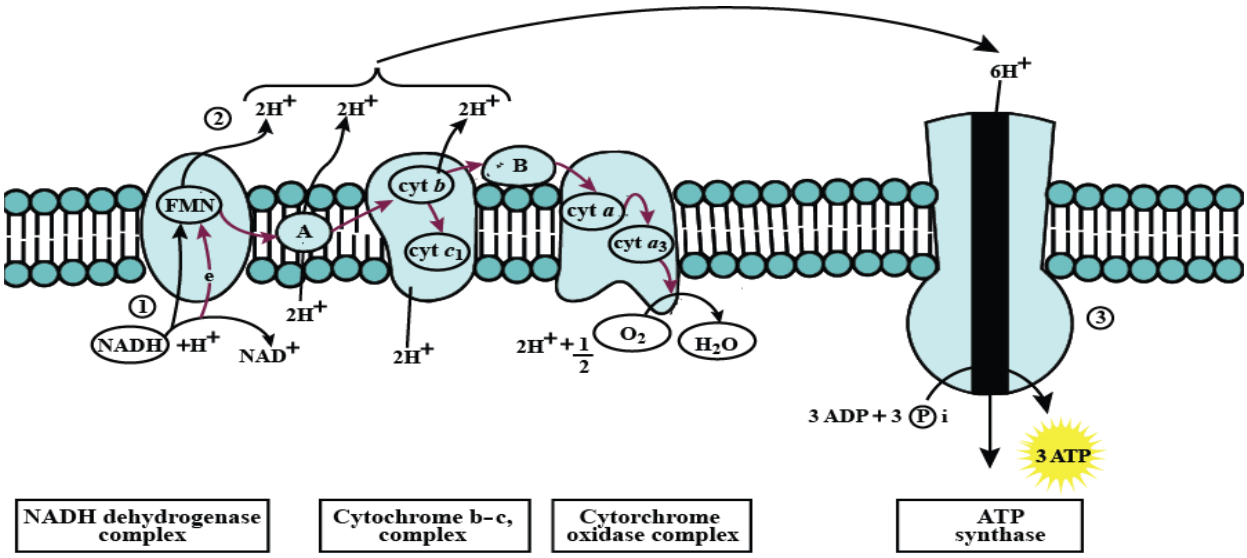

Fig. 23 – Representation of electron transport system

6. Thiamine pyrophosphate

Vitamin B1 i.e., thiamine as thiamine pyrophosphate plays an important role in oxidative phosphorylation and pentose phosphate mechanism acting as cofactor for α-ketoacid dehydrogenases such as pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (KGDH), transketolase and branched-chain α-ketoacid dehydrogenase

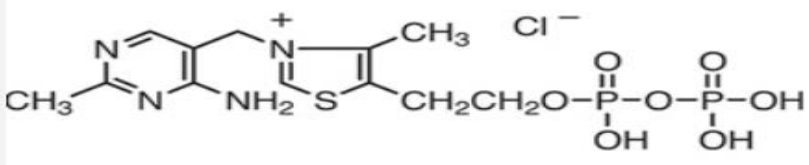

Fig. 24 - Thiamine Pyrophosphate Chloride

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