CC: 13 Unit 4. Enzymes (Part 1)

Amit Kumar Rai
Scientist- C (Fermentation Biotechnology)
Institute of Bioresources and Sustainable Development, Sikkim Centre
Tadong, Gangtok, Sikkim, India

Introduction

Biochemical changes in living cells are regulated by catalytic reactions that are regulated by the function of enzymes. In living cells enzymes catalyze hundreds of biochemical reactions that include degradation of macromolecules or nutrient molecules, conversion of simpler precursor to biological macromolecules and transformation of several molecules to a different form. The study of enzymes is known as enzymology and has numerous practical applications in several areas. The term “Enzymes” was given by Frederick W Kuhne to molecules responsible for conversion of sugar to alcohol. In 1926, urease was the first enzyme isolated and characterised by James Sumner. Sumner found that the enzyme urease consist only of protein and postulated that all enzymes are protein. In 1930, John Northrop and Moses Kunitz crystallized digestive enzymes including pepsin and trypsin and found them to be proteinaceous in nature. It was then conformed that enzymes are proteinaceous in nature. Enzymes can be obtained from different sources such as animal (eg: pepsin, trypsin and chymotrypsin), plants (papain and bromelain) and microorganisms (proteases, lipases, amylase, pectinase, chitinase, etc). Enzymes are very highly specific for their substrates and are central to every biochemical process. we will be studying about enzymes under the following subsections:

- **General characteristics of enzymes**
- **Classification of enzymes**
- **Enzymatic Reactions**
- **Enzyme Kinetics**
- **Application of enzymes in food industry**
- **Immobilised Enzyme**

General characteristics of Enzymes

Enzymes are biocatalyst that catalyses the conversion of a specific set of substrate into a specific product. During their participation in a reaction the enzymes are neither consumed

nor altered permanently. Enzymes are very effective biocatalyst, which enhances the rate of reaction by a factor of 10^5 to 10^{17}. Unlike most of the chemical catalysts, enzymes are highly specific both for the single substrate (or group of very closely related substrate) and the type of reaction catalyzed by them. As most of the enzymes are protein, their catalytic ability depends on the integrity of its native protein conformation. Some RNA molecules also possess ability to catalyse biochemical reactions and are called as Ribozymes. If an enzyme is denatured due to external factors or degraded into smaller peptides/amino acids it usually loses its activity. Some enzymes are completely made up of protein and do not require any functional group for catalysing the reactions. However, other enzymes require a complex organic or metalloorganic molecule called coenzyme or chemical components called cofactor – inorganic ions such as Mg^{2+}, Zn^{2+} or Fe^{2+}. The coenzyme or the metal ion that is linked to the enzyme protein is called as prosthetic group, whereas the protein part of such enzyme is called as apoenzyme or apoprotein. The complete enzyme having catalytic ability is called holoenzyme, which contain both apoenzyme and cofactor/coenzyme. Some of the cofactor and coenzymes of specific enzymes is given in Table 1. In the terms of thermodynamics, the function of an enzyme is to reduce the activation energy for a reaction, enabling them to occur much more readily.

Table 1. Important enzymes and their respective coenzymes and cofactors

Enzymes	**Coenzymes**
Pyruvate dehydrogenase	Thiamine pyrophosphate
Pyruvate carboxylase	Biotin
Glycogen phosphorylase	Pyridoxal phosphate
Acetyl CoA carboxylase	Coenzyme A (CoA)
Thymidylate synthase	Tetrahydrofolate
Lactate dehydrogenase	Nicotinamide adenine dinucleotide
Enzymes	**Cofactors**
Cytochrome oxidase	Cu^{2+}
Cabonic anhydrase, alcohol dehydrogenase	Zn^{2+}
Gluathione peroxidise	Se
Superoxide dismutase	Mn^{2+}
Hexokinase	Mg^{2+}
Urease	Ni^{2+}

Classification of enzymes

Enzymes are named by the addition of the suffix "-ase" to the substrate name. Most of the enzymes catalyse the transfer of functional groups, electrons or atoms. Therefore enzymes names are assigned according to their transfer reaction, the donor group or the group acceptor. According to International Union of Biochemists and Molecular Biology (IUBMB), enzymes are currently grouped into six functional classes, each with subclasses depending on the reaction they catalyse. The different functional classes and their biochemical reactions are given in Table 2. Each enzyme is assigned a systemic name and a four type classification number, which identifies them.

Table 2. International Classification of enzymes and their respective examples

Class No.	Class Name	Biochemical reaction	Example
1	Oxidoreductases	Transfer of electrons or H atoms	Lactate dehydrogenase
2	Transferases	Group transfer rections	Nucleoside monophosphate kinase
3	Hydrolases	Transfer of functional group to water (Hydrolysis reaction)	Chymotrypsin
4	Lyases	Formation of double bonds by removal of groups/Addition of groups to double bond	Fumarase
5	Isomerases	Transfer of a group within a molecule to form an isomer	Triose phosphate isomerase
6	Ligases	Formation of C-C, C-O, C-S and C-N bonds by condensation reaction along with cleavage of Adenosine triphosphate (ATP)	Aminoacyl-tRNA synthetase

Enzymatic Reactions

Any chemical reaction does not occur at the same rate and requires some energy for conversion of substrate to a final product. The enzymatic reaction are characterised by the formation of an enzyme substrate complex, which takes place in the pocket of the enzyme known as active site. The active site of an enzyme contains amino acid residues, which form

a temporary bond with the substrate (called as binding site).whereas, specific residues that catalyses the change in the substrate for the production of the specific product is known as catalytic site. The active site of the enzyme is composed of specific amino acid residues, which forms the base for the binding of the substrate resulting in the enzyme catalysed reaction. Most of the enzyme substrate reaction are weak interactions between enzyme and substrate. Enzymatic reactions are mainly hydrogen bonds, hydrophobic bond and ionic bond in nature.

The chemical reaction of conversion of substrate (S) to product (P) from goes through a higher free energy state called the transition state. The transition state has the higher free energy in comparison to product or substrate (Figure 1). The difference between the free energy of the substrate and the transition is known as the activation energy or the Gibbs free energy of activation. The function of an enzyme in a chemical reaction is to lower the activation energy or facilitate the transition state formation (Figure 1)

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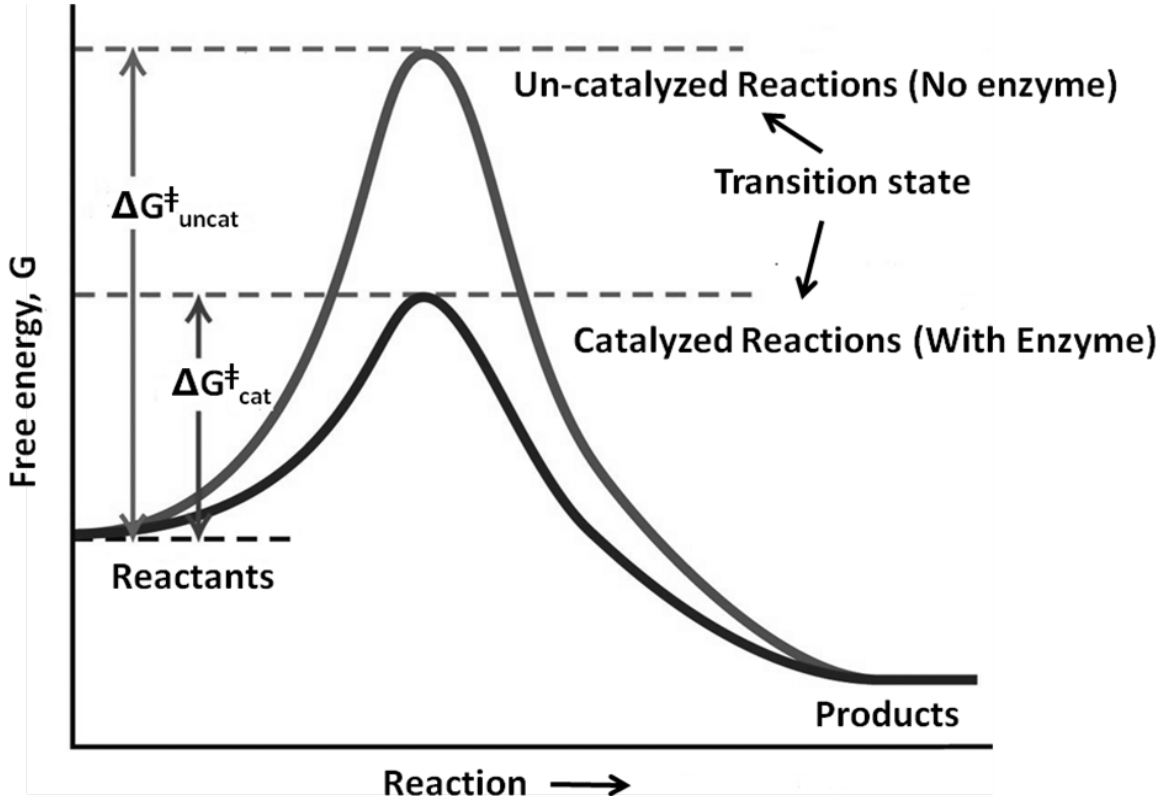


Figure 1. Energy changes during enzymatic catalysed chemical reaction

Factors affecting enzyme activity

There are several factors, which affect the activity of an enzyme, which includes (i) enzyme concentration, (ii) ligand concentration (substrate, product, inhibitor) concentration, (iii) pH (Acidity and Basicity), and (iv) temperature. The activity of an enzyme increases with the increase in temperature until the optimum temperature, beyond which the activity of the enzyme reduces. The temperature or pH at which the activity of the enzyme is the maximum is known as optimum temperature or pH. Apart from these factors, presence of activators, inhibitors and cofactors can also affecting activity of an enzyme. Enzyme inhibitors block enzyme activity by several mechanisms, activators enhances the catalysis whereas cofactors are necessary for normal enzyme activity.

Enzyme substrate reaction

Enzyme substrate reaction is generally explained using two models (i) lock and key model, and (ii) Koshland's induced fit model. In lock and key model of enzyme substrate binding, the active site of the free enzyme is complementary in structure to the substrate (Figure 2A). Thus the substrate easily binds to its specific enzyme and forms an enzyme substrate complex. In case of Koshland's Induced fit model, the formation of the enzyme substrate complex is the result of the specific interaction between the substrate and a flexible active site of the enzyme. The binding of the substrate results in the conformational changes in the enzyme, making it complimentary to the enzyme as shown in Figure 2B.

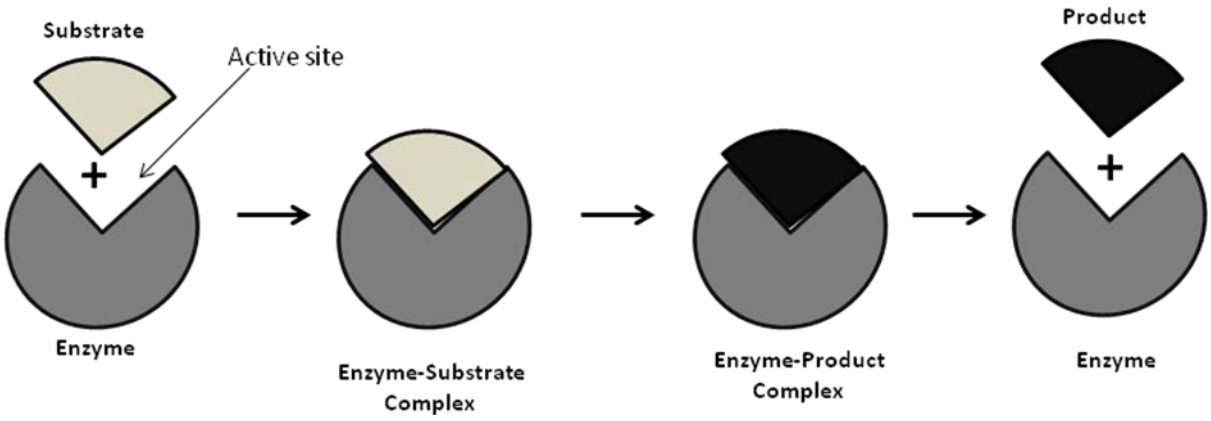


Figure 2A. Lock and key model for enzyme substrate reaction

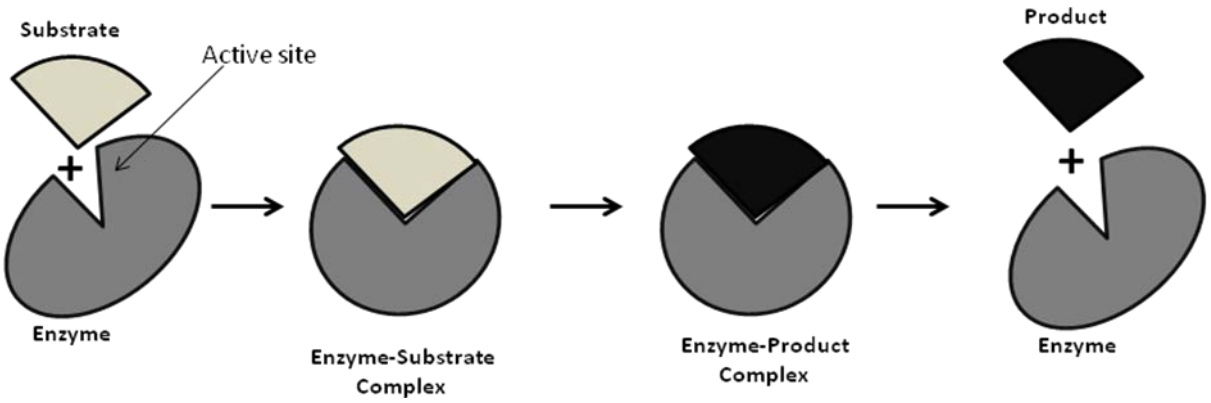


Figure 2B. Koshland's induced fit model for enzyme substrate reaction

Enzyme Kinetics

During an enzyme mediated chemical reaction the substrate is converted to a product. A simple enzyme substrate reaction for the formation of product can be written as shown in equation (1)

$$E + S \underset{}{\overset{\text{Substrate binding}}{\rightleftharpoons}} ES \xrightarrow{\text{Catalytic step}} E + P \quad \text{..... (1)}$$

Where "E" represents the enzyme that catalyzing the chemical reaction, "S" represents the substrate, which undergoes the changes, and "P" the product formed as the result of the reaction. For understanding the function of an enzyme as a biocatalyst, we should understand first that the function of a catalyst is to increase the rate of a reaction. The catalyst does not affect the reaction equilibria.

Michaelis-Mention Equation

Michaelis–Menten Equation is one of the most important equation in enzyme kinetics derived by German biochemist Leonor Michaelis and Canadian physician Maud Menten. The equation was derived based on the basic hypothesis that rate limiting step in an enzyme catalysed reaction is the conversion of the enzyme-substrate complex (ES) in to the product (P) and free enzyme. The Michaelis Menten equation is given below as equation (2)

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \quad \text{..... (2)}$$

In this formula, V_0 represents initial velocity, V_{max} represents maximum velocity, [S] represents concentration of the substrate and K_M represents Michaelis constant. The Michaelis Mention equation assumes a two step enzyme catalysed reaction (i) binding of enzyme and substrate to form an enzyme substrate complex, and (ii) catalysis, where the product is formed and released from the complex. The equation suggests that the initial velocity of the reaction depends on the substrate concentration and K_M. V_{max} reveals the velocity of the enzyme at saturating substrate concentration. For a normal enzyme substrate reaction k_1 signifies the rate constant for the formation of enzyme substrate complex after the reaction between the enzyme and substrate. As the reaction is reversible the dissociation of enzyme and substrate from the enzyme-substrate complex has a rate constant k^{-1}. However, if the reaction proceeds from enzyme-substrate complex to the formation of the product, the rate constant is k_2. The proposed model is given in equation (3)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad \text{..... (3)}$$

K_M is defined as a Michaelis constant, which is derived as the ration of the rate constant as shown in equation (4). K_M values of most of the enzymes vary between 10^{-1} and 10^{-7} and the value depends on specific substrate and factors such as temperature, ionic strength and pH. Also, K_M signifies the concentration of the substrate, which occupies half of the actives sites of the total enzyme concentration.

$$K_m = \frac{k_{-1} + k_2}{k_1} \qquad \text{..... (4)}$$

Units of enzyme activity

Enzyme activity is expressed as unit activity, total activity and specific activity. A unit activity of an enzyme is defined as amount of specific enzyme responsible for conversion of 1 µmol a specific substrate to a final product per minute under a specific set of conditions. The concentration of an enzyme is referred in term of milligrams (mg) or micromoles (µM). Total activity of an enzyme is defined as an activity of enzyme per ml of the extract. Specific activity of an enzyme is defined as unit activity per milligram of protein and represented as µM/mg/min. Specific activity is constant for a purified enzyme at a given set of conditions.

Concentration of an enzyme calculated in mg/ml can be converted to molar units by dividing the value by the molecular weight.

Turnover number

Turnover number (k_{cat}) of a enzyme is defined as the number of substrate molecules converted to a products by one enzyme molecule per second. For example, if turnover number of carbonic anhydrase is $4x10^5$ s^{-1} means that each molecule of carbonic anhydrase produces 400000 molecules of the product per second. The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to 10^7 per second. Turnover number of acetylcholine esterase is 3 x 10^7 per second, which is one of the higher among enzymes. When the concentration of the substrate is much higher than K_M value, the rate of catalysis is equal to turnover number ($k$cat).

Applications of enzymes in Food Processing

Table 4. Application of enzymes in different food industries

Food industry	Enzymes used	Applications
Dairy	Proteases	Production of bioactive peptides, hydrolysis of whey protein
	Lactase	Hydrolysis of lactose for the development of lactose free milk product
	Rennet	Coagulant during production of cheese
	Lipases	flavour enhancement in cheese products
Wine and fruit Juices	Pectinase	Increase in yield and juice clarification
	Glucose oxidase	Removal of oxygen from the bottle drinks
	Acetolactate decarboxylase	Reduction of maturation wine making by the conversion of acetolactate to acetoin
Brewing	Cellulases, β-glucanases, α-amylases, proteases, maltogenic amylases	For liquefaction, clarification and to supplement malt enzymes
Baking	Proteases	Hydrolysis of protein
	α-amylase	Hydrolysis of starch
	Amyloglucosidase	Saccharification
	Xylanase	improve the bread volume, crumb structure and reduce stickiness
	Pentosanase	Breakdown of pentosan, leading to reduced gluten production
	Glucose oxidase	Stability of dough
Inulin	Inulinases	Production of fructose syrups
Meat	Protease	Meat tenderisation, bioactive peptides production

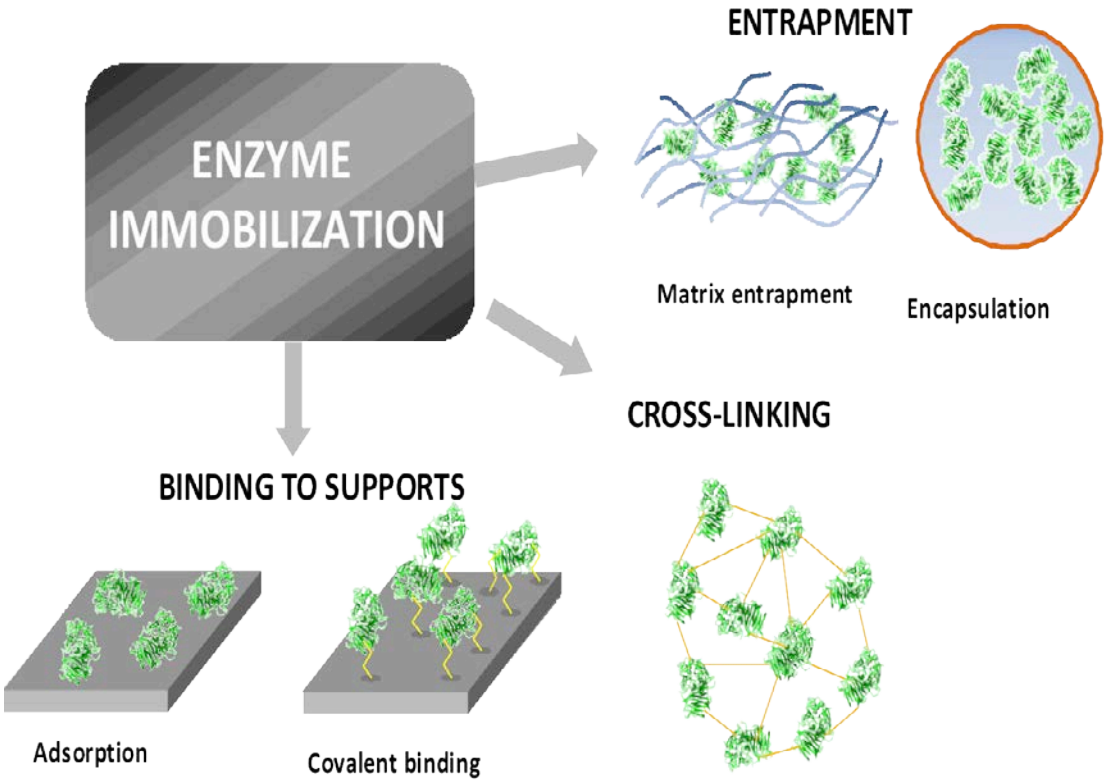
immobilized enzymes

In order to make the enzyme work efficiently and to reuse the enzymes they are immobilized or captured. Immobilised enzyme is defined as cells (alive or dead) or enzyme anchored to a solid

support for use in bioconversion . Such enzyme helps in reducing the wash out rate and increases the turnover rate. This in turn decreases the cost of enzyme and increases the efficiency of the enzyme reactions in food, pharmacology, environmental management industries and in other allied industries. Such immobilization helps in stabilization of enzymes and restricts cross reactions. This would result in the restriction of unwanted metabolite production thereby ensuring the product purity.

Methods used for the immobilization of enzymes fall into four main categories:

1. Physical adsorption onto an inert carrier,
2. Inclusion in the lattices of a polymerized gel,
3. Cross-linking of the protein with a bifunctional reagent, and
4. Covalent binding to a reactive insoluble support.



1)PhysicalAdsorption

Physical adsorption of an enzyme onto a solid is prabably the simplest way of preparing immobilized enzymes. The method relies on non-specific physical interaction between the enzyme protein and the surface of the matrix, brought about by mixing a concentrated solution of enzyme with the solid.

2) Inclusion in the lattices of a polymerized gel

Confining enzymes within the lattices of polymerized gels is another method for immobilization. This allows the free diffusion of low molecular weight substrates and

reaction products. The usual method is to polymerize the hydrophilic matrix in an aqueous solution of the enzyme and break up the polymeric mass to the desired particle size.

3) Cross-Linking

Immobilization of enzymes has been achieved by intermolecular cross-linking of the protein, either to other protein molecules or to functional groups on an insoluble support matrix. Cross-linking an enzyme to itself is both expensive and insufficient, as some of the protein material will inevitably be acting mainly as a support, resulting in relatively low enzymic activity. Generally, cross-linking is best used in conjunction with one of the other methods.

4) Covalent Binding

The most intensely studied of the insolubilization techniques is the formation of covalent bonds between the enzyme and the support matrix. When trying to select the type of reaction by which a given protein should be insolubilized, the choice is limited by the fact that the binding reaction must be performed under conditions that do not cause loss of enzymic activity, and the active site of the enzyme must be unaffected by the reagents used.
A small number of reactions have been designed to couple with functional groups on the protein other than the amino and phenolic residues. As with cross-linking, covalent bonding should provide stable, insolubilized enzyme derivatives that do not leach enzyme into the surrounding solution. The wide variety of binding reactions, and insoluble carriers with functional groups capable of covalent coupling, or being activated to give such groups, makes this a generally applicable method of insolubilization.

Conclusion: Enzymes are biocatalysts which are important for all biological reactions. These reactions may be for the release of energy required for all the cellular activity, like metabolism, repair of cells and its organelle, reproduction and degradation of toxic compounds. In food industries enzymes are fast replacing the inorganic reactions so as to decrease pollution caused by chemicals which otherwise would have been used in food industries.