FIFTH CHAPTER: Enzymology concepts

Enzymology is the branch of biochemistry that focuses on understanding the structure, function, and folding of enzymes. It involves performing various types of experiments to study enzyme catalytic mechanisms and determining the enzyme's three-dimensional structure.

I. Enzymes

Enzymes are biological catalysts (biocatalysts) that accelerate the rate of biological reactions. They are high molecular weight proteinous compounds. These proteins carry out chemical reactions with extraordinary specificity and speed. Specificity is achieved because the binding site for reactants (the active site) is highly complementary in shape to the reactants and products.

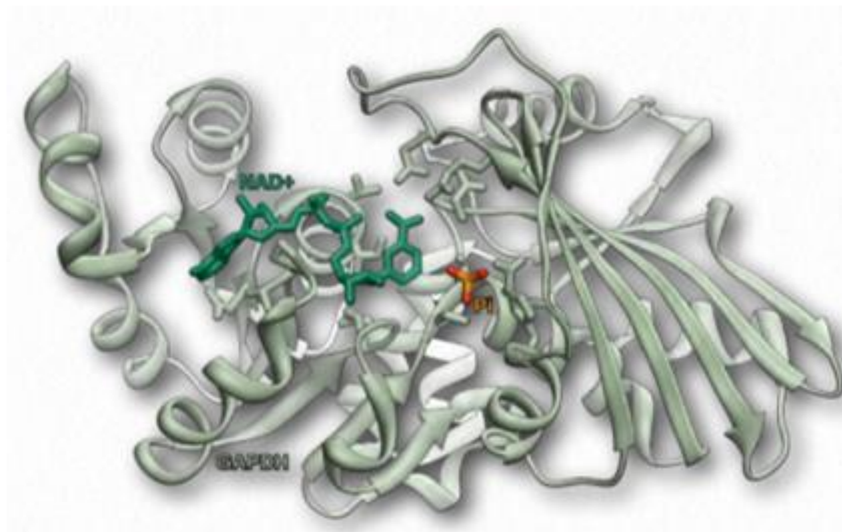
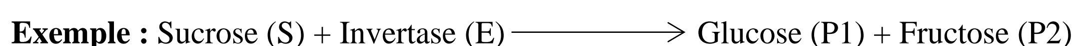


Fig 1. Enzyme's three-dimensional structure.

The enzyme and substrate are both present at the beginning of the reaction, and then form a complex. This allows the product to be formed due to the reduction in activation energy of the enzyme and the product can finally dissociate in the final step.



Enzymes act at low temperatures, and at low concentrations, they remain unchanged at the end of the reaction.

Structurally, there are two main categories of enzymes:

- **Holoenzymes:** purely protein enzymes, they consist only of amino acids. Exp: Ribonuclease.
- **Heteroenzymes:** In addition to protein structures (the apoenzyme), they include non-protein elements called: cofactors or coenzymes.

Cofactors

Some enzymes require the addition of another non-protein molecule to function as an enzyme. These are known as cofactors, and without these enzymes remain within the inactive "apoenzyme" forms. Once the cofactor is added, the enzyme becomes the active "holoenzyme".

Cofactors can either be ions, such as zinc and iron ions, or organic molecules, such as vitamins or vitamin-derived molecules. Many of these cofactors will attach near the substrate binding site to :

- Participate in the recognition and fixation of the substrate to the enzyme.
- Participate in the catalytic transformation of the substrate.
- Stabilize the three-dimensional structure of the reactive site of the enzyme.

II. Nomenclature and classification

Thousands of enzymes have been identified so far; we think that there are thousands still to be discovered in the living world. The nomenclature of enzymes is not standardized, but most often it consists of a radical close to the substrate or the product of catalysis followed by the suffix -ase. According to the International Union of Biochemists (I U B), enzymes are divided into six functional classes and are classified based on the type of reaction in which they are used to catalyze. The six kinds of enzymes are:

- **E.C.1: Oxidoreductases:** The enzyme Oxidoreductase catalyzes the oxidation reaction where the electrons tend to travel from one form of a molecule to the other.
- **E.C.2: Transferases:** The Transferases enzymes help in the transportation of the functional group among acceptors and donor molecules.
- **E.C.3: Hydrolase:** Hydrolases are hydrolytic enzymes, which catalyze the hydrolysis reaction by adding water to cleave the bond and hydrolyze it.

- **E.C.4 : Lyase:** Adds water, carbon dioxide or ammonia across double bonds or eliminate these to create double bonds.
- **E.C.5: Isomerases:** The Isomerases enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule.
- **E.C.6: Ligases:** The Ligases enzymes are known to charge the catalysis of a ligation process.

III. Mechanism of action

Once substrate (S) binds to this active site, they form a complex (intermediate-ES) which then produces the product (P) and the enzyme (E). The substrate which gets attached to the enzyme has a specific structure and that can only fit in a particular enzyme. Hence, by providing a surface for the substrate, an enzyme slows down the activation energy of the reaction. By breaking and making the bonds, the substrate binds to the enzyme (remains unchanged), which converts into the product and later splits into product and enzyme. The free enzymes then bind to other substrates and the catalytic cycle continues until the reaction completes.

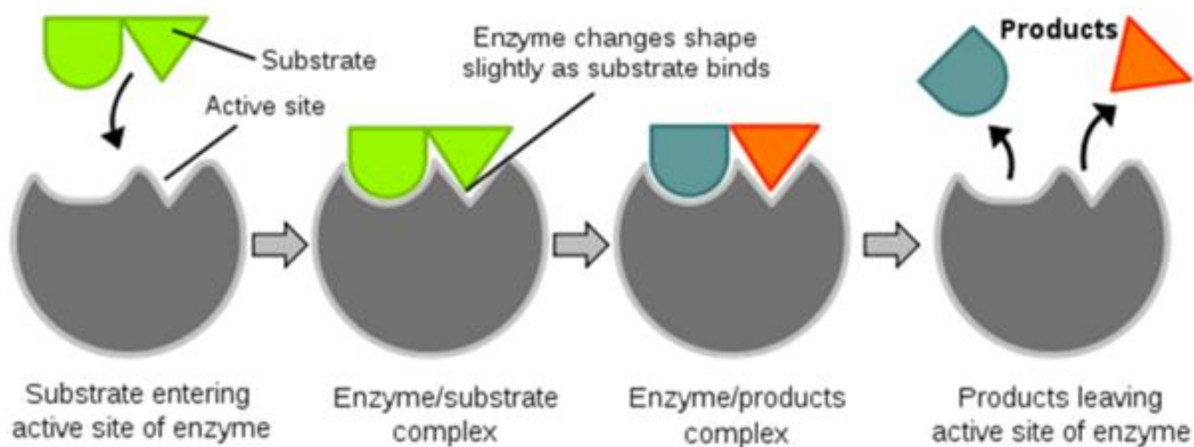


Fig 2. Mechanism of action of enzymes.

IV. Enzyme active sites and enzyme-substrate complexes

Enzymes are a linear chain of amino acids, which give rise to a three-dimensional structure. The sequence of amino acids specifies the structure, which in turn identifies the catalytic activity of the enzyme. Upon heating, the enzyme's structure denatures, resulting in a loss of enzyme activity, which typically is associated with temperature.

Compared to its substrates, enzymes are typically large with varying sizes, ranging from 62 amino acid residues to an average of 2500 residues found in fatty acid synthase. Only a small section of the structure is involved in catalysis and is situated next to the binding sites. The catalytic site and binding site together constitute the enzyme's active site.

The interaction between substrate and enzyme is weak, and these weak interactions induce conformational changes rapidly and strengthen binding and bring catalytic sites close enough to substrate bonds.

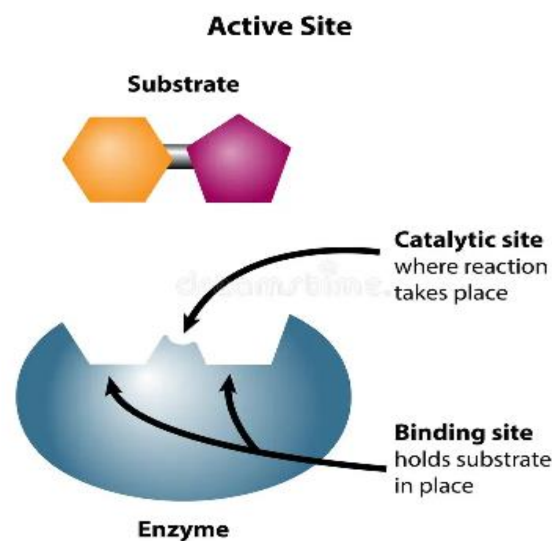


Fig 3. Enzyme active site.

V. Enzyme Kinetics

Enzymes are thought to form a complex with the substrates to catalyze the reaction. This process can be illustrated with the simplified equation, where E is the enzyme, S is the substrate, and P is the product:

$$E + S \Leftrightarrow ES \Longrightarrow E + P$$

The first step of the equation, which is reversible, has the reaction rate constant of k_1 to produce the enzyme substrate complex and k_2 for the reverse reaction. The reaction rate constant for the second step of the equation, which is not reversible, is k_3.

The rate of reaction (v), which is the rate at which the product is formed, is defined by the following equation:

$$v = d[P]/dt = k_3 [ES]$$

The square brackets in the above equation represent the molar concentration of the substance specified within, so [P] refers to the molar concentration of the product and [ES] the molar concentration of the enzyme substrate.

Vmax and Km

Leonor Michaelis and Maud Menten introduced a mathematical illustration to describe the action of enzymes with two constants, Vmax and Km.

The maximal velocity (Vmax) refers to the point at which the increase the concentration of the substrate does not increase the rate of a reaction catalyzed by an enzyme. This occurs because the substrate molecules saturate the active sites of the enzyme and are not able to form more complexes with the enzyme. This value is given as a rate (mmol/s), which is the maximum velocity of the reaction when the enzyme is saturated.

$$V = Vmax [S]/ km + [S]$$

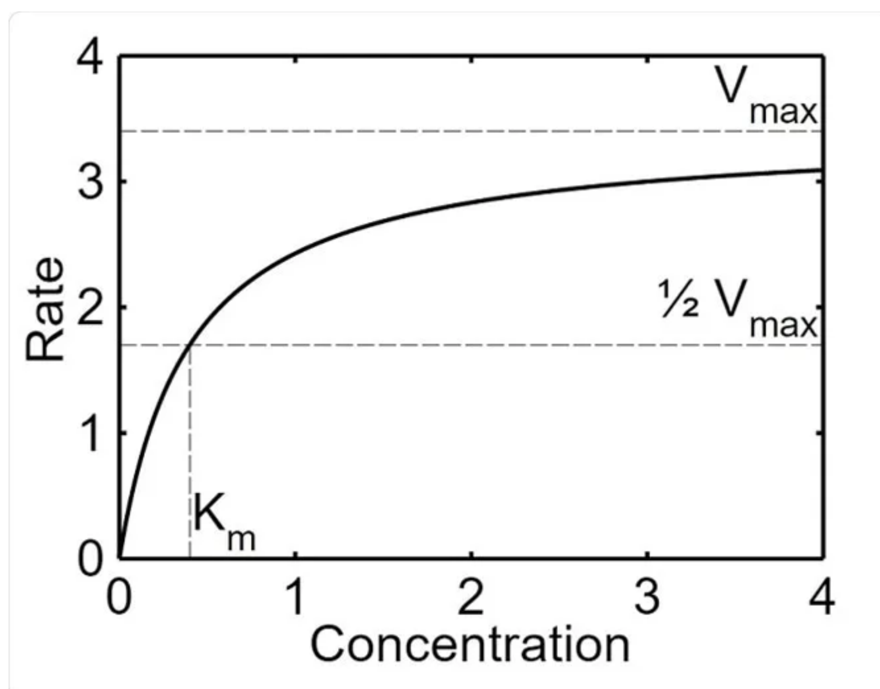


Fig 4. Michaelis-Menten graphical representation.

The Michaelis constant (Km) is the concentration of the substrate when half of the active binding sites of an enzyme are occupied by the substrate. The constant helps to depict the affinity of the enzyme for their substrate. This value is given as the concentration of the substrate (mM) at half of Vmax. An enzyme with a high Km has a low affinity for the substrate,

and a high concentration of the substrate is needed in order for the enzyme to become saturated. Conversely, an enzyme with a high Km has a high affinity for the substrate and the enzyme may become saturated even with a low amount of substrate.

$$Km = [E]\,[S] / [ES] = k2 + k3/ k1$$

To simplify the graphic representation of the Michaleis-Menten equation, we transform the hyperbola into a line.

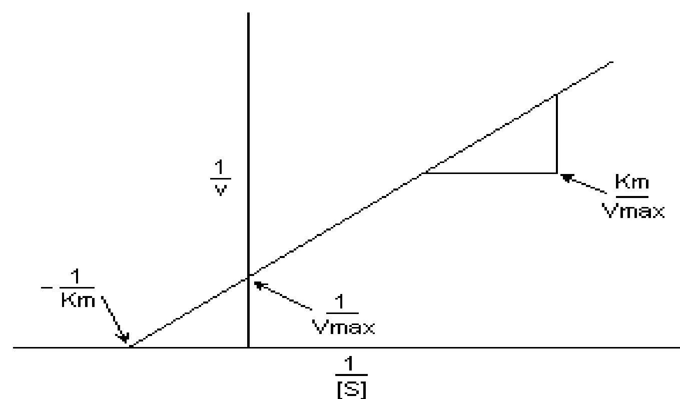


Fig 5. Line Weaver and Burke representation.

VI. Enzyme inhibition

An enzyme inhibitor is a molecule that binds to an enzyme and slows down or blocks its activity. There are different types of inhibitors:

a. Irreversible inhibitors

Irreversible inhibitors covalently bind to an enzyme, and this type of inhibition can therefore not be readily reversed. They do not present structural analogies with the substrate.

b. Reversible inhibitors

Reversible inhibitors attach to enzymes with non-covalent interactions such as hydrogen bonds, hydrophobic interactions and ionic bonds. Multiple weak bonds between the inhibitor and the enzyme active site combine to produce strong and specific binding.

Reversible inhibitors are generally categorized into three types. They are classified according to the effect of the inhibitor on the Vmax and Km as the concentration of the enzyme's substrate is varied.

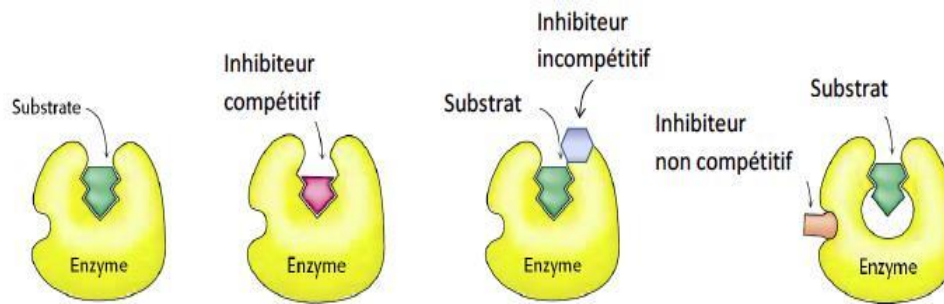


Fig 6. Method of fixing reversible inhibitors

- **Competitive**

In competitive inhibition the substrate and inhibitor cannot bind to the enzyme at the same time. This usually results from the inhibitor having an affinity for the active site of an enzyme where the substrate also binds; the substrate and inhibitor compete for access to the enzyme's active site. This type of inhibition can be overcome by sufficiently high concentrations of substrate. However, the apparent Km will increase as it takes a higher concentration of the substrate to reach the Km point, or half the Vmax. Competitive inhibitors are often similar in structure to the real substrate.

- **Non-competitive**

In non-competitive inhibition the binding of the inhibitor to the enzyme reduces its activity but does not affect the binding of substrate. This type of inhibitor binds with equal affinity to the free enzyme as to the enzyme-substrate complex. The inhibitor may bind to the enzyme whether or not the substrate has already been bound. Vmax will decrease due to the inability for the reaction to proceed as efficiently, but Km will remain the same as the actual binding of the substrate, by definition, will still function properly.

- **Uncompetitive**

In uncompetitive inhibition the inhibitor binds only to the enzyme-substrate complex. This type of inhibition causes Vmax to decrease and Km to decrease. Uncompetitive inhibition is rare.

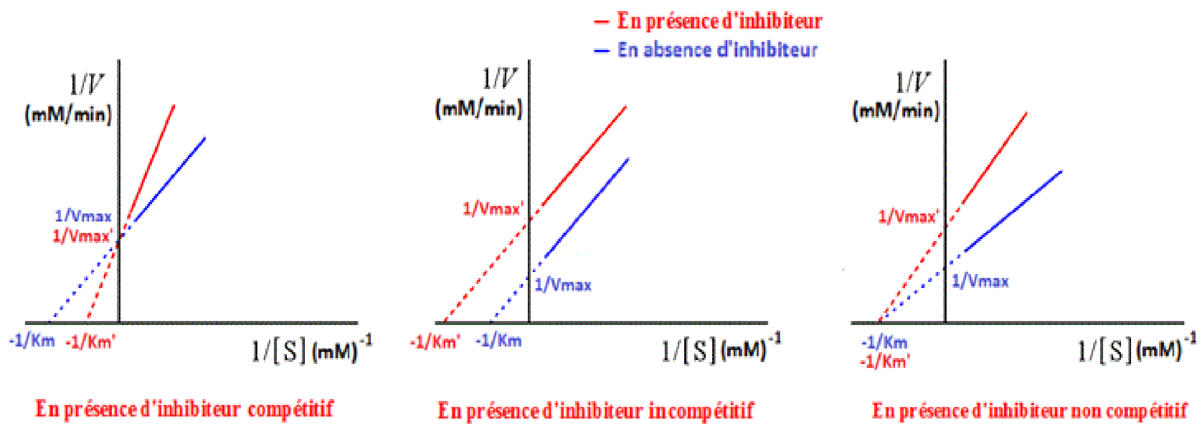


Fig 7. Lineweaver–Burk diagrams of different types of reversible enzyme inhibitors.

In the presence of competitive inhibition:

- Km increases (Km' > Km) therefore the affinity decreases.
- Vmax = Vmax'

In the presence of incompetitive inhibition:

- Km decreases (Km' < Km) therefore the affinity increases.
- Vmax decreases (Vmax' < Vmax)

In the presence of non-competitive inhibition:

- Vmax decreases (Vmax' < Vmax)
- The affinity is not modified (Km' = Km)