ENZYMOLOGY

Introduction

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Thousands of biochemical reactions that take place continuously in living cells have different speeds. Some reactions are fast while others are very slow. To increase the speed of slow reactions to desired level, involvement of catalysis is necessary to make these reactions proceed at a useful rate under physiological conditions. This alteration in the rate of reactions is mediated by biocatalysts called, *Enzymes.* Enzymes are protein catalysts (except ribozymes) that increase the rate of biochemical reactions without itself being changed in the overall process.

Brief history

The beginning of Enzymology can be traced back to the early nineteenth century though biocatalysts have been used for thousands of years to make bread and brew alcoholic beverages. The first clear recognition of an enzyme was made by Payen and Persoz in 1833. They practically isolated the 'active agent' responsible for breaking down starch into sugar and gave it the name *diastase* (now known as *amylase*). In 1834 Schwann isolated the first enzyme from an animal source by acid extraction of animal stomach wall, this is known as pepsin. During the second half of the nineteenth century Liebig held the view that fermentation and other similar processes were due to the action of chemical substances but later Pasteur discovered that fermentation was inseparable from living microorganisms. W. Kuhne in 1879 introduced the name 'enzyme'. In 1897, Buchner obtained the fermentation system from yeast in cell-free extract. The first enzyme obtained in crystalline form was urease from jack bean in 1926 by J.B. Sumner. It was postulated that all enzymes are proteins and this view was further supported by the work of J. Northrop and M. Kunitz when crystallized preparations of pepsin, trypsin and other enzymes by them were found to be proteins. So for all practical purposes all enzymes were regarded as proteins till the beginning of 1981-82. It was in 1982 when Thomas Cech discovered a specialized ribonucleic acid (RNA) molecule with enzyme like properties. This was called ribozyme. For this work, Cech shared the 1989 Nobel Prize in Chemistry with Sidney Altman.

However, the discussions in this chapter as well as in all related chapters, are made on the basis of protein nature of enzymes.

General characteristics

All enzymes are large and highly specialized globular proteins synthesized in cells. Their molecular weight generally ranges from 14000 to 400,000 Da. Enzymes are mostly water soluble colloids but some enzymes remain tightly bound to cell membranes. An enzyme is a catalyst which speeds up the rate of a specific reaction and while doing so it remains chemically unchanged and without loss of activity at the end of the reaction. It is to be emphasized that in an enzyme catalyzed reaction, the chemical equilibrium remains unchanged and the enzyme only speeds up the approach of this equilibrium. As such, enzymes can enhance reaction rates in cells as much as 10^{16} times the uncatalyzed rate. How enzymes help to increase the reaction rate to such a level by functioning as catalyst could be explained with the help of Fig. 1 which illustrates the energy changes that take place during the conversion of reactants into products. The equilibrium of such a reaction is determined by the energy states of reactants and products. These energy states remain unaffected by enzyme action. If the reaction has to proceed then the reactants (or substrate) must be brought to a higher energy level (or state), which is called *transition state.* The amount of energy required to bring the substrate to transition state is called *energy of activation.* This energy acts as a barrier for the progress of the reaction, thereby limiting the rate of the reaction. Catalysts (including enzymes) function by bringing down the activation energy and

hence increase the rate of reaction. The rate of catalyzed reaction both in forward and reverse direction remains same because both these rates have to undergo through same transition state.

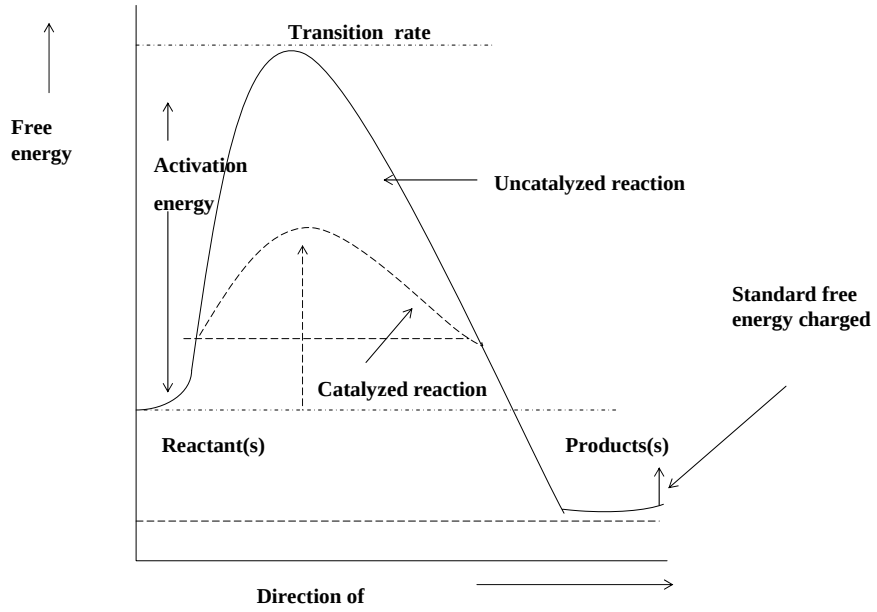
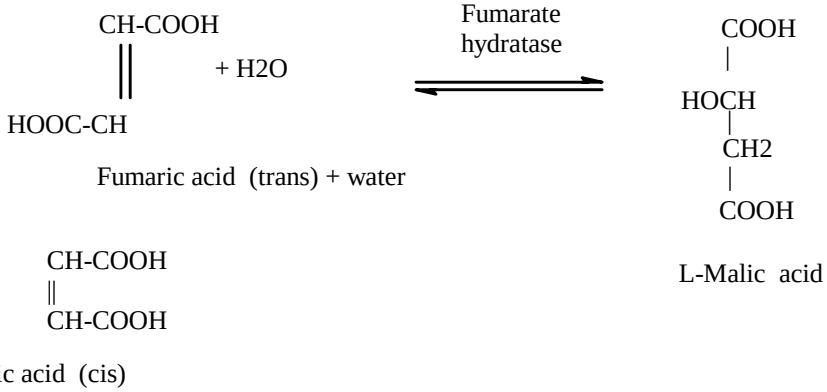


Fig. 1: Need for energy in case of uncatalyzed and enzyme catalyzed reactions

The enzyme catalyzed reactions involve binding of substrate(s) to enzyme (E) at the specific site, called *active site*, to form enzyme substrate complex (ES). This interaction lowers the energy of activation and facilitates formation of new transition state (Fig. 1 broken curve). The substrate while bound to enzyme is converted into product and then released from enzyme. This whole process is represented by following equation:

$$E+S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E+P$$

Each enzyme is very specific in its function though it is generally depicted by the above equation. There are three types of enzyme specificities viz., substrate, stereo chemical and reaction specificity. The extent of substrate specificity varies from enzyme to enzyme. It may be absolute or relative. Most of the biochemical reactions are extremely specific in the production of stereoisomers and the enzymes also show specificity. For example, enzyme fumrate hydratase catalyzes hydration of fumarate to form L-malate (maleic acid, a *cis*-isomer of fumrate is not used).



Enzymes are also reaction specific i.e. one enzyme will catalyze only one of the various reactions which the substrate can undergo. For example, breakdown of ATP into ADP and Pi (H_3PO_4) is catalyzed by enzyme ATPase as hydrolytic cleavage mediated by water where free energy of hydrolysis is dissipated. However, synthetases also catalyze the breakdown of ATP into ADP and Pi but the energy is used in the synthesis of a new bond. The following are the examples, respectively.

$$ATP + H_2O \xrightarrow{ATPase} ADP + Pi + energy$$

$$ATP + A + B \xrightarrow{Synthetase} AB + ADP + Pi$$

Thus both the enzymes are highly specific. Enzyme specificity has been discussed in detail later in this chapter.

Nomenclature

The name of enzyme usually indicates the substrate involved. By convention, an enzyme is named by adding the suffix 'ase' to the name of its substrate. For example, *Lactase, Sucrase* and *Maltase,* catalyze the hydrolysis of lactose, sucrose and maltose, respectively. These are called as *trivial names* of enzymes. This type of nomenclature fails to give a full information regarding the nature of reaction and other factors playing role in the reaction. Sometimes, enzymes are named to indicate the nature of the reaction catalyzed without specifying the substrate, e.g. *dehydrogenase* which mediates removal of hydrogen from substrate. Some names of enzymes indicate neither the substrate nor the reaction type, e.g. *catalase* which mediates the decomposition of hydrogen peroxide.

It is thus clear that a lack of consistency existed in the nomenclature and an urgent need was felt for a systematic method of naming and classifying enzymes. For this purpose, International Union of Biochemistry constituted a Commission which was assigned the job of systematic nomenclature and classification of enzymes. The first report of the Commission was published in 1964 and this has been updated frequently over the years. This report forms the basis of the presently accepted system of enzyme nomenclature.

The Enzyme Commission System of classification

Enzymes are grouped into six main classes in accordance with the nature of reaction catalyzed. Each class represents the type of reaction that is catalyzed by the enzymes of that class. Each enzyme has been given a code number consisting of four digits separated by dots. For example, a code number of the type E.C. 1.1.1.1 has the following meaning.

E.C. stands for Enzyme Commission. The subsequent first three numbers indicate in the order; *major class*, *subclass*, and *sub-subclass*. The fourth digit indicates the serial number of that enzyme in the sub-subclass. Based on this, the classification of enzymes with suitable examples is presented in Table 1.

Table 1: Major classes of enzymes

Enzyme class	**Type of reaction catalyzed**	**Example**
Oxidoreductase	Oxidation/reduction reactions	Alcohol dehydrogenase (EC 1.1.1.1) Alcohol + NAD^+ ⇌ Acetaldehyde + $NADH+H^+$
Transferases	Transfer of functional groups	Hexokinase (EC 2.7.1.1) Glucose + ATP ⇌ Glucose-6-phosphate + ADP
Hydrolases	Hydrolytic reactions	Acid phosphatase (EC 3.1.3.2) β-glycerophosphate+ H_2O ⇌ glycerol + orthophosphate
Lyases	Removal of a group from substrate (No hydrolysis)	Fumarate hydratase (EC 4.2.1.2) L-malate ⇌ Fumarate + H_2O
Isomerases	Isomerization reactions	Aldose-1-epimerase (EC 5.1.3.2) α-D-glucose ⇌ β -D-glucose
Ligases	The joining of two molecules at the expense of bond energy in ATP (or a nucleoside triphosphate)	Glutamamine synthetase L-glutamate+ NH_3 +ATP ⇌ L-glutamine + ADP +orthophosphate

Properties of enzymes

As stated earlier each type of enzyme has a specific 'active site conformation' that is essential for its catalytic activity. The 'active site conformation' includes the presence of some specific amino acid(s) at the active site besides the three dimensional structure of protein. These amino acids are involved in binding of substrate and catalysis of the reaction. In addition, there are some other amino acids at the active site whose side chains help in creating microenvironments at the site. Thus, the function of an enzyme depends on spatial arrangement of binding sites, catalytic sites and their microenvironment. In the process of catalysis, enzyme (E) binds substrate (S) to form enzyme-substrate (ES) complex.

'Lock- and- Key' hypothesis: Fischer in 1890 put forward this concept to explain that complementary structural features between E and S are responsible for the formation of ES complex (Fig. 2).

According to this concept, the structure (or conformation) of enzyme is rigid. The substrate nicely fits into the active site (earlier called binding site) just as key fits into a lock. This model, however, failed to explain many other behavioural features of enzymes such as, high enzyme specificity.

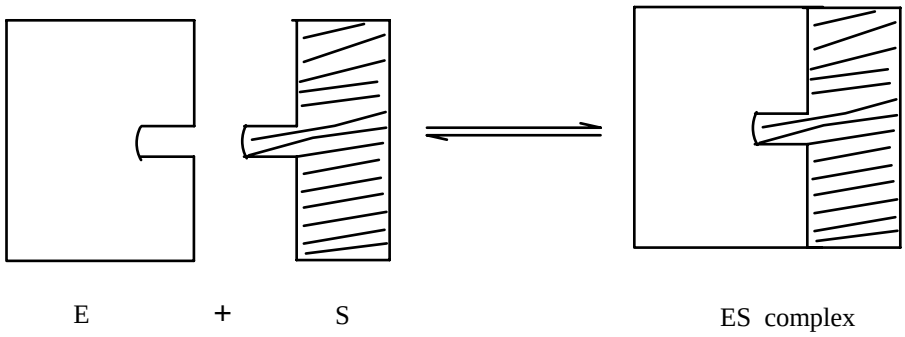


Fig. 2: Enzyme-substrate interaction after 'lock-and-key' hypothesis

'Induced- Fit' hypothesis: Koshland in 1958 suggested that the structures of E and S are complementary to each other only when they exist together in ES form, but not in their free existence (Fig.3).

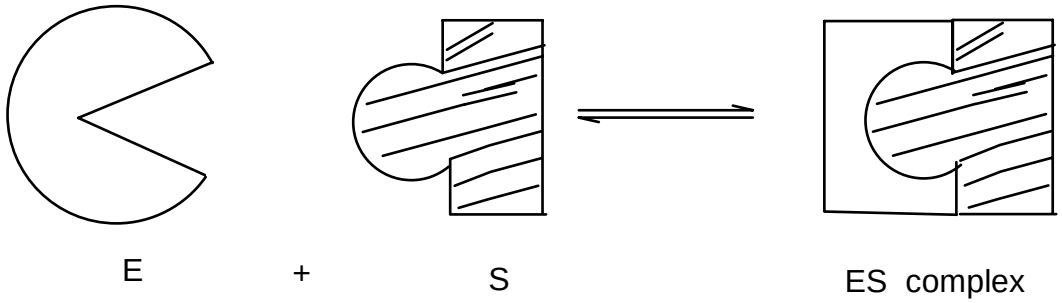


Fig. 3: Enzyme-substrate interaction after 'Induced-Fit' hypothesis

Accordingly, during the binding of substrate to enzyme a conformational change takes place in enzyme and this allows the enzyme to make a grip around substrate. This type of mechanism helps to achieve high degree of specificity for the enzyme.

Substrate- strain theory: As per this theory, substrate on binding to enzyme gets strained due to conformational change in enzyme. The strained substrate gets converted into product.

In reality, the combined effect of 'induced fit' and 'substrate strain' theories is considered to be responsible for enzymatic action. The phenomenon of catalysis is discussed in detail later in this chapter. However, the mechanism of catalysis can be understood better by considering some more facts of enzyme proteins and especially of 'active sites', as would be discussed later in this section.

There are some enzymes which require a 'non-protein' part attached to their molecules for the activity. These are called *cofactors*. The cofactor could be metallic ions or some small molecular weight organic molecules. The nature of binding of a cofactor with the enzyme could be either loose or very tight. In case the binding is loose the 'non-protein' organic part is called *coenzyme.* On the other hand when binding is very tight (sometimes even covalent) then this is called *prosthetic group.* When metal ions are tightly bound to the enzyme then it is called 'metalloenzyme'. The examples of some metalloenzymes are given in Table 2.

Table 2: Some metalloenzymes

Name Enzyme	Metal
Carbonic anhydrase	Zinc
Phenol oxidase	Copper
Carbooxypeptidase A	Zinc
Nitrogenase	Iron and molybdenum
Xanthine oxidase	Molybdenum
Superoxide dismutase	Manganese
Glutathione peroxidase	Selenium
Alcohol dehydrogenase	Zinc
Arginase	Manganese
Ascorbic acid oxidase	Copper
Cytochrome oxidase	Iron, copper
Nitrate reductase	Vanadium
Pyruvate kinase	Potassium, magnesium
Urease	Nickel
Xanthine	Molybdenum, iron
Glutathione peroxidase	Cobalt
Creatine kinase	Manganese
Nickel hydrogenase	Nickel

A fully functional enzyme is called *holoenzyme* which is frequently constituted of a protein-part and a non-protein part. The protein-part of enzyme is called *apoenzyme* and, as stated above, the non-protein (organic) part is coenzyme. Thus,

Apoenzyme + coenzyme ==== Holoenzyme

Table 3 shows the various coenzymes and prosthetic groups involved with enzymes. There are certain substances which modulate the activity of a holoenzyme. If the activity of enzyme is increased the substance is called *activator*. Generally, metal ions are involved in activation of enzyme activity. The activation is caused either by binding with substrate or helping in formation of effective ES-complex by bringing suitable conformational changes in enzyme protein. In addition, the metal ion may participate directly in catalytic process. Some of the enzymes requiring metal ions are listed in Table 3. Example of non-metal ion as activator of enzyme is that of Cl^- for amylase. On the other hand, if the substance decreased the enzyme activity then it is called *inhibitor*. There are a number of substances which cause inhibition of an enzyme catalyzed reaction. The nature of this inhibition is either reversible or irreversible. The details of enzyme inhibition are discussed in other chapter.

Active site of enzyme

It was stated earlier that enzyme proteins are large molecules and a very small region of the enzyme protein is involved in substrate binding and subsequent catalysis of the reaction. This region is called ‘active site’ or ‘active centre’. This site contains certain amino acid residues

whose side chains are in specific conformation and participate in the catalyzed reaction. At the end of the reaction these side chains assume their original conformation. The amino acids present at the active site may appear very close to each other but most often they are widely separated from each other in their location at the level of primary structure of protein. The amino acids are brought close to each other due to folding of protein structure. The side chains of these amino acids are involved to form a part of the pocket located either on the surface of enzyme molecule or forming a deep opening in the enzyme (Fig. 4).

Table 3: Cofactors, coenzymes and prosthetic groups

Cofactor	**Enzyme**
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Cu^{2+}	Cytochrome oxidase
Mn^{2+}	Arginase
Zn^{2+}	Alcoholic dehydrogenase
Coenzyme	
Nicotinamide adenine dinucleotide (NAD^{+})	Alcohol dehydrogenase, Lactate dehydrogenase
Thiamine pyrophosphate (TPP)	Pyruvate dehydrogenase
Flavin adenine dinucleotide (FAD)	Succinate dehydrogenase
Prosthetic group	
Pyridoxal phosphate	Amino transferases, Glycogen phosphorylase
Heme	Cytochrome oxidase

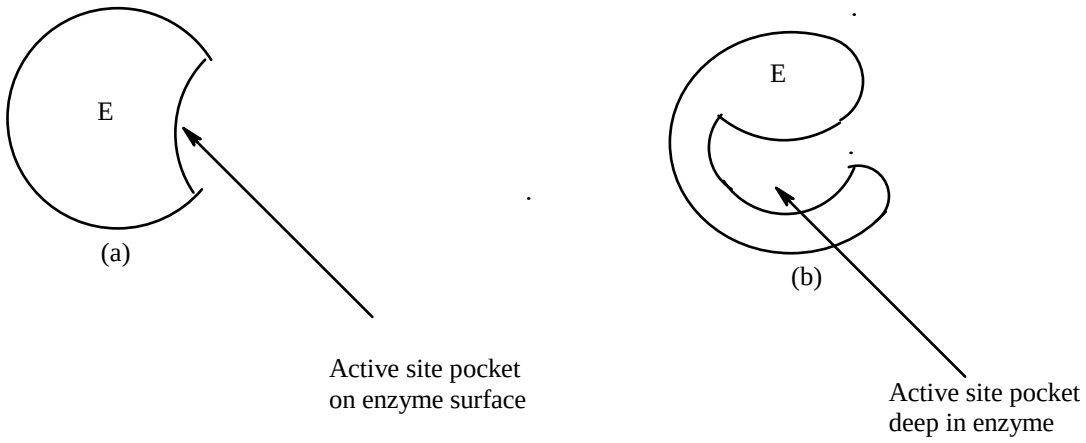


Fig. 4: Location of active sites in enzyme (a) on enzyme surface and (b) deep inside enzyme molecule

The binding of substrate molecule at this site is facilitated by the flexible nature of this site so that an effective ES-complex is formed. The cofactors or coenzymes which are present at the active site facilitate the formation of ES-complex and subsequent catalysis. The binding of

substrate to enzyme at the active site is due to weak interactions (non-covalent bonds) between the two. The amino acids that are generally involved at the active site for substrate binding and catalysis in various enzymes are cysteine, serine, histidine, aspartate, glutamate, tyrosine, arginine, lysine, etc.

Unit of enzyme activity

The activity of an enzyme catalyzed reaction is defined in some quantity which indicates an estimate of the rate of that reaction. Thus, activity is expressed as units in many ways because measurement of number of enzyme molecules or its mass is difficult. According to International Commission on Enzymes, 'One International Unit' of enzyme is defined as the amount of enzyme protein that catalyzes formation of one micromole of product in one minute under the conditions of assay (pH, temperature and ionic strength).

Another definition of unit is in Katal. One Katal is that amount of enzyme which catalyzes the conversion of one mole of substrate into product in one second under the experimental conditions.

Arbitrary Unit has a definition and according to which it is that amount of enzyme which transforms one micromole or nano mole or pico mole of substrate into product per minute under the assay conditions.

However, when enzyme activities are expressed in units as per these definitions then sometimes it is difficult to compare the activities of various enzymes. This becomes relatively a lot easier when activity unit is expressed as specific activity. Accordingly, the specific activity is activity units per mg protein i.e. specific activity of enzyme = $\frac{\text{total activity units}}{\text{total protein in mg}}$.
A general agreement on expression of specific activity is micromoles per min per mg protein.

Turnover number: When enzyme is fully saturated with substrate then number of substrate molecules converted into product in unit time by one molecule of enzyme is called *turnover over number* (k_{cat}). This is also referred to as '*molecular activity'*.

Enzyme specificity

Specificity is most distinctive feature of enzyme catalyzed reaction. There are enzymes which catalyze reactions with absolutely specificity i.e. they are involved in catalysis of a particular reaction, but on the other hand there are enzymes which exhibit broad specificity. The following two examples illustrate these cases:

1. **Glucokinase** - The enzyme is absolutely specific in the catalysis of the following reaction:

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{Glucokinase}} \text{Glucose} - 6 - \text{P} + \text{ADP}$$

2. Unspecific phosphatase – This enzyme has broad specificity in the sense that it catalyzes hydrolysis of phosphate group from a number of phosphate esters:

$$\text{Glucose} - 1 - \text{phosphate} + H_2O \longrightarrow \text{Glucose} + \text{Pi}$$

$$P \sim P + H_2O \longrightarrow 2\,Pi$$
(pyrophosphate)

$$ATP + H_2O \longrightarrow ADP + Pi$$

(Pi = H_3PO_4)

In general, there are four distinct types of specificity of enzymes:

(i) **Absolute specificity** – The enzyme catalyzes only one reaction e.g. glucokinase.

(ii) **Linkage specificity** – The enzyme acts on a particular type of bond in a substrate irrespective of the rest of the molecular structure. For example, phosphatases which hydrolyze phosphate ester bond.

(iii) **Group specificity** –The enzyme acts on certain specific functional groups present in a substrate. For example, methyl groups, amino groups, carboxylic groups, phosphate groups, etc.

(iv) **Stereochemical specificity** – If a substrate can exist in two stereochemical forms then only a particular steric or optical isomer is acted upon by the enzyme.

For example, the enzyme L-amino acid oxidase will act only on L-amino acid and not on D-isomer.

$$\text{L-amino acid} + O_2 + H_2O \xrightleftharpoons{\text{L-amino acid oxidase}} \alpha\text{-keto acids} + NH_3 + H_2O_2$$

$$\text{D-amino acid} + O_2 + H_2O \xrightleftharpoons{\text{D-amino acid oxidase}} \alpha\text{-keto acids} + NH_3 + H_2O_2$$

The specificity is an inherent feature of enzyme catalysis and is facilitated by the active site of the enzyme protein. The precise shape and flexibility of active site and its interaction with a particular substrate are important determining factors of enzyme specificity.

Isoenzymes

In a number of organisms the existence of different molecular forms of an enzymic protein catalyzing same reaction has been shown. These are called *isoenzymes* or *isozymes*. The presence of these isozymes in different organs of an organism suggests the different roles of these enzymic proteins.

For example, lactate dehydrogenase (LDH) present in different human tissues has five isozymic forms. These forms are commonly separated by gel electrophoresis in five different bands (Fig. 5). Every band catalyzes the same reaction and these five forms are known as LDH_1, LDH_2, LDH_3, LDH_4 and LDH_5. These proteins vary in their quaternary structure. All these forms are tetrameric proteins. Two different types of subunits, called H and M are present in variable numbers in each case. There are separate genes for the synthesis of H and M subunits. The relative predominance of these two forms varies in different tissues. For example, H subunits predominate in heart while M subunits predominate in liver and skeletal muscle. The five isozymic forms have the composition as H_4, MH_3, M_2H_2 and M_3H and M_4. The presence of varied proportions of these isozymic forms in different tissues is an indicator of the activities of respective genes responsible for the two subunits.

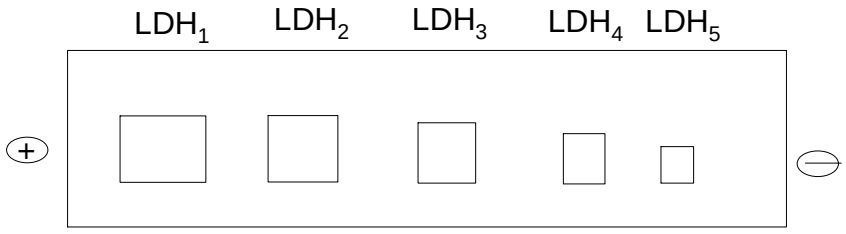


Fig. 5: Schematic presentation of different bands of LDH protein as usually appear on separation by gel electrophoresis

The kinetic properties of different LDH isoforms differ in their relative affinities for the various substrates and their sensitivity to inhibition by the product formed. This is an indicator of the role played by these isozymes in metabolic regulation (This aspect is elaborated under regulation of enzyme activity). In addition, the variation in the relative appearance of these isozymes in blood is indicative of the tissue damage and therefore it is suggestive of a role of isozymes in clinical diagnosis. For example, an increase of H_4 relative to H_3M level in serum is used in the diagnosis of heart attack.

There are many other enzymes whose isozymic forms are known. These are for example; alkaline phosphatase, creatine phosphokinase, isocitrate dehydrogenase, aspartokianse (in bacteria), etc. As seen in case of LDH that the enzyme is made up of different polypeptide units, but this is not so in case of all enzymes. There are enzymes having only one polypeptide unit as structural entity and there are enzymes having more than one polypeptide units. These are named accordingly as;

Monomeric enzymes*:* If an enzyme is made up of a single polypeptide unit it is called monomeric enzyme.

Oligomeric enzymes: These enzymes having quaternary structure are made up of two or more polypeptide chains which are linked to each other by non-covalent interactions. These proteins are also called *multimeric* proteins having high molecular weight (usually more than 40,000 Da) and their component polypeptides are called *subunits*. All regulatory enzymes are oligomeric enzymes showing the property of *allosteric regulation*. If these enzymes are made up of single type of monomer subunits, they are called *homooligomers*. When subunits are of different kinds then they are termed *hetero-oligomers*. Most of the oligomeric enzymes are made up of either 2 or 4 subunits. The following are examples of some exceptions:

(i) Glutamine synthetase in *E. coli* has 12 identical subunits.
(ii) Aspartate transcarbamoylase has two types of subunits in 6+6 numbers.

Multienzyme complexes

The reaction sequences in metabolic pathways are linked together by a common ‘product substrate’ relationship. A number of enzymes, catalyzing various reaction sequences, are sometimes intimately associated giving rise to what is known as ‘multienzyme complex’. It can also be defined as a complex having catalytic domains on more than one type of protein. If such a system is subjected to enzyme purification steps then all the associated activities also get purified. A few examples of such complexes are following:

(i) Enzymes involved in fatty acid synthesis.
(ii) Activities associated with pyruvate dehydrogenase system.
(iii) Enzyme system involved in tetrahydrofolate (FH_4) biosynthesis.
(iv) Enzyme system of α-ketoglutarate dehydrogenase.
(v) Activities of tryptophan synthase.

For any enzyme system to qualify to be called *multienzyme system* it should not catalyze different reactions by using the same catalytic centre. Also the system should not include the regulatory binding domains because as per definition given above it should possess multiple catalytic functions. In most of the multienzyme systems the various domains are joined by peptidase-sensitive linker regions and this is demonstrated by limited proteolysis. Genetic methods are also used, sometimes, to demonstrate that a single gene encodes several autonomous functional protein domains. The following are the advantages of multienzyme systems:

(i) *Catalytic enhancement of overall process* - The diffusion time of an intermediate product from one enzyme to the next during intermediary metabolism is reduced when the component activities are part of a multienzyme complex. This enhances the overall catalytic rate.
(ii) *Substrate channeling* – The multienzyme system excercises a control over a biosynthetic route by directing an intermediate metabolite to a specific enzyme rather than allowing competition from other enzymes in solution. Thus metabolic efficiency is increased.
(iii) *Sequestration of reactive intermediates* - Multienzyme complexes provide protection to chemically unstable intermediate metabolites from aqueous solution or say non-enzymic degradation.

Catalysis

The fundamental role of an enzyme is that it acts as a catalyst in biological system i.e. it speeds up the rate of a biochemical reaction. In considering a chemical reaction (**S** $\rightarrow$**P**) in laboratory it can be stated that the reaction proceeds through the following stages:

(i) Mutual collision of reactants.
(ii) Formation of an 'activated intermediate' at higher energy level.
(iii) Formation of product due to decay of 'activated intermediate'.

The mutual collision of reactants increases when energy input is increased i.e. it increases the rate of reaction. The formation of 'activated intermediate' is the result of reorganization of electron orbitals at higher energy level. This leads to reorganization of bonds which is a transient state. Subsequently, the decay must follow leading to rearrangement of orbitals – resulting in formation of product. The free energy at the end of reaction is less than that of initial state. At some point of time the system attains equilibrium where the concentrations of reactants and products are in such proportion that the overall free energy of the system is least.

Non-enzymatic catalysis

If the incorporation of a chemical species in the above system increases the rate of reaction with lower input of energy and, at the same time, this chemical is not used up in the process then we call it a catalyst. Thus, a catalyst modifies the mechanism of a reaction but never changes the equilibrium state it only makes approach of the equilibrium faster. Therefore, it can be stated that a catalyst reduces the energy of activation (Eα) for both, forward and

reverse reactions. In such a catalyzed reaction there could be many intermediate steps involved and each step is affected differently by the catalyst. However, there is no change in ΔH. Figure 6 illustrates these points.

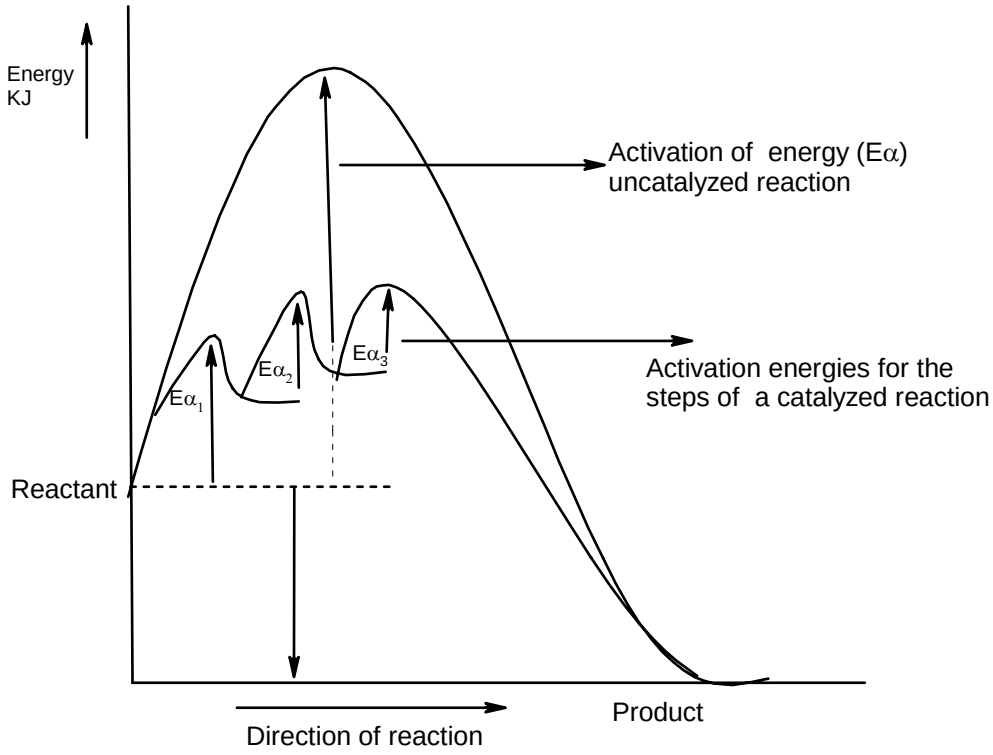


Fig. 6: Figure comparing the quantum of energy of activation in case of uncatalyzed and various steps of catalyzed reaction.

Two types of catalysis are generally seen:

1. ***Homogeneous catalysis*:** In this, the catalyst and the reactants are in the same phase and this is encountered in liquid phase.
2. ***Heterogeneous catalysis*:** In this catalyst and the reactants are in different phases.

Example of catalysis

Hydrogenation of unsaturated fat catalyzed by Ni or Pd or Pt, etc.

$$\text{Unsaturated oil} \xrightarrow[\text{Pt or Pd}]{\text{Hydrogenation}} \text{Saturated product (fat)}$$

Nature of catalysis

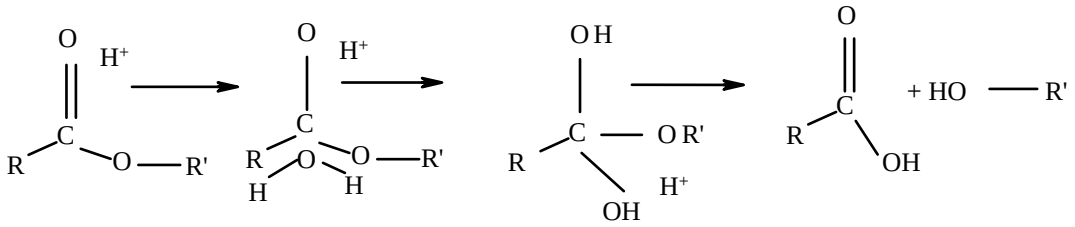
Non-enzymic catalysis that is generally encountered is of following types:

(i) **Acid base catalysis**: In this either acid or base catalyzes a process by donating or accepting a proton, respectively. This transfer is of temporary nature just to stabilize the transition state. An example is the hydrolysis of an ester as given below:

In this reaction (under neutral conditions) the incorporation of H_2O to ester forms a transition-state compound having unstable regions of positive and negative changes. Interaction either with acid or base stabilizes the transition state and catalyzes the hydrolysis of ester.

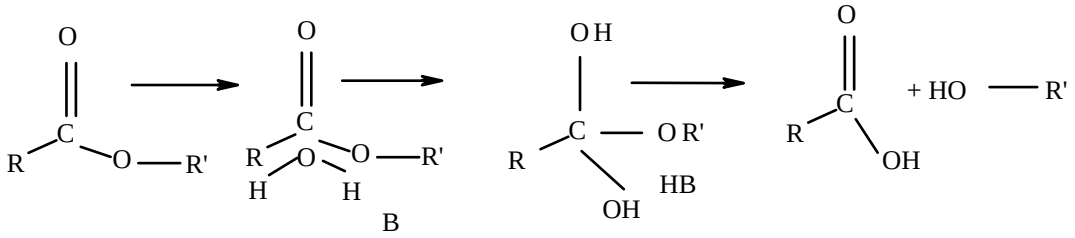
Under acidic conditions, a proton is donated to the transition state compound to stabilize it and increase the rate of reaction

General acid catalysis

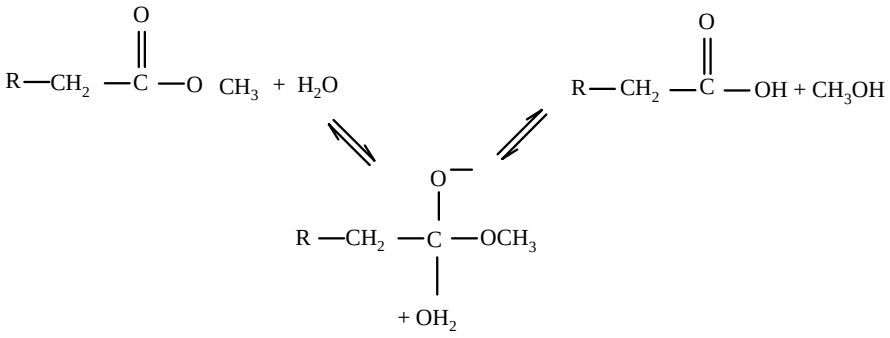


Under basic conditions, the transition state is stabilized by extracting a proton by the bases and nucleophilic character of the attacking group is increased. This increases the rate of reaction.

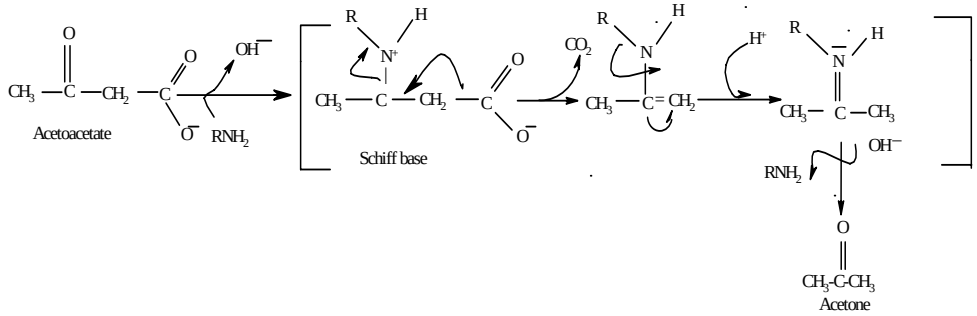
General base catalysis



(ii) **Electrostatic catalysis:** In this the electrostatic interaction between charged groups of a catalyst and charged groups of a transition-state compound may stabilize the state. In the following example of hydrolysis of an ester proceeding through a transition state the negative charge on oxyanion is stabilized by the positive charge of a metal ion and the positive charge on a carbonium ion can be stabilized by interaction with a negatively charged ion.



(iii) **Covalent catalysis:** In this a covalent bond is formed between the catalyst and the reactant(s) during the reaction forming an intermediate which rapidly breaks down to yield products. For example, breakdown of acetoacetate is catalyzed by amines



The above reaction involves the following steps:

(i) The catalyst (e.g. amino group) attacks substrate nucleophilically forming an intermediate (e.g. Schiff base).
(ii) Electrophilic withdrawal of electrons from the substrate.
(iii) Nucleophilic group leaves, regenerating catalyst.

Enzyme catalysis

Enzymes are biological catalysts and they accelerate the rate of reactions by factors which are sometimes more than a million. There exists a lot of diversity in the nature of enzyme catalyzed reactions. The mechanisms of catalysis as discussed earlier for non-enzymic reactions are also observed in enzyme catalyzed reactions though additional factors also play the role. Since enzymes are large in size (high molecular weight compounds) possessing a variety of groups that participate in forming a non-covalent complex with substrate (ES complex) and subsequently involved in the reaction mechanism, therefore they are able to influence the catalysis to a far greater extent than seen in case of non-enzyme catalysts.

These additional factors are following:

(i) *Entropy loss in ES formation*: The binding of substrate(s) on to the enzyme at active site brings about more order in system thereby decreasing the entropy.
(ii) *Proximity and orientation*: This refers to the precise arrangement of catalytic groups at active site. Proximity is important because all the required groups are provided by the

enzyme to increase their "effective concentration" at the vicinity. Orientation is important because the required groups in side chains of amino acids are brought in to participate in catalysis.

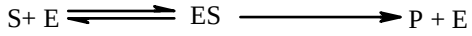
(iii) *Metal ions*: Metal ions are used in catalysis. The metal ions are either tightly bound to enzymes, e.g. Cu, Fe, Zn (metalloenzymes) or there is loose binding to form transient complexes e.g. Mg – ATP in case of kinases.
The metal ions are involved in following fashion:
   a) By using their charge they influence orientation of substrate binding.
   b) The metal ions cause electrostatic stabilization of negative charges of substrate or transition state.
   c) Metal ions influence oxidation-reduction reactions by transfer of electrons.

(iv) *Preferential transition state binding*: This refers to interactions between enzyme and transition state which do not exist in ES complex. Increased enzyme transition state interactions are essential to increase catalysis by the enzyme.

In conclusion, the enzymes are responsible for creating a suitable environment for the reactions to proceed smoothly. This is caused by lowering the energy of activation and simultaneously the overall energy requirement is spread over several stages of the reaction catalyzed.

Measurement of enzyme activity

The measurement of enzyme activity is called enzyme assay. As we know that an enzyme (E) catalyzes the transformation of a substrate (S) into products (P) which can be represented as per the following reaction



It is obvious that E regenerates at the end of the reaction. The rate at which S is converted into P represents the activity of E. If higher is the rate of formation of P from S, higher is the activity of E. The measurement of this quantity (activity) of E is made on the basis of either rate of formation of P or rate of disappearance of S. This is the basic principle of measurement of activity. A variety of methods are available for quantitatively following the enzyme catalyzed reactions. These are following:

(i) Spectrophotometric method: This method is used under conditions where substrate and/or products absorb light.

(ii) Flourescence method: The method is applicable when a substrate/product possesses the property of fluorescence, i.e. it absorbs visible light of a specific wave length and then emits it at a longer wave length. This method is more sensitive and specific than absorption measurement.

(iii) Manometric method: This method is applicable for the reaction system where at least one of the components is gas. In reactions where O_2 is consumed and/or CO_2 is produced this method is applicable.

(iv) Electrode method: In reactions where there is production or consumptions of H^+, O_2, etc., specific electrodes, such as pH-electrode, O_2-electrode, etc. are used to monitor the change in their concentrations.

(v) Polarimetric method: Many enzymes act on the optical isomer of their substrate. In such a case, if the product is optically active, the extent of reaction can be followed by recording the change in optical rotation.

(vi) Sampling method: This method is applicable in cases where any of the above properties are missing. In this the reaction is followed by withdrawing samples of reaction mixture at time intervals and chemical estimation of the leftover substrate or product formed is done.

Time course of enzyme catalyzed reaction

In order to evaluate the activity of an enzyme catalyzed reaction, we need to measure the initial rate of the reaction. If we record the concentration of product formed, [P], with time and plot a graph between them then it is likely that we may obtain any of the following types, a or b or c, curves (Fig. 7).

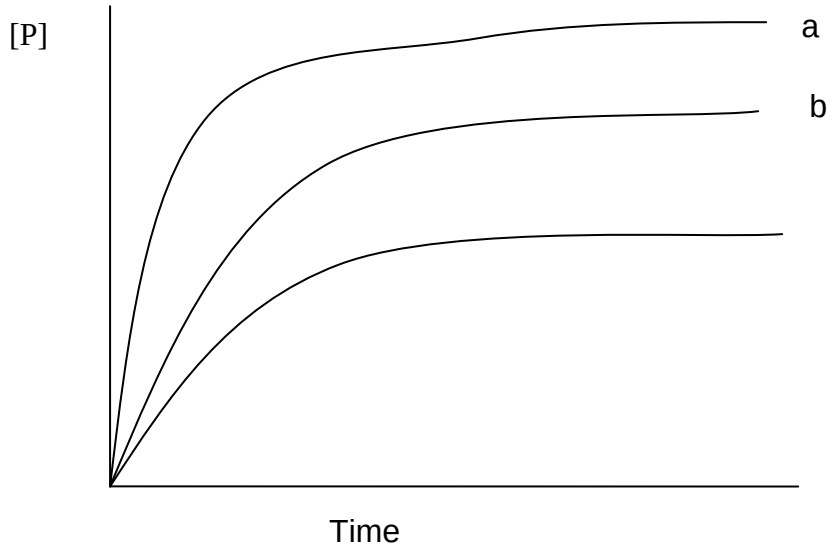


Fig. 7: Time-dependent-course of product formation on enzyme catalyzed reactions. Curve a – is obtained when rate of reaction is very high (K_m and V_{max} high) so that the enzyme saturation falls constantly; Curve b – indicates that K_m and V_{max} are much lower and ES complex is relatively stable and Curve c – indicates that enzyme is unstable and there is constant loss of activity.

As such, the most ideal case to measure the initial rate of reaction is curve b. Since enzyme activity is influenced by pH, temperature (shall be discussed in detail in next chapter) and time (as seen above), therefore maintenance of these factors at constant value is a standard pre-requisite for evaluation of activity. The measurement of true value of initial rate is achieved either from the curves where there is prolonged linearity or it can be calculated from the time period in which less than 10-20 percent of the total substrate consumption has occurred.

Experimental approaches

The following approaches are used:

(A) *Direct and continuous assays:* In cases where changes in properties of reactants take place the measurement is relatively easier and can be done directly as reaction proceeds. For example, the experimental methods (i) to (v) listed earlier in this section

are applicable. In reactions where – SH groups are generated, the treatment with a chemical, viz., 5,5'-dithiobis-2-nitrobenzoate (DTNB), yields a yellow coloured complex which has a characteristic absorption maxima at 412 nm. In reaction catalyzed by *citrate synthase* (CS)

$$\text{Acetyl} - \text{CoA} + \text{oxaloacetic acid} \xrightarrow{\text{CS}} \text{citrate} + \text{CoA} - \text{SH}$$

the measurement of -SH group of CoA by above method would be a basis of assay of enzyme activity.

(B) *Indirect assays:* In this, the reaction is stopped after a definite time period either separate the products for analysis or produce changes in properties of substrate or products for quantitation. These assays are of following types:

Discontinuous: As stated above one can use methods of rapid separation and quanitation of products formed.

For example, measurement of luminescence can form the basis of a highly sensitive assay. If a reaction system consumes or produces NADPH + H^+ then changes in its quantity can be determined by using the following reaction system:

$$\text{NADPH} + \text{H}^+ + \text{FMN} \longrightarrow \text{NADP}^+ + \text{FMNH}_2$$

$$\text{FMNH}_2 + \text{RCHO} + \text{O}_2 \xrightarrow[\text{luciferase}]{\text{bacterial}} \text{H}_2\text{O} + \text{RCOOH} + \text{light (luminescence)}$$

The measurement of luminescence could be a basis of NADPH quantitation. RCHO in above is generally a long chain aldehyde.

Similarly, luciferin and luciferase system has been used to monitor the quantity of ATP in any system. Since ATP is involved either as reactant or product in a variety of enzyme catalyzed reactions, therefore its quantitation by the above system would be rapid and sensitive for the measurement of activity of enzymes responsible for the changes in its concentration. The system, used is following:

$$\text{ATP} + \text{luciferin} + \text{O}_2 \xrightarrow{\text{firefly luciferase}} \text{oxyluciferin} + \text{AMP} + \text{PPi} + \text{CO}_2 + \underset{\text{(luminescence)}}{\text{light}}$$

(C) *Coupled assays:* These methods are used in cases where direct measurement either of substrate disappearance or product formation can not be done easily and accurately. In these cases the product formed is coupled to a second enzymic activity so that while acting as substrate it yields a product that can be measured easily and accurately.

For example, use of enzyme glucose-6-phosphate dehydrogenase is made in the assay of hexokianse (HK) activity, as per following sequence of reactions:

$$\text{Glu cos e} + \text{ATP} \xrightarrow{\text{HK}} \text{Glu cos e} - 6 - \text{phosphate} + \text{ADP}$$

$$\text{Glu cos e} - 6 - \text{phosphate} + \text{NADP}^+ \xrightarrow[\text{dehydrogen ase}]{\text{Glu cos e}-6-\text{phosphate}} 6 - \text{phosphoglu conic acid} + \text{NADPH} + \text{H}^+$$

The end product, NADPH + H^+, is quantitated by recording the increase in absorption at 340 nm (λ_{max} of NADPH). Since, the chemical stoichiometry in the above reactions is strictly followed, therefore, quantity of NADPH + H^+ formed becomes the basis of HK activity.

However, in such systems the reactant(s) used in second reaction (e.g. $NADP^+$ in this case) should not be limiting.

Choice of assay method

Since a variety of approaches are available for the assay of enzyme activities, therefore it is important to consider the nature of enzyme, substrate and product formed in selecting the method for enzyme assay. For example, if product formed is not very stable then we should follow a method of direct assay if the product gives characteristic absorption.

Choice of buffers

It is also very important to have a proper choice of buffer solutions so that the components of buffer do not interfere either in enzyme assay or in product estimation. For example, in selecting buffer solution for the assay of enzyme phosphatase, phosphate buffer is never used because it interferes with estimation of phosphate released by enzyme action.

Preparation of assay mixture

As most of the reactants of assay mixture and enzyme preparation are maintained at 2-4^oC just before assay, therefore pre-equilibration of reactants at assay temperature is required. It is very important to prepare enzyme assay mixture in such a fashion that the amount of reactant(s) does not become limiting throughout the course of assay. In addition, running a proper control is also important where addition of either substrate is omitted or, if not, then denatured enzyme is added. In case if substrate is of labile nature then reaction is initiated by its addition. Throughout the assay period there should be proper mixing of the contents of assay mixture.

Termination of reaction

The termination of enzymic reaction, whenever required, is done by denaturing the enzyme. The method of denaturation should be such that it does not affect the nature of products formed and at the same time it should not cause subsequent interference in the estimation of product. Trichloroacetic acid (5% in final concentration) is generally used to stop the reaction by denaturing the enzyme and it also precipitates the protein which can be separated by centrifugation. In some studies, reaction is stopped by transferring the assay mixture, at the end of reaction, to a higher temperature to denature the enzyme protein. In cases where either reactants or products have characteristic absorption, the direct time-dependent measurement can be done without terminating the reaction. This is particularly useful in enzyme assays involving NAD (P)H.

Role of vitamins as coenzymes

Vitamins are small organic molecules. As is known that on the basis of their solubility, these are classified as fat-soluble and water-soluble vitamins. The water-soluble vitamins are members of vitamins B and C. These vitamins, in most of the cases, act as precursors of coenzymes which are essential components of many enzyme catalyzed reactions in all types of cells. The deficiency of any of these vitamins affects the efficiency of the biochemical reaction where they participate besides causing other deficiency diseases. Table 4 depicts the various vitamins, their respective coenzyme forms, the type of reaction mediated by them and the name of the disease associated with their deficiency.

Table 4: Vitamins as coenzymes

Vitamin	Coenzyme form	Type of reaction mediated	Example	Deficiency disease
B_1(Thiamine)	Thiamine pyrophosphate (**TPP**)	Aldehyde transfer	Catabolism and biosynthesis of branched chain amino acids	Beri-beri
B_2(Riboflavin)	Flavin mononucleotide (**FMN**)	Oxidation-reduction reaction	L-amino acid se	Dermatitis and cheilosis
	Flavine adenine dinucleotide (**FAD**)	Oxidation-reduction reaction	D-amino acid oxidase, xanthine oxidase, succinate dehydrogenase, etc.	
B_3(Niacin, Nicotinamide)	Nicotinamide adenine dinucleotide (**NAD^+**)	Oxidation-reduction reaction	Lactate dehydrogenase, alcohol dehydrogenase	Pellegra (dermatitis, diarrhea)
	Nicotinamide adenine dinucleotide phosphate (**$NADP^+$**)	Oxidation-reduction reaction	Glucose-6-phosphate dehydrogenase	
B_5 (Pantothenic acid)	Coenzyme A	Carbon activation and Acyl group transfer	Thiokinase	Dermatitis in some birds, hypertension
B_6 (Pyridoxine)	Pyridoxal phosphate	Transfer of amino group (Transamination)	Glutamate-oxalate aminotransferase	Dermatitis, Convulsions, etc
B_7 (Biotin)	Biocytin (Bioinylated enzymes)	Carbondioxide transfer or ATP dependent carboxylation	Acetyl CoA carboxylase, Pyruvate carboxylase, etc.	Dermatitis, muscular pain, etc.

Folic acid	Tetrahydrofolate (**FH_4**)	Transfer of one carbon group, e.g. methenyl, formyl, etc.	Serine hydroxy methyltrans-ferase	Aneamia
Lipoic acid	Lipoamide	Carrier of hydrogen, acetyl group, etc.	Dihydrolipoyl transferase (E_2 of the pyruvate dehydrogenase complex)	
B_{12} (Cyanobalamin)	Methyl cobalamin, Deoxyadenosyl cobalamin	Mutase, methyl transfer	Methylmalonyl CoA mutase, homocysteine methyl transferase	Pernicious anemia
Vitamin C	Ascorbate/dehydro ascorbate	Oxidation reduction reaction	Hydroxylation of proline	Scurvy

Suggested Readings

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Presentation transcript: 1 Clinical Enzymology Introduction. 2 Enzyme Characteristics Catalytic power Increase reaction rates by up to 1014 times Highly specific for their substrates No catalysis for closely related compounds Specific active site for substrate binding No by-products produced Regulation â€“ regulated by interactions with Inhibitors â€“ reversible or irreversible Activators â€“ via presence of an activator or by permanent modification of the enzymeâ€™s structure Huda S.AlBake, KSU, CAMS WordPress Shortcode. Link. Enzymology- introduction and classification. 3,708 views. Share. Like. IMS & SUM Hospital, Bhubaneswar. Follow. Published on Oct 23, 2017.Â JBS Haldane wrote a treatise-â€™Enzymesâ€™. Enzymology- introduction and classification. 1. ENZYMES Prof. Dr. V.P. Ach. 2. introduction and classification. 3. What shall we learn? â€¢ Why should we learn about enzymes? â€¢ Why medicos should know about them? â€¢ Enzymology - Science topic. The study of the properties and action of enzymes. Questions (352).Â Introduction: Several pharmacoepidemiological studies indicate that proton pump inhibitors (PPIs) significantly increase the risk of dementia. Yet, the underlying mechanism is not known. Here, we report the discovery of an unprecedented mode of action of PPIs that explains how PPIs may increase the risk of dementia. Enzymology is the study of enzymes, their kinetics, structure, and function, as well as their relation to each other. In the diagram on the right, hexokinase is displayed as an opaque surface with a pronounced open binding cleft next to an unbound substrate (top) and the same enzyme with more closed cleft that surrounds the bound substrate (bottom). MOLECULAR ENZYMOLOGY, BECAUSE OF ITS CHEMICAL AND MATHEMATICAL content, is often regarded Python Programming: An Introduction to Computer Science. 261 PagesÂ·2012Â·1.2 MBÂ·112,897 Downloads. Python Programming: An Introduction to Computer Science John M. Zelle, Ph.D. Version 1.0rc2 Fall The Rules of Work: A Definitive Code for Personal Success. 273 PagesÂ·2012Â·827 KBÂ·190,428 Downloads.