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Paper 05: Molecular Enzymology and Protein Engineering

Module No. 02: Mechanism of Enzyme Action, Activation energy, Collision & Transition state theories

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Objective:

Objective of this module is to understand the mechanism of enzyme action. We will discuss role of activation energy in enzyme functions as well as assumption of the different model given for the enzyme catalysis. We will also discuss the limitation of these models. This module is divided into following sections-

1. Introduction
2. Activation energy and its role in the enzyme catalysis
3. Theories of the catalysts
    3.1 Collision theory
    3.2 Transition state theory
4. Summary
5. Question
6. Resources and suggested reading

1. Introduction

Enzymes are biocatalysts that have efficiency, specificity and mode of regulation. 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. 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. Enzymatic reactions can be increased up to 10^{17}- fold. The efficiency of the enzyme will be explained by the collision theory. Specificity of the enzyme will be explained by the assumption of transition state theory. In the present module, we will discuss about the catalytic mechanism of the enzyme and different theories that help in the study of catalytic mechanism of the enzyme.

2. Activation energy and its role in the enzyme catalysis

Activation energy is a term introduced in 1889 by the Swedish scientist Syante Arrhenius to describe the minimum energy that must be available to a chemical system with potential reactants to result in a chemical reaction. Activation energy may also be defined, as the minimum energy required starting a chemical reaction. The activation energy is the energy difference between reactant and transition state. The activation energy of a reaction is usually denoted by Ea and given in units of kilojoules per mole (kJ/mol) or Kilocalories per moles (Kcal/mol) and is defined as follows:

$$K = Ae^{-\frac{E_a}{RT}}$$

Where:

- *K* is the reaction rate constant or coefficient which is equal $K = \frac{V_{max}}{[E_t]}$
- *A* is the product of the collision frequency and the fraction of molecules with enough kinetic energy to cause a reaction to occur the likelihood of molecules with sufficient kinetic energy will collide, causing a reaction to occur or number of collisions (leading to a reaction or not) per second occurring with the proper orientation to react.
- E_a is the activation energy of the reaction
- *T* is the temperature (in Kelvin)
- *R* is the gas constant, 8.314 J mol^{-1} K^{-1}

- $e^{-\frac{E_a}{RT}}$ is the probability that any given collision will result in a reaction

Linear form of the reaction Arrhenius equation is

$$\log K = -\frac{E_a}{2.3R}.\frac{1}{T} + \log A$$

Therefore a plot of log K or $\log\frac{V_{max}}{[E_t]}$ versus 1/T yield Ea for the catalytic step. Ea calculated from the Arrhenius plot will be an apparent or average value.

The integrated form of the Arrhenius equation is

$$\log\frac{K_2}{K_1} = \frac{E_a}{2.3R}.(\frac{T_2 - T_1}{T_2.T_1})$$

Where K_2 and K_1 are the specific reaction rate constants at T_2 and T_1 respectively.

This can be transformed into

$$Ea = \frac{2.3R.T_2.T_1}{(T_2 - T_1)}.\log\frac{K_2}{K_1}$$

2. Theories of the catalysts

There are two theories given to explain the process of enzyme catalysis. First theory is collision theory and second one is transition state theory.

2.1 Collision theory

The collision theory is based on the kinetic theory and assumes a collision between reactants before a reaction can take place. Collision theory basically states for a bimolecular reaction to occur, molecules must Collide with one another to initiate a reaction, collide with a sufficient amount of energy and collide in a specific orientation. Max Trautz and William Lewis propose this theory in 1900s. Collision theory is an aspect of kinetic-molecular theory; it is strongly interconnected with chemical kinetics. The theory is used to explain how different variables affect the rate of reaction. This theory is built on the idea that reactant particles must collide for a reaction to occur, but only a small percentage of the total collisions have appropriate energies and "orientations" to effectively cause the reactants to form into the products. In order for molecules to react, a physical chemist named Svante Arrhenius explained, the colliding molecules must possess enough kinetic energy to overcome the repulsive and bonding forces of the reactants. The minimum amount of energy required for a chemical reaction to occur is known as the activation energy (Eα). The higher the activation energy of a reaction, smaller the amount of energetic collisions present and slower the reaction. Energetic collisions are collisions between molecules with enough kinetic energy to cause the reaction to occur. Not all collisions are energetic collisions because they do not provide the necessary amount of activation energy, so not all collisions lead to reactions and product formation. In contrast, the lower the activation energy of a reaction, the greater the amount of energetic collisions present, and the faster the reaction.

A reaction cannot occur if the particles do not collide with the activation energy of the reaction. If the atoms collide with less energy, the atoms simply bounce away from each other. Only collisions with energy that is equal to or greater than the activation energy will create a reaction. Ultimately, chemical reactions involve the breaking of some bonds (which takes energy) and making new bonds (which releases energy). Activation energy is the key to breaking the initial bonds. When collisions are too gentle, the adequate amount of energy is not brought to the bonds and a reaction fails to occur. The activation energy is crucial in the reaction rate because depending on how much kinetic energy is brought to the collision, the reaction will vary in speed and frequency. Important criterion is that colliding molecules must have sufficient energy to overcome a potential energy barrier (the activation energy) to react.

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 having in the order of 10^9 M^{-1} s^{-1}. 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. Therefore, we can say that enzyme is more efficient than the catalyst.

2.2 Transition state theory

This theory is developed in 1935 by Henry Eyring, Meredith Gwynne Evanus, Michael Polanyi. Transition state theory is also known as ‘activated-complex theory’ ‘absolute-rate theory’ and ‘theory of absolute reaction rates’. The transition state theory suggests that as reactant molecules approach each other closely they are momentarily in a less stable state than either the reactants or the products. For a bimolecular reaction, a transition state is formed when the two molecule’s old bonds are weakened and new bonds begin to form or the old bonds break first to form the transition state and then the new bonds form after. The theory suggests that as reactant molecules approach each other closely they are momentarily in a less stable state than either the reactants or the products. It takes a lot of energy to achieve the transition state, so the state is a high-energy substance. The potential energy of the system increases at this point because the approaching reactant molecules must overcome the mutual repulsive forces between the outer shell electrons of their constituent atoms. Atoms must be separated from each other as bonds are broken

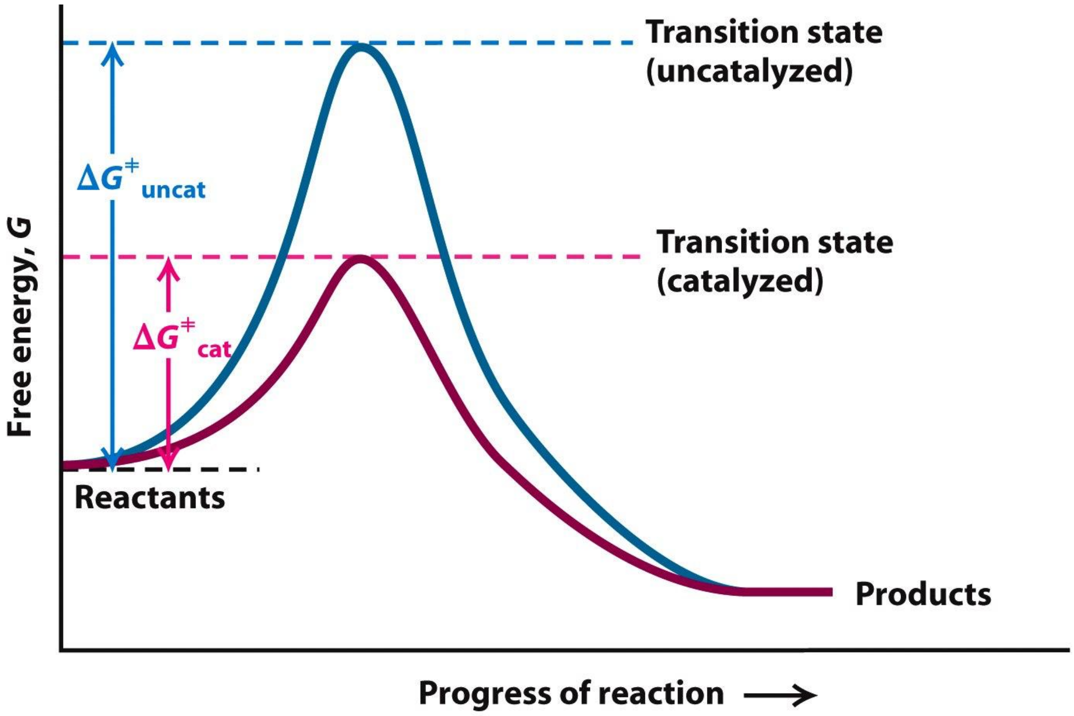

Figure 1: Enzyme catalyzed reaction

This increase in potential energy corresponds to an energy barrier over which the reactant molecules must pass if the reaction is to proceed. The transition state occurs at the maximum of this energy barrier. The transition state is an unstable transitory combination of reactant molecules that occurs at a potential energy maximum. The combination can either go on to form products or fall apart to return to the unchanged reactants. The energy difference between the reactants and the potential energy maximum is referred to as the activation energy. The basic ideas behind transition state theory are as follows:

- Rates of reaction can be studied by examining activated complexes that lie near the saddle point of a potential energy surface. The details of how these complexes are formed are not important. The saddle point itself is called the transition state.

- The activated complexes are in a special equilibrium (quasi-equilibrium) with the reactant molecules.
- The activated complexes can convert into products, and kinetic theory can be used to calculate the rate of this conversion.

Limitations of Transition State Theory

Transition state has following limitations

- When applied to each elementary step of a multi-step reaction, the theory assumes that each intermediate is long-lived enough to reach a Boltzmann distribution of energies before continuing to the next step. When the intermediates are very short-lived, however, then transition state theory fails. In such cases, the momentum of the reaction trajectory from the reactants to the intermediate can carry forward to affect product selectivity
- Transition state theory is also based on the assumption that atomic nuclei behave according to classic mechanics. It is assumed that unless atoms or molecules collide with enough energy to form the transition structure, then the reaction does not occur. However, according to quantum mechanics, for any barrier with a finite amount of energy, there is a possibility that particles can still tunnel across the barrier. With respect to chemical reactions this means that there is a chance that molecules will react even if they do not collide with enough energy to traverse the energy barrier. While this effect is expected to be negligible for reactions with large activation energies, it becomes a more important phenomenon for reactions with relatively low energy barriers, since the tunneling probability increases with decreasing barrier height.
- Transition state theory fails for some reactions at high temperature. The theory assumes the reaction system will pass over the lowest energy saddle point on the potential energy surface. While this description is consistent for reactions occurring at relatively low temperatures, at high temperatures, molecules populate higher energy vibrational modes; their motion becomes more complex and collisions may lead to transition states far away from the lowest energy saddle

point. This deviation from transition state theory is observed even in the simple exchange reaction between diatomic hydrogen and hydrogen radical.

Significance of the transition state theory for enzyme catalysis

Each catalytic event requires a minimum of three or often more steps, all of which occur within the few milliseconds that characterize typical enzymatic reactions. According to transition state theory, the smallest fraction of the catalytic cycle is spent in the most important step i.e transition state. Linus Pauling proposed that the powerful catalytic action of enzymes could be explained by specific tight binding to the transition state species. According to transition state theory, rate of reaction is proportional to the fraction of the reactant in the transition state complex. Wolfenden hypothesized that the rate increase imposed by enzymes is proportional to the affinity of enzyme for the transition state structure relative to the Michaelis complex i.e enzyme-substrate complex. Because enzymes typically increase the non-catalyzed reaction rate by factors of 10^{10}-10^{15}, and Michaelis complexes often have dissociation constants in the range of 10^{-3}-10^{-6} M, it is proposed that transition state complexes are bound with dissociation constants in the range of 10^{-14}-10^{-23} M. As substrate progresses from the Michaelis complex to product, chemistry occurs by enzyme-induced changes in electron distribution in the substrate. Enzymes alter the electronic structure by protonation, proton abstraction, electron transfer, geometric distortion, hydrophobic partitioning, and interaction with Lewis acids and bases. These are accomplished by sequential conformation changes in enzyme and substrate. When a combination of individually weak forces are brought to bear on the substrate, the summation of the individual energies results in large forces capable of relocating bonding electrons to cause bond-breaking and bond-making. Analogs that resemble the transition state structures should therefore provide the most powerful noncovalent inhibitors known, even if only a small fraction of the transition state energy is captured.

The transition states for chemical reactions are proposed to have lifetimes near 10^{-13} seconds, on the order of the time of a single bond vibration. Enzymes function to stabilize transition states lying between reactants and products, and that they would therefore be expected to bind strongly any inhibitor that closely resembles such a transition state. Substrates and products often participate in several enzyme reactions, whereas the transition state tends to be characteristic of one particular enzyme, hence inhibitor analogous to transition state, tends to be specific for that particular enzyme. The identification of numerous transition state inhibitors supports the transition state stabilization hypothesis for enzymatic catalysis. Currently there is a large number of enzymes known to interact with transition state analogs, most of which have been designed with the intention of inhibiting the target enzyme. Examples include HIV-1 protease, racemases, β-lactamases, metalloproteinases, cyclooxygenases and many others.

4. Summary:

In this module, we have discussed about the different characteristics of collision and transition state theory of the enzyme. We have also discussed about the limitation of these methods as well as its significance in the process of enzyme catalysis. We have also discussed about activation energy and its determination using Arrhenius equation. The efficiency and different specificity of the enzymatic reactions is explained using these two models of enzyme catalysis. We have also compared at the required places how enzyme is different from the catalyst.