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Paper 05: Molecular Enzymology and Protein Engineering
Module No. 01: Enzyme Definition and characteristics

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Objective: Objective of this module is to understand the basic of enzyme and its characteristics. We will discuss the enzyme efficiency, specificity and role of the coenzyme in the enzyme functions. In the present module, we will also discuss the structure of the active site as well as its characteristics. This module is divided into following sections-

Introduction:

1. Enzyme Definition:

During the chemical reaction substrate molecule is converted into the product. The rate of conversion of the substrate into the product is slow because of

thermodynamic and kinetic barrier that is present to facilitate the reaction. Substrate is chemical species that is modified in a chemical reaction and produces the product. Transition state is the state observed during the chemical reaction having highest free energy and minimal stability. This is observed during the conversion of the substrate into the product (Figure 1). The specific form of the transition state depends on the mechanisms of the particular reaction. Activation energy is the energy difference between substrate and transition state. Most of the reactions are slow because the substrate does not have sufficient energy to cross the activation energy barrier or transition energy barrier. Catalyst and enzymes facilitate the reaction and speed-up the reaction. Catalyst and enzyme facilitates the substrate towards product conversion. Enzyme differs from the catalyst in many ways such as efficiency, specificity and regulation therefore, enzymes is defined as 'biocatalysts that have efficiency, specificity and mode of regulation'.

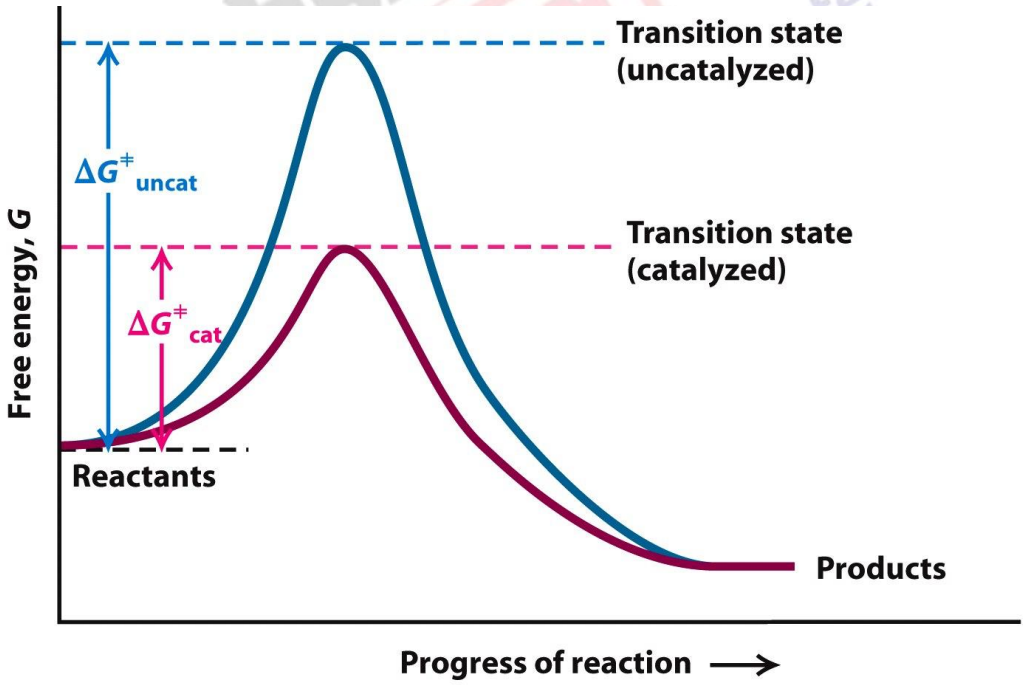

Figure 1: Enzyme catalyzed reaction. The activation energy is higher in the absence of the catalyst or enzyme. Catalyst or enzymes facilitate the enzymatic reaction by altering the activation energy.

2. Characteristics of the enzymes

As the definition of enzyme states that it has following characteristics such as efficiency, specificity and regulation. We will discuss each one by one.

2.1 Enzyme Efficiency

According to the collision theory, the rate of product formation is directly proportional to the effective collision. The maximum collision that takes place in any reacting system is known as diffusion limit or kinetic perfection or catalytic perfection. The diffusion limit is have in the order of 10^9 M^{-1} s^{-1}. Most of the uncatalysed reactions have the efficiency constant in order of 10^2 or 10^3. Kinetically perfect enzymes have efficacy constant (k_{cat}/K_m) in the order of 10^8 M^{-1} s^{-1}. For example catalase enzyme have the efficiency constant of $3.6x10^7$ which is very close to the diffusion limit therefore, we can say that the enzyme increase the efficacy of the catalyzed reaction. The enhancement in the efficacy of the enzymatic reaction is achieved because of the presence of active site in the enzyme. The active site creates the environment in such a way that most of the collisions taking place in the active site are effective. The efficacy constant of the enzyme is better than the metal catalyzed reaction. Therefore, we can say that enzyme is more efficient than the catalyst.

2.2 Enzyme Specificity

Enzymes also have specificity towards the substrate. The Following are the three types of specificity present in the enzymes-

2.2.1. Stereo-specificity-

Enzyme are very much specific towards the stereoisomer of the substrates for example an enzyme which utilizes the D-Carbohydrate can not utilize L-carbohydrate (figure 2) and enzyme which utilizes the L-amino acid can not use the D-amino acid. D-form and L-form differ due to the orientation of the specific group at a penultimate carbon. This small rotation of the group is easily distinguished by the enzyme hence we can say that enzymes are more stereo specific as compared to metal catalyst which cannot even distinguish between meta as well as ortho- and para- position of group at different carbon. It can be seen in the metal catalyzed reaction where one condition product one major and minor product while other condition produces other major and minor product. Stereo-specificity will lead to the absolute specificity of enzyme. Absolute specificity is the property of enzymes that react specifically with only one substrate.

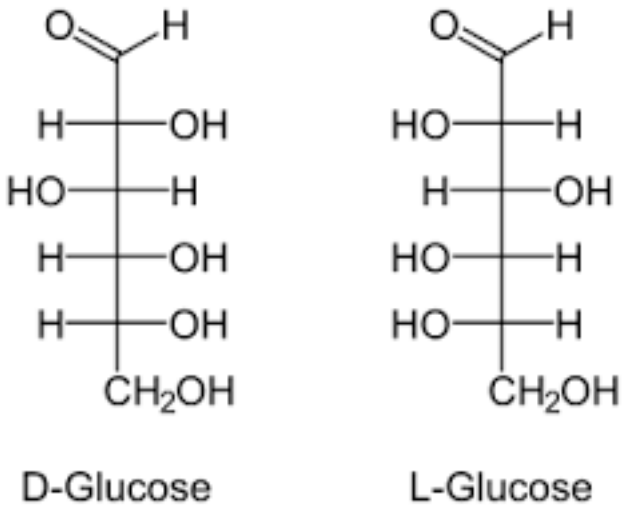

Figure 2: Structure of D-Glucose and L-Glucose

2.2.2. Bond-specificity

Every enzyme requires specific bond for the cleavage that is known as bond specificity of the enzymes. For example Proteases requires amide bond (peptide bond), Glycosidase require ether linkage (glycoside bond), lipases requires ester bond and nuclease requires phosphordiester bond.

2.2.3. Group-specificity

Some enzymes requires specific group in the substrate for their action that is known as group specificity of the enzymes. One such example of the group specificity is the requirement of the hydroxyl group (-OH of serine, threonine, methionine or any other substrate as glucose etc) by the kinases enzyme. This is responsible for the Reaction specificity of the enzyme. Reaction specificity means enzyme will only be involved in the specific reaction.

2.3 Other characteristics of enzymes

In addition to the characteristics explained above, enzymes also have other characteristics, which are explained below

i) Enzyme activity can be controlled i.e. regulated but the catalyst can not regulate the activity.

ii) Most of the enzymes are protein in nature because protein can exist in the tertiary folded structure that is one of the requirements of the enzyme. This is the reason why naturally accuring RNA can also exist as enzyme i.e. Ribozyme, but only designed DNA can exist as enzyme i.e DNAzyme.

iii) Enzyme works at a given pH and temperature known as optimal pH and temperature.

iv) A specific substrate concentration is required by the enzyme to be functional. The concentration of the substrate required will be decided by the Km of enzyme for a given substrate.

v) The inhibitors influence enzyme activity.

vi) Enzyme is either allosteric or non-allosteric.

3. Cofactor and Co-enzyme

The complete enzyme is known as holo-enzyme. The peptide part of the holo-enzyme is known as apo-enzyme while non-peptide part is known as cofactor. Based on the strength of interaction, co-factor is either prosthetic group (if there is strong interaction) or co-enzyme (if there is weak interaction). Most of the metals fall in the category of the prosthetic group while organic molecule such as vitamin as co-enzyme. Different co-enzymes and their functions have been listed in the table 1. Please note that, there is another classification for this, which is less common the based on the non-peptide part (prosthetic group) decides the cofactor (metal) or coenzyme (organic) but it is less accepted.

Table 1: Vitamin and co-enzyme function

S.N.	Vitamin	Co-enzyme	Function
1	Thiamine (Vitamin B1)	TPP	Cleavage and formation of the carbon-carbon single bond adjacent to carbonyl carbon
2	Riboflavin (Vitamin B2)	FMN, FAD	Oxidation and reduction reaction, hydride ion will be taken from the adjacent carbon (-CH2-CH2-)
3.	Niacin (Vitamin B3)	NAD, NADP	Oxidation and reduction reaction, hydride ion will be taken from the same carbon (-HC(OH)-)
4	Pantothenic Acid (Vitamin B5)	CoASH	Acyl group carrier such as acetyl, fatty acyl etc.
5	Pyridoxine (Vitamin B6)	PLP	Carrier of the amino group i.e transamination reaction

6.	Biotin (Vitamin B7)	BCP	Carboxyl group carriers i.e carboxylation, decarboxylation and trans-carboxylation
7.	Folic Acid (Vitamin B9)	THF	One carbon carrier such as methyl group, formyl group, formimidino group
8.	Cynocobalamin (Vitamin B12)	Cyno-cobalamin	Carrier of the hydrogen atom or alkyl group i.e. rearrangement reaction
9	Ascorbate (Vitamin C)	Ascorbate	Carrier of the hydroxyl group i.e hydroxylation

Deficiency of the different vitamins with coenzyme functions leads to different diseases such as thiamine deficiency leads to the beriberi and Wernicke-Korsakoff syndrome; riboflavin deficiency causes ariboflavinosis, glossitis and angular stomatitis; niacin deficiency leads to pellagra; pantothenic acid deficiency causes paresthesia; pyridoxine deficiency causes anemia peripheral neuropathy; biotin deficiency leads to the dermatitis and enteritis; folic acid deficiency leads to megaloblastic anemia; cynocobalamin deficiency causes megaloblastic anemia and ascorbate deficiency produces scurvy. The above-mentioned diseases are due to the defective function of enzymes involving the above-mentioned vitamin as co-enzymes. In the absence or deficiency of the vitamin the normal function of the enzyme is not takes place that leads to the particular diseases such as deficiency of the vitamin-c result into the inhibition of the activity of the hydroxylase enzymes. Hydroxylation of pro to hydroxyl-pro, lys to hydroxyl-lys is required for the stability of the collagen. The deficiency of this vitamin leads to the weak collagen that result into the scurvy. Therefore, the coenzyme function of the vitamins are very important for normal functioning of the different enzymes that are are important for the survival of the human being.

4. Active site characteristics

Active site is a groove like region of the enzyme that is responsible for the binding of the substrate and its chemical reaction. Active site consists of two types of residues, the binding residue and the catalytic residues. Binding residues are mainly involved in the binding of the substrate to the active site while catalytic residues are involved

in the actual catalysis. The following model models that will explain binding of the substrate with the active site are 'lock and key model' as well as 'induced fit model'. Both the models are differing from each other in-term of the substrate interaction and conformational changes that takes places when substrates bind to the active site.

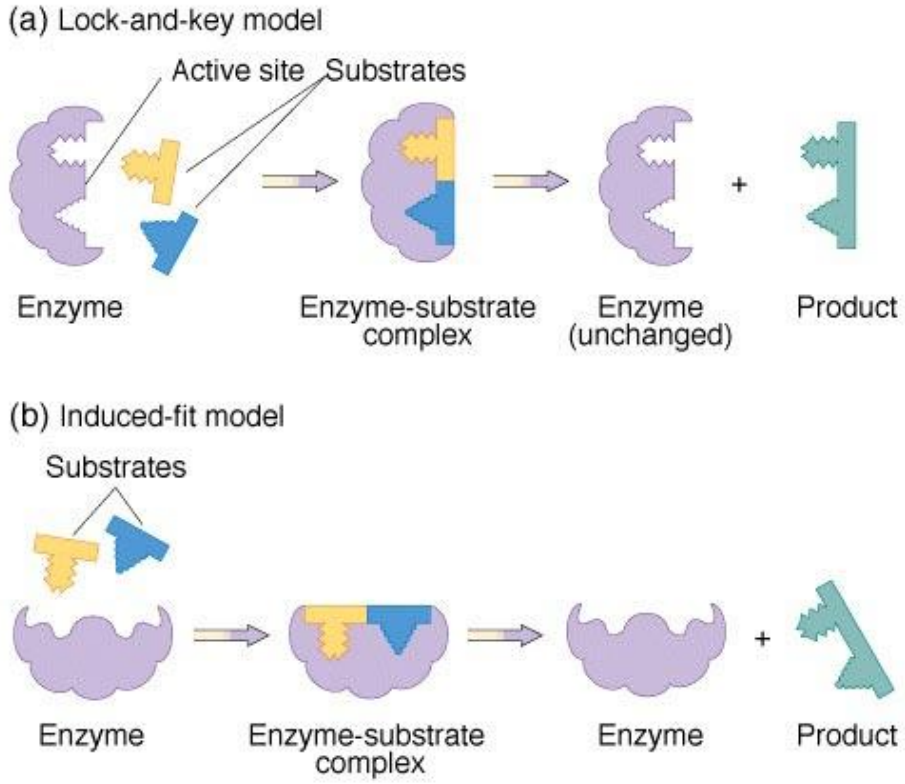

Figure 2. Lock-key model and induced fit model

Binding site residues form non-covalent bond (such as hydrogen bond, ionic bond, van der Waals force, hydrophobic interaction) between side chain of the binding residue and atoms substrate. The strength of total interaction between substrate and active site is responsible for the binding affinity. The binding affinity is responsible for the selection of the particular substrate. Catalytic residues of the active site help in the lowering the activation energy (energy difference between transition state and substrate) of the reaction hence, accelerate the reaction. This is achieved by the different mechanism such as acid-base mechanism, covalent mechanism, intra-molecular mechanism etc that will be discussed in the other modules. Attractive electrostatic forces on the enzyme that entice the substrate to the active site is called as Circe effect. Active site (Binding and Catalytic) residues regain its structure after reaction is over.

4. Summary:

In this module, we have discussed about the different characteristics of the enzyme as well as active sites. We have also discussed about the efficiency and different specificity of the enzymatic reactions. We have discussed the difference different type of the specificity such as stereo-specificity, bond-specificity and group-specificity. We have also seen that the stereo-specificity of the enzyme comes from the arrangement of the different amino acid side chain group in the active site residues. We have also compared at the required places how enzyme is different from the catalyst. We have also discussed about the co-enzyme and co-enzymatic function of the vitamins. We have seen that the vitamins are very important for the catalytic function of the enzymes. In the end, we have explained about the binding and catalytic residues of the active site and their role in the catalysis.