PAPER 5: MOLECULAR ENZYMOLOGY & PROTEIN ENGINEERING

Module 16: Hill's coefficients, Cooperativity, positive and negative Cooperativity

Introduction:

Enzyme Cooperativity is a phenomenon in which the shape of one subunit of an enzyme consisting of several subunits is altered by the binding of the ligand. This ligand can be either the substrate itself or some other molecule which alters the substrate binding on the enzyme. If the ligand induced change in shape facilitates the binding of substrate to the same or other subunit, the effect is called positive cooperativity. In negative cooperativity, the binding of a molecule to the first subunit inhibits the binding of substrate to the same or other subunit. Therefore, in multi subunit enzymes, binding of substrate is cooperative and it shows sigmoidal curve unlike the hyperbolic curve in Michaelis-Menten model.

Objectives:

In this chapter, we will learn about following aspects of Enzyme cooperativity

- **Hill's Coefficient**
- **Cooperativity**
- **Positive Cooperativity**
  - **The Concerted model**
  - **The sequential Model**
- **Negative Cooperativity**

16.1 Hill's Coefficient

The biochemical reaction kinetics of those enzymes which have single active site follows Michaelis-Menten model. However, the same model does not apply for the kinetic properties of those enzymes which are allosteric in nature, because they bind their substrates in a cooperative fashion analogous to the binding of oxygen by haemoglobin. These enzymes consist of multiple subunits and multiple active sites with binding ability of substrates at different binding sites. In allosteric enzymes, the binding of substrate to one active site can affect the properties of other active sites in the same enzyme molecule. Due to this reason, the binding of substrate becomes *cooperative;* that is, the binding of substrate to one active site of the enzyme facilitates substrate binding to the other active sites. Binding of the first substrate molecule at one site affects the binding of other substrate molecules in both positive and negative manner. For enzymes that display positive cooperativity in binding substrate usually display sigmoidal (because it resembles "S") curve, when the curve is plotted between the reaction velocity V_i and substrate concentration [S] (Figure 1); as compared to the hyperbolic plots predicted by the Michaelis-Menten model.

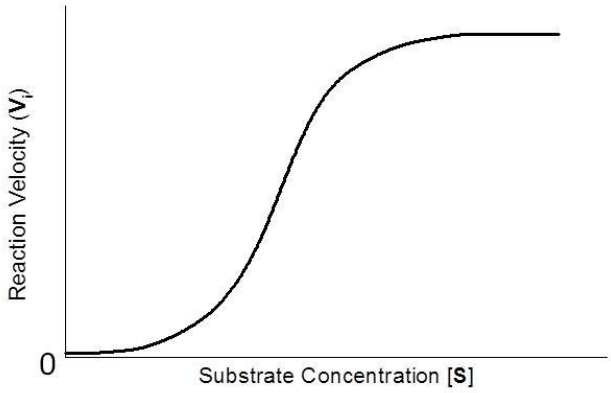

Figure 16.1: The sigmoid curve of reaction kinetics by allosteric enzymes

The Michaelis-Menten expression cannot be used to evaluate such positive cooperative saturation kinetics of allosteric enzymes. Therefore, the graphic representation of the Hill equation (equation 1) was employed, which was originally derived by A V Hill in 1910 to describe the cooperative binding of O_2 by haemoglobin (multi-site, allosteric protein).

$$\frac{\log v_1}{V_{max} - v_1} = n \log [S] - \log k' \quad \text{------ Equation 1}$$

Equation (1) states that when [S] is low relative to k', the initial reaction velocity increases as the nth power of [S]. Here the K' is complex constant. Using this equation a graph was plotted between log $V_1/(V\text{max} - V_1)$ and log [S] which linearizes the sigmoidal curve (Figure 2). The slope, **n** of this linear curve is called **Hill's coefficient**. A perpendicular line dropped on x-axis from the point where the Y axis, log $V_1/(V\text{max} - V_1)$ is zero, intersects at a substrate concentration termed S50 (Figure 2), the substrate concentration that results in half-maximal velocity. S50 thus is analogous to the P50 for oxygen binding to haemoglobin.

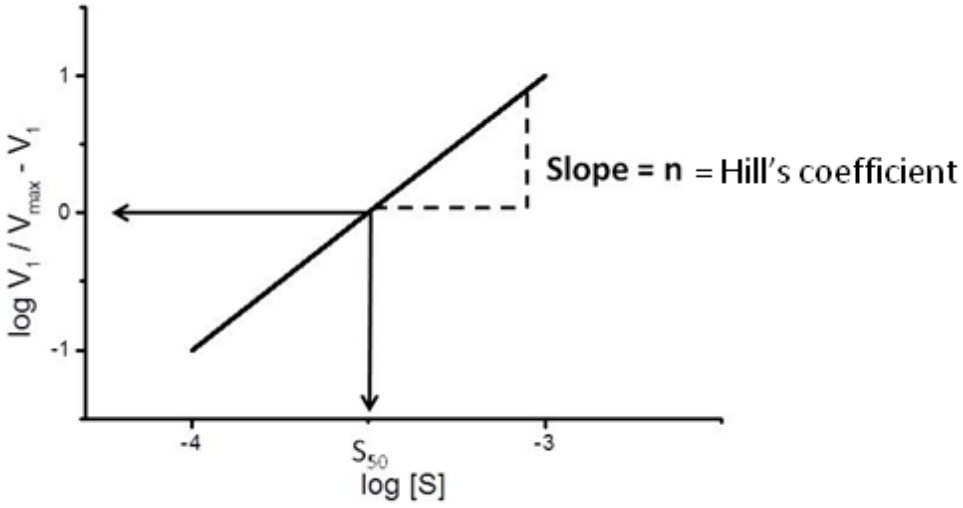

Figure 16.2: The diagram depicts the linear representation of the Hill eqation, which is used to calculate the substrate concentration at which enzymes attain half maximal velocity, and the degree of cooperativity or Hill's coefficient, n.

Hill's coefficient is an empirical parameter whose value is a function of the number, kind, and strength of the interactions of the multiple substrate-binding sites on the enzyme. When the value of n = 1, then it means the reaction is not cooperative, all binding sites behave independently, and the substrate binding to one site does not affect the binding on the other site. If n>1, the enzyme shows positive cooperativity; where, binding of the first substrate molecule then enhances the affinity of the enzyme for binding other substrate molecules. The greater the value for n, the higher the degree of cooperativity and the more sigmoidal will be the plot of V_1 versus [S]. However, if n<1 that means the reaction is negatively cooperative, meaning the binding of one substrate will inhibit the binding on other site. It is important to note here that the Hill's coefficient n is always greater than 0. The activity of an allosteric enzyme may be altered by regulatory molecules that are reversibly bound to specific sites other than the catalytic sites. Therefore, allosteric enzymes play an important role in regulating the biochemical reactions in the cell.

This kinetic behaviour of enzyme molecules is supported by structural changes in the enzymes. In the absence of substrate, the enzyme exists almost entirely in the T state. However, the binding of substrate molecules to the enzyme shifts the enzyme toward the R state. A transition from T to R favored by substrate binding to one site will increase the enzymatic activity of the remaining five sites, leading to an overall increase in enzyme activity. This important property is called *cooperativity* because the subunits cooperate with one another. An increase in substrate concentration favors a transition from the T-state curve to the R state curve.

16.2 Cooperativity

In multi-meric proteins, the binding of a ligand/substrate to one site/ subunit may alter the binding of the same or other molecule on other site/subunit. The binding events therefore "cooperate" because they act together. This phenomenon in multimeric proteins is called cooperativity. It can also be described as the increasing or decreasing affinity for binding of the other sites affected by the original binding site. Cooperativity can be seen in both enzymes and receptors having multiple substrate/ ligand binding sites. For an enzyme that shows a cooperative behavior, the relation between the substrate concentration and velocity of reaction shows a sigmoidal curve (Figure 1) instead of Michaelis and Menten behavior. Enzymes that demonstrate cooperativity are known as allosteric enzymes.

The concept of cooperativity is applicable only in the case of molecules having more than one substrate/ ligand binding sites. Cooperativity is called homotropic, if a ligand influences the binding of same substrate/ ligands, or heterotropic, if it influences binding of other substrate/ ligands. If the ligand binding to any one site does not affect the others then the receptor is said to be non-cooperative. The cooperativity can be of following two types.

16.3 Positive cooperativity:

When the binding of a substrate/ ligand molecule facilitates the binding of other substrate/ ligand then it is called positive cooperativity. The value of Hill's coefficient $n > 1$, in this condition. One of the most studied models of positive cooperativity is the binding of oxygen to the haemoglobin. Systems exhibiting positive cooperativity have the advantage that they can be very responsive to small changes in substrate/ ligands concentration.

A Danish physician, Christian Bohr was the first who studied the binding of hemoglobin to oxygen under different physiological conditions. When plotting hemoglobin saturation with oxygen as a function of the partial pressure of oxygen, he obtained a sigmoidal curve (Figure 1). This indicates that the oxygen binding to haemoglobin facilitates the binding of more oxygen, until all binding sites are saturated. He also noticed that the increased CO_2 concentration in the tissues inhibits the binding of O_2 to haemoglobin. This latter phenomenon, together with the observation that hemoglobin's affinity for oxygen increases with increasing pH, is known as the "Bohr effect". In the case of haemoglobin, Bohr observed homotropic positive cooperativity (binding of oxygen facilitates binding of more oxygen) and heterotropic negative cooperativity (binding of CO_2 reduces hemoglobin's affinity to bind oxygen).

Careful analysis of the binding curve for haemoglobin indicates that the apparent affinity for oxygen changes with oxygen concentration (the affinity for oxygen is low at low concentrations of oxygen, and then increases when oxygen concentration increases). This behaviour of haemoglobin shows typical of positive cooperativity.

There are many models have been proposed to describe the molecular basis of cooperativity. Majority of the models propose that cooperative proteins exist in equilibrium between two different states, Tense (T) and Relaxed (R), where the R state has a higher affinity for the ligand. Binding of ligands or effector molecules alters the equilibrium between the T and R states.

16.3.1 The concerted model: According to this model, which is also known as symmetry model or MWC model, enzyme subunits are connected in such a way that a conformational change in one subunit is necessarily conferred to all other subunits. Thus all subunits must exist in the same conformation. Example: In hemoglobin, the tetramer changes conformation together (R state) after four oxygen molecules bind to all four monomers. The transition from the T state to the R state occurs in one step. At each level of oxygen loading, an equilibrium exists between the T-state and R-state. The equilibrium shifts from strongly favoring the T-state (no oxygen bound) to strongly favoring the R-state (fully loaded with oxygen). Overall, oxygen binding shifts the equilibrium toward the R state. This means that at high oxygen levels, the R form will be prevalent and at lower oxygen levels, the T form will be prevalent.

16.3.2 The sequential model: In this model subunits are not connected in such a way that a conformational change in one induces a similar change in others. It s not necessary that all enzyme subunits posses the same conformation in multi subunit structure. The sequential model states that molecules of substrate bind through an induced fit. Example: In hemoglobin, the four monomers change conformation (R state) one at a time as oxygen binds to each monomer. This allows hemoglobin to have R state monomers and T state monomers. This model follows the concept that after binding occurs at one site in the active site, the binding affinity in the other sites around the protein will increase as well. Hence, the plot of substrate concentration versus reaction rate is of a sigmoidal shape.

The difference between the sequential model and concerted model is that the T states do not have to convert to R states all at one time. In this model, the ligand will change the conformation of the subunit that it is bound to and induce changes in the neighboring subunits. However, neither the sequential model nor the concerted model fully explains the nature of hemoglobin. Properties from both models appear in a real system.

Another good example of positive cooperativity is Aspartate transcarbamoylase (ACTase) that catalyzes the first step in the biosynthesis of pyrimidines, bases that are components of nucleic acids. The reaction catalyzed by this enzyme is the condensation of aspartate and carbamoyl phosphate to form *N*carbamoylaspartate and orthophosphate ATCase catalyzes the committed step in the pathway that will ultimately yield pyrimidine nucleotides such as cytidine triphosphate (CTP). ATCase is inhibited by CTP, the final product of the ATCase-controlled pathway. The rate of the reaction catalyzed by ATCase is fast in the absence of high concentrations of CTP but decreases as the CTP concentration increases. Thus, more molecules are sent along the pathway to make new pyrimidines until sufficient quantities of CTP have accumulated. Actually, CTP binds to allosteric or regulatory sites of the ACTase. CTP is an example of an allosteric inhibitor. In ATCase, the larger catalytic subunit and smaller regulatory subunit are separate polypeptide chains. Therefore, it is observed that the allosteric binding of CTP at higher concentration brings large changes in the quaternary structure of the enzyme (ACTase).

16.4 Negative cooperativity: When the binding of a substrate/ ligand molecule inhibits the binding of other substrate/ ligand then it is called negative cooperativity. The value of Hill's coefficient n < 1, in this condition. Phosphofructokinase, a tetrameric enzyme of glycolytic pathway is the best example of negative cooperativity. Each monomer of the phosphofructokinase complex has two binding sites for ATP; the active site, which has a high affinity for ATP, and a lower affinity allosteric regulatory site. Binding of ATP to the regulatory site stabilizes the T state of the enzyme and inhibits the substrates binding and therefore the velocity of reaction.

16.5 Summary

Allosteric enzymes shows cooperativity, represented by sigmoidal curve.

A linear form of the Hill equation is used to evaluate the cooperative substrate-binding kinetics exhibited by some multimeric enzymes. The slope **n,** the Hill coefficient, reflects the number, nature, and strength of the interactions of the substrate-binding sites.

If Hill's coefficient n = 1, It will be a hyperbolic curve

If n > 1, then it is positive cooperativity

If n < 1 (but greater than 0), then it is negative cooperativity.

Multimeric proteins can exhibit cooperativity, in which one substrate alters the binding of others. Hemoglobin is a well-characterized example of positive cooperativity (binding of oxygen increases the affinity for other oxygen molecules).

Both types of cooperativity are involved in regulation of enzymatic activity. The changes in substrate/ ligand concentration exert direct changes in enzymatic activity or in ligand binding.

Binding of small molecules can cause conformational changes of varying degrees; even small conformational changes can have significant effects on the activity of the protein.

Molecules other than the substrate can regulate cooperative proteins; an example of this is the regulation of hemoglobin by 2,3-bisphosphoglycerate and hydrogen ion concentration.

For some enzymes, increasing concentrations of substrate can decrease the enzymatic activity. This is illustrated by the effect of ATP on phosphofructokinase, where ATP acts both as a substrate and as a negative effector.