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Paper 05: Molecular Enzymology and Protein Engineering

Module No. 03: Type of Enzymatic Catalysis;
Acid-Base, Nucleophilic-Electrophilic Covalent Catalysis

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Objective: Objective of this module is to understand the different type of the catalysis that will help to understand the enzymatic reactions and kinetics. We will discuss the involvement of the different side chain residues in the different type of catalysis. This module is divided into following sections-

1. Introduction:

The substrate is converted into the product by an enzyme. Substrate binds to the active site and undergoes change that leads to the formation of the transition state and finally 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. Enzyme catalysis increases the rate of reaction with the help of the active site of the enzyme/protein which is made of catalytic residues and binding residues. The enzyme may be part of the multi-subunit complex may or may not be permanently associated with a co-factor. A key driver of the protein evolution is the optimization of such catalytic activities via protein dynamics. The specific form of the transition state depends on the mechanisms of the particular reaction i.e. type of the catalysis. There are different type of catalysis such as acid-base catalysis, covalent catalysis and intra-molecular catalysis. Different type of catalysis follow different path to convert substrate into the product but enzyme remains unaltered after the completion of the reaction. The kinetics of the enzymatic reaction is also dependent on the type of catalysis. Therefore it is important to understand the different catalytic mechanism.

2. Type of Catalysis

2.1 Acid Base catalysis

Acids are molecules that can donate the proton while base are one that can accept proton. The acid and base donate and accept proton in order to stabilize developing charges in the transition state. The ionizable functional groups of aminoacyl side chains and (where present) of prosthetic groups contribute to catalysis by acting as acids or bases known as acid base catalysis. Based on the proton taken or given by substrate, the acid base catalysis is either acid catalysis (hydrogen is taken by the enzyme and given by substrate) or base catalysis (hydrogen is given by the enzyme or taken by substrate).

2.1.1 Specific Acid base catalysis

The acid base catalysis is further divided into specific acid-base catalysis or general acid base catalysis. In specific acid-base catalysis only proton H_3O^+ or OH^- is involved and rate of reaction is sensitive to changes in the concentration of protons but independent to the concentration of other acids (proton donor) or base (proton acceptor) present in solution. Protonation by H_3O^+ lowers the free energy of the

transition state (specific acid) while abstraction of a proton (or nucleophilic attack) by OH- lowers the free energy of the transition state (specific base). Decrease in the pH increase the specific acid catalysis wile increase in the pH enhances the specific base catalysis.

2.1.2 General Acid base catalysis

In general acid base catalysis, rate of reaction are responsive to all the acids or base present in the solution such as side chain of amino acid etc. Partial transfer of a proton from a Brønsted acid to substrate lowers the free energy of the transition state (General acid) while partial abstraction of a proton by a Brønsted base (proton acceptor) lowers the free energy of the transition state (General base). Rate of reaction (General Acid) increases with decrease in pH and increase in Brønsted acid while rate of reaction (General Base) increase with increase in pH and increase in Brønsted base. The protonated forms of His, Asp, Glu, Tyr, Cys, Lys can function as general acids. While unprotonated (ionised) forms of Asp, Glu, His, Tyr, Cys, Lys can function as general base. Histidine is generally involved in the acid-base catalysis because it has pKa close to neutral pH hence can accept and donate protons. Environment also have influences on the catalysis by acid or base such as hydrophobic environment increase pKa of acid and decrease pKa of base, Adjacent residues of like charges increase the pKa of acids and decrease the pKa of base. Similarly salt bridge and Hydrogen bond formation decrease the pKa of acid and increase the pKa of bases.

Chymotrypsin follows acid base catalysis. Histidine at active site (catalytic triad) of enzyme gain (act as general base) and loses protons (general acid) mediated by the pKa of histidine in protein being close to physiological pH. Serine of the catalytic triad form a covalent link with the substrate hence follow the covalent catalysis that is discussed in next section.

2.2 Covalent catalysis

Covalent catalysis involves the formation of the transient covalent bond between substrate and the residues in the enzyme active site or with the co-factor. Formation of covalent intermediate help in reducing the energy of later transition state formed in reaction. The contributor group is regenerated after catalysis. The covalent catalysis not only lowering the activation energy but also provides the alternate pathway via covalent intermediate that is energetically more favorable and therefore faster than

its normal reaction pathways. Chemical modification of the enzyme is, however, transient & enzyme returns to its original unmodified state on completion of the reaction. Covalent catlysis involves ε-amino group lysine (schiff base formation), imidazole of histidine, thiol of cysteine, carbonyl of aspartic acid and hydroxyl of serine. Several families of enzymes have been demonstrated to form covalent intermediates such as Serine proteases (acyl-serine intermediates), Cysteine proteases (acyl-cysteine intermediates), Protein kinases & phosphatase (phospho-amino acid intermediates), and Pyridoxal phosphate-utilizing enzymes (pyridoxal-amino acid Schiff bases). The covalent catalysis is either electrophilic catalysis (involve Lys of enzyme that have positively charged ε-amino group and leads to the formation of Schiff's base between substrate and enzyme) or nucleophilic catalysis (involved serine residues which have negative charge on the hydroxyl group and lead to the formation of acyl-adduct).

2.2.1. Nucleophilic Catalysis

Nucleophilic reactions involve donation of electrons from the enzyme nucleophile to a substrate with partial formation of a covalent bond between the groups in the transition state of the reaction. The reaction rate in nucleophilic catalysis depends both on the strength of the attacking nucleophile and on the susceptibility of the substrate group (electrophile) that is being attacked (i.e., how good a ''leaving group'' the attacked species has).

2.2.2. Electrophilic Catalysis

In electrophilic catalysis covalent intermediates are formed between the cationic electrophile of the enzyme and an electron-rich portion of the substrate molecule (nucleophile). The amino acid side chains do not provide very effective electrophiles, hence, electrophilic catalysis most often require electron-deficient organic cofactors or metal ions. Most common mechanism: Substrate and catalytic group combine to generate an electrophile containing a cationic nitrogen atom (schiff base formation). Nitrogen itself is not a strong electrophile, but it can act as an effective electron sink because of its ease of protonation & it can form cationic unsaturated adducts with ease.

For example covalent bond is contributed by hydroxyl group of serine found in catalytic triad of trypsin and chymotrypsin (serine protease). Similarly, Schiff base

formation takes place-using Lysine of the enzyme Aldolase of glycolysis. Some enzymes also involve cofactors such as pyridoxal phosphate (PLP) or TPP (thiamine pyrophosphate) in the formation of covalent intermediates with substrate. The cofactor based covalent catalysis includes PLP-dependent aspartate transaminase, TPP-dependent pyruvate dehydrogenase.

2.3 Intra-molecular catalysis (Proximity and orientation effect)

When enzyme and substrate bind with each other and act as a single molecule. The rate of reaction of this ES complex is fast because this is probably due to the reduction in the entropy of the reactants and thus makes reaction such as ligation or addition more favorable. Therefore, we can say that enzyme can accelerate a reaction between two species by holding two reactants together in appropriate orientation. This is known as proximity and orientation effect that is important for the intra-molecular catalysis. The concentration and orientation effects associated with substrate binding are referred to as the proximity effect or the propinquity effect. *Orbital steering* is related to proximity effects and states that juxtaposition of reactive groups among the substrates and active site residues is not sufficient for catalysis. In addition to this positioning, the enzyme needs to precisely steer the molecular orbitals of the substrate into a suitable orientation. When the substrates are sequestered within the active site of the enzyme, their effective concentrations are greatly increased with respect to their concentrations in solution. Structure of the enzyme active site is designed to bind the substrates in a specific orientation that is optimal for reaction. Enzyme active site groups have evolved to optimize this steering involved in substrate binding.

2.4 Conformational distortion or strain catalysis

It is reported that transition state has relatively better interaction with enzyme as compared to substrate itself. This induces the structural arrangement which strain substrate bond into a position closer to the conformation of the transition state that decrease the energy difference between transition state and substrate i.e lower the activation energy hence enhances the reaction. Enzymes that catalyze lytic reactions which involve breaking a covalent bond typically bind their substrates in a conformation slightly unfavorable for the bond that will undergo cleavage. The

resulting strain stretches or distorts the targeted bond, weakening it and making it more vulnerable to cleavage. Lysozyme shows this type of catalysis.

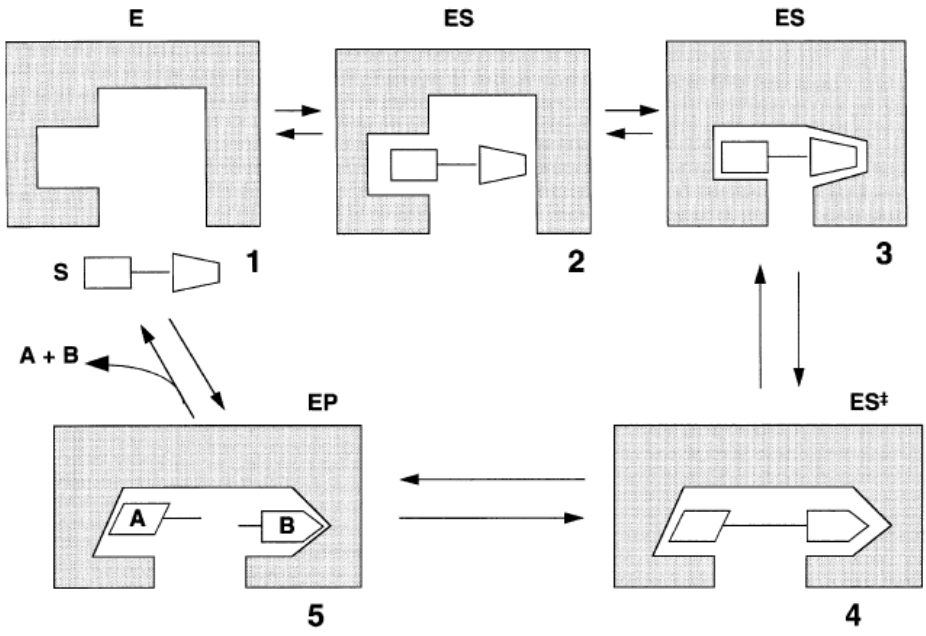

Figure: Model showing conformational distortion or strain catalysis

2.5 Electrostatic catalysis

The charged residue of the enzyme such as Arg, Lys, Asp and Glu are also involved in the stabilization of the charged transition state by forming the ionic interaction. This type of catalysis is known as electrostatic catalysis. Displacement of the water from the active site will enhance the dielectric constant of the active site that further enhances the strength of electrostatic interaction between polar substrate and active site hence enhances the stabilization effect on the transition state. Sometime, metal are also involved in the electrostatic catalysis such as metal ion of carboxypeptide decrease the pKa of water in such a way that makes water as effective nucleophile.

3. Summary

In this module, we have discussed about the different type of the catalysis such as acid-base catalysis, covalent catalysis, intra-molecular catalysis (Proximity and orientation effect), conformational distortion or strain catalysis and electrostatic catalysis. We have discussed the role of different size chain in the active site, metal ion and water molecules etc. Each catalysis mechanism involve different residue of the active site. For example covalent catalysis involves Ser and Lys residues. We

have also discussed about the general and specific acid-base catalysis. Histidine is important residues for the acid base catalysis because has pKa close to the physiological pH i.e pH 7 hence it can accept the proton and donate the proton therefore histidine is important for the acid-base catalysis. They have specific requirement for the catalysis. The enzyme can adopt more than one type of the catalysis mechanism such as Chymotrypsin involves acid-base catalysis and covalent catalysis. We have also discussed about role of the electrostatic and intramolecular catalysis. The study of the type of catalysis will help to understand the process of catalysis in more detail.