Chapter 4
Enzymes

4.1 Introduction

Enzymes are proteins that act as biological catalysts, i.e. these alter the rates of biochemical reactions without undergoing any permanent change in themselves. These have a high degree of specificity besides high efficiency or rate of reactions. The term enzyme was introduced by W. Kühne in 1878 to refer to certain substances in yeast (in *zymin*) that made it act as a fermentative—an agent causing fermentation. In nature, enzymes help millions of chemical reactions to occur at extraordinary speeds and under moderate conditions. In the absence of enzymes, most chemical reactions that maintain a living organism would occur only under very drastic conditions say at a temperature of the order of 100° or above which would 'kill' the fragile cell. At normal body temperatures, these reactions would often proceed at an extremely slow rate. For example, the dissociation of carbonic acid into CO_2 and H_2O that takes place in the lungs proceeds only at a rate of about 10^{-7} mol dm^{-3} s^{-1} at room temperature in a test tube. On the other hand, in the cells, the enzyme *carbonic anhydrase* accelerates the reaction by more than a million times.

$$H_2CO_3 \xrightarrow{carbonic\ anhydrase} CO_2 + H_2O$$

Similarly, a single molecule of the enzyme, *catalase*, catalyses the decomposition of about 10^7 molecules of hydrogen peroxide—a toxic by-product of metabolism, in one second. A comparison of the rates of the catalysed and uncatalysed reactions gives an idea of the capability of the enzymes. For example,

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2 \quad K_{295} = 3.5 \times 10^7$$

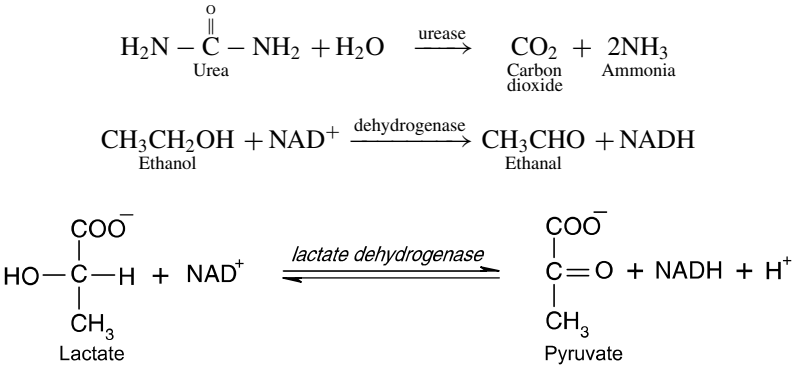
$$2H_2O_2 \xrightarrow{Fe^{2+}} 2H_2O + O_2 \quad K_{295} = 56$$

V. K. Ahluwalia et al., *Chemistry of Natural Products*,
https://doi.org/10.1007/978-3-030-86698-3_4

The macromolecular components of almost all enzymes are composed of proteins, except for a class of RNA modifying catalysts known as **ribozymes**. These are molecules of ribonucleic acid that catalyse reactions on the phosphodiester bond of other RNAs. Besides sustaining life, enzymes are important in industry also. These have been in use for ages in the production of wine, curd, cheese, etc. In recent times, the synthetic applications of enzymes have revolutionised the area of synthetic Organic Chemistry.

4.2 Nomenclature and Classification of Enzymes

Conventionally, the trivial or common names of the enzymes were derived from the names of the substrate they acted on or the reaction they catalysed or both and ended in a suffix '**-ase**'. For example, *urease* is an enzyme acting on urea, *dehydrogenase* catalyses the reaction involving removal of hydrogen and *lactate dehydrogenase* refers to an enzyme that catalyses the removal of hydrogen from the lactate ions to give pyruvate.



The system worked well initially with fewer enzymes but as the number of enzymes known increased, their naming in this way proved far from satisfactory. In many cases, the same enzyme was known by several different names, while sometimes same name was given to different enzymes. Further, many of the names conveyed little or nothing about the nature of the substrate or reactions catalysed, e.g. catalase, chymotrypsin etc. At times, similar names were given to enzymes of quite different types. To meet this situation, an International Commission on enzymes was set up by the **International Union of Biochemistry** (IUB) in 1957. The **Commission on Enzymes (EC)** considered the question of a systematic and logical nomenclature for enzymes and recommended two types of nomenclature: one systematic and the other working or trivial. The nomenclature has been revised and updated from time to time in consultation with IUPAC, earlier by the EC and later by the **Nomenclature**

Committee of International Union of Biochemistry and Molecular Biology (NC-IUBMB). The currently used nomenclature (NC-IUBMB; 1992 recommendations) is briefly described below.

4.2.1 Systematic and Recommended Names

The systematic name of an enzyme is given in accordance with definite rules and describes the action of an enzyme as exactly as possible thereby identifying the enzyme precisely. The **systematic name** of an enzyme ends in *-ase* and consists of two parts. The first part contains the name of the substrate, while the second part indicates the nature of the reaction, e.g. *alcohol dehydrogenase*. However, in case of a bimolecular reaction, the first part contains the names of both the substrates separated by a colon and has small and equal spaces before and after the colon. For example, for the bimolecular reaction,

$$\text{ATP} + \text{Creatine} \rightarrow \text{ADP} + \text{Phosphocreatine}$$

the systematic name of the enzyme is ATP: creatine phosphotransferase. The enzyme catalyses the transfer of a phosphate group from ATP to creatine. The trivial name for this enzyme is *creatine kinase*. Though systematic names are quite logical, these are quite cumbersome and inconvenient to use; therefore, the commission later decided to give more prominence to the trivial names. However, the systematic names are still retained as the basis for classification of enzymes (discussed below) which gives a code number to every enzyme. All the known enzymes are given code numbers depending on the their classes and subclasses, etc. These are collected in a list called as **enzyme list**, in which the common names follow immediately after the code number, and are described as **recommended names**. In assigning recommended names, a name in common use is taken as such if it gives some indication of the reaction and is not incorrect or ambiguous. Otherwise a recommended name is based on the same general principles as the systematic name but with only essential details so as to produce a name short enough for convenient use.

4.2.2 Classification Numbers and Code Names

The enzyme commission has devised a system for classification of enzymes that also serves as a basis for assigning code numbers to them. In this system, each enzyme is classified by assigning an enzyme commission (EC) number to it which consists of four parts. Three general principles have been used by the commission for this purpose.

1. An enzyme is to be named by adding the suffix '**-ase**' to the name of the substrate on which it acts and refers only to single enzymes, i.e. single catalytic entities. In cases where a number of enzymes are involved in catalysing an overall reaction, the word *system* is to be included in the name. For example, the oxidation of succinate by molecular oxygen involves succinate dehydrogenase, cytochrome oxidase and several other intermediate carriers. These enzymes are named as a group called *succinate oxidase system* and not as *succinate oxidase*.
2. Since the chemical reaction catalysed is the specific property that distinguishes one enzyme from another, it is logical to use it as the basis for the classification and naming of enzymes. Therefore, according to the second general principle, the enzymes are principally classified and named according to the reaction they catalyse. For example, *dehydrogenases* catalyse a reaction involving removal of hydrogen.
3. According to the third guiding principle, the enzymes divided into groups on the basis of the type of reaction catalysed, along with the name(s) of the substrate(s) provide a basis for naming individual enzymes. For example, *lactate dehydrogenase* acts on the substrate lactate in catalysing the removal of hydrogen atoms.

An advantage of this method of classification is that the chemical reactions catalysed by the enzymes fall into relatively smaller number of types, whereas the large number of the available enzymes is due to their high specificity towards the part of the substrate molecule other than the group actually undergoing the reaction.

In the light of above mentioned principles, the enzymes have been classified into six main classes, which are further divided into subclasses and sub-subclasses. Presently more than 3500 different enzymes have been classified and assigned code numbers by the Nomenclature Committee of International Union of Biochemistry and Molecular Biology (NC-IUBMB). These code numbers, which are now widely in use, are prefixed by EC and contain four digits or figures (a.b.c.d) separated by points, for example, EC 1.3.2.6, with the following meaning:

(i) the first digit shows to which of the six main classes do the enzyme belong
(ii) the second digit indicates the subclass
(iii) the third digit gives the sub-subclass
(iv) the fourth digit is the serial number of the enzyme in its sub-subclass.

The six main classes and their division into subclasses and sub-subclasses are described below.

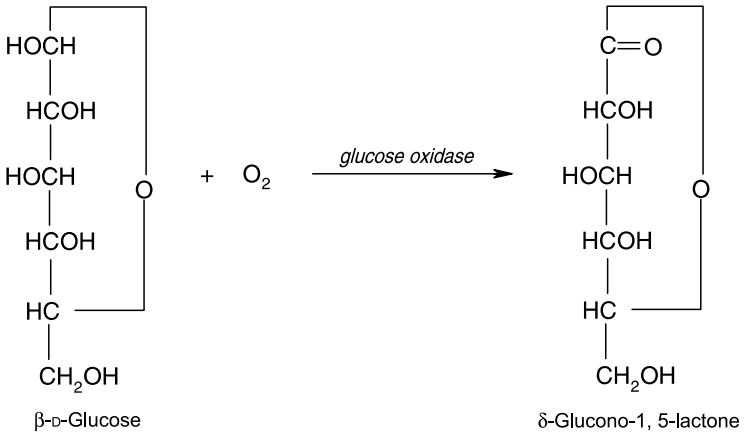
Class 1

Oxidoreductases: The enzymes belonging to this class catalyse oxidation–reduction reactions. The substrate that is oxidised is regarded as hydrogen donor and the systematic name is created as donor: acceptor oxidoreductase. The recommended name will be a *dehydrogenase* or *reductase* (if transfer of hydrogen from the donor is not obvious) or *oxidase* (if O_2 is the acceptor) as the case may be. This class is assigned a number 1 or a code number EC 1, the second figure in the code number

of the oxidoreductases indicates the group in the hydrogen donor which undergoes oxidation and the third figure indicates the type of acceptor involved. The last digit refers to the substrate in question.

Table 4.1 shows the subclasses, sub-subclasses for a subclass (1.1) and the enzyme list (truncated) of the sub-subclass (EC 1.1.3) for oxidoreductases (class 1). Let us take an example to understand this classification scheme. Suppose the code for an enzyme is EC 1.1.3.4. Here, EC stands for Enzyme Commission, 1 stands for the main class, i.e. *oxidoreductases*, next 1 stands for acting on CH–OH group of donors, 3 stands for oxygen as acceptor and 4 stands for the substrate in question, i.e. β-D-glucose.

In fact, this enzyme catalyses the following reaction.



The systematic name given to this enzyme would be:

$$\beta - \mathrm{D} - \text{glucose} : \text{ oxygen 1-oxidoreductase}$$

As can be seen in the table, the recommended name for the enzyme is *glucose oxidase*.

Table 4.1 Subclasses, sub-subclasses and the enzyme list (truncated) for the class, *oxidoreductases* (EC 1)

Number			
EC 1.1	**Acting on the CH–OH group of donors**		
EC 1.1.1	With NAD or NADP as acceptor		
EC 1.1.2	With a cytochrome as acceptor		
EC 1.1.3	With oxygen as acceptor	**Individual enzymes**	**Recommended names**
		EC 1.1.3.1	deleted, included in EC 1.1.3.15
		EC 1.1.3.2	now EC 1.13.12.4
		EC 1.1.3.3	malate oxidase
		EC 1.1.3.4	**glucose oxidase**
		EC 1.1.3.5	hexose oxidase
		EC 1.1.3.6	cholesterol oxidase
		EC 1.1.3.7	aryl-alcohol oxidase
		EC 1.1.3.8	L-gluconolactone oxidase
		EC 1.1.3.9	galactose oxidase
		EC 1.1.3.10	pyranose oxidase
EC 1.1.4	With a disulphide as acceptor		
EC 1.1.5	With a quinone or similar compound as acceptor		
EC1.1.99[a]	With other acceptors		
EC 1.2	**Acting on the aldehyde or oxo group of donors**		
EC 1.3	**Acting on the CH–CH group of donors**		
EC 1.4	**Acting on the CH–NH_2 group of donors**		
EC 1.5	**Acting on the CH–NH group of donors**		
EC 1.6	**Acting on NADH or NADPH**		
EC 1.7	**Acting on other nitrogenous compounds as donors**		
EC 1.8	**Acting on a sulphur group of donors**		
EC 1.9	**Acting on a heme group of donors**		
EC 1.10	**Acting on diphenols and related substances as donors**		
EC 1.11	**Acting on a peroxide as acceptor**		
EC 1.12	**Acting on hydrogen as donor**		
EC 1.13	**Acting on single donors with incorporation of molecular oxygen (oxygenases)**		
EC 1.14	**Acting on paired donors, with incorporation or reduction of molecular oxygen**		

(continued)

Table 4.1 (continued)

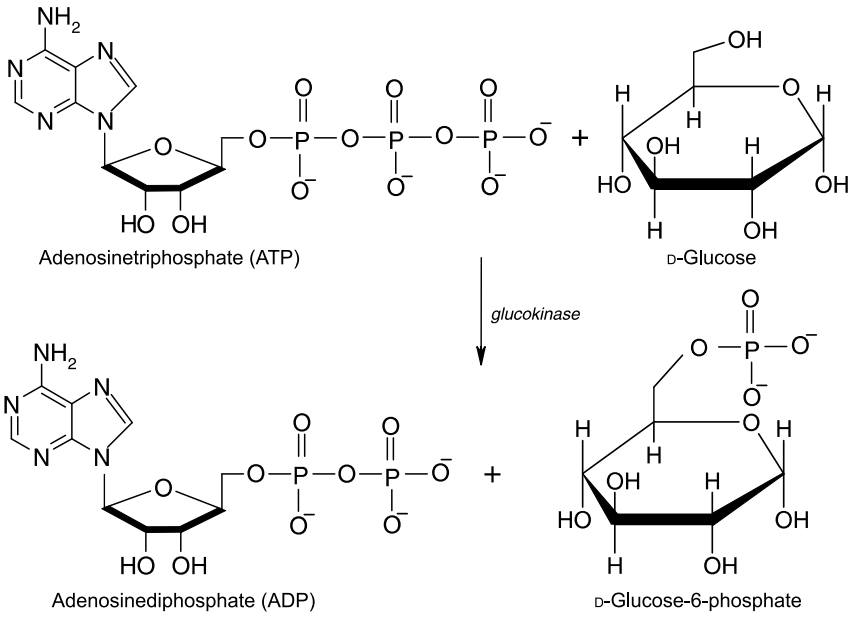
Number	
EC 1.15	**Acting on superoxide as acceptor**
EC 1.16	**Oxidising metal ions**
EC 1.17	**Acting on CH_2 groups**
EC 1.18	**Acting on iron-sulphur proteins as donors**
EC 1.19	**Acting on reduced flavodoxin as donor**
EC 1.20	**Acting on phosphorus or arsenic in donors**
EC 1.97	**Other oxidoreductases**

[a] In a few cases of subclasses or sub-subclasses, the subdivision is given a number 99 and is designated as 'others' so as to leave space for the new subdivisions

Class 2

Transferases: Transferases are enzymes transferring a group, e.g. a methyl group, a glycosyl group or a phosphate group, from one compound (donor) to another compound (acceptor).

For example, the following reaction,



is an example of a reaction involving the transfer of a phosphate group. Here, ATP is the donor of phosphate group which is accepted by D-glucose. The **systematic names** of the enzymes belonging to this class are formed according to the scheme

donor : acceptor group-transferred-transferase

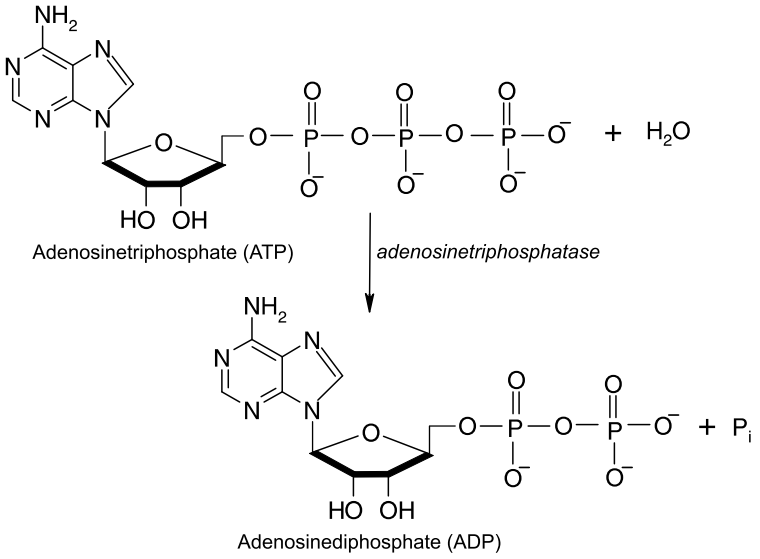
Thus, the full systematic name of the enzyme catalysing the above reaction would be:

ATP : D-glucose 6-phosphotransferase

This enzyme is commonly known as *glucokinase*. The EC code for this enzyme is 2.7.1.2. The first digit refers to the class transferases while the second figure in the code number indicates the group transferred (phosphate) and the third figure gives further information on the transferred group. The last digit refers to the substrate in question. The recommended names for this class are normally formed according to *acceptor grouptransferase* or *donor grouptransferase*.

Class 3

Hydrolases: These enzymes catalyse the hydrolytic cleavage of C–O, C–N, C–C and some other bonds including phosphoric anhydride bonds. Although the systematic name always includes *hydrolase*, the recommended name is, in many cases, formed by the name of the substrate with the suffix *-ase*. For example, the enzyme catalysing the following reaction,

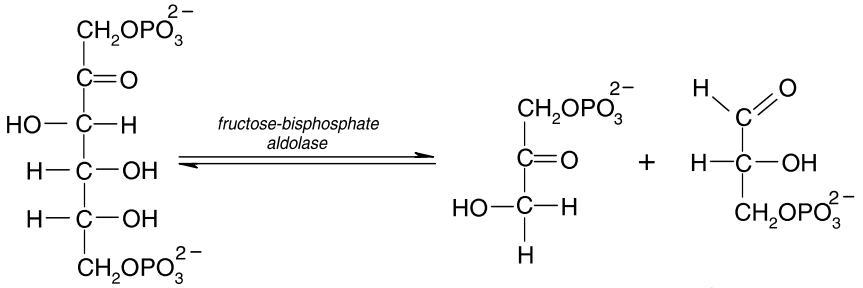


is *adenosinetriphosphatase*. The name of the substrate with this suffix implies a hydrolytic enzyme. The systematic name of the enzyme is ATP: phosphohydrolase. The EC code for this enzyme is EC 3.6.1.3. The first digit as usual refers to the class, the second figure in the code number of the hydrolases indicates the nature of the

bond hydrolysed and the third figure normally specifies the nature of the substrate. The last figure refers to the substrate (ATP in the given example) in question.

Class 4

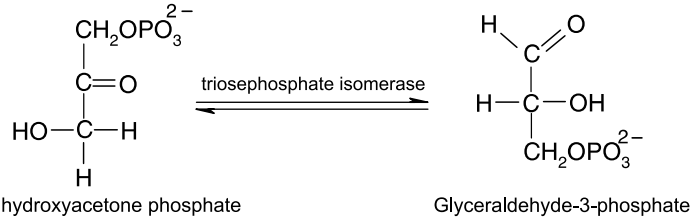
Lyases: Lyases are enzymes cleaving C–C, C–O, C–N and other bonds by elimination, leaving double bonds or rings, or conversely adding groups to double bonds. The systematic name is formed according to the pattern substrate group-lyase. For example, the enzyme fructose-bisphos-phate aldolase catalyses the following reaction.



The systematic name of the enzyme is D-fructose-1,6-bisphosphate D-glyceraldehyde-3-phosphate-lyase. The hyphen is an important part of the name, and to avoid confusion this hyphen should not be omitted, e.g. *phosphate-lyase* not '*phosphatelyase*'. The EC code for the enzyme is EC 4.1.2.13. In this, the second figure indicates the bond broken and the third figure gives further information on the group eliminated. The last digit refers to the substrate in question.

Class 5

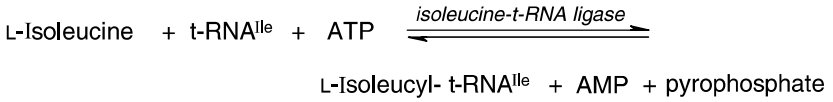
Isomerases: These enzymes catalyse geometric or structural changes within one molecule. According to the type of isomerism, they may be called *racemases, epimerases* or *tautomerases etc.* For example, triosephosphate isomerase catalyses the following reaction.



The systematic name of the enzyme is D-glyceraldehyde-3-phosphate ketol-isomerase and the code is EC 5.3.1.1. Here, the second digit refers to the type of

isomerism while the third digit indicates the type of substrate. The last digit refers to the substrate in question.

Class 6
Ligases: Ligases are enzymes catalysing the joining together of two molecules coupled with the hydrolysis of a diphosphate bond in ATP or a similar triphosphate. The systematic names are formed on the system *X : Y ligase* (*AMP or ADP-forming*) where *X* and *Y* are the two molecules to be joined together. For example, isoleucyl-t-RNA ligase catalyses the linking of L-isoleucine to t-RNA^{Ile} i.e. the isoleucine acceptor t-RNA.



The systematic name of the enzyme would be L-Isoleucine : t-RNA^{Ile} ligase (AMP-forming) and the code is EC 6.1.1.5. The second figure refers to the type of bond formed (carbon–oxygen in the present case) while third figure indicates the type of the compound formed. The last figure refers to the substrate.

Table 4.2 gives a summary of major subclasses of different enzyme classes.

4.3 Characteristics of Enzymes

Most enzymes, as mentioned earlier, are proteins that are synthesised by living cells and act as catalysts for the large number of biochemical reactions in a cell. Enzymes display certain characteristic properties which are in contrast to those of their chemical counterparts that is, chemical catalysts. The significant characteristics are

- Extent of rate enhancement or catalytic power
- Specificity and
- Regulation.

4.3.1 Catalytic Power

As mentioned in Sect. 4.1, the enzyme catalysed reactions may have extremely high rates. Typically, enzymes **accelerate** the reactions by a factor of 10^6 to 10^{12} and allow millions of reactions necessary in our body to proceed in very short time. An example is provided by the enzyme *urease* that catalyses the hydrolysis of urea. Here, the catalysed reaction has a rate that is roughly 13 orders of magnitude higher.

Table 4.2 Enzyme classes and their major subclasses

Class no.	Enzyme class name	Major subclasses
1	Oxidoreductases	Dehydrogenases
		Oxidases
		Reductases
		Peroxidases
		Catalases
		Oxygenases
		Hydroxylases
2	Transferases	Transaldolases and transketolases
		Acyl-, methyl-, glucosyl- and phosphoryl-transferases
		Kinases
		Phosphomutases
3	Hydrolases	Esterases
		Glycosidases
		Peptidases
		Phosphatases
		Thiolases
		Phospholipases
		Amidases
		Deaminases
		Ribonucleases
4	Lyases	Decarboxylases Aldolases
		Hydratases
		Dehydratases
		Synthases
		Lyases
5	Isomerases	Racemases
		Epimerases
		Isomerases
		Mutases (not all)
6	Ligases	Synthetases
		Carboxylases

$$\underset{\text{Urea}}{H_2N-\overset{\overset{O}{\|}}{C}-NH_2} + H_2O \xrightarrow{\text{urease}} CO_2 + 2NH_3; \quad K \sim 5 \times 10^{6}$$

$$H_2N-\overset{\overset{O}{\|}}{C}-NH_2 + H_2O \xrightarrow{H^+} CO_2 + 2NH_3; \quad K \sim 5 \times 10^{-7}$$

In extreme cases, the increase in the rate could be as high as 10^{17} times. The enzymes achieve these high rates by altering the thermodynamics of a reaction in such a way that the reactants and products of a reaction reach equilibrium much faster than otherwise would, without altering their equilibrium concentrations. The enzymes, like chemical catalysts, are not altered during their reactions and are released unchanged after catalysing the reaction. Therefore, these are required only in small amounts in the cell.

4.3.2 Enzyme Specificity

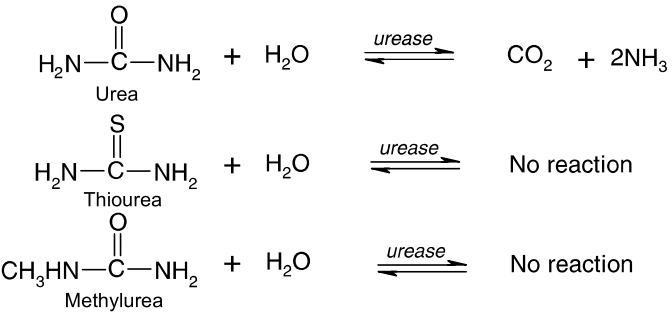
Enzymes show a high degree of specificity towards their substrates, the products and to the type of chemical reaction being catalysed as compared to their chemical counterparts. For example, the rate of oxidation of β-D-glucose by *glucose oxidase* is about 150 times faster than that for the α-anomer. Further, if a given substrate can give two different types of products, then it would be acted over by two different enzymes, each giving a specific product. Formation of a side product is very rare in an enzyme catalysed reaction. Thus, *specificity of enzymes refers to the ability of an enzyme selectively recognise and transform a given substrate out of a mixture of a number of substrate types.*

Different enzymes demonstrate different types and extents of specificities. These can arbitrarily be put into following groups.

- Absolute specificity
- Stereochemical specificity
- Linkage specificity and
- Group specificity.

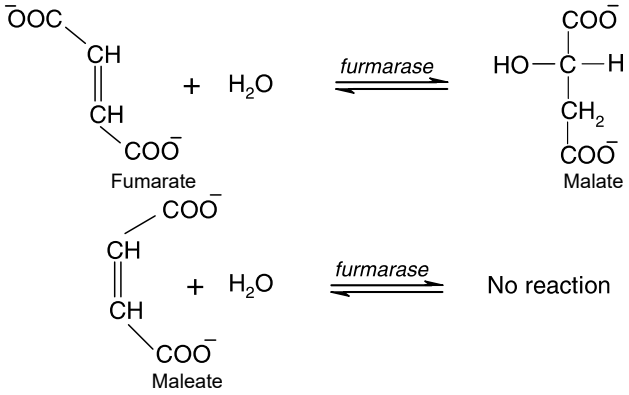
Absolute Specificity

As the name suggests certain enzymes specifically catalyse a given reaction on a particular substrate. *Urease* is an example of this class as it catalyses the hydrolysis of urea only and does not show any reaction with closely related substrates like, thiourea or methylurea.



Stereochemical Specificity

Many enzymes show specificity towards the stereochemical nature of the substrate. For example, enzyme *fumarase* catalyses addition of water to fumarate (*trans* isomer) to give malate whereas maleate (the *cis* isomer) remains unaffected.



Linkage Specificity

Some enzymes have specificity towards a kind of bond in the substrate rather than the substrate as a whole. For example, *peptidases* are specific to the peptide bond.

Group Specificity

Some enzymes, however, show group specificities, i.e. acting on a group instead of a particular bond or substrate. For example, the enzyme *carboxypeptidase A* selectively removes the C-terminal amino acid in a peptide containing a free C-terminal or *pepsin* hydrolyses peptide bonds having adjacent aromatic amino acids.

As discussed earlier, the specificity of enzymes has provided a convenient system for classifying and naming them**.** The enzymes are sensitive to the changes in pH, temperature and nature and concentration of salt and work best under optimum conditions (Sect. 4.5).

Enzymes have a somewhat broad range of substrate specificity, i.e. a given substrate may be acted upon by a number of different enzymes, each of which uses the same substrate(s) and produces the same product(s). The individual members of a set of enzymes acting at same substrate and producing same product are known as **isozymes**. Isozymes are a set of closely related enzymes that are produced genetically in the organism. These differ slightly in terms of their primary structure (the amino acid sequence) or higher-order (conformation) structures. Sometimes these may differ even in terms of certain covalent modifications. In a set of isozymes, the individual members are distinguished on the basis of their electrophoretic mobilities. These are designated by numbers, the most mobile isozyme is given number l and the others follow in the sequence. Various isozymes of a group are often found in different tissues of the body. The best studied set of isozymes is the ***lactate dehydrogenase* (LDH) system**. A number of genetically related forms of *lactate dehydrogenase* are found in the serum and muscle extracts.

4.3.3 Enzyme Regulation

In addition to the specificity, the capacity to be **regulated** is another important feature of enzymes. The catalytic activity of many enzymes is found to depend on the concentrations of the substances other than their substrates. These substances may be small molecules or ions or substrate analogues or at times totally unrelated to the substrate. This characteristic of the enzymes is extensively exploited by the cell to make the most effective use of the cellular enzymes.

4.4 Mechanism of Enzyme Action

In general, catalysts increase the rate of product formation by lowering the activation energy for the reaction and facilitating favourable orientation of colliding reactant molecules for product formation. The mechanisms by which enzymes lower the energy of activation are still not totally understood. However, the mechanisms are believed to be directly or indirectly related to achievement of a **transition state** for the reaction. A portion of the overall tertiary structure of the enzyme is responsible for its activity and is called **active site** (Fig. 4.1). The active site is quite small as compared to the overall size of the enzyme. The side chains of some of the amino acids in the active site help in holding the substrate to the enzyme and are called **binding groups**, while side chains of other amino acids are involved in the catalytic process and are called **catalytic groups**. For example, in the enzyme *trypsin*, its complex tertiary structure brings together a histidine residue from one section of the molecule with glycine and serine residues from another. The side chains of the residues in this particular geometry produce the site that accounts for the enzyme's reactivity. The catalytic activity of most of the enzymes can be accounted for by the

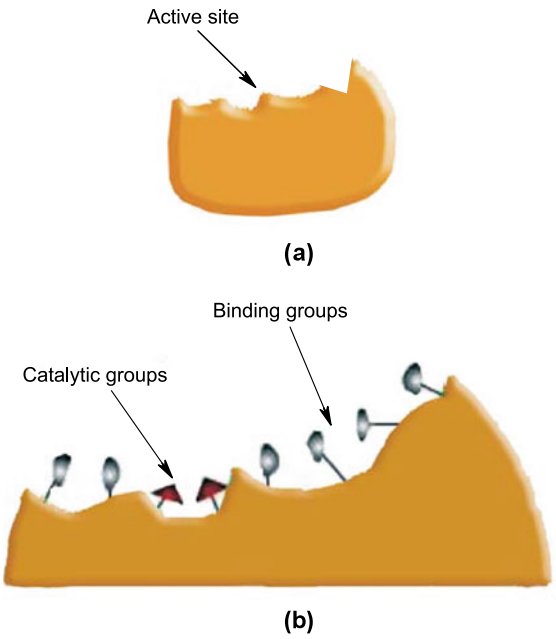
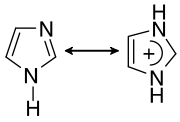


Fig. 4.1 **a** A schematic representation of the active site of an enzyme and **b** a part of active site showing the binding and catalytic groups

presence of five functional groups given in Table 4.3. It is not necessary that the active sites of all the enzymes contain all the five functional groups.

Binding of substrates to the active site of the enzyme brings them close together, thereby raising their effective concentration in the active site to many times the concentration in the surrounding solution. In this enzyme-substrate complex, the polar and non-polar groups of the active site may also bring the substrate molecules into an arrangement in which they can collide at the correct positions and orientations so as to form the transition state. This facilitates the bond breaking and making,

Table 4.3 Catalytically active functional groups

Functional group	Amino acid	pK_a
COOH, COO^-	Asp, Glu	4
OH	Ser	14–15
SH	Cys	10
NH_3^+	Lys	10
	His	7

leading to conversion of the reactants to the products. Once the reaction is complete, the product molecule separates itself from the active site and makes the site available for another incoming substrate molecule. This process occurs in a highly efficient manner measured by **turnover number** of the enzyme which refers to the number of molecules of substrate upon which a given molecule of the enzyme acts per second. These numbers can vary over about five orders of magnitude. The turnover numbers of some enzymes are compiled in Table 4.4.

The mechanism described above is based on the well-known **lock and key** model for the specificity of enzyme action (Fig. 4.2) proposed by Emil Fischer in l890. The key (substrate) has a specific shape (arrangement of functional groups and other atoms) that allows it and no other key to fit into the lock (the active site of enzyme). Thus, the enzyme catalysed reactions proceed through a number of steps. These are illustrated in Fig. 4.3.

This model assumes the structure of the enzyme to be rigid. However, the developments in the understanding of protein structure suggest it to be dynamic in nature. To accommodate the flexibility of protein structure, the lock and key model was modified by Daniel E. Koshland Jr. in l958. According to the **induced fit** or **hand and glove model** proposed by Koshland, the active site of the enzyme is not rigid but has a certain amount of flexibility whereby it can expand or contract to some extent so as to accommodate the substrate molecule. That is, when the substrate molecule approaches the enzyme, the active site acquires a shape complimentary to that of the substrate as shown in Fig. 4.4. It is somewhat like a hand fitting into a glove. The glove adjusts in shape and size to fit different hands within a certain range of sizes. Further, in the E-S complex, the enzyme forces the substrate molecule to be distorted

Table 4.4 Turnover numbers of some enzymes

Enzyme	Turnover number (s^{-1})
DNA polymerase	1.5×10^1
Chymotrypsin	1.0×10^2
Laatate dehydrogenase	1.0×10^3
3 Ketoisomerase	2.8×10^4
Carbonic anhydrase	6.0×10^S

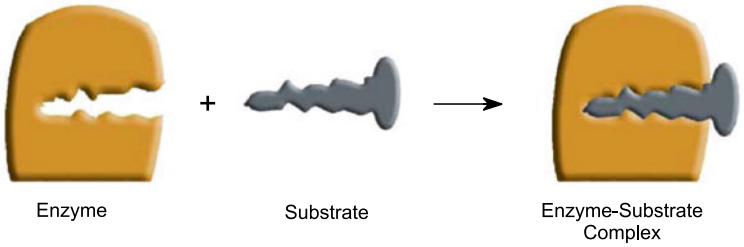


Fig. 4.2 A diagrammatic representation of the lock and key model for enzyme specificity. The structure of the active site of the enzyme is complimentary to that of the substrate

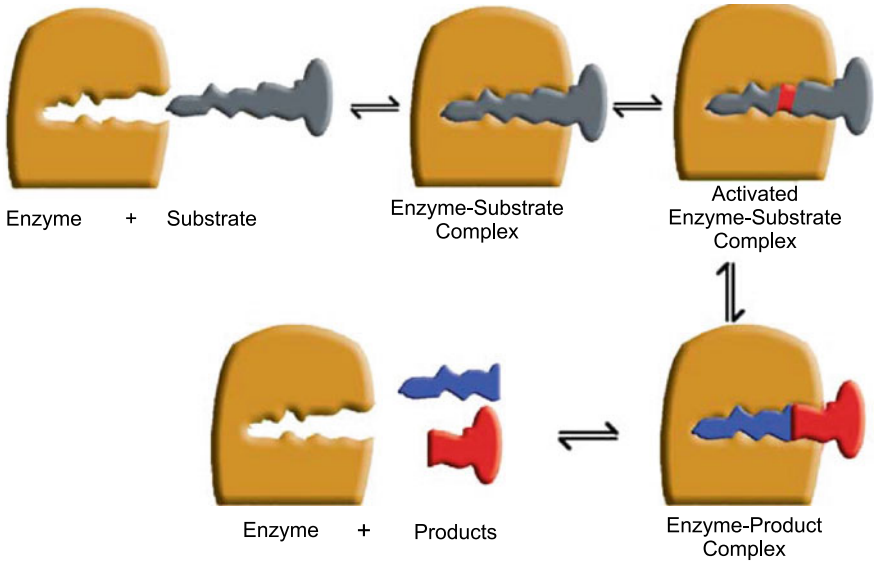


Fig. 4.3 A diagrammatic representation of various steps in enzyme catalysed reactions according to lock and key model

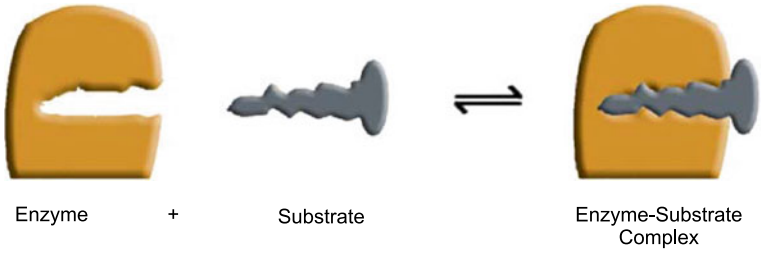


Fig. 4.4 A diagrammatic representation of the induced fit model for enzyme specificity. The active site of the enzyme gets ‘ induced’ to take a shape complimentary to that of the substrate

to acquire a transition state. In other words, the induced fit model envisages changes in the enzyme as well as the substrate. It may be noticed here that the two models differ only in terms of the mechanism of E-S complex formation; the other steps in the catalytic mechanism remain the same.

4.5 Factors Affecting Enzyme Action

The activity of enzymes is quite sensitive to the factors like temperature, pH, concentration of substrate, enzyme itself and the salt. These are discussed below.

Temperature

Every enzyme has an optimum range of temperature (usually ranging from about 30–40 °C) wherein it shows its maximum activity. The activity of the enzyme decreases or is completely lost outside this range. This occurs because the changes in temperature disrupt the forces stabilising the enzyme (protein) structure, which in turn may alter the active site to an extent that it is unable to accommodate the substrate molecules. At low body temperature (hypothermia) or very high body temperatures (hyperthermia), most of the enzymes within the human cells may lose activity or stop functioning and thereby hamper the functioning of the cell leading to severe physiological consequences.

Hydrogen Ion Concentration (pH)

The enzyme catalysed reactions are strongly influenced by the hydrogen ion concentration or the pH of the reaction mixture. Usually small changes in the pH of the cell can also affect the normal functioning of the enzyme. The pH dependence of an enzyme catalysed reaction is given in Fig. 4.5. The bell-shaped curve shows that the activity of the enzyme is maximum over a very narrow range of pH. This is called **optimal pH** range. The enzyme activity decreases at pH values lower or higher than this range.

Most of the enzymes have an optimal pH range around 7.0; however, some enzymes can have their optimal pH in extremely acidic or basic range. For example, the enzymes like *pepsin* and chymotrypsin present in the stomach can operate effectively at a very low pH. While the enzymes like α-*amylase* found in the saliva of the mouth operate most effectively near neutral pH. On the other hand, certain enzymes like the *lipases* function most effectively at basic pH values. The optimal pH values for some of the common enzymes are given in Table 4.5.

The change in pH from the optimal value can affect the structure of the enzymes by altering the ionic state of different side chains of the enzyme molecules. This in turn may alter the structure of the active site which as a consequence can no longer

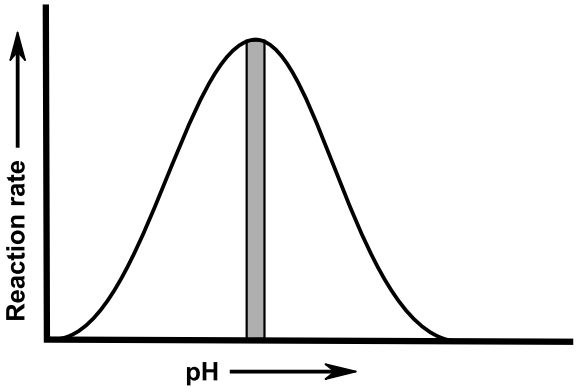


Fig. 4.5 Effect of pH on the activity of enzyme

Table 4.5 Optimal pH values of some common enzymes

Enzyme	Optimal pH value
α-Amylase	7.0
Alk aline phosphatase	9.5
Carboxypeptidase	7.5
α-Gluaosidase	5.4
Pepsin	1.5
Trypsin	7.8
Urease	6.7

accommodate the substrate. Alternatively, the amino acid side chains in the active site itself may have its ionic state altered. In case of metabolic disorders leading to **acidosis** (a decrease in the blood pH) or **alkalosis** (an increase in the blood pH), the enzymes may lose their activity leading to undesired consequences. Correcting pH or temperature imbalances usually allows the enzyme to resume its original shape and function.

Concentration of the Enzyme

In the presence of a sufficient concentration of the substrate, increasing enzyme concentration will increase the reaction rate, as more active sites are available to the substrate molecules.

Concentration of the Substrate

At a given concentration of the enzyme, the reaction rate is controlled by the substrate concentration at lower substrate concentrations. The rate of the reaction increases with an increase in the concentration of the substrate. However, at high concentrations of the substrate, the enzyme gets saturated with the substrate, and any increase in the concentration of substrate does not increase the reaction rate any further. A typical curve showing the variation of the reaction rate with an increase in the concentration of the substrate is given in Fig. 4.6.

Salt Concentration

Each enzyme has an optimal working salt concentration. Changes in the salt concentration may also denature the enzyme and hamper its performance.

4.6 Chymotrypsin: An Enzyme in Action

Chymotrypsin is a proteolytic enzyme belonging to a broad group of enzymes called *serine proteases* that use **serine** side chain as a reactive nucleophile in the catalysed reaction. Chymotrypsin specifically cleaves the peptide bond next to an aromatic side chain and to a lesser extent the peptide bond next to a hydrophobic side chain like

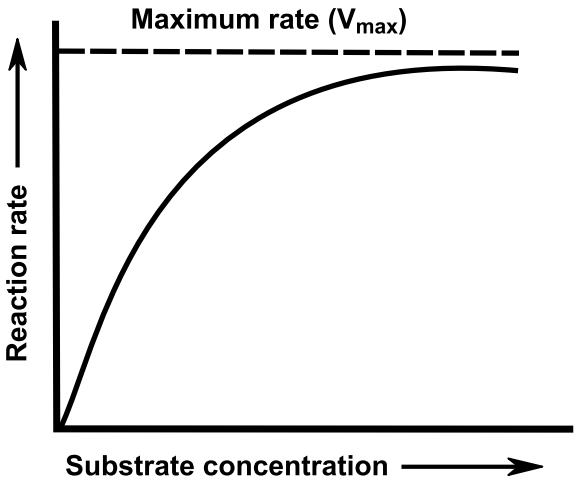
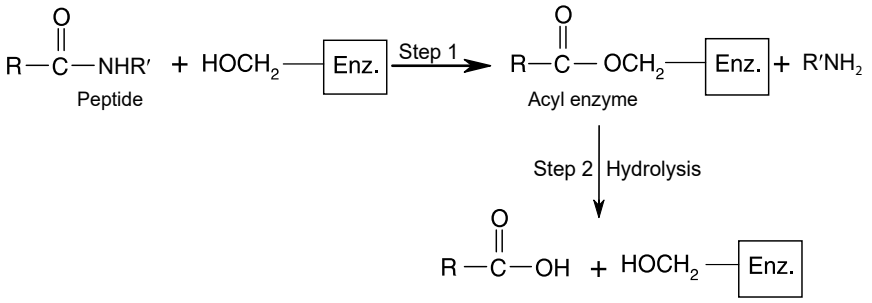


Fig. 4.6 Variation of reaction rate with concentration of the substrate at a given concentration of enzyme

methionine or leucine. Since *proteases* are destructive in nature, these are normally synthesised in their inactive form called **zymogen**. These are suitably activated as per the requirements. Chymotrypsin is synthesised in the mammalian pancreas as an inactive precursor called **chymotrypsinogen**. This precursor is secreted in the intestine where it is activated by proteolytic cleavage by *proteases*. The structure and mechanism of action of chymotrypsin are probably the most extensively studied and understood system. It catalyses the hydrolysis of the amide (or peptide) bond with the help of a strong nucleophile in the form of $-CH_2OH$ group of a specific serine residue. The overall hydrolysis is split into two steps. In the first step, an acyl-enzyme ester is formed as an intermediate, which is then hydrolysed by water in the second step to yield free carboxylic acid and the enzyme is regenerated. The detailed mechanism is explained later (Sect. 4.6.3).



4.6.1 Structure of Chymotrypsin

The precursor to chymotrypsin (i.e. chymotrypsinogen) consists of a 245-residue long single chain polypeptide, which is held in its natural conformation by five intramolecular disulphide linkages. The linkages are between cysteine residues 1 and l22; 42 and 58; l36 and 20l; l68 and l82; l9l and 220. A schematic structure of chymotrypsinogen showing the disulphide linkages and cleavage sites is given in Fig. 4.7.

Chymotrypsinogen is converted into the active enzyme in a sequence of interesting proteolytic cleavages. This process is initiated by an enzyme called *enterokinase* that specifically activates trypsinogen which in turn produces trypsin. Trypsin then executes the first cleavage (between residues Arg-l5 and Ile-l6) of chymotrypsinogen. This yields a two chain active enzyme called **π-chymotrypsin**. It digests itself in terms of a secondary cut between Leu-l3 and Ser-l4 residues to generate what is called **δ-chymotrypsin**. It is followed by two more cleavages (between Tyr-l46 and Thr-l47 and between Asp-l48 and Ala-l49) to give the active **α-chymotrypsin**. α-Chymotrypsin consists of three polypeptide chains held in place by three intramolecular and two intermolecular disulphide linkages as shown in Fig. 4.8.

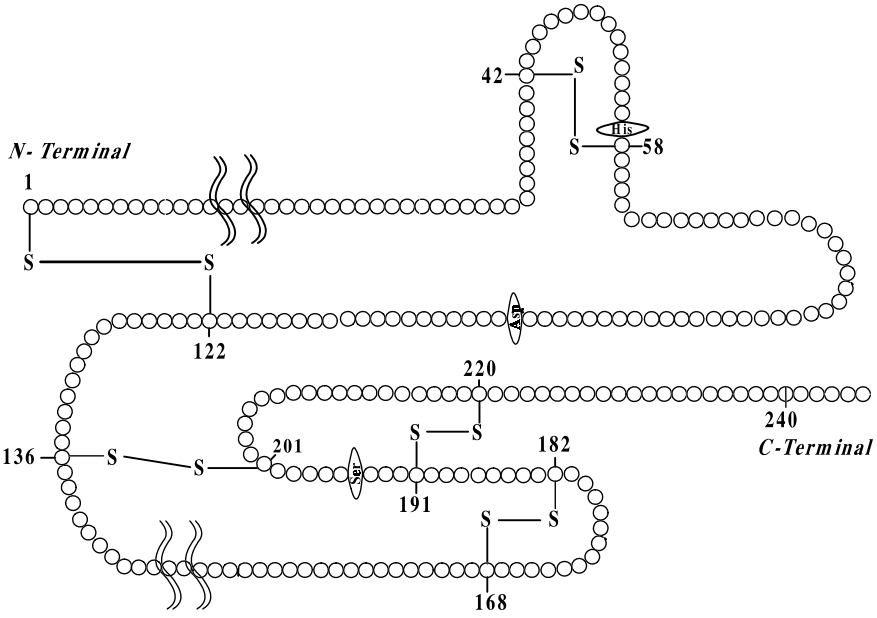


Fig. 4.7 Schematic representation of the structure of chymotrypsinogen. Each circle represents an amino acid, and three amino acids directly involved in the activity of chymotrypsin are shown as ovals. The vertical wavy lines indicate the positions where the chain is cleaved to get the active enzyme

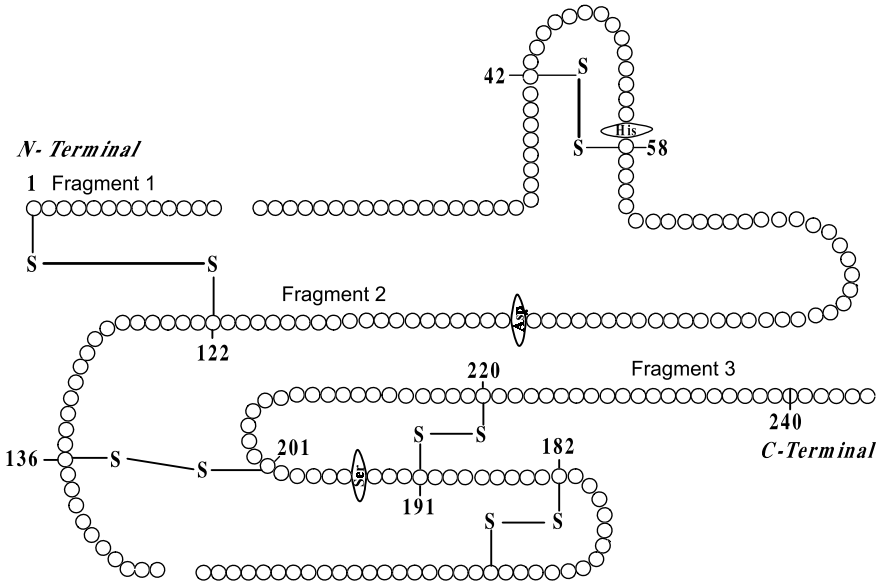


Fig. 4.8 Schematic representation of the structure of chymotrypsin. Each circle represents an amino acid. The ovals indicate three amino acids directly involved in the activity of chymotrypsin. The three fragments of chymotrypsin have slightly different conformations than that in chymotrypsinogen

Though a number of cleavages take place in the activation process, it is the cleavage after residue l5 that is the most crucial. The amino group of the Ile-l6 residue is very important as it gets into the formation of a salt link with the Asp-l94 side chain. Such a possibility does not exist in the zymogen. The salt link in turn introduces conformational changes in the fragment l89–l94 whereby the backbone amide bond of Gly-l93 residue acquires an important position from the mechanism point of view.

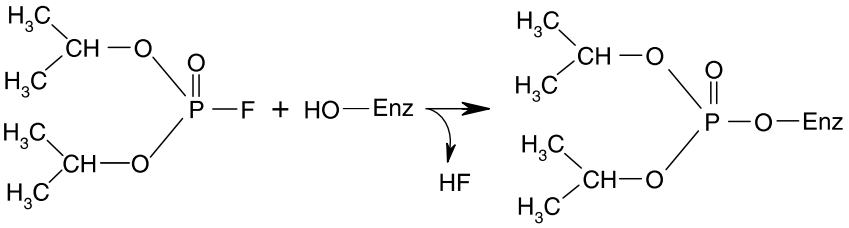
4.6.2 Important Amino Acid Residues of Chymotrypsin

In terms of the activity of chymotrypsin, three amino acids, viz. Ser-l95, His-57 and Asp-l02 are the most important; serine-l95 being the central residue. In addition, Ile-l6 also has a role to play. Other residues provide the right geometry for its action.

Role of Serine-195

It is interesting to note that chymotrypsin contains as many as 28 serine residues but only one of these, i.e. Ser-l95 has a direct role in the activity of the enzyme. This has been established on the basis of the reaction of chymotrypsin with diisopropylfluorophosphate (a nerve gas) that leads to the loss of activity of the enzyme. This inhibitor reacts specifically and irreversibly by forming a covalent linkage with

the side chain of Ser-l95 residue only. The enzyme—inhibitor complex contains a phosphate ester having a structure similar to that of an acyl enzyme.



Diisopropylfluorophosphate Diisopropyl-phosphate derivative of enzyme

This specificity is due to the spatial proximity of His-57 and Asp-l02 residues with serine-l95 as revealed by X-ray crystallography. The specificity of Ser-l95 was further established by reacting chymotrypsin with *p*-nitrophenylacetate followed by end group analysis.

Role of His-57

The importance of His-57 in the enzyme activity was suggested by the inhibition of its activity by a phenylalanine derivative containing reactive chloromethylketone group.

In the primary sequence of the enzyme, His-57 and Ser-l95 are quite far from each other but in the tertiary structure these two residues are in close proximity. Affinity labelling studies also point out to the same. The orientation of His-57 side chain is such that the serine—OH group can form a hydrogen bond with the imidazole group of the histidine residue.

Role of Asp-102

Asp-l02 residue in chymotrypsin is buried inside the globular fold of the protein—a rare energetically unfavourable feature in proteins. It is so oriented that it comes closer to the imidazole ring of His-57 residue from the side opposite to that of Ser-l95. The histidine residue is thus flanked between aspartic acid and serine. The carboxylate ion in the aspartic acid side chain interacts with the nitrogen proton on the imidazole ring.

Site directed mutagenesis suggests that these three amino acid residues (Asp-l02, His-57 and Ser-l95) act synergistically to increase the rate of the enzyme catalysed reaction. The combination of His-57 and Asp-l02 residues with Ser-l95 create what is called a **charge-transfer relay system** or **catalytic triad**. These three residues are so arranged in space that these lead to a partial ionisation of the hydroxyl group in the Ser-l95 side chain.

The interaction of carboxylate group of Asp-l02 with imidazole side chain facilitates the ionisation of serinyl—OH group in the charge relay system as shown below (Fig. 4.9).

Substrate Binding Pocket

In addition to the three residues discussed above, a few more residues are also important as these aid in the binding of the substrate to the enzyme. The binding site (or pocket) contains a number of non-polar side chains, which favourably accommodates a hydrophobic side chain (e.g. Tyr, Trp, Phe, etc.). The bottom of the binding pocket is occupied by Ser-l89 while two glycyl residues (Gly-2l6 and Gly-226) are located near the opening of the pocket (Fig. 4.10).

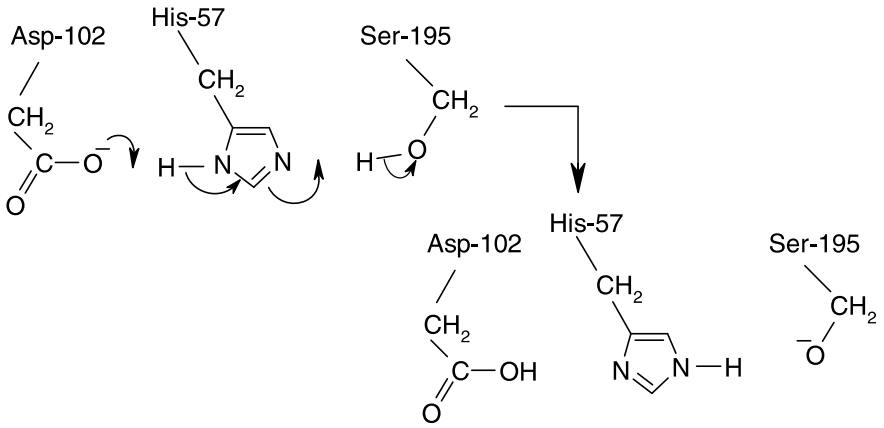


Fig. 4.9 Charge-transfer relay system or catalytic triad operative in chymotrypsin and some other serine proteases like trypsin, etc.

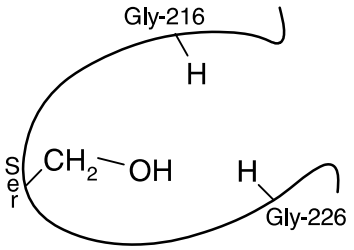


Fig. 4.10 Substrate-binding pocket of chymotrypsin. A serine residue lies at the bottom of the pocket while two glycyl residues are located near the neck region

4.6.3 Mechanism of Action

The mechanism of action of chymotrypsin has been well studied. The sequence of steps showing the mechanism is depicted in Fig. 4.11a–g along with their explanation.

When the substrate (a peptide or protein containing an aromatic or highly hydrophobic side chain) approaches the enzyme, the aromatic residue slips into the binding pocket of the enzyme where it is held in place by hydrophobic interactions with the residues constituting the pocket. In this enzyme-substrate complex, the carbonyl group of the peptide bond to be hydrolysed gets into hydrogen bonding with the peptide –NH groups of Ser-l95 and Gly-l93 residues. These two amide groups form what is referred to as **oxyanion binding site** or **hole**.

The reaction is then initiated by the nucleophilic attack of the Ser-l95 side chain that carries a negative charge due to the charge relay system created by the participation of Asp-l02 and His-57 side chains as explained earlier.

This leads to the formation of a tetrahedral intermediate, which is stabilised by the amide protons of Ser-195 and Gly-193. These amides stabilise the intermediate by accommodating the negative charge on the carbonyl carbon in the so-called oxanion. The tetrahedral intermediate has not been observed directly, and its existence is

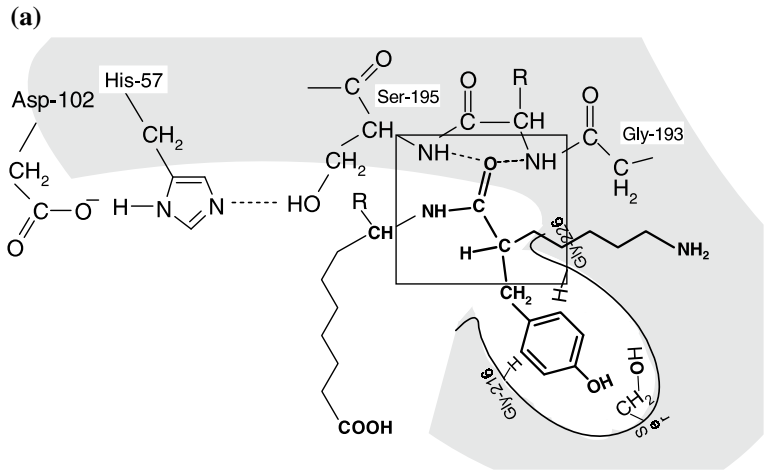


Fig. 4.11 **a** Substrate-chymotrypsin complex; the relevant side chains of the enzyme molecule are shown schematically. The schematic sequence shown in dark colour and smaller font represents the peptide substrate. The aromatic amino acid side chain of tyrosine is in the binding pocket of the enzyme. **b** Nucleophilic attack of Ser-195 facilitated by the participation of Asp-102 and His-57 residues in the charge-transfer relay system. **c** Formation and stabilisation of the tetrahedral intermediate. The negatively charged oxanion gets into hydrogen bonding with amide protons of Ser-195 and Gly-193. **d** Formation of acyl-enzyme intermediate accompanied by elimination of a peptide fragment with a new N-terminal. **e** Nucleophilic attack of water molecule facilitated by aspartate and histidine side chains. **f** Formation of second tetrahedral intermediate. **g** Formation of enzyme-product complex

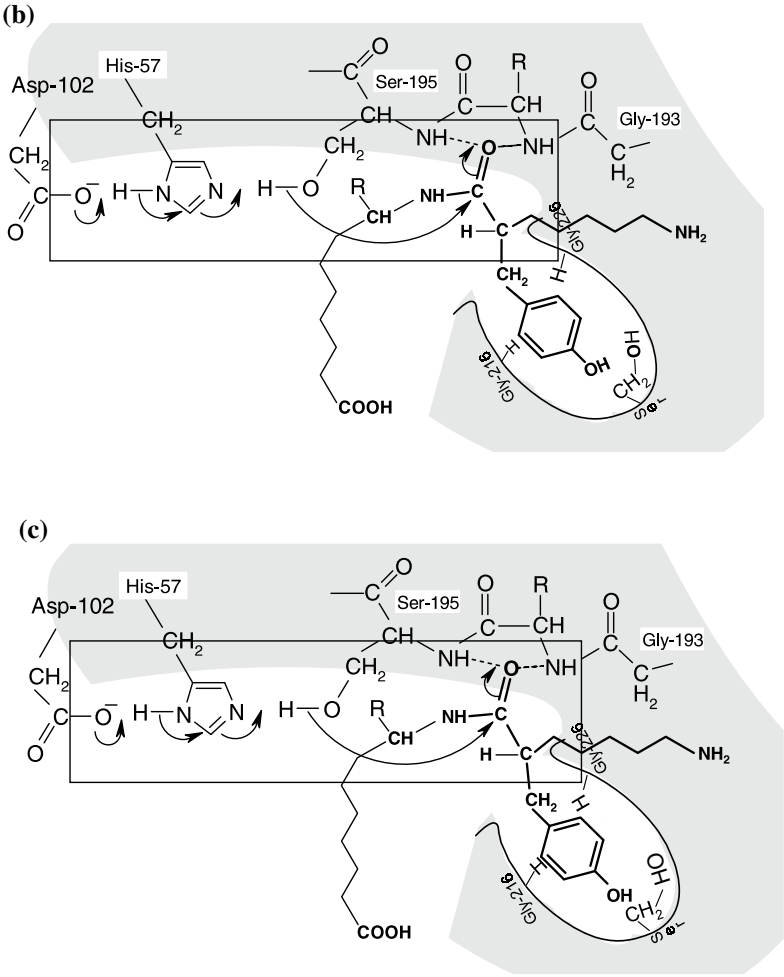


Fig. 4.11 (continued)

attributed by analogy to the tetrahedral adducts obtained on binding of the inhibitors to the enzyme in the active site.

This short-lived intermediate is decomposed in an acid catalysed elimination of a peptide fragment with a new N-terminal. In this process, another intermediate is obtained in which the serine residue is linked to the enzyme as an acyl ester. Here, histidine residue acts as an acid and aids the formation of the acyl intermediate.

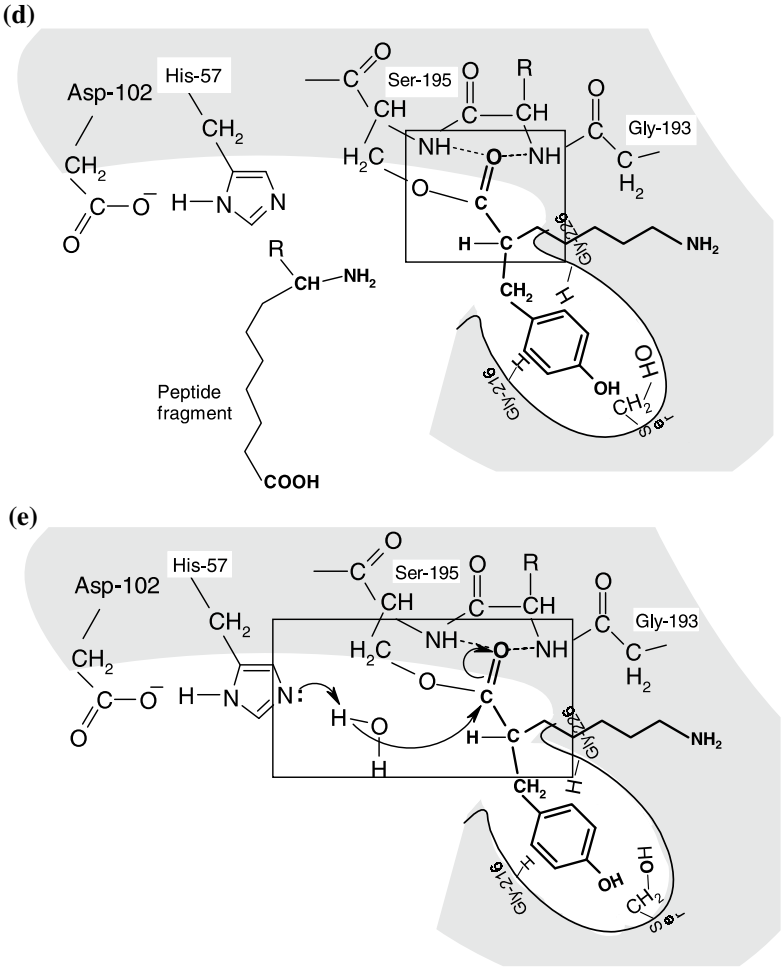


Fig. 4.11 (continued)

Up to this stage, half of the reaction is over. The acyl-enzyme intermediate undergoes deacylation by a nucleophilic attack of a water molecule. The sequence of reactions is reverse of the ones explained above. The attack of water is facilitated by the participation of aspartate and histidine side chains.

The deacylation also proceeds through the formation of a tetrahedral intermediate.

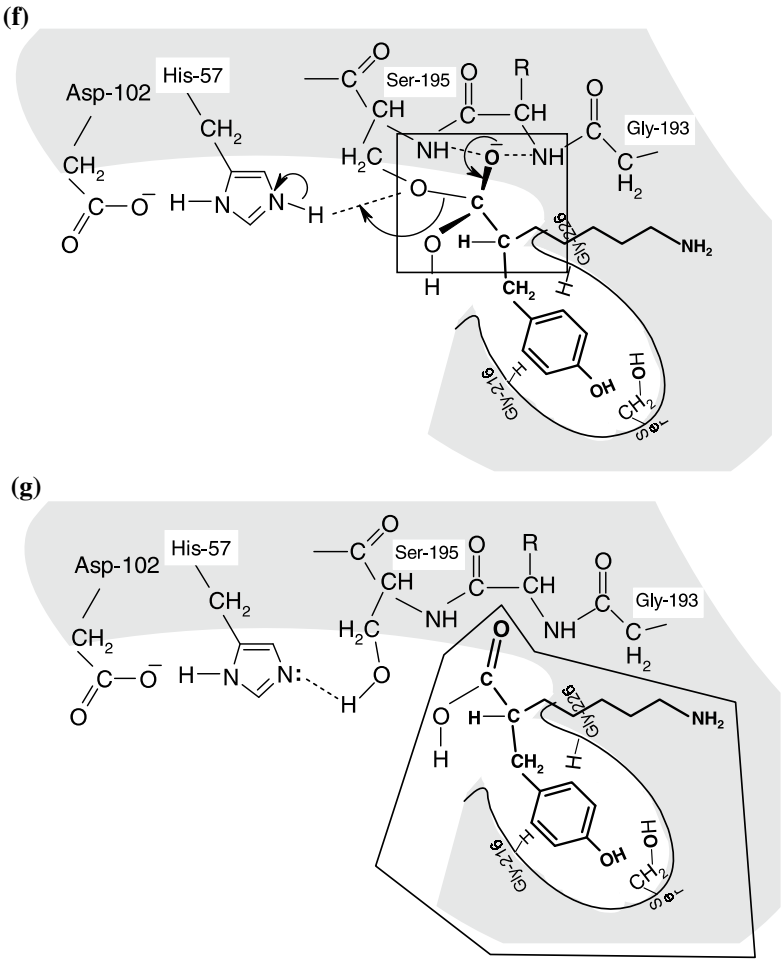


Fig. 4.11 (continued)

This tetrahedral intermediate breaks down to yield an enzyme-product complex in which the second fragment of the protein (or peptide) having a new C-terminal is bound to the binding pocket of the enzyme.

The enzyme-product complex finally dissociates to give free enzyme and the peptide fragment.

4.7 Cofactors (or Coenzymes)

As discussed earlier, enzymes perform their functions with the help of functional groups of the amino acid side chains present in their active sites. However, these side chain functional groups are not capable of catalysing all the reactions needed by a cell to perform its functions. Certain enzymes need the help of small non-protein organic molecules for catalysing these essential reactions.

These non-protein groups, if covalently linked, are referred to as **prosthetic groups**. The protein part in this combination is called **apoenzyme** while the other component is referred to as **cofactor**. The cofactors may be metal ions or complex organic molecules called **coenzymes**. The coenzyme binds to the apoenzyme to give **holoenzyme** that binds the substrate to perform the reaction. The coenzymes normally are derived from water-soluble vitamins (Fig. 4.12).

Enzymes of IUB classes l, 2, 5 and 6, catalysing oxidoreductions, group transfers, isomerisations and ligation reactions, respectively, often need coenzymes. The coenzymes may either be attached by covalent bonds to the enzyme (prosthetic group) or may exist freely in solution. Enzymes that require a metal in their composition are known as **metalloenzymes** if they bind and retain their metal atom(s) under all conditions, that is with very high affinity for the metal ion. Those enzymes which have a lower affinity for metal ion but still require the metal ion for their activity are known as **metal-activated enzymes**. Some of the metal ions commonly participating as cofactors for enzymes are given in Table 4.6.

The coenzymes perform two important functions. Firstly, on binding to the enzyme, these create an active site suitable for the substrate molecule to bind and secondly provide some functional groups to aid in the catalytic action of the enzyme. The functional role of coenzymes is to act as transporters of chemical groups from one reactant to another. The chemical groups carried can be as simple as the hydride ion ($H^+ + 2e^-$) carried by nicotinamide adenine dinucleotide (NAD^+) or the molecule of hydrogen carried by flavin adenine dinucleotide (FAD); or they can be even more complex than the amine ($-NH_2$) carried by pyridoxal phosphate. These coenzymes are often altered structurally in the course of these reactions, the change being exactly opposite to the one taking place in the substrate. However, these are always restored to their original form in subsequent reactions catalysed by other enzyme systems.

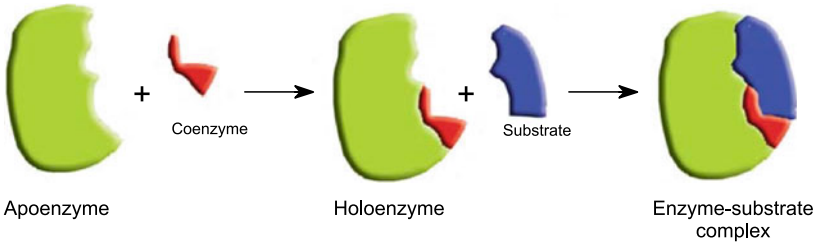


Fig. 4.12 Role of coenzyme in a stable complex formation between the enzyme and substrate

Table 4.6 Important metal ion cofactors

Enzyme	Metal ion cofactor	Function of the metal ion
Cytochrome oxidase	Fe	Oxidation and reduction
Alcohol dehydrogenase	Zn	Binding of coenzyme; NAD^+
Urease	Ni	Part of catalytic site
Glutamate mutase	Co	Part of the coenzyme, cobalmin
Ascorbic acid oxidase	Cu	Oxidation and reduction

Human body is unable to synthesise most of the coenzymes from elementary precursors available in the cell and needs dietary support in terms of vitamins, etc. All vitamins are not coenzymes per se; however, some are precursors that are transformed into coenzymes after metabolic modifications. Constitutionally, most coenzymes are obtained by modification of the water soluble vitamins ingested in food. If a given vitamin was not available in the diet, a deficiency with obvious clinical symptoms would arise.

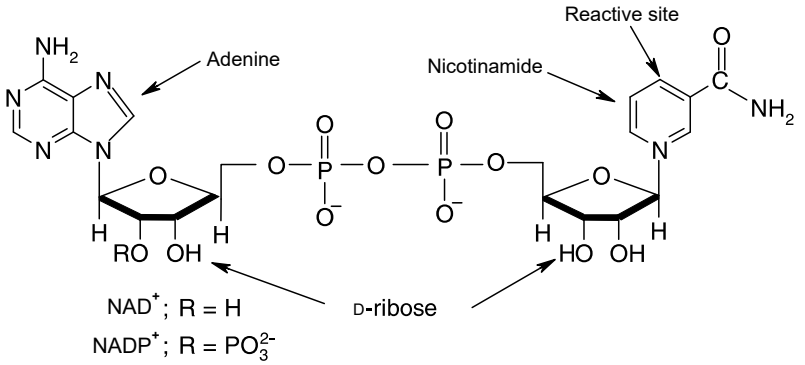
Since coenzymes are chemically changed as a consequence of enzyme action, these are often considered to be a special class of substrates, or **second substrates**, which are common to many different holoenzymes. In all cases, the coenzymes donate the chemical group carried from the substrate to an acceptor molecule and get regenerated to their original form. This regeneration of coenzyme (unlike the usual substrates, which are consumed during the course of a reaction) and holoenzyme fulfils the definition of an enzyme as a chemical catalyst. The cofactors assist the enzyme in a number of ways. For example

- As interenzyme carriers
- As intermediates
- As templates or as primers in DNA synthesis
- As modifiers of the shape of enzymes
- As stabilisers of enzymes, etc.

Some of the cofactors act in more than one way in a reaction. Of the various possible modes of action, the most common mode of cofactor action is as interenzymic carrier. In this mode, the carrier combines with the enzyme and a part of the substrate is transferred to the carrier. This loaded carrier then migrates to another enzyme to transfer the load to the substrate on the other enzyme and then the cofactor dissociates from the enzyme. These interenzymic carriers can be classified in terms of the species or the group being carried. Some of the important coenzymes and their mode of action is described below.

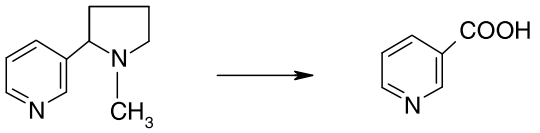
4.7.1 *Nicotinamide Adenine Dinucleotide (NAD^+) and Nicotinamide Adenine Dinucleotide Phosphate ($NADP^+$)*

NAD^+ and $NADP^+$ are two closely related coenzymes, derived from niacin (nicotinic acid and nicotinamide) or vitamin B_3. NAD^+ is a dinucleotide containing nicotinamide, adenosine, ribose sugar and phosphoric acid while $NADP^+$ contains an additional phosphate group in the 2' position of ribose sugar attached to adenine.



Structure of $NAD^+/NADP^+$

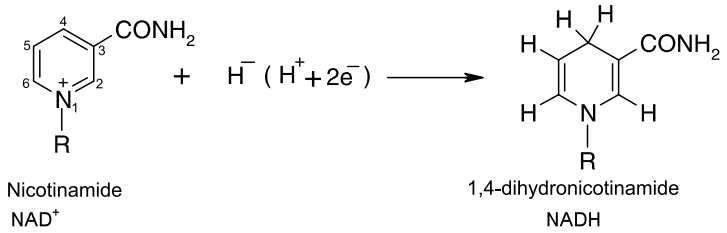
Nicotinatamide is the amide of nicotinic acid which is closely related to nicotine, an alkaloid found (~2 to 8% of dry weight) in tobacco leaves. Nicotinic acid can be obtained by the oxidation of nicotine.



Nicotine Nicotinic acid

The deficiency of vitamin B_3 may result in physiological and psychological disorders. Mild deficiency leads to headache and dizziness whereas in case of acute deficiency there may be metabolic disorders like diarrhoea or even neurological symptoms like hallucinations.

These coenzymes perform the role of accepting equivalent of a hydride ion (a proton and two electrons) from the substrate to affect its oxidation. The nicotinamide ring is the functional part of the coenzymes; the para position (C-4) of the ring being the site of hydrogen transfer. As a consequence of hydrogen transfer, the positive charge on the nitrogen atom is neutralised and the pyridine ring is reduced and loses its aromaticity. This gives a compound called l,4-dihydronicotinamide adenine dinucleotide, NADH (or NADPH).



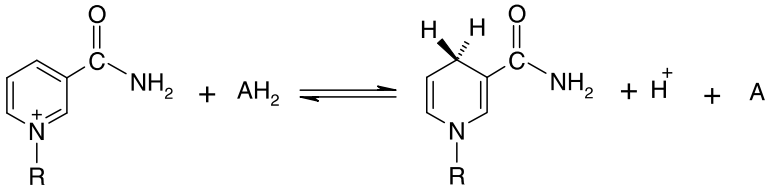
Nicotinamide NAD^+ 1,4-dihydronicotinamide NADH

R = remaining part of the coenzyme molecule

The general reaction of NAD^+ with the substrate (AH_2) may be summarised as:

$$NAD^+ + AH_2 \rightleftharpoons NADH + H^+ + A$$

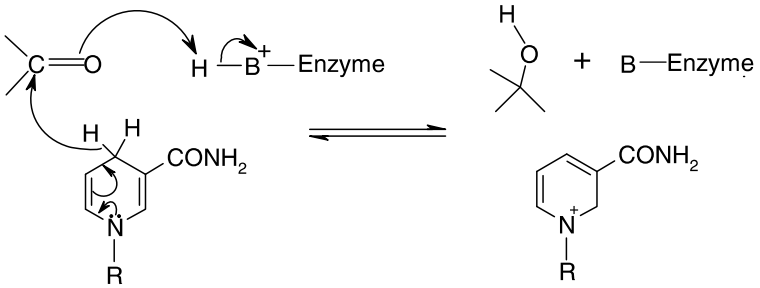
The transfer of hydrogen between the substrate and NAD^+ is direct and occurs with stereospecificity. The two hydrogens at C-4 position of NADH are chemically equivalent; it is the difference in their topology that is responsible for the stereospecificity of the reaction.



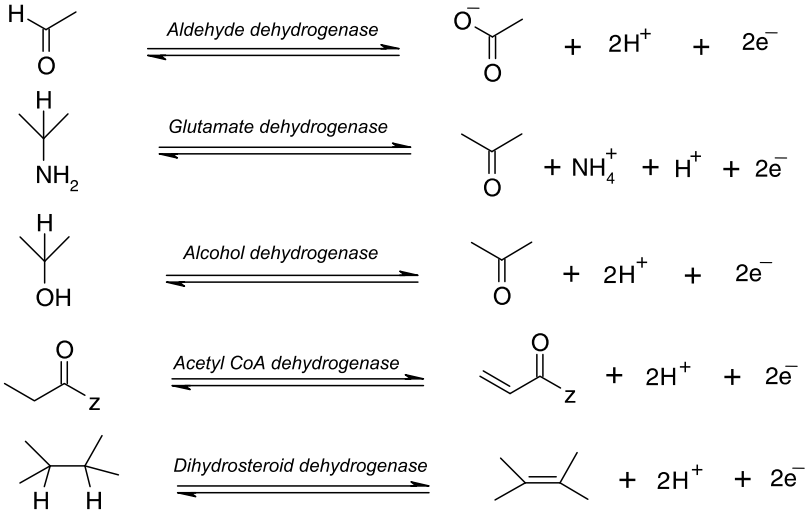
The reduced coenzymes are later reoxidised with the help of electron transport chain in the mitochondria accompanied with the formation of adenosine triphosphate (ATP), the energy carrier of the cell. The conversion of alcohol to aldehyde by *alcohol dehydrogenase* is a typical example of the reaction in which NAD^+ acts as a coenzyme.

$$\text{Alcohol} + NAD^+ \rightleftharpoons \text{Aldehyde} + NADH + H^+$$

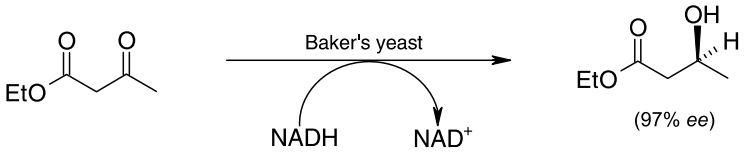
The reverse reaction, i.e. the reduction of aldehyde to alcohol in the fermentation process is also catalysed by the same enzyme and is accompanied by the oxidation of NADH to NAD^+. The proposed mechanism of the reaction involves the transfer of a hydride from the reduced coenzyme to the substrate.



Nicotinamide adenine dinucleotide plays the role of coenzyme in many other enzymes. Some important reactions where NAD^+ plays the role of a coenzyme are given below.



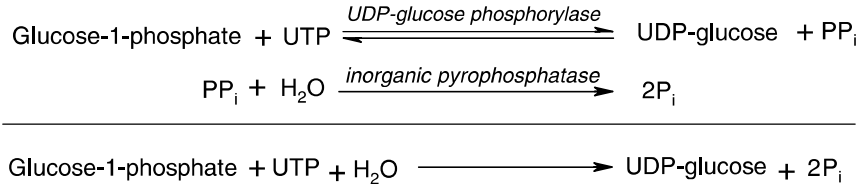
Since the hydrogen transfer reactions using NAD^+ display a high degree of stereoselectivity, they have been used extensively in organic synthesis. Chiral compounds requiring highly selective reagents to achieve enantiomeric purity can be made easily by using NADH and an appropriate enzyme, for example,



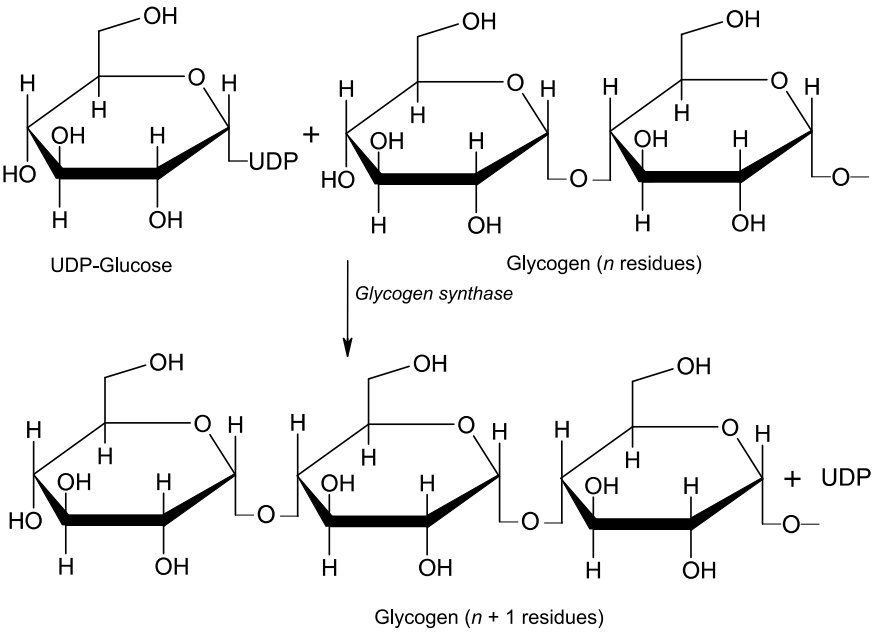
Certain enzymes catalyse elimination, epimerisation or aldolisation reactions on such substrates which do not have requisite chemical groups to perform the said reaction. In such cases, the coenzyme NAD^+ performs a special function of transiently providing such chemical groups. The epimerisation of UDP-galactose to UDP-glucose, essential for the entry of galactose into the glycolytic pathway (related to the breakdown of glucose) or glyconeogenesis (synthesis of glycogen) in the cell, is a typical example of this type.

4.7.2 UDP-Glucose

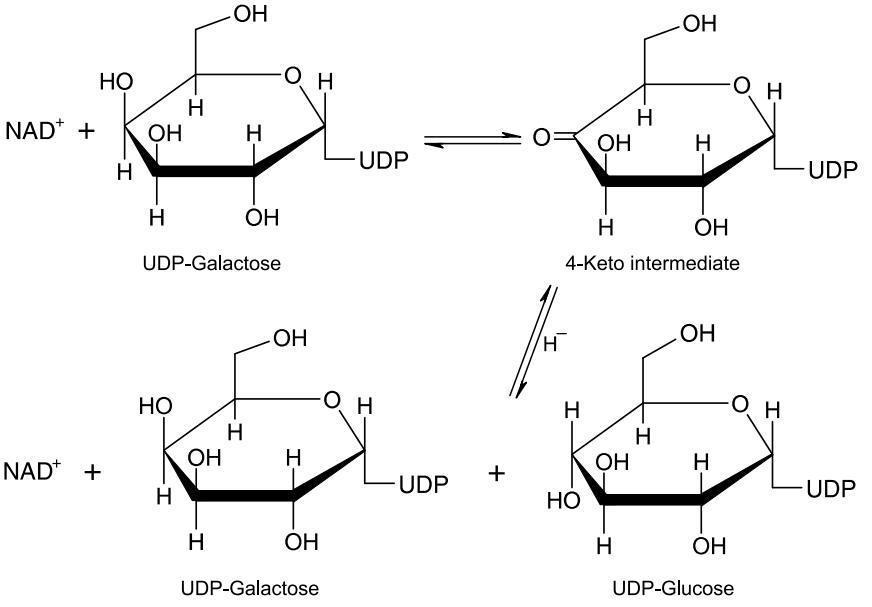
UDP-glucose (uridine diphosphate glucose) is a glucosyl donor for the biosynthesis of glycogen from glucose or other hexoses. It is an activated form of glucose like coenzyme A—an activated form of acetyl group or ATP which is an activated form of orthophosphate. UDP-glucose is formed by a reaction of glucose-l-phosphate with UTP catalysed by *UDP-glucose phosphorylase*. The reaction is reversible but is driven to the right by irreversible hydrolysis of the pyrophosphate to orthophosphate by an enzyme, *inorganic pyrophosphatase*. This hydrolysis continuously removes pyrophosphate, the product of the first reaction, thereby driving it to the formation of UDP-glucose.



This activated group of UDP-glucose can be transferred to the C-4 end of the elongating glycogen chain through a $\alpha(1 \rightarrow 4)$ linkage.



Since UDP-glucose can be obtained by the epimerisation of UDP-galactose, it plays another important role. It provides a means of storing excess galactose as glycogen in the body as UDP-glucose obtained can be stored as glycogen in the body.



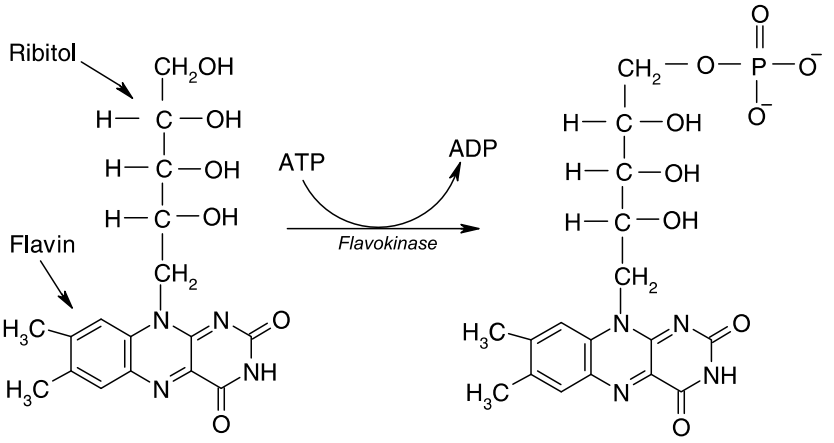
The enzyme, *UDP-galactose-4-epimerase* associated with this reaction contains a tightly bound molecule of the coenzyme NAD^+ or NADP. It has been demonstrated that NAD^+ is transiently reduced to NADH in the reaction. Based on these observations, it has been proposed that the oxidised coenzyme NAD^+ accepts a proton from the—OH group at C-4 atom which gets transiently oxidised to a carbonyl group in the process.

In the next step, the carbonyl group is reduced by accepting a hydride ion from NADH. Since this can happen from either side of the carbonyl group, it generates UDP-galactose and its 4-epimer, UDP-glucose.

4.7.3 Flavin Mononucleotide (FMN) and Flavin Adenine Dinucleotide (FAD)

FMN and FAD are derived from **riboflavin** (also known as **vitamin B_2**) and act as coenzymes in electron transfer or oxidation–reduction reactions catalysed by certain enzymes. The word flavin has been derived from the Latin word *flavus* meaning yellow, the colour of the oxidised isoalloxazine ring present in the coenzyme. The enzymes that require FMN or FAD as cofactors are termed **flavoproteins**. These catalyse redox reactions acting as dehydrogenases, oxidases and monooxygenases (also called hydroxylases). Several flavoproteins also contain metal ions and are termed **metalloflavoproteins**. Flavoproteins function in different ways like hydride transfer and substrate carbanion addition or by free radical mechanism. Therefore, these provide link between two electron and one electron transfer processes.

Structurally, FMN is formed by phosphorylation of riboflavin by the action of ATP with the help of enzyme *flavokinase.*

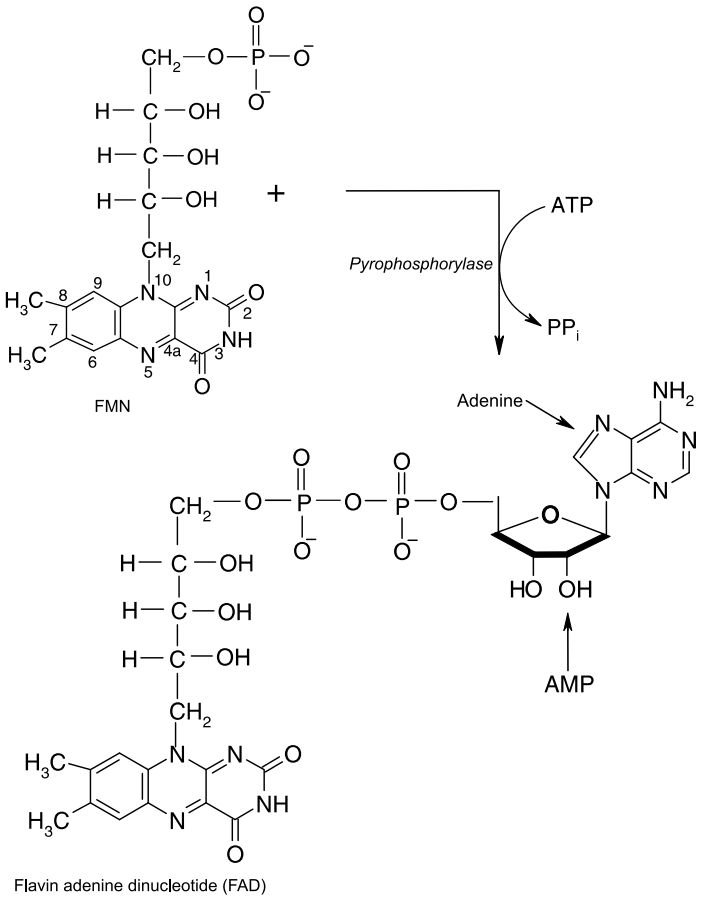


Riboflavin (vitamin B_2) Flavin monophosphate (FMN)

Strictly speaking FMN is not a nucleotide, it is a derivative of ribitola pentahydroxy sugar while nucleotide contains ribose or deoxyribose sugar.

FAD, on the other hand, is synthesised by the reaction of FMN with ATP wherein the AMP moiety of ATP is transferred to FMN. The synthesis of FAD is controlled by enzyme flavin nucleotide pyrophosphorylase.

$$FMN + ATP \rightarrow FAD + PP_i$$



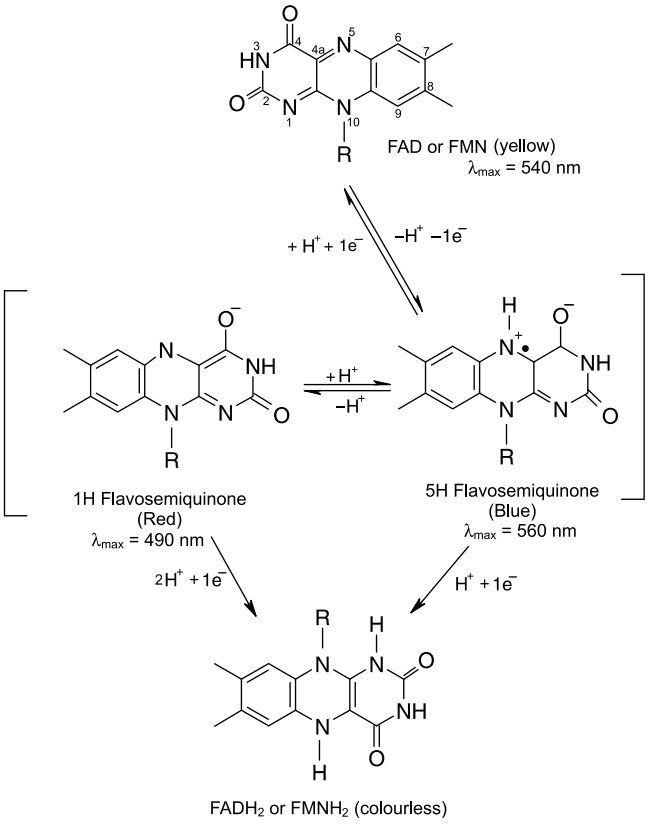
Flavin adenine dinucleotide (FAD)

The mechanism of action of these coenzymes is still under investigation. According to one of the proposed mechanisms, $FADH_2$ and $FMNH_2$ act as transient intermediates in a number of reactions and the **isoalloxazine ring** system of the riboflavin moiety is the functional part of this coenzyme.

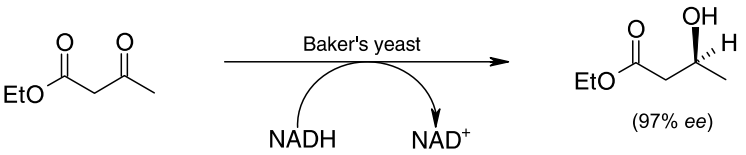
N-5, C-4a, N-l0, N-l and C-2 can be regarded as the catalytic entity; N-5 and C-4a positions being specifically important. The chromophore N=C–C=N plays the same

role as played by nicotinamide ring in NAD^+. Together, this structure operates in the transfer of hydrogens (or electrons).

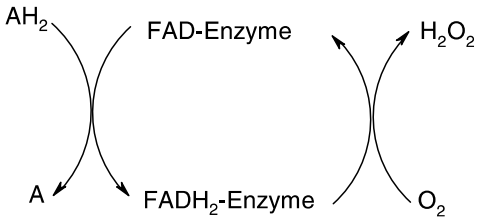
This catalytic entity can exist in three spectrally distinguishable redox states. One of these is an oxidised form (yellow) and another a reduced form (colourless); and there are two one-electron reduced forms (red and blue). The flavin coenzymes work as a switch between these one and two electron processes. Two-electron reduction of flavoquinone gives rise to flavo-hydroquinone while one-electron oxidation can give one of the two semiquinone radicals. The red coloured lH isomer is unstable while the blue coloured 5H isomer is quite stable and is of great importance in the one-electron transfer processes.



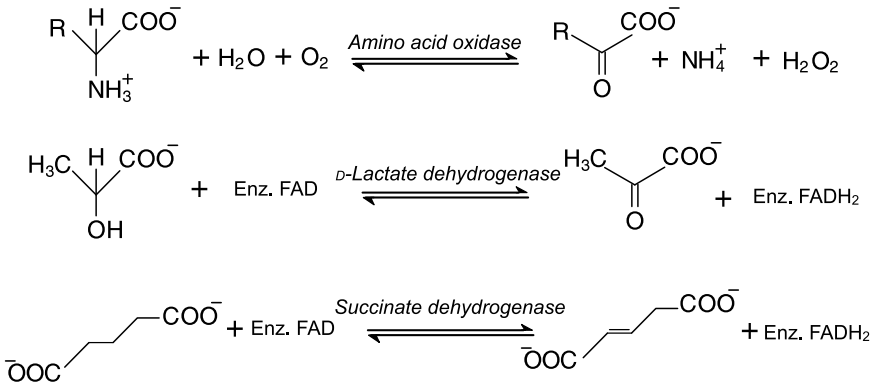
Fatty acyl-CoA desaturase is an example of flavin-dependent enzyme, which catalyses an important step in the biosynthesis of unsaturated fats.



The reaction is actually more complex than shown and involves other cofactors, but FAD is the key cofactor for the enzyme. The most important aspect of the involvement of flavin coenzymes in hydrogen transfer is their ability to transfer hydrogen to oxygen as part of respiration process. In other words, they utilise molecular oxygen.

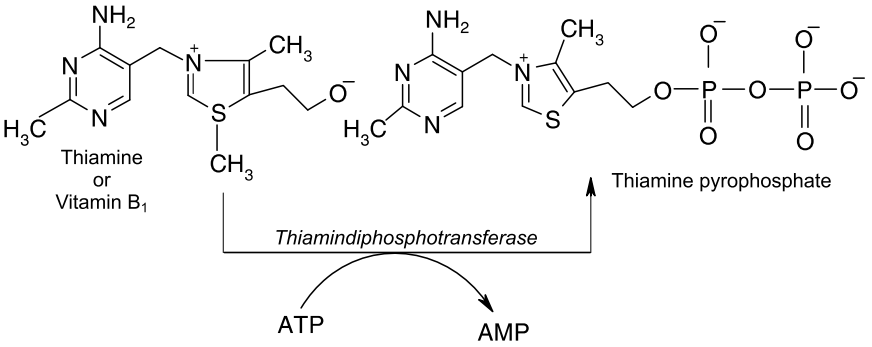


The involvement of FMN and FAD in variety of reactions on a range of substrates shows their versatility. Some representative examples are given below.



4.7.4 Thiamine Pyrophosphate (TPP)

Thiamine pyrophosphate (TPP) is produced in the brain and liver cells by phosphorylation of thiamine or vitamin B_1 with the help of the enzyme, *thiamine diphosphotransferase* or *TPP synthase*. In this process, a pyrophosphate group is transferred from ATP to the vitamin and a molecule of AMP is generated as a by-product.

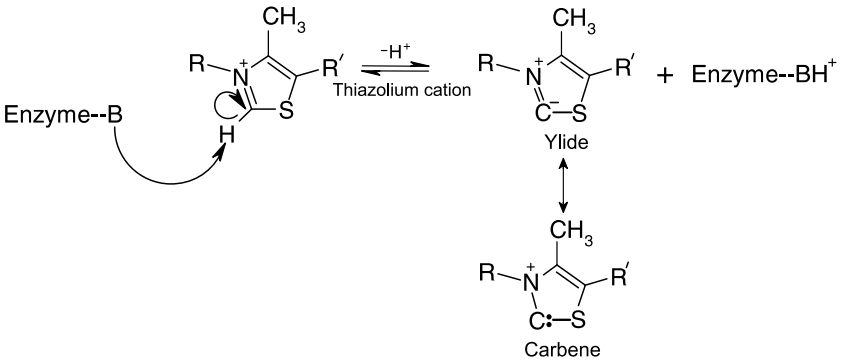


Thiamine in turn consists of a substituted pyrimidine and a thiazole group coupled by a methylene bridge. TPP can stabilise carbanions and in doing so facilitates a number of crucial reactions in the cell.

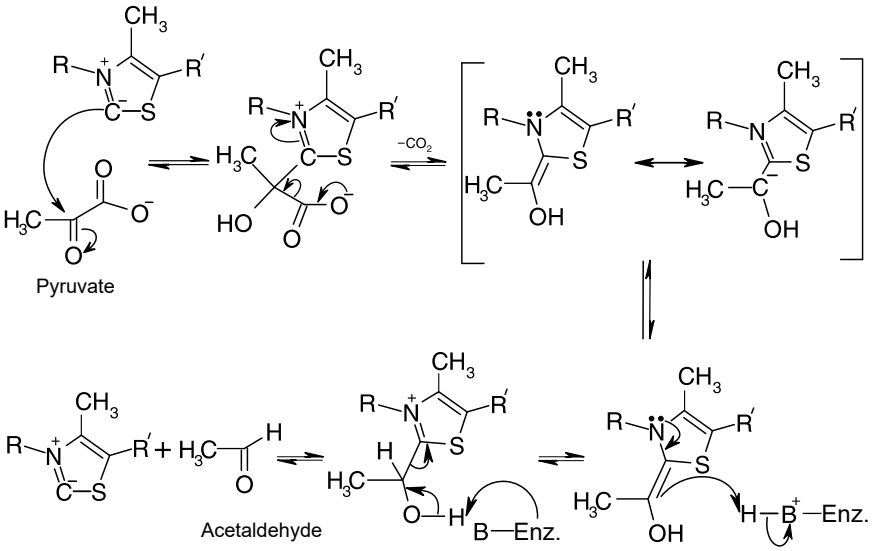
TPP is necessary as a coenzyme for the enzymes catalysing decarboxylations of α-keto acids and the formation of C–C bonds between a carbanion stabilised compound on TPP and a carbonyl carbon of another compound. TPP catalyses the following types of reactions on α-keto acids produced by pyridoxal phosphate

- Oxidative carboxylation
- Transketolisation
- Non-oxidative decarboxylation
- Acetoin formation.

The active portion of the TPP is thiazolium cation which can lose the acidic proton on the carbon between the S and the N atoms of the ring in a base catalysed mechanism to form a ylide.



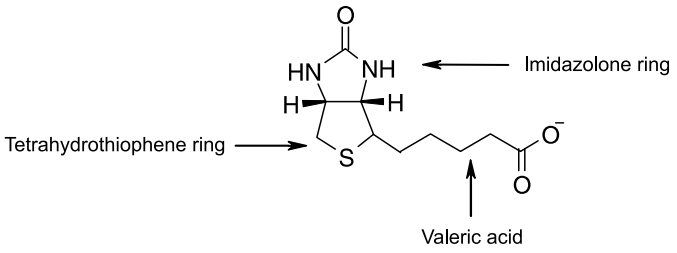
The negative charge of the carbanion is stabilised by the adjacent positive charge on the quaternary ammonium ion and the participation of *d*-orbitals of sulphur. The resonance stabilised carbanion so obtained is the reactive entity of the coenzyme. The reactions of TPP involve the attack of this stabilised carbanion (nucleophile) on the electrophilic carbonyl of α-keto acids. The thiazolium ring being electrophilic in nature stabilises the carbanionic intermediates. Eventually, the thiazolium ring acts as a good leaving group and is regenerated. TPP, together with *pyruvate dehydrogenase*, catalyses the cleavage of a carbon–carbon bond in pyruvate to give acetaldehyde. The mechanism of this key reaction in the metabolism of glucose is shown below,



Since direct decarboxylation would give unstable carbonyl carbanion, it is unfavoured instead the enzyme stabilises the system to produce an aldehyde. This reaction occurs as part of the alcoholic fermentation of sugar to eventually produce ethanol.

4.7.5 Cocarboxylase

Coenzymes that work with the enzymes catalysing transfer of carboxyl groups are called as cocarboxylases. **Biotin** (Vitamin H) is one such coenzyme acting as a component of multisubunit enzymes involved in carboxylation reactions, e.g. *acetyl-CoA carboxylase* and *pyruvate carboxylase* etc. Biotin is an imidazole derivative in which an imidazolone ring is *cis*- fused to a tetrahydrothiophene ring to which a valeric acid molecule is attached at position 2.

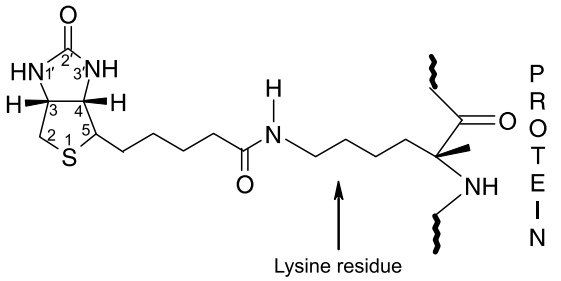


Biotin

The main function of biotin is to accept a carboxyl group and transfer it to a suitable substrate. All the steps of the reaction take place in the same multisubunit complex. Biotin mediates two kinds of carboxylation reactions namely direct carboxylations and transcarboxylations.

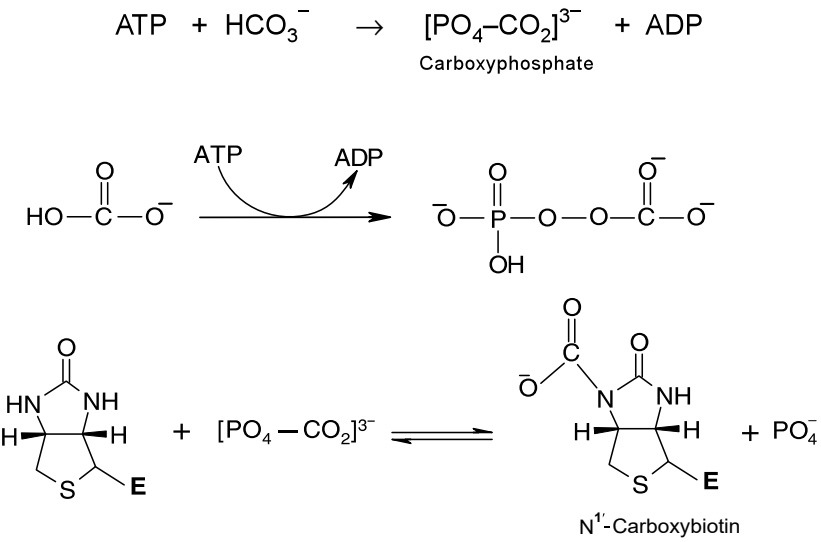
Direct Carboxylation

As the name indicates, this involves direct carboxylation of the substrate. Conversion of acetyl-CoA to malonyl-CoA by *acetyl-CoA carboxylase* is a typical example. The multienzyme *acetyl-CoA carboxylase* contains three components, a biotin carboxyl carrying protein (BCCP), biotincarboxylase and carboxytransferase. In BCCP, the carboxylic acid group of biotin is anchored to the ε-NH_2 group of a lysine residue on the enzyme via. an amide bond.



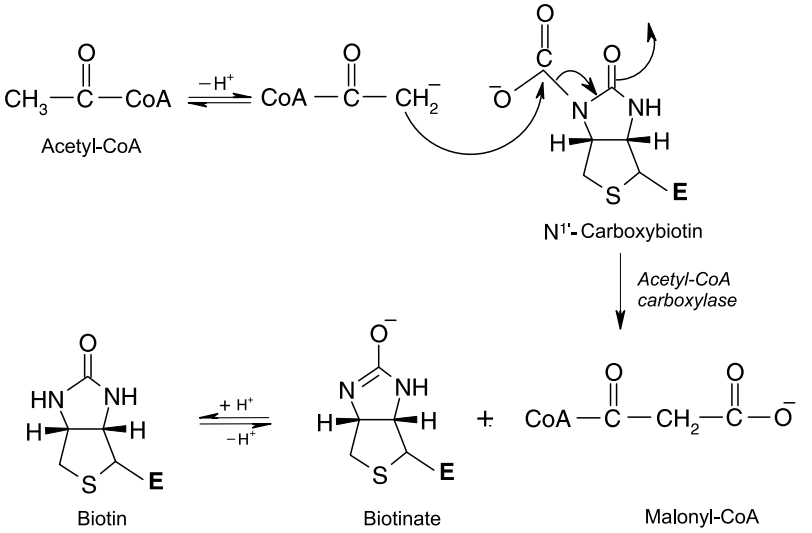
Biotin carboxyl carrying protein (BCCP)

In the first step, $N^{1'}$ atom of the imidazolone ring, the seat of action, gets a carboxyl group from carbonic phosphoric anhydride to generate an activated intermediate called **carboxybiotin**. The carbonic-phosphoric anhydride (or carboxyphosphate) is formed by the action of ATP on bicarbonate ion and acts as a source of the carboxyl group. The addition of CO_2 group from carboxyphosphate to $N^{1'}$ atom of biotin is catalysed by *biotincarboxylase*.

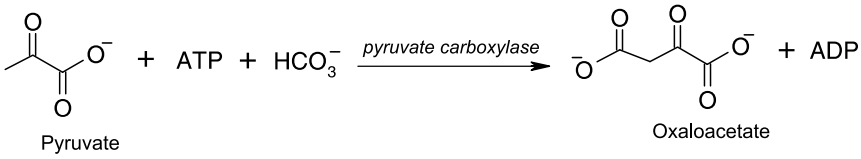


In the next step, the activated carboxybiotin transfers the carboxylic acid group to acetyl-CoA with the help of the enzyme *carboxytransferase.* In the probable mechanism of the reaction, acetyl-CoA loses a proton to generate a carbanion that attacks the carboxyl group of the activated carboxybiotin followed by the removal of biotinate ion which picks up a proton to regenerate biotin attached to the protein.

These reactions can be summarised as

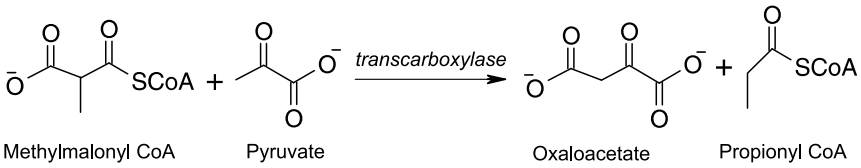


The generation of oxaloacetate from pyruvate also proceeds by a similar mechanism.



Transcarboxylation

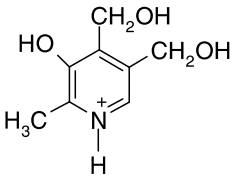
Transcarboxylation refers to the transfer of a carboxyl group from one species to the other, e.g. methylmalonyl CoA transfers a carboxyl group to pyruvate to give oxaloacetate,



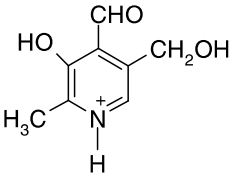
Biotin is found in numerous foods and is also synthesised by intestinal bacteria and as such deficiencies of this vitamin are rare. Deficiencies are generally seen only after long antibiotic therapies which deplete the intestinal fauna or following excessive consumption of raw eggs. The latter is due to the affinity of the egg white protein, avidin, for biotin which prevents intestinal absorption of biotin.

4.7.6 Pyridoxal-5-Phosphate

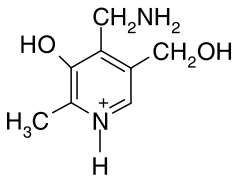
Pyridoxal-5-phosphate is a versatile coenzyme engaged in a variety of reactions required for the synthesis and catabolism of α-amino acids. The reactions include transamination, racemisation, decarboxylation, α-β and β-γ eliminations etc. It is derived from vitamin B_6 which in fact is a group of three derivatives of pyridine, namely **pyridoxal, pyridoxamine** and **pyridoxine**. In diet these are present as pyridoxine, pyridoxal phosphate and pyridoxamine phosphate. All the three forms are important as these can be interconverted in the body. There may be some hydrolysis of the phosphate groups during digestion; however, these are phosphorylated back by *pyridoxal kinase* present in the cell, with the help of ATP.



Pyridoxine

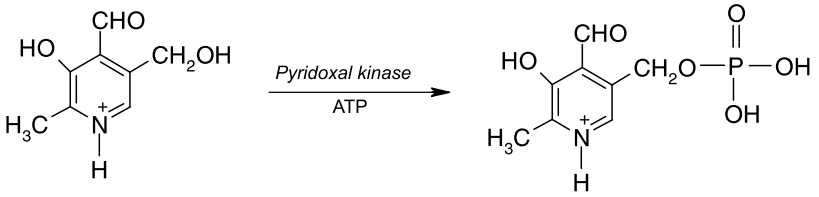


Pyridoxal



Pyridoxamine

Of the three constituents of vitamin B6, it is pyridoxal-5-phosphate (PLP) that is active as coenzyme.

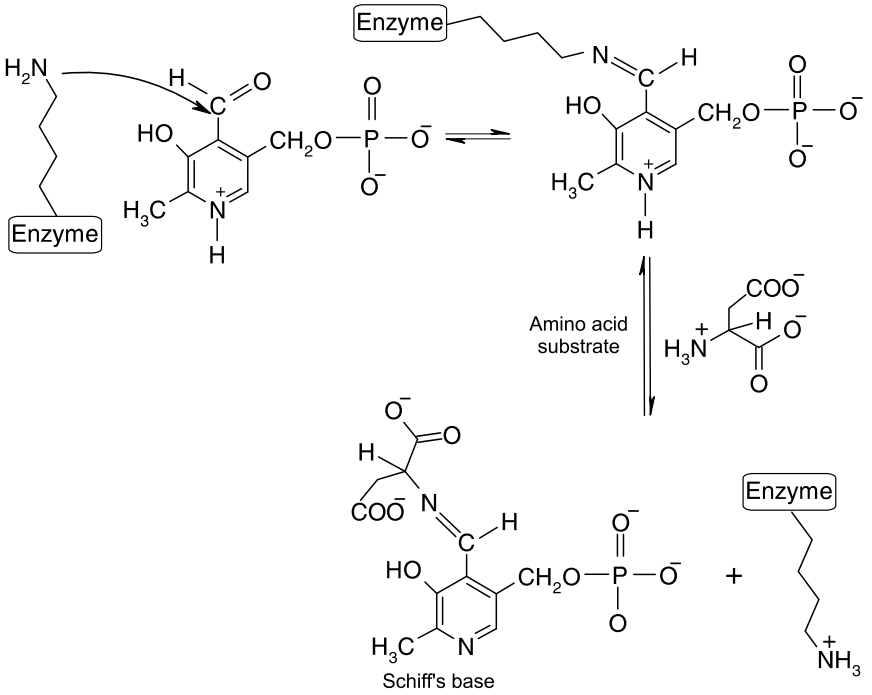


Pyridoxal Pyridoxal-5-phosphate

Pyridoxal phosphate acts by forming an **aldimine** or **Schiff's base** adduct with the amino group of the α-amino acid and catalyses bond cleavages by stabilising the electronic environment of the α- and β-carbons of the adduct intermediates. The adduct can undergo different changes depending on the enzyme involved and cause different overall changes. Similar to TPP, loss of a H as a proton on the α-carbon yields a carbanion that is resonance stabilised by bond shifts in the adduct. When β-carbon has –OH, –SH or phosphate as functional group, pyridoxal phosphate can act to effect β-elimination and so on.

The role of pyridoxal phosphate in transamination reaction in the metabolism of α-amino acids is quite well studied and interesting. Transamination refers to the transfer of an α-amino group from an α-amino acid to a α-keto acid to give a new amino acid and a different α-keto acid. These reactions are catalysed by aminotransferases also called transaminases. More than 50 aminotransferases are known and all need pyridoxal phosphate as coenzyme. One of the most important aminotransferases is *aspartate aminotransferase* which catalyses the transfer of α-amino group of aspartate to α-ketoglutarate producing oxaloacetate and glutamate. The reaction can be represented as follows.

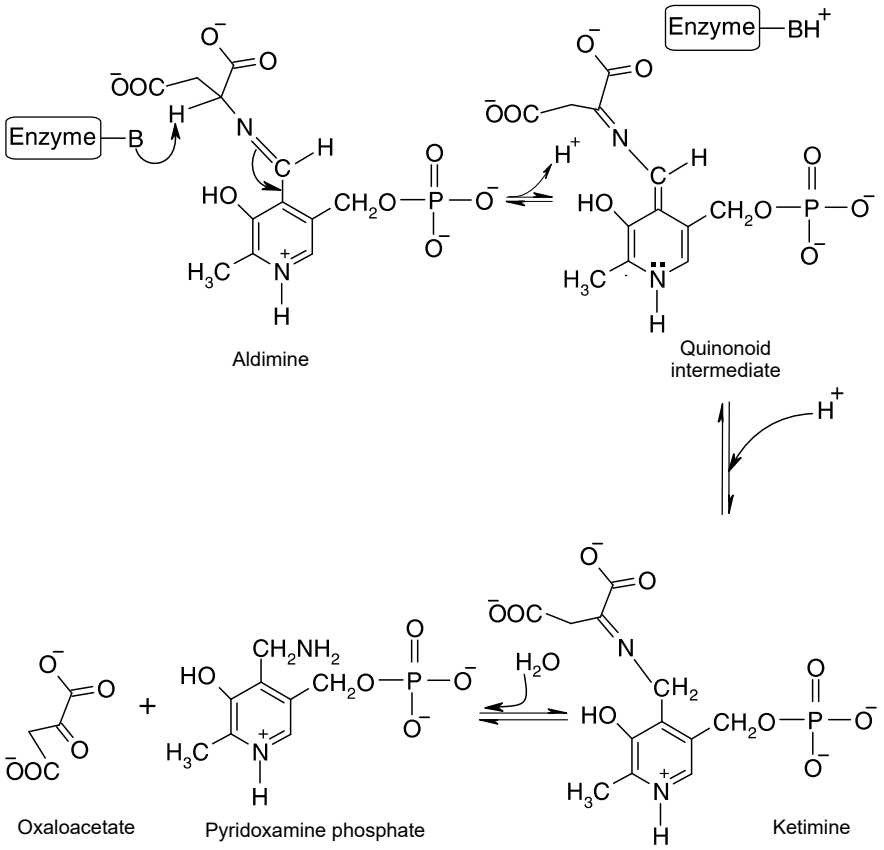
$$\text{Aspartate} + \alpha\text{-Ketoglutarate} \rightarrow \text{Oxaloacetates} + \text{glutamate}$$



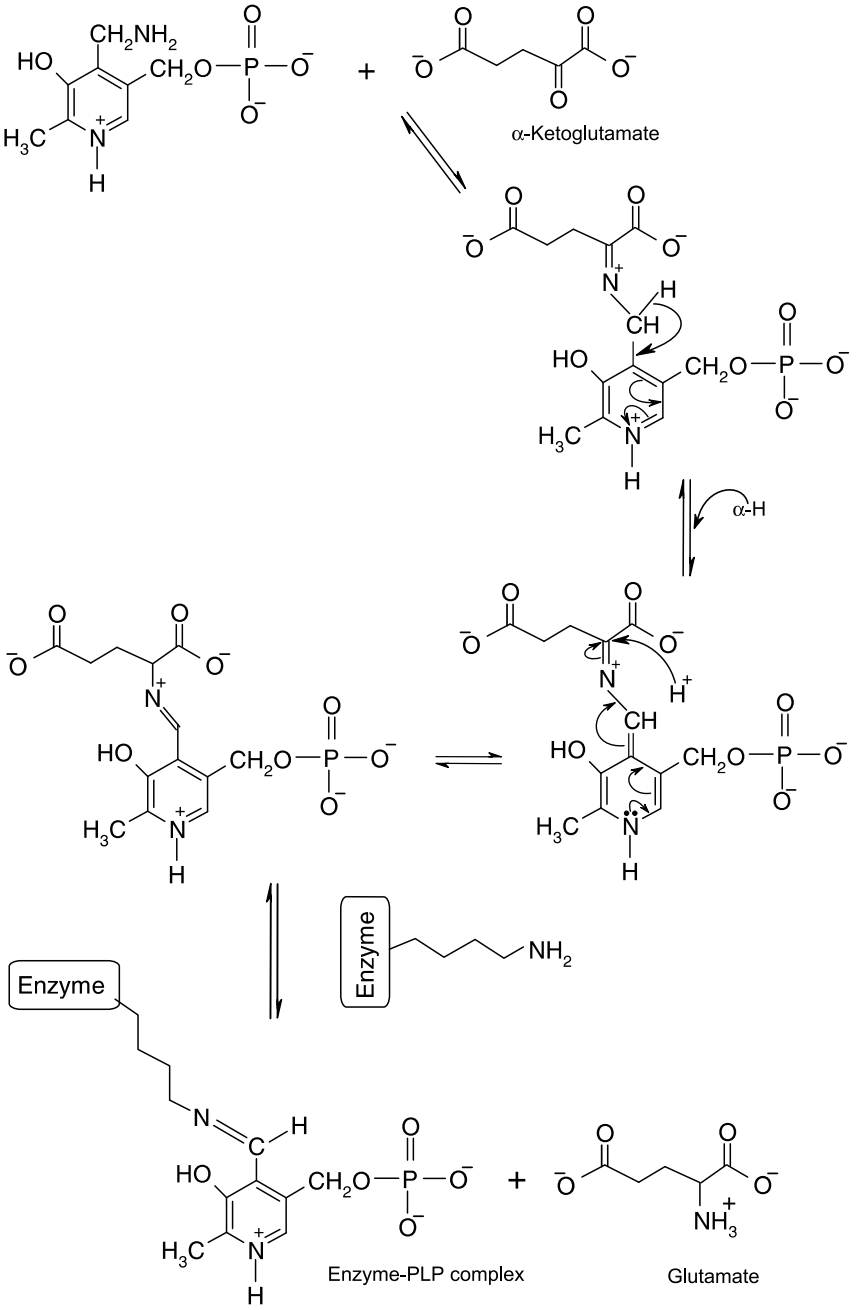
Transamination reaction is proposed to have the following mechanism. The coenzyme, pyridoxal phosphate, is initially bound to the ε-amino group of a lysyl residue of the enzyme.

The incoming substrate forms a schiff's base with pyridoxal phosphate bound to lysyl residue, by displacing the ε-NH_2 group and releasing the lysyl residue of the enzyme. This intermediates (schiff's base) called an aldimine contains a double bond between the α-N of the amino acid and the carbon of the coenzyme. The proton attached to the α-carbon atom is lost to give rise to a quinonoid structure which on reprotonation forms a ketimine containing unsaturation between Cα-N bond. This ketimine on hydrolysis yields an α-keto acid and pyridoxamine phosphate.

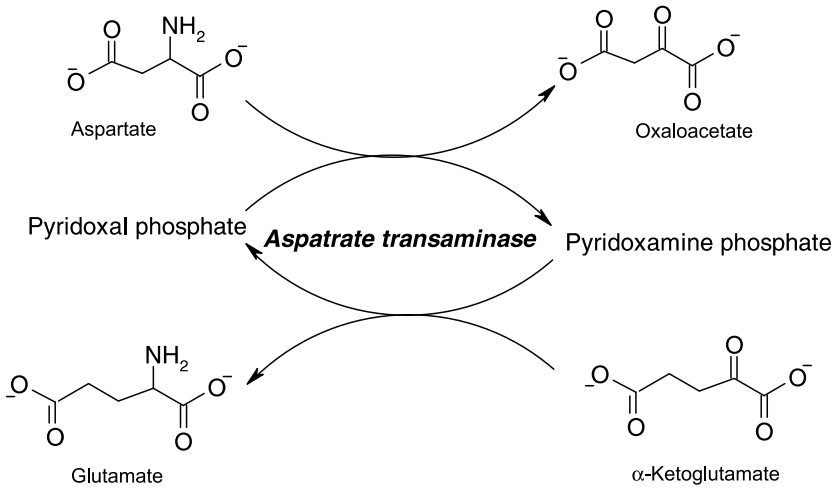
Effectively, the net result of these steps amounts to the transfer of α-NH_2 group of the amino acid to the coenzyme and releasing α-keto acid (oxaloacetate).



In the next set of reactions, the amino group is transferred from the coenzyme to another α-keto acid. The enzyme-pyridoxamine complex now binds to α-etoglutarate and hands over the above-mentioned NH_2 group to α-ketoglutarate yielding back pyridoxamine phosphate-enzyme complex. To execute this, pyridoxamine phosphate condenses with the α-keto group of α-ketoglutarate to give an adduct. A proton from C_4' atom is lost to yield a quinonoid structure. Accepting a H at the Cα facilitates the release of glutamate with a concomitant regeneration of enzyme-pyridoxal phosphate complex.



The overall changes in the transamination reaction between aspartate and α-ketoglutarate to give oxaloacetate and glutamate can be summarised as



The ketimine formed, may as well lose a β carboxyl group instead of an α hydrogen and generate an α-carbanionic intermediate. In the presence of the enzyme aspartate β-decarboxylase, the protonation of this intermediate followed by hydrolysis can generate alanine.

Deficiencies of vitamin B_6, however, are rare and usually are related to an overall deficiency of all the B-complex vitamins. Isoniazid and penicillamine are two drugs that complex with pyridoxal and pyridoxal phosphate resulting in a deficiency in this vitamin.

4.8 Enzymes in Organic Synthesis

Enzymes are an important tool in organic synthesis. This is especially due to:

- Easy availability
- Good catalytic properties
- Mild reaction conditions
- Being free of undesirable reactions.

The earliest enzymatic conversion known to mankind is the manufacture of ethyl alcohol from molasses. This conversion is brought about by the enzyme 'invertase' (present in yeast) which converts sucrose into glucose and fructose and finally by the enzyme 'zymase' (also present in yeast) that converts glucose and fructose into ethyl alcohol. This process is used even today for the manufacture of ethyl alcohol. The reactions are as given below.

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} \xrightarrow{\text{invertase (in yeast)}} \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}$$

$$\underset{\text{Glucose and Fructose}}{C_6H_{12}O_6} \xrightarrow{\text{zymase (in yeast)}} 2\underset{\text{Ethyl alcohol}}{CH_3CH_2OH} + 2CO_2$$

Two other conversions, known since early times, are the enzymatic conversion of ethyl alcohol into acetic acid by bacterium acetic in the presence of air (process is known as quick-vinegar process) and the conversion of lactose into lactic acid by *Bacillus acidic lactic.*

$$\underset{\text{Ethyl alcohol}}{CH_3CH_2OH} + O_2 \xrightarrow{\text{Bacterium acetic}} \underset{\text{Acetic acid}}{CH_3COOH} + H_2O$$

$$\underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{Bacillus acetic lactic}} 4\underset{\text{Lactic acid}}{CH_3CH(OH)COOH}$$

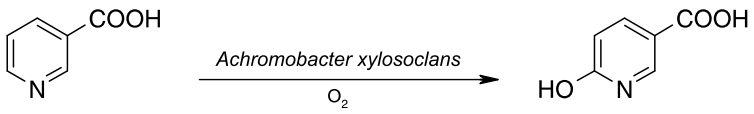
The enzymatic transformations given above are referred to as **fermentations.** These and some of the other earlier applications used organisms as such for the transformations. Due to the associated messy procedures, these processes did not find many uses. Once the isolation of enzymes from the organisms became possible at good scale, these were used in numerous industrial and synthetic processes. Recent advances in the area of genetic engineering have facilitated the production of large quantities of purified enzymes which in turn has increased the scope of industrial and synthetic applications of enzymes.

The enzymatic conversions or transformations have definite advantages; some of these are:

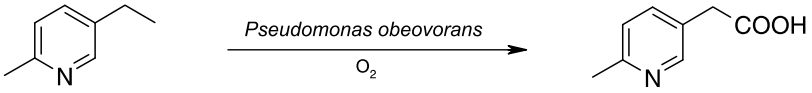
- Generally the reactions are performed in aqueous medium and at ambient pH, temperature and pressure.
- The reactions involve only one step and are much faster than non-enzymatic reactions.
- The conversions are regio-, stereo- and chemospecific

A significant advantage of enzymatic reaction is that same conversions or transformations, which are not possible by conventional chemical means, can be achieved by enzymes. Some examples of this type are given below.

Electrophilic substitution next to nitrogen in heterocycle



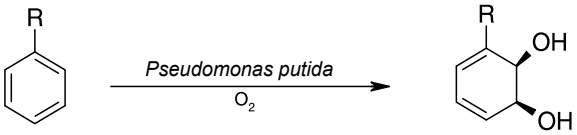
Oxidation of hydrocarbon in the side chain of heterocycle to carboxylic acid.



The most important application of enzymes in organic synthesis involves enzymatic oxidations, hydroxylations, hydrolysis, reductions and isomerisations. A brief discussion on these is given below.

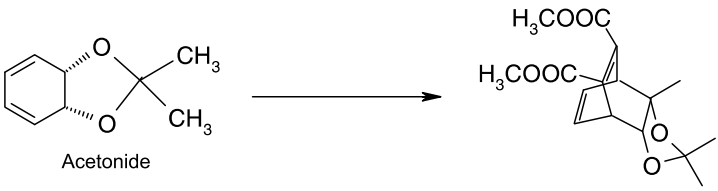
4.8.1 Enzymatic Oxidations

Considerable amount of work has been reported on enzymatic oxidations of aromatic nucleus. Benzene and substituted benzenes give corresponding *cis*-diols on oxidation with *dioxygenases* from *Pseudomonas putida* in the presence of oxygen.

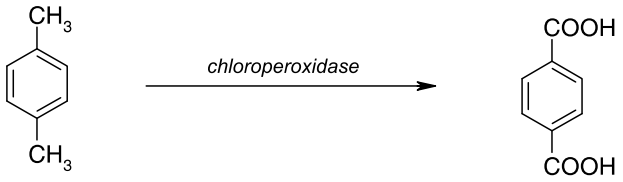


benzene	R = H
toluene	R = CH_3
chlorobenzene	R = Cl
styrene	R = —CH=CH_2
phenylacetylene	R = —C≡CH

The products of the reaction are often obtained in optically pure form and their derivatives (e.g. acetonides) may act as substrate for various cycloadditions to give important molecules. For example, the acetonide of the cyclohexa-3, 5-diene-l, 2-cis-diol obtained from the oxidation of benzene in presence of *Pseudomonas putida* can undergo Diels-Alder [4+2] addition to give tricyclic compounds.

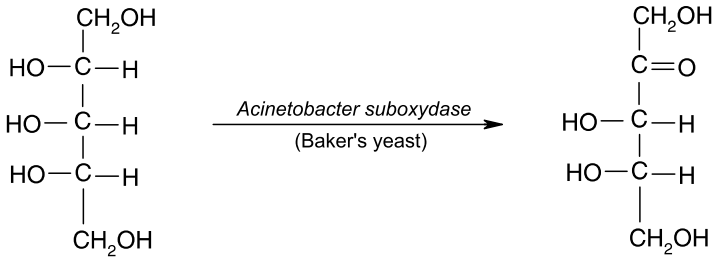


The oxidation of hydrocarbons is an important transformation whose product is used as feedstock in chemical industries. Generally, it is possible under harsh conditions only, however, with the help of enzymes these oxidations can be performed under mild conditions, for example, oxidation of *p*-xylene to terephthalic acid by chloroperoxidase enzyme extracted from *caldariomyces fumago.*

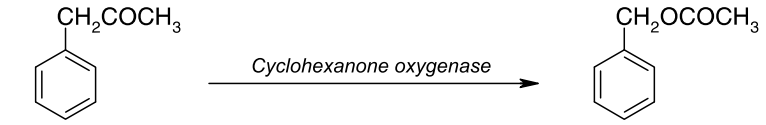


p-Xylene Terephthalic acid

Secondary alcohols on enzymatic oxidation give the corresponding ketones. A secondary alcohol group can be oxidised to the corresponding ketone even in the presence of primary alcoholic group as shown below.

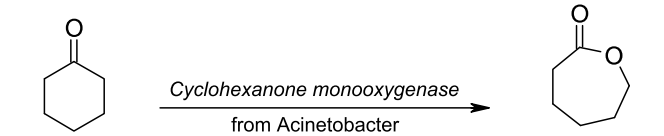


Baeyer–villiger oxidations can also be conveniently performed using enzymes. The monooxygenases from *Acinetobacter* are used in association with its cofactor NADPH. The following are two such examples:

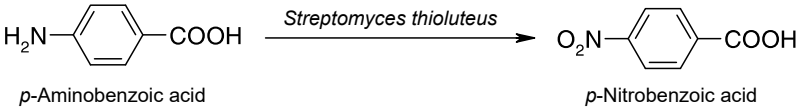


Phenylacetone Benzyl acetate

Ring expansion of cyclic ketones by microorganisms containing an oxidative flavin-based enzyme is used to generate chiral lactones. For example:

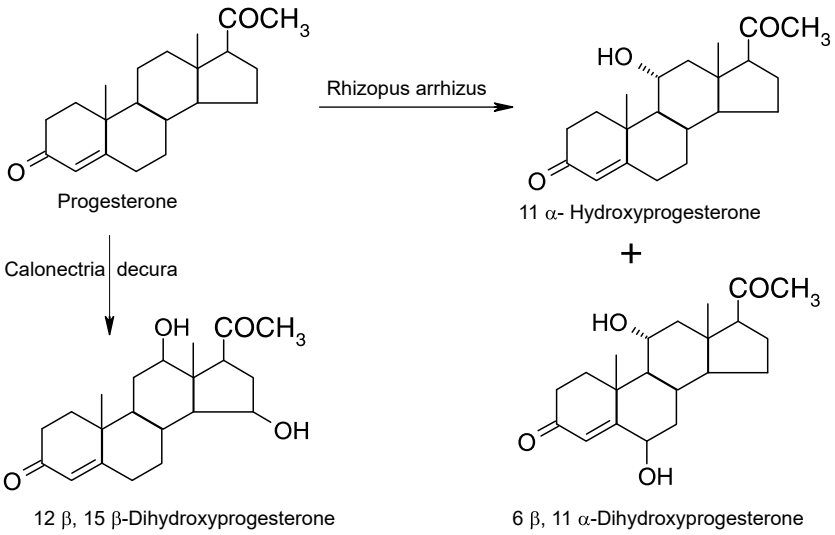


Amino groups can be oxidised into nitro group by enzymes.



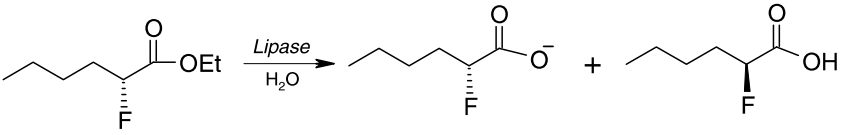
4.8.2 Enzymatic Hydroxylation

Hydroxylation in steroids has been affected in different positions in a regio- and steroselective manner. The most favoured positions in hydroxylation are llα followed by llβ, 6β, 7α, l0β, l2α, l2β, l3β, l4α, l5α, l5β, l7α and 7β positions. As an illustration, hydroxylation of progesterone gives the products shown in the following scheme.



4.8.3 Enzymatic Hydrolysis

Hydrolysis using enzymes results in the formation of almost pure enantiomer. For example using lipase the following reaction can be carried out to get 99% enantiomeric excess (ee) of the enantiomer which does not react with the enzyme while the isomer which reacts with the enzyme also gives the hydrolysed product in 69% ee.

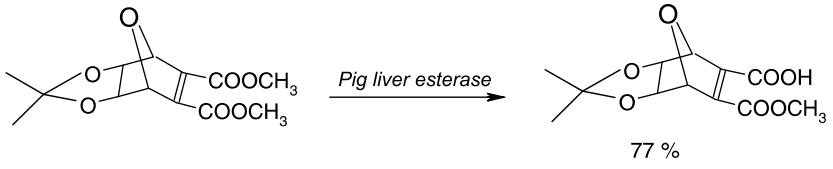


Ethyl (±)-2-fluorohexanoate

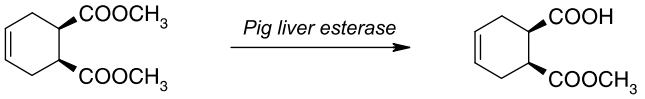
Ethyl (*R*)-2-fluorohexanoate
99% ee

(S)-(-)-2-fluorohexanoic
acid > 69% ee

Enzymes have been successfully used for selective hydrolysis as shown in the examples given below.



77 %

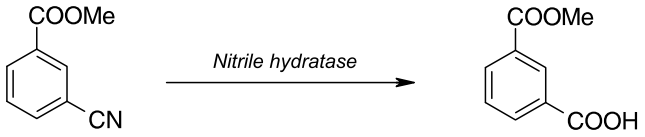


96 %

Enantioselective hydrolysis of the following has been affected by hog pancreatic lipase.

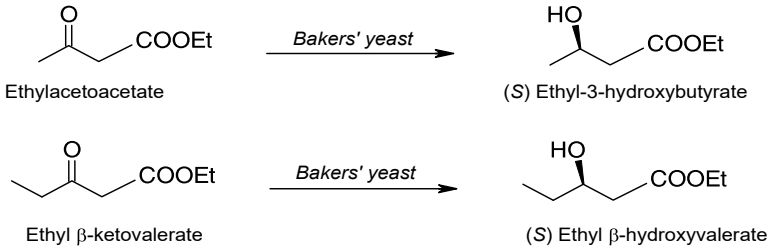
95 % ee

Selective hydrolysis of nitrile group can be carried out with the help of the enzyme *nitrile hydratase* under very mild conditions.

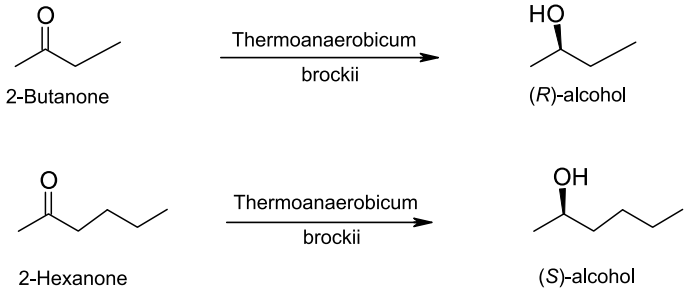


4.8.4 Enzymatic Reductions

The organisms like bakers' yeast and isolated enzymes have been extensively used for the reduction of carbonyl groups to the corresponding alcohols. Bakers' yeast has been used to reduce a wide range of simple ketones, β-ketoesters and cyclic diketones, etc. Yeast alcohol dehydrogenase (YAD) and horse liver alcohol dehydrogenase (HLADH) are commonly employed for reductions. In the reductions, change of enantioselectivity is observed with the change of substrates.

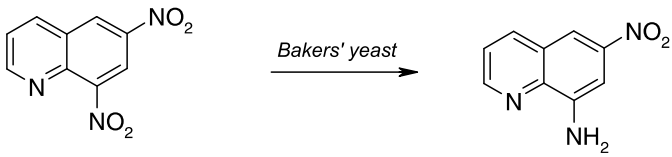


The selectivity of the reactions as given above was inconsistent with the predictions based on Prelog's rule. Enzymatic reduction of 2-butanone gave (*R*)-alcohol but reduction of 2-hexanone gave (*S*)-alcohol as shown below.

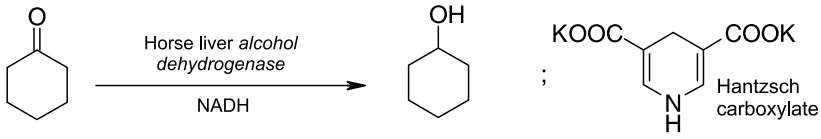


The above examples illustrate that the enantioselectivity of the reductions and its selectivity is dependant on the size and nature of the group around the carbonyl group.

Bakers' yeast has also been used to cause reduction of aromatic nitro groups to the corresponding amines.



The reduction of cyclohexanone to cyclohexanol using horse liver alcohol dehydrogenase is another example of enzymatic reduction. This requires the presence of a stoichiometric amount of the reducing cofactor (NADH) which is provided by simultaneously oxidising a sacrificial substrate like Hantzsch carboxylate that regenerates NADH from NAD^+.

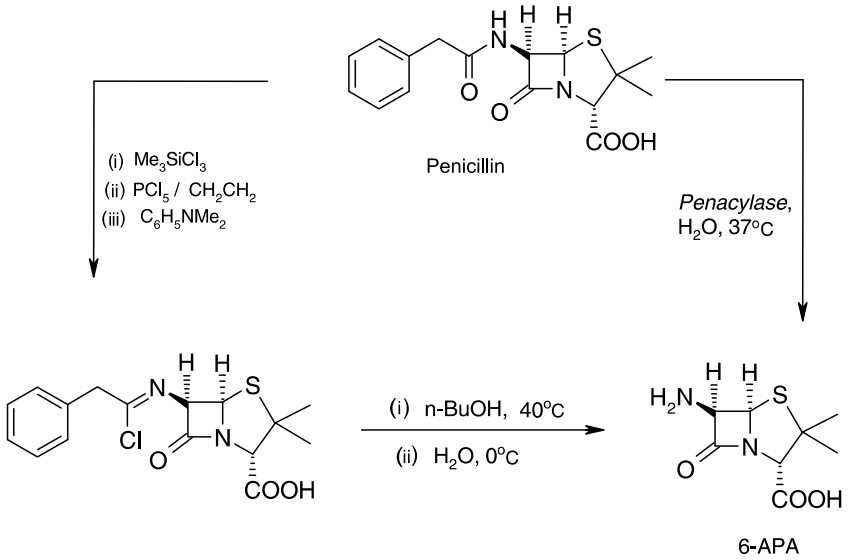


4.8.5 Enzymatic Isomerisations

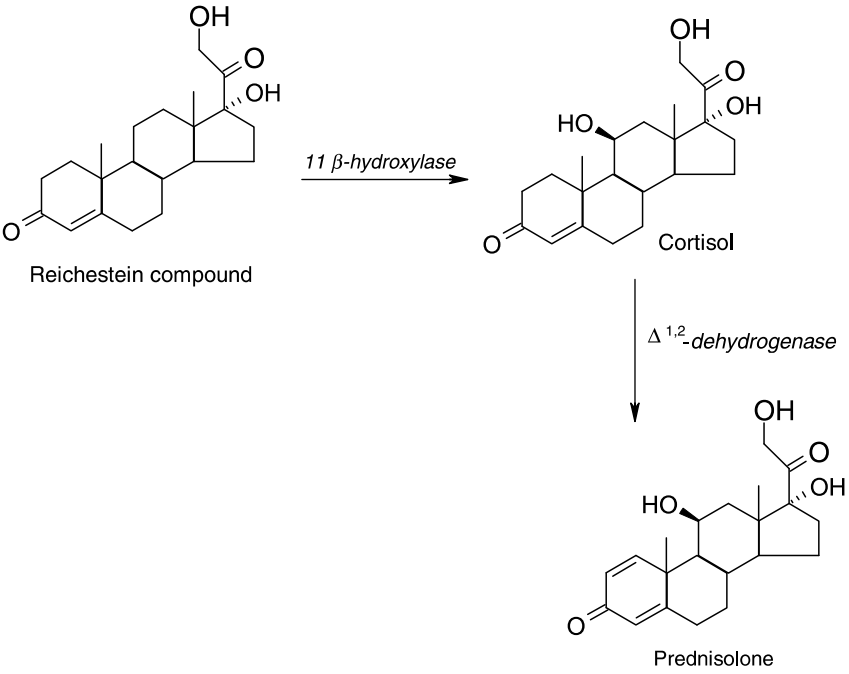
Only few examples of enzymatic isomerisation have been recorded. The most important is the production of high-fructose corn syrup from glucose using glucose isomerase.

4.8.6 Pharmaceutical Applications of Enzymes

One most common example is the enzymatic conversion of penicillin into 6 aminopenicillanic acid (6APA) by the enzyme '*penacylase*'. The hydrolytic product, 6APA, is obtained in one step on a large scale. The product is free of any impurities and is used to prepare semisynthetic penicillins. The chemical conversion, however, requires a number of steps as follows.



Another well-known example of enzymatic conversion is the conversion of Reichsteins compound into cortisol by the enzyme '*11β-hydroxylase*' into cortisol and finally conversion of cortisol into prednisolone with enzyme $\Delta^{1,2}$*-dehydrogenase*'.



The above enzymatic conversions are rapid, regio- and stereoselective.

Exercises

1. What are enzymes? List important characteristics of enzymes.
2. Briefly discuss the factors affecting the enzyme action.
3. Clearly define the following terms.
   (a) apoenzyme
   (b) holoenzyme
   (c) coenzyme
   (d) cofactor
   (e) prosthetic group.
4. What do you understand by the specificity of enzymes? Briefly describe the 'induced fit' model for the enzyme action.
5. What are serine proteases? What kind of reactions do these catalyse?
6. Explain the importance of charge-relay system in chymotrypsin.
7. What are zymogens? How is the zymogen of chymotrypsin activated?
8. Explain the mechanism of action of α-chymotrypsin.
9. What are cofactors? In what ways do the cofactors help the enzymes in performing their functions?
10. Discuss some synthetic applications of enzymes with the help of examples.

Suggested Readings

Organic Chemistry; I. L. Finar Fifth edition, ELBS and Longman group ltd. (1974).
Chemistry and Biochemistry of Amino Acids Ed. G. C. Barrett Chapman and Hall (1985).
Proteins: Structure and Molecular properties; Thomas E Creighton; Second Edition W.H. Freeman and company (1993).
Bioorganic chemistry; Herman Dugas; Springer (1999).