ENZYMES

Enzymes, often referred to as biocatalysts, are predominantly proteins that facilitate and accelerate biochemical reactions within living cells. Enzymes, identified as proteinaceous entities by Sumner in 1926, were predominantly recognized for their catalytic role in biological processes. However, it's worth noting that two RNA enzymes, exceptions to the protein norm, have been recently discovered.

- **Ribozyme:** In 1981, Cech and colleagues isolated ribozyme from Tetrahymena, showcasing that catalytic properties are not exclusive to proteins but can also be exhibited by certain RNA molecules.
- **Ribonuclease P:** Discovered by Altman in bacteria, this RNA enzyme further expanded the understanding of non-proteinaceous catalysts in living organisms.
- Similar to other proteins, enzymes possess both secondary and tertiary structures. In the tertiary structure, the protein's backbone folds upon itself, creating a crisscross pattern with various crevices or pockets. These pockets, known as "active sites," play a pivotal role in enzyme function.

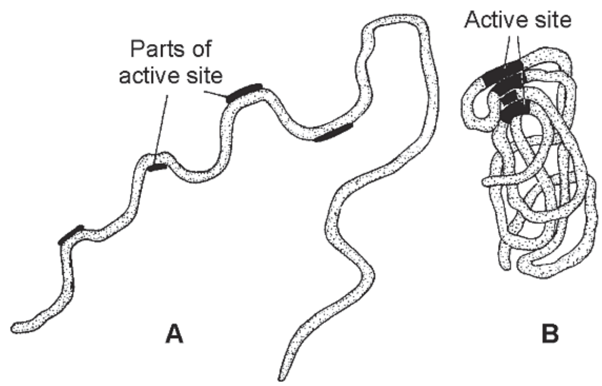


Fig. : Grouping of amino acids of a polypeptide during the formation of tertiary structure to produce an active site

- An active site is a specific crevice or pocket where the enzyme binds with its substrate, a particular compound upon which the enzyme acts. Through these active sites, enzymes catalyze reactions at an accelerated rate.
- Enzymes, serving as organic catalysts, distinguish themselves from inorganic catalysts, which lack a presence in living cells. The specificity of enzymes in recognizing and interacting with particular substrates is a crucial aspect of their catalytic efficiency.
- A fascinating subset of enzymes is derived from thermophilic organisms, which inhabit environments characterized by extremely high temperatures, such as hot vents and sulfur springs. Enzymes isolated from these organisms exhibit thermal stability, retaining their catalytic activity even at elevated temperatures, typically ranging from 80° to 90°C. This thermal stability becomes a defining and advantageous characteristic of enzymes originating from thermophilic organisms..

Chemical Reactions:

- Chemical compounds exhibit two primary types of changes: physical changes and chemical changes. A physical change entails alterations in shape without bond breakage, representing a purely physical process. Another aspect of physical processes includes changes in the state of matter, such as the transition from ice to water or water to vapor.
- In contrast, a chemical reaction involves the breaking and formation of bonds, resulting in a transformation of substances. An example of an inorganic chemical reaction is represented by the equation:

$$Ba(OH)_2 + H_2SO_4 \longrightarrow BaSO_4 + 2H_2O$$

- This reaction features inorganic compounds, specifically barium hydroxide and sulfuric acid, producing barium sulfate and water.
- Conversely, the hydrolysis of starch into glucose exemplifies an organic chemical reaction, as both compounds involved are organic in nature.
- The rate of a physical or chemical process is defined as the amount of product formed per unit time. This rate can be expressed mathematically as:

$$\text{Rate} = \frac{\text{Change in concentration of reactant or product}}{\text{Change in time}}$$

- The rate can also be referred to as velocity if the direction is specified. Temperature is a crucial factor influencing the rates of both physical and chemical processes. Generally, the rate doubles or decreases by half for every 10°C change in either direction.
- Enzymes play a pivotal role in catalyzing chemical reactions. For instance, the reaction involving carbon dioxide and water to form carbonic acid is significantly accelerated in the presence of the enzyme carbonic anhydrase. Without this enzyme, the reaction is notably slower, producing 200 molecules of carbonic acid in an hour. However, in the presence of carbonic anhydrase, the rate dramatically increases, yielding about 600,000 molecules every second. This demonstrates the remarkable catalytic effect of enzymes, enhancing reaction rates by several orders of magnitude.
- Enzymes, with thousands of types identified, each catalyze unique chemical or metabolic reactions. A sequence of enzyme-catalyzed steps in a chemical reaction, known as a metabolic pathway, characterizes processes like glycolysis. Glycolysis, for instance, involves ten distinct enzyme-catalyzed metabolic reactions converting glucose into pyruvic acid.
- The end products in a metabolic pathway can vary under different conditions. In skeletal muscles under anaerobic conditions, pyruvic acid transforms into lactic acid. In aerobic conditions, pyruvic acid is converted into Acetyl CoA, further yielding CO_2 and H_2O. In yeast, during anaerobic conditions, pyruvic acid undergoes fermentation to produce alcohol. These examples highlight the complexity and versatility of metabolic pathways in different biological contexts.

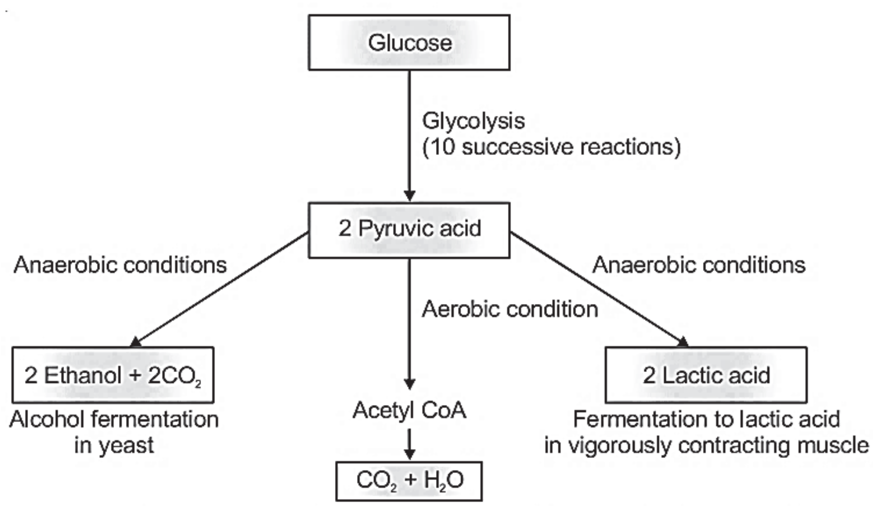


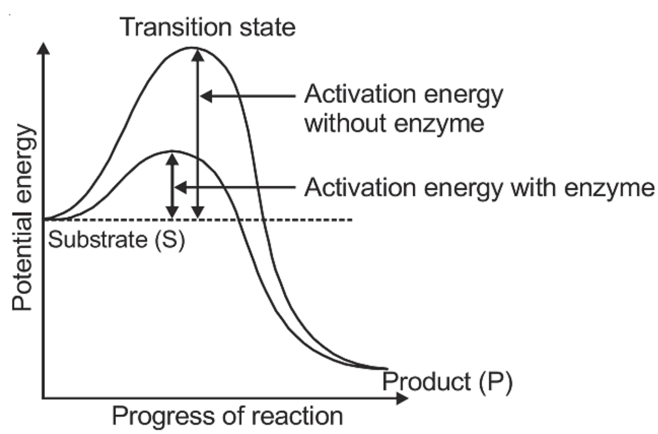
Fig. : Three possible fates of pyruvic acid formed in the glycolysis

How do Enzymes bring about such High Rates of Chemical Conversions?

- Understanding how enzymes achieve high rates of chemical conversions involves delving into their intricate mechanisms. We have previously explored the concept of the active site, a crucial element in enzyme function. Now, let's delve deeper into the process.
- Enzymes, which are proteins possessing three-dimensional structures incorporating an active site, facilitate the conversion of a substrate (S) into a product (P). This transformation can be represented as:

$$\text{S (Substrate)} \longrightarrow \text{P (Product)}$$

- The substrate 'S' binds to the enzyme's active site, necessitating its diffusion toward this specific region. This binding results in the formation of an 'ES' (Enzyme-Substrate) complex, a transient phenomenon lasting only a short duration.
- Upon binding to the enzyme's active site, the substrate undergoes a structural transformation, forming a transition state structure. The molecules of the substrate experience chemical changes, involving the breaking or formation of bonds, ultimately leading to the formation of the product, which is then released from the active site. In essence, the enzyme transforms the structure of the substrate into the product.
- Throughout this process, the transformation of substrate into product must traverse the so-called transition state structure. Numerous other 'altered structural states' exist between the stable substrate and the product, but all these intermediate states are inherently unstable. The stability of a structure is closely tied to its energy status.
- Initiating a reaction demands an external supply of energy, known as activation energy. However, the vast number of reactions occurring in living systems cannot rely on external sources for this activation energy. Enzymes play a pivotal role by reducing the activation energy required for a reaction to commence.



- In a graphical representation where the Y-axis signifies potential energy content and the X-axis illustrates the progression of structural transformation from substrate to product, or the progression of the reaction, the energy level difference between 'S' and 'P' determines the nature of the reaction.
  Exothermic Reactions: If the energy level difference results in 'P' being at a lower level than 'S,' the reaction is exothermic. In exothermic reactions, the energy content of the product is lower than that of the substrate, as heat is released during the process. External energy supply is not required for product formation.
  Endothermic Reactions: Conversely, when 'S' is at a lower level than 'P,' the reaction is endothermic. In endothermic reactions, the energy content of the products is higher, and heat is absorbed during the reaction. External energy input is essential for product formation.

The difference in energy levels between the substrate (S) and the transition state represents the activation energy required to initiate the reaction. Enzymes play a pivotal role in minimizing this activation energy, facilitating the efficiency and speed of biochemical reactions within living organisms.

Nature of Enzyme Action

- The mechanism of enzyme action involves a series of well-defined steps, centering around the concept of an active site where substrates and enzymes interact. To initiate the catalytic process, substrates bind to the active site, establishing a transient and highly reactive enzyme-substrate complex (ES). Subsequently, this complex undergoes a transformation into the enzyme-product complex (EP). The final step involves the dissociation of the enzyme-product complex into products (P), leaving the enzyme unchanged and available to bind with additional substrate molecules.

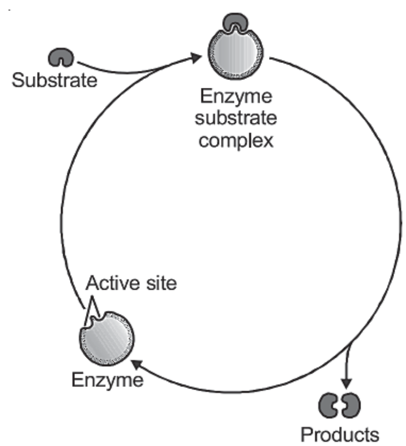


Fig. : The method by which the same enzyme molecule can be used again and again

The formation of the enzyme-substrate complex (ES) is crucial for the catalytic activity of enzymes, serving as a key intermediate step in the overall reaction.

$$E + S \rightleftharpoons ES \longrightarrow EP \longrightarrow E + P$$

The catalytic cycle of enzyme action unfolds in a systematic manner:

- Substrates initially attach to the active site of the enzyme.
- The binding of substrates induces a conformational change in the enzyme, causing it to enclose the substrate more tightly.
- The enzyme's active site, positioned in close proximity to the substrate, catalyzes the breaking of chemical bonds within the substrate, resulting in the formation of the enzyme-product complex.
- The enzyme liberates the reaction products, and the free enzyme, remaining unaltered, is now prepared to engage with another molecule of substrate, perpetuating the enzymatic process.

Factors Affecting Enzyme Activity

- Enzyme activity is highly influenced by various factors due to the intricate tertiary structure of these proteins. Changes in this tertiary structure can significantly impact enzyme action. The factors affecting enzyme activity include
- **Temperature:** Enzymes typically operate within a specific temperature range. The optimal temperature, at which an enzyme exhibits its highest activity, is crucial. Enzyme activity diminishes both below and above this optimum temperature. Lower temperatures temporarily inactivate

enzymes, and upon returning to normal temperatures, they regain their lost activity. However, higher temperatures lead to denaturation, where the increased kinetic energy breaks the weak hydrogen bonds maintaining the enzyme's tertiary structure, resulting in the loss of catalytic activity. Once denatured, enzymes remain inactive even if the temperature is subsequently lowered..

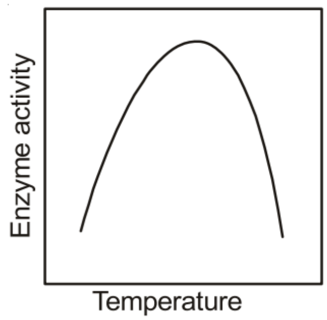


Fig. : Effect of change in temperature on enzyme activity

- **Hydrogen Ion Concentration (pH):** Each enzyme operates optimally within a specific pH range. Deviations from this optimum pH can reduce enzyme activity. Some enzymes function best in acidic environments, while others prefer alkaline conditions. Each enzyme has an optimum pH where its catalytic action is maximal.

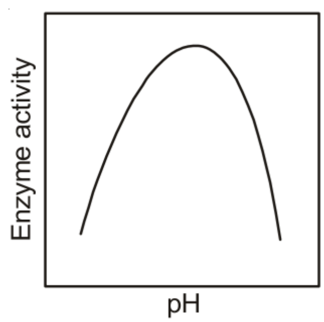


Fig. : Effect of change in pH on enzyme activity

- **Concentration of Substrate:** The velocity of an enzymatic reaction increases with an increase in substrate concentration. However, there is a point of saturation, known as Vmax, where further substrate concentration does not enhance the reaction rate. At this stage, all enzyme molecules become fully saturated, and no active sites remain available to bind additional substrate molecules. The Michaelis constant (Km) is a mathematical expression representing the substrate concentration at which the enzyme-catalyzed reaction achieves half of its maximum velocity.

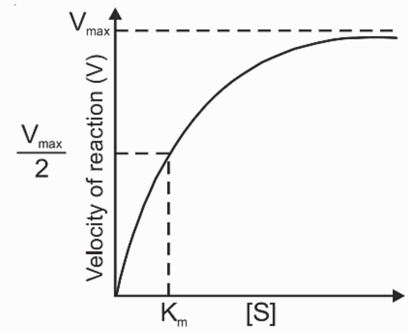


Fig. : Effect of change in : Concentration of substrate on enzyme activity

Allosteric enzymes, in contrast, deviate from the typical Michaelis-Menten constant behavior. Their regulation involves the binding of molecules at sites other than the active site, influencing their activity in a distinctive manner.

Classification and Nomenclature of Enzymes Co-factors

The Enzyme Commission (EC), established by the International Union of Biochemistry (IUB) in 1961, devised a comprehensive system for the classification and nomenclature of enzymes. The IUB system categorizes enzymes into six major classes, with further subdivisions into subclasses and sub-subclasses. Each enzyme is assigned a unique four-digit EC number (e.g., 5.2.1.7), where each digit conveys specific information:

- The first digit signifies the enzyme class.
- The second digit designates the enzyme subclass.
- The third digit represents the sub-subclass.
- The fourth digit identifies the individual enzyme.

The six major classes of enzymes are as follows:

- **Oxidoreductases / Dehydrogenases:** These enzymes facilitate oxidation-reduction reactions involving the transfer of electrons or H+ from one molecule to another. Such reactions result in the oxidation of one compound and the reduction of another. Examples of enzymes in this class include dehydrogenases, oxidases, reductases, catalase, and peroxidase.
  Example Reaction:

$$S_{reduced} + S'_{oxidised} \longrightarrow S_{oxidised} + S'_{reduced}$$

$$\text{Alcohol} + \text{NAD} \xrightarrow{\text{alcohol dehydrogenase}} \text{Aldehyde} + \text{NADH2}$$

- **Transferases:** Transferases catalyze the transfer of specific groups, excluding hydrogen, from one substrate to another. Examples include transaminases (transfers amino groups) and kinases (catalyze phosphorylation by transferring phosphate groups, typically from ATP).
  Example Reaction:

$$S - G + S' \rightarrow S + S' - G$$

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{glucohexokinase}} \text{Glucose 6 phosphate} + \text{ADP}$$

- **Hydrolases:** Hydrolases facilitate the breakdown of larger molecules into smaller ones through the addition of water. They catalyze the hydrolysis of ester, ether, peptide, glycosidic, C-C, C-halide, or P-N bonds. Examples include proteases, amylases, lipases, maltase, nucleases, and digestive enzymes.

Example Reaction:

$$\text{Sucrose} + H_2O \xrightarrow{\text{sucrase/invertase}} \text{Glucose} + \text{Fructose}$$

- **Lyases:** Lyases catalyze the cleavage of substrates into two parts without using water or removing groups without hydrolysis. This process often leads to the formation of a double bond at the site of group removal. Examples include aldolase, decarboxylase, and carbonic anhydrase.
  Example Reaction:

$$\text{Fructose 1, 6, diphosphate} \xrightarrow{\text{aldolase}} \text{Dihydroxy acetone phosphate} + \text{Glyceraldehyde phosphate}$$

- **Isomerases:** Isomerases catalyze the rearrangement of molecular structures to form isomers. Isomers are molecules with the same molecular formula but different arrangements of atoms or groups. Isomerases facilitate the interconversion of optical, geometric, or positional isomers.
  Example Reaction:

$$\text{Glucose} - 6 - \text{phosphate} \overset{\text{isomerase}}{\rightleftharpoons} \text{Fructose } 6 - \text{phosphate}$$

- **Ligases:** Ligases catalyze the covalent bonding of two substrates to form a larger molecule. They facilitate the joining of C-O, C-S, C-N, P-O bonds, among others, using the energy derived from ATP.

Properties of Enzymes:

- **Protein Nature:** Enzymes predominantly consist of proteins, and while they may incorporate additional inorganic or organic substances to facilitate their activity, their fundamental nature is proteinaceous.
- **Chemical Reaction:** Enzymes do not initiate chemical reactions; instead, they accelerate the rate of a chemical reaction. Importantly, enzymes do not alter the equilibrium of the reaction; rather, they expedite the attainment of equilibrium.
- **Efficiency:** The efficiency of an enzyme is quantified by its turnover number, which refers to the number of substrate molecules that a single enzyme molecule can convert in one minute. A higher turnover number indicates a more efficient enzyme, capable of catalyzing a greater number of substrate transformations in a given time.
- **Unchanged Form:** Enzymes undergo no transformation or depletion during a chemical reaction; they emerge from the reaction unaltered. Despite their active involvement in catalysis, enzymes remain unchanged and available for subsequent reactions.
- **Enzyme Specificity:** Enzymes exhibit remarkable specificity in their catalytic actions. Each enzyme is highly selective in targeting a particular substrate or group of substrates. For instance, the enzyme maltase specifically acts on the sugar maltose, distinguishing it from other sugars like lactose or sucrose. This specificity is a defining characteristic of enzyme activity, ensuring precise and selective catalysis in biological systems.

Co-factors:

- Enzymes, typically composed of one or more polypeptide chains, often require the association with specific non-protein substances to exhibit catalytic activity. These essential non-protein components are known as co-factors, and in enzymes with co-factors, the protein portion is referred to as the apoenzyme.

Three distinct types of co-factors have been identified:

- **Prosthetic group:** Prosthetic groups are organic compounds tightly bound to the apoenzyme. An example is the prosthetic group Haem in enzymes like peroxidase and catalase, which catalyze the breakdown of hydrogen peroxide into water and oxygen. Haem, serving as a prosthetic group, becomes an integral part of the enzyme's active site.

- **Co-enzymes:** Co-enzymes, also organic compounds, transiently associate with the apoenzyme for a short duration, typically during catalysis. Many coenzymes, crucial for enzymatic activity, are composed of vitamins. For instance, coenzyme nicotinamide adenine dinucleotide (NAD) and NADP contain the vitamin niacin.
- **Metal ions:** Several enzymes rely on metal ions for their activity, forming coordination bonds with side chains at the active site and simultaneously forming coordination bonds with the substrate. An example is zinc acting as a co-factor for the proteolytic enzyme carboxypeptidase.

The removal of a co-factor from an enzyme results in the loss of its catalytic activity, underscoring the crucial role these co-factors play in enzyme function.

- **Functions of Nucleotides:** Nucleotides, the building blocks of nucleic acids, undergo polymerization to form these essential biological macromolecules. Higher purine and pyrimidine nucleotides, particularly adenosine triphosphate (ATP), store energy in their high-energy phosphate bonds. Generated during photosynthesis and respiration, the hydrolysis of ATP's phosphate bonds releases energy for driving various energy-dependent reactions and processes.
- Certain nucleotides, such as those containing nicotinamide and riboflavin, serve as coenzymes for oxidizing enzymes, participating in redox reactions that play critical roles in cellular metabolism. In this way, nucleotides contribute to both the structural integrity of nucleic acids and the energetic and catalytic functions of coenzymes in biochemical processes.