Enzymes

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Enzymes are biological molecules, typically proteins, that act as catalysts to speed up chemical reactions within living organisms. They facilitate these reactions by lowering the activation energy required for the reaction to occur, thus increasing the rate at which the reactions proceed without being consumed in the process. Enzymes are crucial for various physiological processes such as digestion, metabolism, and cellular signalling. They are highly specific, each enzyme typically catalysing a specific chemical reaction or a set of closely related reactions.

Functions of Enzymes

Enzymes perform a wide range of functions in living organisms. Some of the key functions of enzymes include:

- **Catalysis**: Enzymes catalyze biochemical reactions by lowering the activation energy required for the reaction to occur. They accelerate reactions without being consumed in the process.
- **Metabolism:** Enzymes play essential roles in metabolic pathways, including the breakdown of nutrients such as carbohydrates, proteins, and fats to produce energy, as well as the synthesis of complex molecules necessary for growth, repair, and reproduction.
- **Digestion:** Digestive enzymes break down large food molecules into smaller, absorbable components that can be utilized by the body. For example, amylase breaks down carbohydrates, proteases break down proteins, and lipases break down fats.

- **Cellular signaling**: Enzymes are involved in cellular signaling pathways, regulating processes such as cell growth, differentiation, and apoptosis (programmed cell death).
- Kinases, for instance, phosphorylate proteins to activate or deactivate them, thereby transmitting signals within cells.
- **DNA replication and repair**: Enzymes are involved in DNA replication, ensuring accurate copying of genetic information during cell division. Enzymes also participate in DNA repair mechanisms, correcting errors and damage to the DNA molecule.
- **Detoxification:** Enzymes such as cytochrome P450 enzymes are involved in detoxifying harmful substances by metabolizing drugs, pollutants, and other foreign compounds in the body. Defense: Some enzymes have antimicrobial properties and contribute to the immune response by breaking down the cell walls of pathogens or producing toxic compounds that inhibit their growth.
- **Homeostasis**: Enzymes help maintain internal balance (homeostasis) by regulating biochemical reactions and metabolic pathways in response to changes in the internal or external environment

Classification of Enzymes

Enzymes are mainly classified based on the reactions that they catalyse. These are:

- **Oxidoreductases:** Catalyze oxidation-reduction reactions by transferring electrons from one molecule (the reductant) to another (the oxidant). Examples include dehydrogenases and oxidases.
- **Transferases:** Transfer functional groups (such as amino, methyl, or phosphate groups) from one molecule to another. Examples include kinases and transaminases.
- **Hydrolases:** Catalyze hydrolysis reactions, breaking bonds by adding water molecules. Examples include proteases, lipases, and nucleases. Lyases: Catalyze the addition or

removal of groups to form double bonds or break double bonds without hydrolysis. Examples include decarboxylases and synthases.

- **Isomerases:** Catalyze the rearrangement of atoms within a molecule to form isomers. Examples include isomerases and mutases.
- **Ligases**: Catalyze the joining of two molecules, often coupled with the hydrolysis of ATP. Examples include DNA ligase and RNA ligase.
- **Translocases**: – Catalysing the translocation of hydrogen ions, inorganic cations and anions, amino acids, carbohydrates, or other compounds

Chemical Nature of Enzymes

Most enzymes consist of proteins, with a small number being catalytic RNA molecules known as ribozymes. Enzymes are primarily composed of chains of amino acids connected by peptide bonds, forming high molecular weight compounds. They can undergo denaturation and precipitation when exposed to salts, solvents, or other agents. Their molecular weights typically range from 10,000 to 2,000,000 Da.

Numerous enzymes necessitate the presence of additional compounds known as cofactors for their catalytic function to be activated. This complete active entity is termed the holoenzyme, comprising the apoenzyme (the protein component) along with the cofactor(s) (which can be a coenzyme, prosthetic group, or metal-ion activator).

Cofactor

- Bound to some enzymes is an additional chemical non-protein component called a cofactor, which is a direct participant in the catalytic event and thus is required for enzymatic activity. A cofactor may be either a coenzyme—an organic molecule (which is dialyzable, thermostable, and loosely attached to the protein part), such as a vitamin—or an inorganic metal ion; some enzymes require both.

- A cofactor may be either tightly or loosely bound to the enzyme. If tightly connected, the cofactor is referred to as a prosthetic group (- an organic substance which is dialyzable and thermostable which is firmly attached to
- The protein or apoenzyme portion. This entire active complex is referred to as the holoenzyme; i.e., apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group, or metal-ion-activator) is called the holoenzyme.

    - Fe^{2+} or Fe^{3+} – Catalase, peroxidase, cytochrome oxidase.
    - K^{+} – Pyruvate kinase
    - Zn^{2+} – Carbonic anhydrase

Apoenzyme: Apoenzyme or apoprotein is an enzymatically inactive protein part of an enzyme, which requires a cofactor for its activity.

Holoenzyme: Holoenzyme is a complete, functional enzyme, which is catalytically active. Holoenzyme consists of an apoenzyme together with its cofactors. Holoenzyme contains all the subunits required for the functioning of an enzyme, e.g. DNA polymerase III, RNA polymerase.

Cofactors can be of three types:

(i) Coenzyme: This refers to a non-protein organic compound that is dialyzable, thermostable, and loosely bound to the protein component.

(ii) Prosthetic group: Describes an organic compound that is dialyzable, thermostable, and firmly attached to the protein or apoenzyme segment

(iii). Metal-ion activator: This category encompasses various metal ions such as K^{+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, and Mo^{3+}.

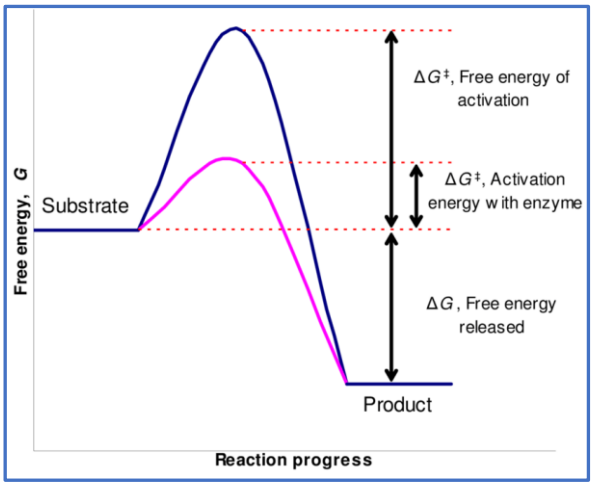
$$Apoenzyme + Cofactor = Holoenzyme$$

Zymogen, also called Proenzyme, any of a group of proteins that display no catalytic activity but are transformed within an organism into enzymes, especially those that catalyse reactions involving the breakdown of proteins.

Enzyme Catalysis

The enzymes increase the rate of a biochemical reaction by providing the reaction, a pathway with a lower activation energy than the uncatalyzed reaction.

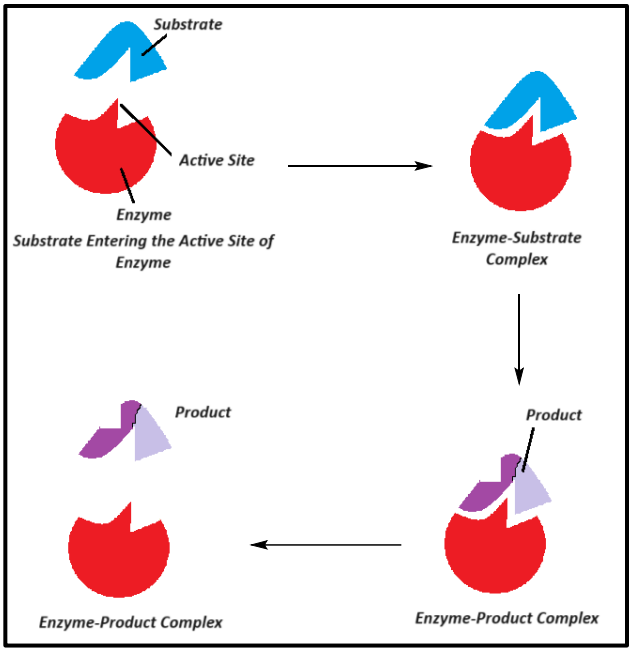
Active Site of Enzyme



The **active site** is the region of an enzyme where substrate molecules bind and undergo a chemical reaction. The active site consists of amino acid residues that form temporary bonds with the substrate, the binding site, and residues that catalyse a reaction of that substrate, the **catalytic site**. Although the active site occupies only ~10–20% of the volume of an enzyme, it is the most important part as it directly catalyses the chemical reaction.

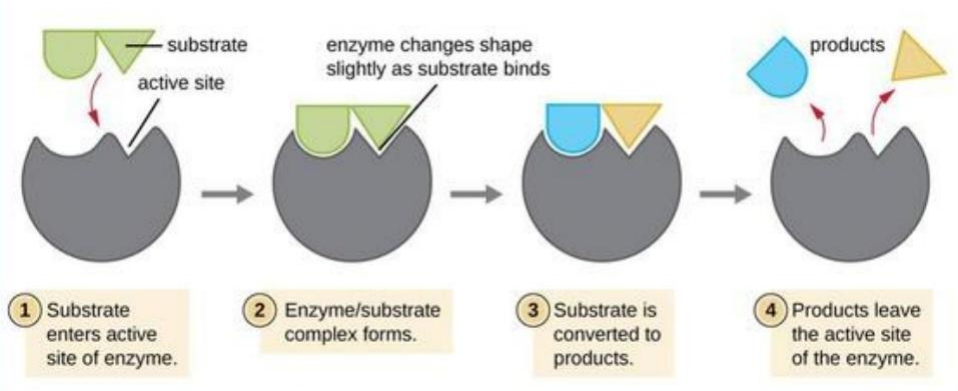
Mechanism of enzyme action

Fischers's Lock and Key Theory



- According to this model, shape of active site of enzyme is complementary to the shape of substrate molecules, i.e., the substrate is like a key whose shape is complementary to the enzyme which is supposed to be lock and they fit perfectly.
- Enzymes catalyse only those substrates which fit perfectly on the active site of that enzyme.
- Most enzymes are far larger than the substrates molecules that act on and the active site is usually a very small portion of the enzyme, between 3 and 12 amino acids. The remaining amino acids which make the bulk of the enzyme, function to maintain the correct globular shape of the enzyme.
- Once the product is formed, they no longer fit into the active site and escape into surrounding medium. According to lock and key model, enzymes behave as rigid molecules. However, most enzymes are globular and are flexible with varying shape.

Koshland's Induced Fit Model



In 1959, Koshland suggested a modification to the 'Lock and Key' hypothesis which is known as 'Induced fit' hypothesis. Working from evidence that suggested that some enzymes and their active site are more flexible. To this, he proposed that the active site can modify its shape as the substrate interact with the enzyme. The amino acids which make up the active site are moulded into precise shape which enable the enzyme to perform its catalytic function most efficiently. For instance, a suitable analogy to describe Induced fit model would be that of a hand changing the shape of the glove as the individual put on the glove. Therefore, in this case, glove is the active site of enzyme and the hand is substrate. However, in some cases, the substrate molecules changes slightly as it enters the active site before binding.

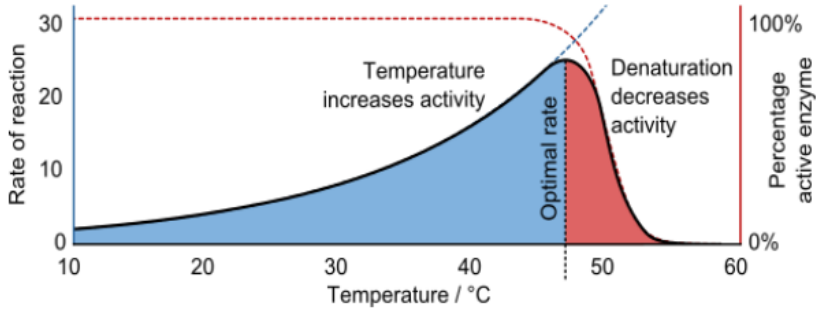
Factors Affecting the Activity of Enzymes

(1) Effect of Temperature

As the temperature increases the rate of chemical reaction will increase, but just up to a certain point, then it will sharply drop. The optimal temperature it occurs when the rate of the reaction is at its highest, each enzyme has a certain temperature at which it is more active, ranges between **37** to **40 ºC**. The protein nature of the enzymes makes them extremely sensitive; so, after the

optimal temperature the rate starts to drop that is because the protein starts to denature so it loses its shape and do not function well.

The enzyme activity gradually lowers as the temperature rises more than the optimal temperature until it reaches a certain temperature at which the enzyme activity stops completely due to the change of its natural composition. On the other hand, if the temperature lowers below the optimal temperature, the enzyme activity lowers until the enzyme reaches a minimum temperature at which the enzyme activity is the least. The enzyme activity stops completely at 0 ºC, but if the temperature rises again, and then the enzyme re-activate once more.



(2) Effect of pH

Enzymes are protein substances that contain acidic carboxylic groups (COOH–) and basic amino groups (NH2). So, the enzymes are affected by changing the pH value. Each enzyme has a pH value that it works at with maximum efficiency called the optimal pH. If the pH is lower or higher than the optimal pH, the enzyme activity decreases until it stops working. For example, pepsin works at a low pH, i.e., it is highly acidic, while amylase works at a high pH, i.e., it is basic. Most enzymes work at neutral pH 7.4.

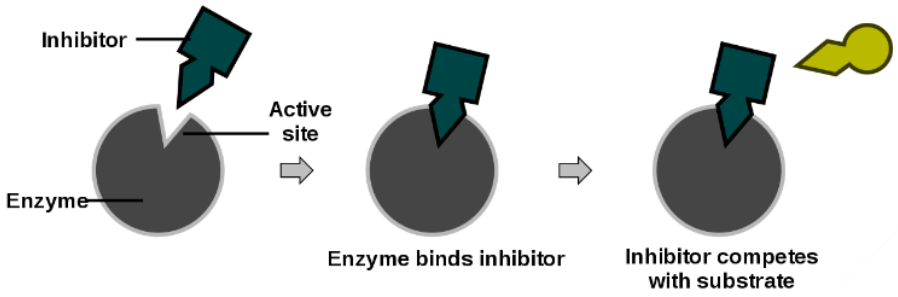
Inhibition of Enzyme Action

In addition to concentration, pH, and, temperature; the presence of **inhibitors** will also affect enzyme activity. Inhibitors are compounds that cause enzymes to lose activity, either by slowing or stopping the chemical reaction. Some inhibitors cause temporary loss of activity, while others cause permanent loss of activity.

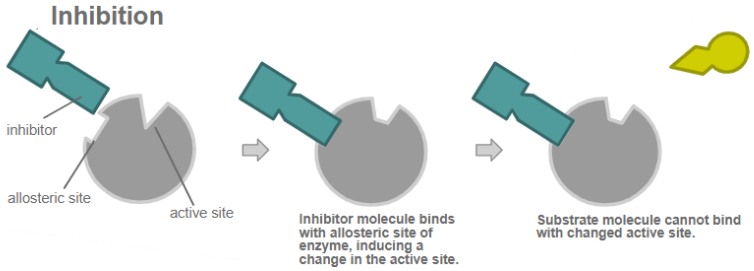
Reversible Inhibitors

A **reversible inhibitor** is one that will cause a temporary loss of enzymatic activity. This substance forms a non-covalent interaction with the enzyme. Reversible inhibitors can be competitive of noncompetitive.

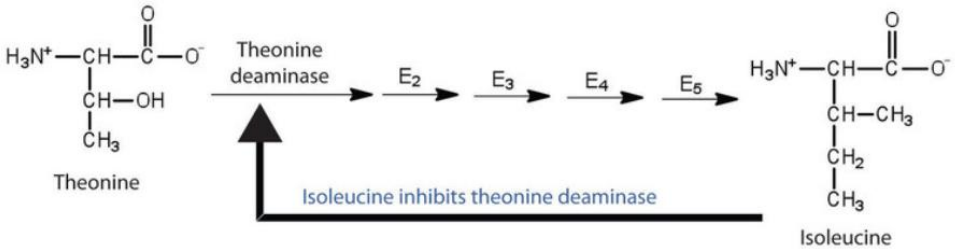
A **competitive inhibitor** is any compound that bears a structural resemblance to a particular substrate and thus competes with that substrate for binding at the active site of an enzyme. The inhibitor is not acted on by the enzyme but does prevent the substrate from approaching the active site. The degree to which a competitive inhibitor interferes with an enzyme's activity depends on the relative concentrations of the substrate and the inhibitor. If the inhibitor is present in relatively large quantities, it will initially block most of the active sites. But because the binding is reversible, some substrate molecules will eventually bind to the active site and be converted to product. Increasing the substrate concentration promotes displacement of the inhibitor from the active site. Competitive inhibition can be completely reversed by adding substrate so that it reaches a much higher concentration than that of the inhibitor.



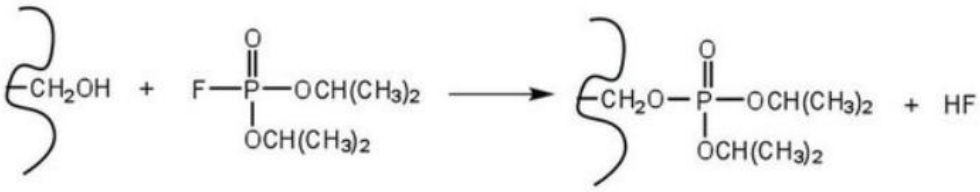
A **noncompetitive inhibitor** attaches at an allosteric site, which is any site on the enzyme that is not the active site. The attachment of the non-competitive inhibitor to the allosteric site results in a shift in three-dimensional structure that alters the shape of the active site so that the substrate will no longer fit in the active site properly. A noncompetitive inhibitor can combine with either the free enzyme or the enzyme-substrate complex because its binding site on the enzyme is distinct from the active site. Because the inhibitor does not structurally resemble the substrate, the addition of excess substrate does not reverse the inhibitory effect.



Feedback inhibition is a normal biochemical process that makes use of noncompetitive inhibitors to control some enzymatic activity. In this process, the final product inhibits the enzyme that catalyzes the first step in a series of reactions. Feedback inhibition is used to regulate the synthesis of many amino acids.



An **irreversible inhibitor** is one that will cause a permanent loss of enzymatic activity. An irreversible inhibitor inactivates an enzyme by bonding covalently to a particular group at the active site. The inhibitor-enzyme bond is so strong that the inhibition cannot be reversed by the addition of excess substrate. The nerve gases, especially Diisopropyl fluorophosphate (DIFP), irreversibly inhibit biological systems by forming an enzyme-inhibitor complex with a specific OH group of serine situated at the active sites of certain enzymes. The peptidases trypsin and chymotrypsin contain serine groups at the active site and are inhibited by DIFP.



Specificity of Enzyme

The specificity of enzymes refers to their ability to recognize and selectively bind to specific substrates, catalysing particular chemical reactions. Enzymes exhibit various levels of specificity:

(i) **Substrate specificity**: Enzymes typically recognize and bind to specific substrates based on their chemical structure and properties. This specificity ensures that enzymes only catalyse reactions involving their designated substrates, preventing unwanted side reactions.

(ii) **Stereochemical specificity:** Enzymes can distinguish between stereoisomers, which are molecules with the same molecular formula and connectivity but differing in spatial arrangement. Enzymes often exhibit stereochemical specificity by selectively interacting with one stereoisomer while ignoring others.

(iii) **Reaction specificity:** Enzymes catalyse specific types of chemical reactions. For example, some enzymes specifically catalyse hydrolysis reactions, while others facilitate oxidation-reduction reactions or transfer functional groups between molecules. This reaction specificity ensures that enzymes contribute to specific metabolic pathways and processes within cells.