# ENZYMOLOGY

## Enzyme kinetics

P.C. Misra
Professor
Department of Biochemistry
Lucknow University
Lucknow-226 007

12-May-2006 (Revised 17-Aug-2006)

**CONTENTS**

**Introduction**
**Effect of substrate concentration**
**Derivation of Michaelis-Menten equation**
**Significance of $K_m$**
**Lineweaver-Burk double reciprocal plot**
**Bi-substrate reactions**
**Kinetics of first and zero order reactions**
**Effect of temperature**
**Effect of enzyme concentration**
**Effect of pH**
**Effect of inhibitors**
**Allosteric enzymes**

**Keywords**

*Enzyme kinetics; E*nzyme activity; Michaelis-Menten equation; Bi-substrate reactions; $K_m$, $V_{max}$, Kinetics of reaction, Reversible inhibition; Irreversible inhibition; Allosteric enzymes; Lineweaver-Burk plot

## Introduction

The basic function of enzymes is to increase the rates of biochemical reactions so that the needs of cell/organism are suitably met. However, the actual rates at which enzyme catalyzed reactions proceed are governed by the cellular conditions. In order to understand this function of enzymes, one has to follow the kinetic description of their activity. The word 'kinetic' has Greek origin (*Kinetikos*, means 'moving'). Its application to an enzyme catalyzed reaction is concerned with the initial rate of that reaction and how this rate is influenced by a variety of factors.

The factors that influence the initial rate of an enzyme catalyzed reaction are following:

1. Concentration of substrate
2. Concentration of enzyme
3. pH of the medium
4. Temperature of the medium
5. Concentration of inhibitors
6. Concentration of activators
7. Concentration of products

In this chapter we shall restrict our discussion on the influence of first five factors on kinetics of enzyme catalyzed reaction.

## Effect of substrate concentration

In general the rate of an enzyme catalyzed reaction (v), involving a single substrate, is influenced by increasing substrate concentration, [S], as depicted in Fig.1. Initially the rate of enzyme catalyzed reaction increases linearly as substrate concentration increases and then it gradually levels off to attain a maximum value at relatively higher substrate concentrations. The shape of this curve is of a hyperbola. The interpretation of this relationship between v and [S] can be given by considering that an enzyme catalyzed transformation of a substrate, S, into product, P follows a pathway as depicted in reaction (i).

$$E+S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E+P \qquad \text{(i)}$$

where $k_1$, $k_2$ and $k_3$ are respective rate constants. Accordingly, enzyme binds substrate to form an effective enzyme-substrate complex, ES, which in turn is converted into free enzyme, E, and product (s), P.

In 1913 Leonor Michaelis and Maud Menten proposed a theory to explain the above relationship and it became known as 'Michaelis-Menten hypothesis'. This hypothesis became very useful in the quantitative analysis of almost all aspects of enzyme kinetics.

## Derivation of Michaelis-Menten equation

### A. *Under equilibrium assumption*

The enzyme catalyzed reaction as shown by equation (i) is assumed to take place in the conditions where; (a) the fixed concentration of enzyme, [E], is much less than that of substrate concentration, [S], (b) the initial concentration of product, [P], is effectively zero

because the reaction of E with S to form ES is freely reversible and is much faster to dissociate back to form E and S than to form product, P.

Under the above conditions, the rates of formation of ES complex and its dissociation back into E and S can be calculated. Accordingly, the rate of formation of

$$ES = k_1 [E] [S] \quad \text{(ii)}$$

and the rate of dissociation of

$$ES = k_2 [ES] \quad \text{(iii)}$$

where, [ES] is concentration of ES complex.

Since at equilibrium both the rates are equal, therefore

$$k_1 [E] [S] = k_2 [ES] \text{ or } [E] = [ES]\frac{k_2}{k_1[S]} \quad \text{(iv)}$$

The amount of total enzyme, $[E]_t$, in the system at any time can be given as

$$[E]_t = [E] + [ES] \quad \text{(v)}$$

On substituting values for [E] from equation (iv)

$$[E]_t = [ES]\frac{k_2}{k_1[S]} + [ES]$$

or

$$[E]_t = [ES]\left[1 + \frac{k_2}{k_1}.\frac{1}{[S]}\right] \quad \text{(vi)}$$

In case, if reverse reaction in equation (i) is ignored then the over all rate of the reaction, v, is given as

$$v = k_3 [ES] \quad \text{(vii)}$$

on substituting value of [ES] from equation (vi)

$$v = \frac{k_3[E]_t}{\left(1 + k_2/k_1.\frac{1}{[S]}\right)} \text{ or } = \frac{k_3[E]_t.[S]}{[S] + k_2/k_1} \quad \text{(viii)}$$

At high substrate concentration when all the enzyme is bound to substrate then $[E]_t = [ES]$ and under these conditions the velocity of the reaction is maximum, $V_{max}$, therefore,

$$v = V_{max} = k_3 [E]_t \quad \text{(ix)}$$

Since the system depicted by equation (i) is in equilibrium, therefore $k_2/k_1 = K_S$ (called dissociation constant). Under these conditions, the equation (viii) can be written as

$$v = \frac{V_{max}.[S]}{[S] + K_S}$$

This is the form of Michaelis-Menten equation under equilibrium assumption. Under these conditions, $K_S$ is called $K_m$

When [S] >> $K_S$ then v = $V_{max}$

When [S] << $K_S$ then v = $\frac{V_{max}}{K_S}$.[S]

Since $\frac{V_{max}}{K_S}$ is a constant entity, therefore,

v $\alpha$ [S]

In case [S] = $K_S$ then $v = \frac{1}{2} V_{max}$. This means, $K_S$ is equal to the substrate concentration at which the reaction rate is half of the maximum value.

***B. Under steady-state approach***

In the previous section, we had examined the relation between velocity, v, and kinetic constants ($K_m$ or $K_S$ and $V_{max}$) under the equilibrium assumption. However, in reality the rate of enzyme catalyzed reaction as determined by formation of products (P), howsoever small, always influences the formation of ES. As such, the following derivation is based on the postulate that in a system represented by equation (i), at any moment, the rates of formation and breakdown of ES complex are essentially equal. Therefore,

Rate of ES formation = $k_1$ [E] [S]

Rate of ES disappearance = $k_2$ [ES] + $k_3$ [ES] = ($k_2$ + $k_3$) [ES]

At steady state

$$k_1 [E] [S] = (k_2 + k_3) [ES]$$

$$\text{or } [E] = \frac{(k_2 + k_3)}{k_1} . \frac{[ES]}{[S]}$$

$$\text{or } \frac{[E][S]}{[ES]} = \frac{k_2 + k_3}{k_1}$$

$$\text{or } [E] [S] / [ES] = K_m \qquad \text{(x)}$$

where $\frac{k_2 + k_3}{k_1} = K_m$ (Michaelis-Menten constant)

At any time the amount of total enzyme, $[E]_t$, is defined as

$[E]_t = [E] + [ES]$ or $[E] = [E]_t - [ES]$

Substituting the value of [E] in equation (x)

$$\frac{([E]_t - [ES])[S]}{K_m} = [ES] \qquad \text{(xi)}$$

On solving the above equation

$$[E]_t [S] - [ES] [S] = [ES] K_m$$

or

$$[E]_t [S] = [ES] (K_m + [S])$$

or

$$[ES] = \frac{[E_t][S]}{K_m + [S]} \qquad \text{(xii)}$$

since $v = k_3 [ES]$
therefore, by substituting the value of [ES] from equation (xii)

$$v = k_3 \frac{[E]_t[S]}{K_m + [S]} \qquad \text{(xiii)}$$

and when all the enzyme is bound to **S** then $[E_t] = [ES]$ and at that the velocity of the reaction, v, is maximum, i.e. $V_{max}$ .

Accordingly, $V_{max} = k_3 [E]_t$ and therefore when $V_{max}$ is substituted for $k_3 [E]_t$ in equation (xiii) then it turns into the following form:

$$v = \frac{V_{max}[S]}{K_m + [S]} \qquad \text{(xiv)}$$

which is the form of Michaelis-Menten equation under *steady-state*. As stated earlier under equilibrium assumption, when $[S] << K_m$

$$v = \frac{V_{max}}{K_m} [S]$$

i.e., velocity is directly proportional to substrate concentration.

On the other hand, when $[S] >> K_m$

$$v = V_{max}$$

i.e., velocity is independent of substrate concentration, and when $[S] = K_m$ then $v = \frac{1}{2} V_{max}$.

Thus $K_m$ and $V_{ma}$, called kinetic constants, are characteristics of an enzyme catalyzed reaction under specified conditions with a particular substrate.

**Significance of $K_m$**

As has been shown that $K_m$ value of an enzyme for a substrate represents that concentration of a substrate at which the velocity of the reaction is half of the maximum attainable velocity ($V_{max}/2$). Thus, if an enzyme acts on different substrates then the observed differences in the concentrations of substrates required to attain $½V_{max}$ value is an indicator of the relative strength of binding of enzyme and substrate to produce fruitful ES complex. Thus higher is the strength of substrate binding to enzyme quicker will be the time required to attain the value of $V_{max}/2$. Earlier it has been noticed that $K_m$ is related to rate constants under 'equilibrium assumption' and 'steady-state' as equal to $\frac{k_2}{k_1}$ and $\frac{k_3 + k_2}{k_1}$, respectively. Thus, to associate $K_m$ with the strength of binding of substrate to enzyme, the value of $k_3$ must be very small. As such, according to the reaction (i) $k_2$ is much smaller than $k_1$ and therefore $K_m$ value is small. Conversely, a large $K_m$ means $k_2$ is much greater than $k_1$. It is therefore inferred that smaller is the value of $K_m$, higher is the affinity of enzyme for that substrate.

In the above relationship the constant $k_3$ is a direct measure of the transformation of substrate into product, i.e. the larger is the value of $k_3$ the faster would be the rate of enzyme

catalyzed reaction. Thus, this constant is also, sometimes, referred to as $k_{cat}$ and it is expressed as per second. It therefore suggests that reciprocal of $k_{cat}$ indicates the time taken by an enzyme molecule to transform one substrate molecule into product. Thus, $k_{cat}$ is an index of the transformation of number of substrate molecules by one enzyme molecule per second. Therefore $k_{cat}$ is also referred as the 'turnover number' or 'molecular activity' of the enzyme.

According to equation (xiii) where the relation between the velocity, v, and kinetic constants, $K_m$ and $V_{max}$ is given by $v = \frac{k_3[E]_t[S]}{K_m + [S]}$ or $\frac{k_{cat}[E]_t[S]}{K_m + [S]}$ , therefore under physiological conditions if [S] << $K_m$ then most of the enzyme is in free state so that $[E]_t$ = [E], and the above equation can be written as

$$v = \frac{k_{cat}[E]_t[S]}{K_m} \qquad \text{(xv)}$$

The ratio $\frac{k_{cat}}{K_m}$ is a direct measure of enzyme efficiency and specificity. The significance of this ratio can be noticed when $k_{cat} >> k_2$, indicating that catalytic process is very fast and the efficiency of enzyme is totally dependent on rate of substrate binding to enzyme.

**Lineweaver-Burk double reciprocal plot**

It was noticed from Fig. 1, describing hyperbolic shape of graph between v and [S], that ½ $V_{max}$ value can be used to determine the $K_m$ of enzyme from the corresponding value of [S]. In some enzyme catalyzed reactions however, the measure of the true $V_{max}$ by this method is difficult and thereby the determination of a reliable $K_m$ value becomes a problem.

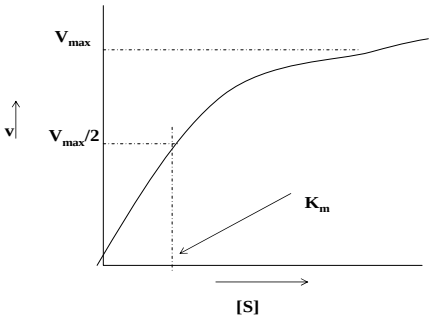



**Fig. 1: Effect of substrate concentration, [S], on the initial velocity of an enzyme catalyzed reaction**

The Michaelis-Menten equation (xiv) can be rearranged by several ways to transform it into a straight-line equation. The most common method is to get Linewever-Burk double reciprocal equation as follows:

Taking the reciprocal of both sides of equation (xiv), we get

$$\frac{1}{v} = \frac{K_m + [S]}{V_{max}[S]} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \qquad \text{(xvi)}$$

The above equation is of the type $y = mx + C$ (the straight-line equation), where y = 1/v; m is the slope, which in this case $K_m/V_{max}$; x = 1/[S]; and C = $1/V_{max}$.

A graph of 1/v versus 1/[S] gives a straight-line called Lineweaver-Burk plot (Fig. 2) where intercept on x-axis is $-1/K_m$, the intercept on y-axis is 1/Vmax and the slope of the line is $K_m/V_{max}$.

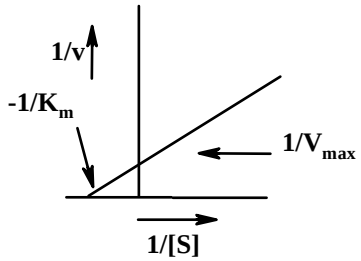



**Fig. 2: A double-reciprocal plot, between velocity (v) and [S]**

Thus, experimental measurement of initial velocity of enzyme catalyzed reaction at different substrate concentrations would provide a set of data that is used to obtain the above graph which provides a quick test for the enzyme which follows Michaelis-Menten kinetics.

A limitation of Linewever-Burk plot is that a long extrapolation is generally needed to determine $K_m$, causing uncertainty in the result. However there are other ways to plot data to ascertain the result. Sometimes, the kinetics of the reaction disobeys the Michaelis-Menten equation and this is indicated by a departure from linearity in these plots. Such deviations from linearity are the characteristics of the kinetics of regulatory enzymes known as allosteric enzymes (discussed at the end of this chapter).

**Bi-substrate reactions**

Earlier, the discussions on enzyme kinetics have been limited to simple reactions involving one substrate binding to an enzyme and subsequently undergoing catalytic reaction. This situation in reality is not common. A majority of biochemical reactions catalyzed by enzymes involve two or more substrates taking part in the reactions. For example, an enzyme, E, catalyzing a reaction involving two substrates A and B and resulting the production of P and Q as products is of the type

$$A + B \xrightarrow{E} P + Q \qquad \text{(xvii)}$$

This type of reaction is called a bi-substrate reaction. In general, these reactions proceed by either of the two possible ways:

1. Both the substrates, A and B, bind to the enzyme, E, and then reaction proceeds to yield products, P and Q

$$E + A + B \rightarrow EAB \rightarrow EPQ \rightarrow E + P + Q \qquad \text{(xviii)}$$

Reactions of this type are called sequential or simple-displacement reactions. These types of reactions are further subdivided into following groups:

**a. Ordered substrate binding:** In this case one substrate must bind before a second substrate can bind to a meaningful extent. Thus we have

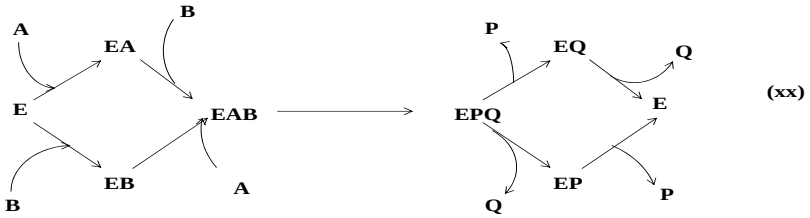
$$E + A \rightarrow \underset{+B}{EA} \rightarrow EAB \rightarrow EPQ \rightarrow^{P} +EQ \rightarrow Q + E \qquad \text{(xix)}$$

indicating a sequential binding of substrates as well as sequential release of products.

This type of mechanism is observed in reactions catalyzed by oxidoreductases involving $NAD^+$. For example, lactate dehydrogenase catalyzed dehydrogenation of lactic acid by $NAD^+$

$$\text{Lactic acid} + NAD^+ \xrightarrow{E} \text{Pyruvic acid} + NADH + H^+$$

**b. Random substrate binding**: In this case either A or B may bind to the enzyme first, followed by the other substrate and the release of the products is also in random fashion. Thus,

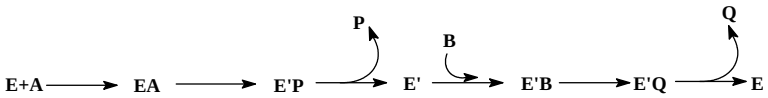



(xx)

This type of mechanism is observed in reactions catalyzed by transferases. For example, hexokinase catalyzed phophorylation of glucose by ATP.

$$\text{Glucose} + ATP \xrightarrow{E} \text{Glucose-6-P} + ADP$$

2. The other possibility in bi-substrate reaction is that one substrate, A, binds to the enzyme and on reacting with it a product, P, is released and enzyme turns into a modified form, E′. The second substrate, B, comes in and reacts with E′ to yield second product, Q, and regeneration of enzyme, E occurs.
Thus,




(xxi)

The reactions following the above mechanism are called Ping-Pong or double-displacement reactions. This type of mechanism is observed in reactions catalyzed by aminotransferases. These enzymes catalyze the transfer of an amino group from an amino acid to an α-keto acid.

The products formed are a new amino acid corresponding to keto acid and a new keto acid corresponding to carbon skeleton of amino acid, as below:

$$\text{amino acid}_1 + \text{keto acid}_2 \xrightarrow{\mathbf{E}} \text{keto acid}_1 + \text{amino acid}_2$$

**Kinetics of first and zero order reactions**

Chemical kinetics is the study of rates of chemical reactions. Let us consider the following conversion reaction where a substrate, S, is converted into a product, P:

$$S \rightarrow P$$

We assume the conversion $S \rightarrow P$ as an elementary reaction and it is spontaneous and irreversible, i.e. conversion of P to S is very slow. The velocity, v, or rate of the reaction $S \rightarrow P$ is the amount of S consumed per unit time, t. So that

$$v = -\frac{d[S]}{dt}$$

The relationship between reaction rate, v, and concentration of reactant, [S], is the rate law and in this case

$$v = k\,[S]$$

From this it is clear that the rate is proportional to the concentration of S, and k is the proportionality constant or *rate constant.* It has the units of $(\text{time})^{-1}$, usually $\text{sec}^{-1}$, v is a function of [S] to the first power or in the terminology of kinetics, v is first order with respect to S. For such a reaction, the order of reactant is given by its exponent in the rate equation. Therefore, the reaction $S \rightarrow P$ is a first-order reaction. In reactions where the rate of reaction is independent of the reactant concentration then it is a case of zero-order reaction. A graphical presentation of zero-order and first-order reaction in the system depicted by $S \rightarrow P$ is presented in Fig. 3 (i & ii).

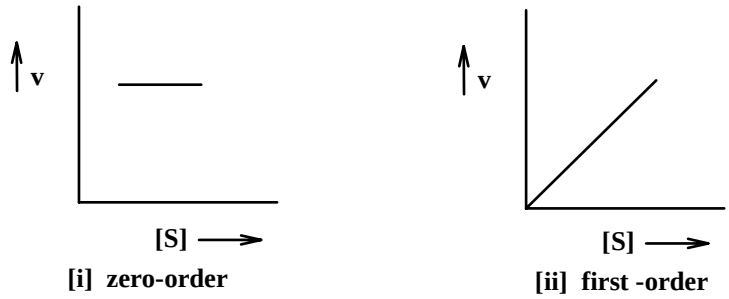



**Fig. 3: Graphical presentation of initial velocity against substrate concentration**

Extending these arguments to enzyme catalyzed reactions involving a single substrate we see quite different results (Fig. 4). At low [S], v is proportional to [S] as is normally expected of first-order reaction. However, as shown at high [S], v does not increase in proportion to the increase in [S] instead it levels off. Thus, at high [S], v is independent of [S] and attains a maximum value, called $V_{max}$. Since the rate of enzyme catalyzed reaction is no longer dependent on [S] at these high concentrations, therefore it can be stated that the enzyme catalyzed reaction, at these high [S], is obeying zero-order kinetics.

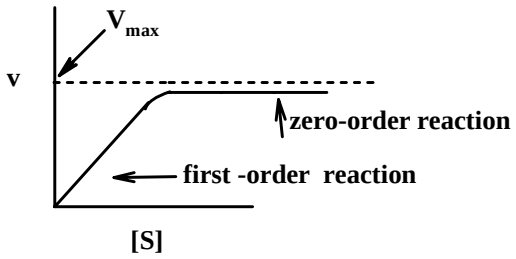



**Fig. 4: Graphical presentation of velocity against substrate concentration in an enzyme catalyzed reaction. The graphical segments depicting first-order and zero-order kinetics are marked**

### Effect of temperature

The rates of chemical reactions are highly dependent on temperature. The rate constant of a reaction is affected by temperature but there is no affect on the order of reaction. In enzyme catalyzed reactions as the temperature is increased the velocity of the reaction initially increases and then it may or /may not decrease immediately depending on temperature and time of assay. Therefore, the effect of temperature on the velocity of enzyme catalyzed reactions may be due to several causes. This could be due to the effect on the stability of enzyme and/or an effect on the velocity of breakdown of the ES complex (i.e. affect on $k_3$) which is determined by heat of activation of the reaction; it is also likely to affect affinity of enzyme substrate complex (i.e. effect on $k_2$ and $k_1$), and there are many more factors that are influenced.

If you measure velocity of an enzyme catalyzed reaction at different temperatures as a function of time and plot the data of your findings as v versus time, you may get the following graph (Fig.5).

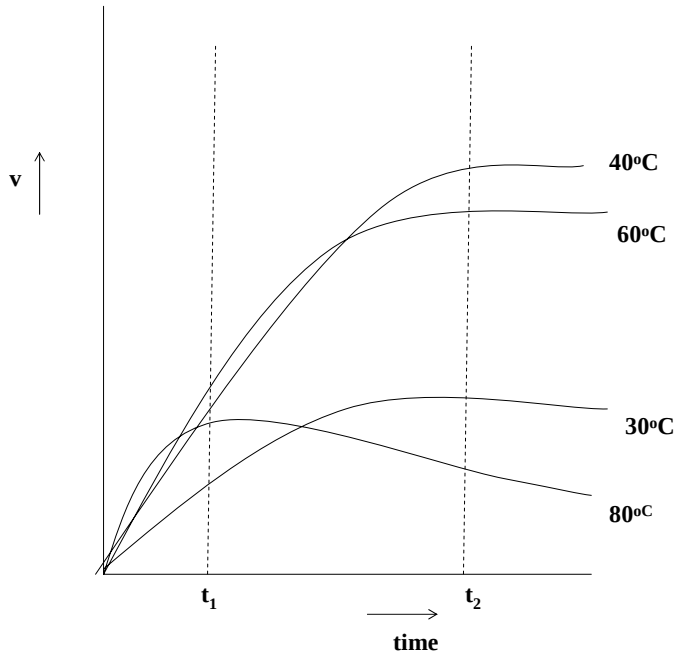



**Fig. 5: Temperature dependent progress of enzyme catalyzed reaction with time**

It is to be noticed from the Fig. 5 that although the initial velocity increases as the temperature is raised, but depending on temperature the velocity of the reaction dies down at different time points. Thus it is evident that the optimum temperature of an enzyme catalyzed reaction is a function of time. However, for most of the mammalian enzymes this value is $37^oC$ and for plants $30^oC$ with some exceptions. For example, muscle adenylate kinase is active at $100^oC$. Enzymes of thermophilic bacteria are also heat stable. If one measures the activity of an enzyme catalyzed reaction at different temperatures for a fixed duration and plots of a graph between the rate of reaction (v) and temperature of measurement (temp.) the type of curve shown in Fig. 6 is obtained.

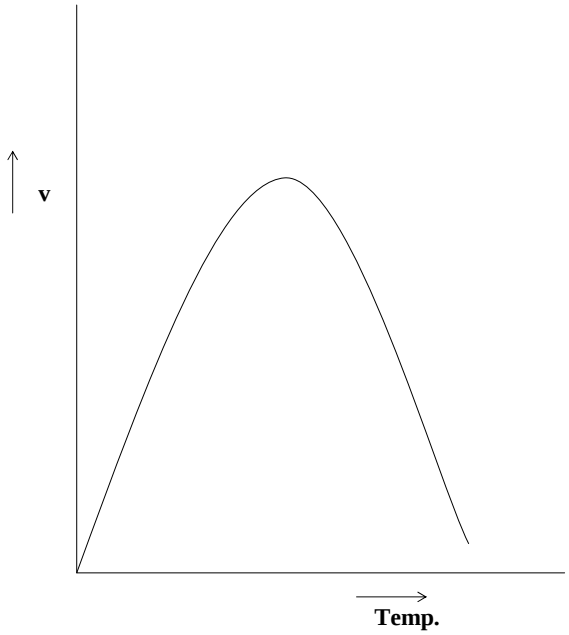



**Fig. 6: Variation in rate of enzyme catalyzed reaction as a function of temperature**

It is clear that an increase of temperature increases the rate of reaction. It is generally noticed that for a rise in temperature by $10^oC$ the rate of most of the enzymatic reactions is doubled. This is called $Q_{10}$ value of enzymes. Increase in temperature results in higher activation energy of reacting molecules which is followed by more collision and interaction between enzyme and substrate molecules. This results the reaction to proceed faster.

The shape of the curve (Fig. 6) is primarily determined by two factors: (i) the rate of activation of enzyme activity with increase of temperature and (ii) the rate of denaturation of enzyme protein with rise in temperature. So long the former rate is higher than the latter the apparent effect is the 'increased rate' as the net effect. On the other hand when rate of enzyme denaturation takes over the rate of activation then net rate registers a decrease. Use of such studies is made to determine the energy of activation of an enzyme catalyzed reaction.

The energy of activation as has described in previous chapter (Fig. 1) is the amount of free energy required to bring the reactants to the transition state. The temperature dependent measurements of initial velocity of an enzyme catalyzed reaction (as shown in Fig. 6) make use of Arrhenius equation to calculate the energy of activation.

**Effect of enzyme concentration**

When [S] is not limiting then under these conditions any increase in [E] has a direct effect on the rate of reaction. A plot of v versus [E] results a straight line (Fig. 7) which indicates a first order reaction. In the event of presence of any impurity or accumulation of high [P] or [S] becoming limiting the above linear relationship may not hold.

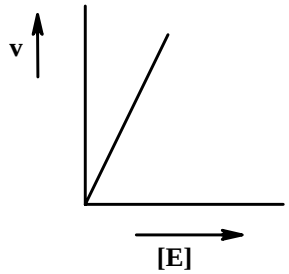



**Fig. 7: Effect of [E] on v**

**Effect of pH**

Enzyme activity is affected by the pH of the assay medium. A typical graph obtained on plotting v versus pH is shown in Fig. 8.

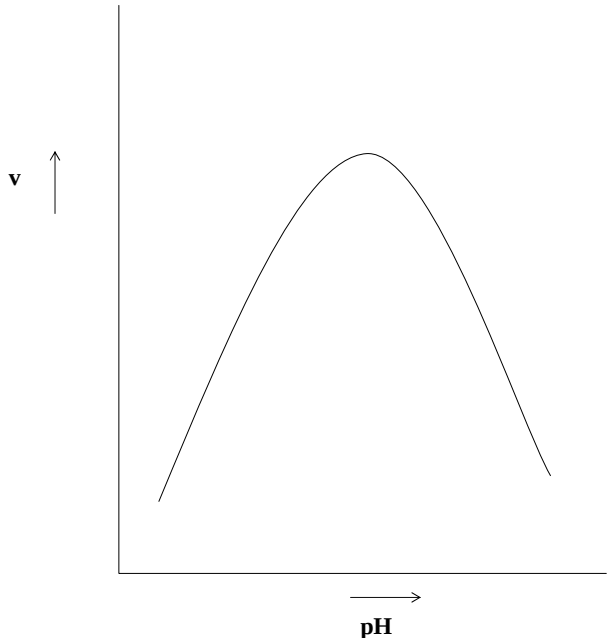



**Fig. 8: Effect of pH on velocity of enzyme catalyzed reaction**

The pH value(s) at which the obtained activity is maximum, is called the optimum pH of enzyme. This could be a particular pH value in case of most of the enzymes where shifting pH on either side of this value would lower the activity, v. In some cases there could be a pH range where the maximum value of v remains unaffected within the pH range. For example, the optimum pH of pepsin is 1.5 and that of trypsin is 6.8, whereas the enzyme

lysozyme has the optimum pH in the range from 5-8. As the side chains of amino acids present at the active site of enzymes are involved in the enzymic action and these may act as weak bases and acids depending on their state of ionization which in turn is dependent on the pH of the medium. As such, a change in pH may influence either binding of substrate to enzyme or the catalytic process or the both. These studies help to gather information regarding the mechanism of enzyme action. Sometimes a graph of v versus pH for an enzyme activity gives two or more peaks (Fig. 9). This suggests the existence of isozymes in the enzyme preparation.

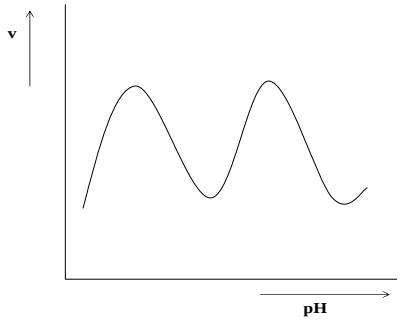



**Fig. 9: Enzyme activity showing more than one pH optima**

**Effect of inhibitors**

The inhibition (or decrease) in the rate of an enzyme catalyzed reaction in presence of a compound qualifies it to be called an inhibitor. The inhibitors are broadly classified into two categories: reversible inhibitors and irreversible inhibitors. The reversible inhibitors show the inhibitory effect by non-covalent association/ dissociation reaction with enzyme molecule. The irreversible inhibitors, as the name indicates, usually exert their effect by causing covalent alterations in the enzyme molecule thus making it ineffective as a catalyst. The net result of irreversible inhibitor or inhibition is to cause irretrievable decrease in the amount of active enzyme in a system.

Examples of:

(i) Irreversible inhibition – $CN^-$ reacting with metal containing enzymes, iodoacelate reacting with –SH groups of enzymes.

$$\text{E-SH} + \text{ICH}_2\text{COOH} \longrightarrow \text{HI} + \text{E-S-CH}_2\text{COOH}$$

Enzyme Iodoacetate Irreversibly inhibited enzyme

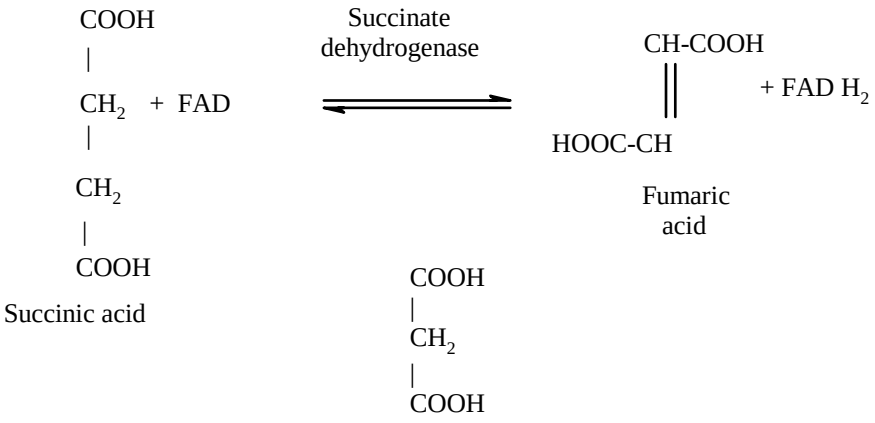
Sometimes addition of excess reducing material, e.g. cysteine, to the reaction system makes the inhibitory reaction reversible. This is seen e.g. in enzyme inhibition by chloromercuribenzoate

$$\text{E-SH} + \text{ClHg}_2\text{-C}_6\text{H}_4\text{COOH} \longrightarrow \text{HCl} + \text{E-S-HgC}_6\text{H}_4\text{-COOH}$$

Enzyme Chloromercuribenzoate (Reaction reversed by addition of excess reducing material, e.g. cysteine)

These are non-specific inhibitors. These inhibitors bind to –SH, -OH, -$COO^-$ groups of protein.

(ii) Reversible inhibition –the inhibitors have specific inhibitory function generally shown by substrate analogues reacting with enzymes. For example, succinate dehydrogenase is inhibited by malonate.



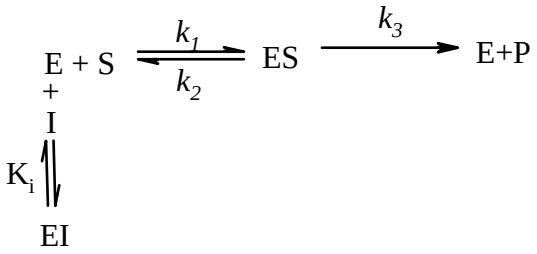

Malonic acid (inhibitor)

The similarity in the structure of substrate and inhibitor makes this type of inhibition as competitive inhibition.

***Reversible inhibition***

The reversible inhibition of enzymes is of three types: competitive, non-competitive and uncompetitive

***Competitive inhibition*:** Considering the following reaction




(xxii)

it can be stated that the inhibitor I competes with substrate **S**, to bind reversibly with the enzyme at active site. $K_i$ is the dissociation constant for inhibitor binding with enzyme and is defined as,

$$K_i = \frac{[E][I]}{[EI]} \qquad \text{(xxiii)}$$

where [I] and [EI] represent inhibitor and enzyme inhibition complex concentrations, respectively. When [S] is increased relative to [I], the equilibrium of the reaction

$$E+I \rightleftharpoons EI$$

shifts in the direction of dissociation of **EI** so that the liberated enzyme is able to bind with more **S**. Converse is also true when [I] is more than [S].

One can solve the rate equation for the above, and accordingly at any time

$$[E]_t = [E] + [ES] + [EI]$$

where $[EI] = \frac{[E][I]}{K_i}$ from equation (xxiii). Now, by proceeding in a manner similar to that of substrate binding to enzyme to derive Michaelis-Menten equation (xiv), we get a similar equation of the form

$$v = \frac{V_{max}[S]}{[S] + K_m\left(1 + \frac{[I]}{K_i}\right)} \qquad \text{(xxiv)}$$

On comparing this with Michaelis-Menten equation (xiv), it is observed that the term, $K_m$, in the denominator, in presence of competitive inhibitor is increased by a factor $\left(1 + \frac{[I]}{K_i}\right)$ thereby suggesting that v is less in presence of the inhibitor. Since the two equations (xiv) and (xxiv) are nearly identical therefore the corresponding double reciprocal equations as shown below (in the absence and presence of I) compared as straight lines under Linewever-Burk plots (Fig. 10).

$$\frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \qquad \text{(xv)}$$

$$\frac{1}{v} = \frac{K_m(1 + [I]/K_i)}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \qquad \text{(xxv)}$$

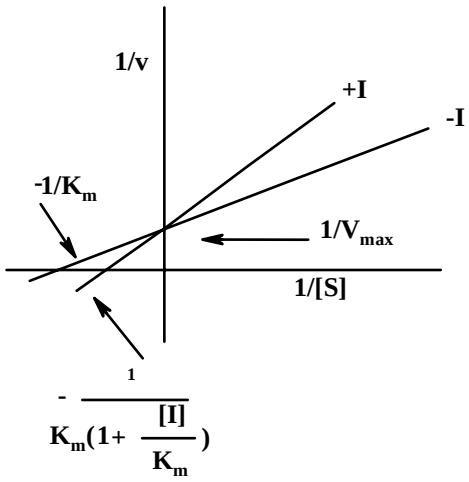



**Fig. 10: Comparison of double reciprocal plots in presence of a competitive inhibitor**

It is thus clear from Fig.10 that in presence of I the intercept value in x-axis is decreased suggesting the increase in apparent $K_m$ (or $K_m^{app}$). At the same time the value of $1/V_{max}$ in y-

axis remains unaffected, thus leading to the conclusion that the $V_{max}$ value in presence of competitive inhibitor, I, does not change.

Experimentally, if one plots a graph between v and [S] in absence and presence of **I** (Fig.11), it shows that the rate of reaction is decreased in presence of **I** but $V_{max}$ in attainable at high [S]. The value of $K_m^{app}$ is higher in presence of I.

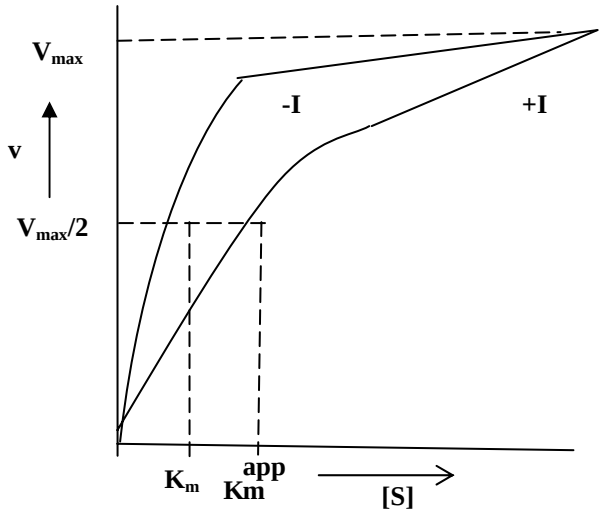
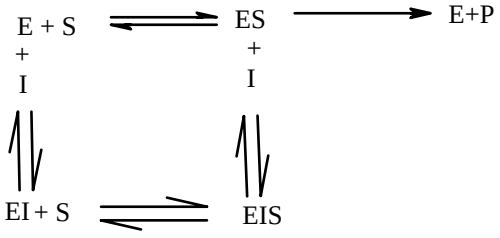



**Fig. 11: Comparison of the course of velocity as a function of increase in [S] in absence and presence of a competitive inhibitor**

The most common example of competitive inhibition is of succinate dehydrogenase inhibition by malonate.

***Non-competitive inhibition:*** A non-competitive inhibitor can combine with both **E** and **ES** as shown in the following equation




(xxvi)

In this case, the catalytic activity of enzyme is destroyed by inhibitor either by binding to the catalytic site or by resulting in a conformational change that affects the catalytic site, but this does not affect substrate binding. So inhibitor and substrate combine independently.

The Michaelis-Menten rate equation for such a situation can be obtained by proceeding exactly as earlier. The equation is

$$v = \frac{V_{max}[S]/(1+[I]/K_i)}{K_m + [S]} \qquad \text{(xxvii)}$$

and the corresponding double reciprocal equation obtained in similar fashion is

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left(1+\frac{[I]}{K_i}\right)\frac{1}{[S]} + \frac{1}{V_{max}}\left(1+\frac{[I]}{K_i}\right) \qquad \text{(xxviii)}$$

Lineweaver-Burk plots in absence and presence of non-competitive inhibitor compare as shown in Fig.12.

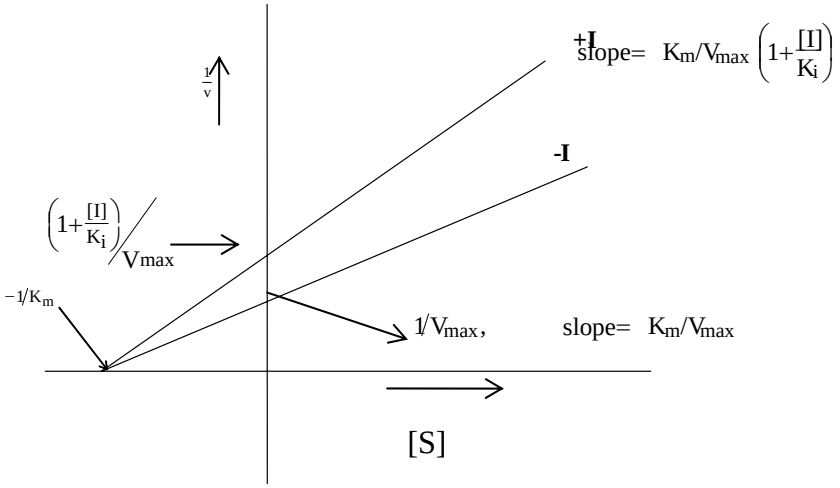


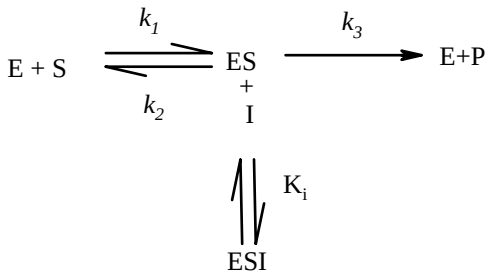

**Fig. 12: Comparison of Lineweaver-Burk double reciprocal plots in absence and presence of a non-competitive inhibitor**

As is seen from the Figure that there is no change in the value of intercept on x-axis in presence of inhibitor, but intercept value on y-axis is increased. It is thus evident that a non-competitive inhibitor does not change $K_m$ but decreases $V_{max}$. It should be noted that unlike competitive inhibition, non-competitive inhibition cannot be overcome by increasing the [S].

**Example:** Inhibition of O-diphenol oxidase by phenylthiourea. Some compounds and heavy metal ions which bind to SH groups of the enzymes sometimes give non-competitive kinetics. Similar kinetics is also obtained in case of irreversible inhibition. This uncertainty is due to the effect of reduction in the amount of active enzyme in both the cases.

***Uncompetitive inhibition***

An uncompetitive inhibitor, unlike competitive and non-competitive inhibitors, does not bind with **E**. It binds only with **ES** complex, as shown in the following reaction:




(xxix)

It is thus clear that this inhibitor binds at a site which is different from the active site. ESI is a dead end complex, it cannot form product. It has only one option to dissociate into ES and I. Under these conditions the Michaelis-Menten rate equation applicable has the form

$$v = \frac{\left( V_{max} \Big/ 1 + \frac{[I]}{K_i} \right)[S]}{\left( K_m \Big/ 1 + \frac{[I]}{K_i} \right) + [S]} \qquad \text{(xxx)}$$

The corresponding double reciprocal equation obtained by proceeding as stated earlier has the following form:

$$\frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{(1 + [I]/K_i}{V_{max}} \qquad \text{(xxxi)}$$

Lineweaver-Burk plots in absence and presence of inhibitor compare as shown in the Fig.13.

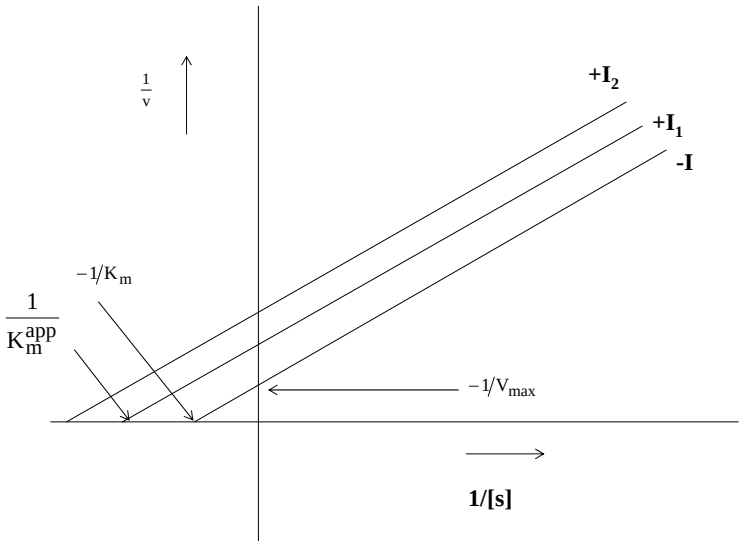



**Fig. 13: Comparison of Linewever-Burk double reciprocal plots in absence and presence of uncompetitive inhibitor. $I_2 > I_1$. $K_m^{app} = (1+[I]/K_i)/K_m$**

It is evident from the graph that the straight-lines are obtained both in absence and presence of inhibitor. These lines are all parallel indicating that there is no change in slope of double-reciprocal plot in presence of an uncompetitive inhibitor. Since the straight lines are all parallel even at different concentrations of inhibitor thus indicating that values both in x-axis and y-axis are affected by the same factor. The value of this change can be calculated at any given [I]. Since the value of intercepts as shown in Fig.13 increased as [I] is increased, therefore it is conclusive that the presence of uncompetitive inhibitor lowers the values of $V_{max}$ and apparent $K_m$ $\left(K_m^{app}\right)$. This change in values is by a factor $1 \Big/ 1 + \frac{[I]}{K_i}$ in both cases.

A comparison in the values of kinetic constants in absence and presence of various inhibitors is presented in Table 1.

**Table 1: Comparison of various intercepts of Linewever-Burk plots in absence and presence of different inhibitors**

| Nature of inhibitor | Intercept on x-axis | Intercept on y-axis | Slope of straight line |
|---|---|---|---|
| No inhibitor (control) | $1/K_m$ | $1/V_{max}$ | $K_m/V_{max}$ |
| Competitive | $1\Big/ K_m\left(1+\frac{[I]}{K_i}\right)$ | $1/V_{max}$ | $K_m\left(1+\frac{[I]}{K_i}\right)\Big/V_{max}$ |
| Non-competitive | $1/K_m$ | $\frac{1+[I]/K_i}{V_{max}}$ | $\frac{Km\left(1+\frac{[I]}{K_i}\right)}{V_{max}}$ |
| Uncompetitive | $\frac{1+[I]/K_i}{K_m}$ | $\frac{1+[I]/K_i}{V_{max}}$ | $K_m/V_{max}$ |

**Allosteric enzymes**

It has been observed earlier that the rate of an enzyme catalyzed reaction was affected by binding of S, and non-substrate ligands called inhibitors (or even activators). The hyperbolic graph (Fig.1) indicated that the substrate binding to the enzyme was 'isostearic'. This is primarily because the active sites of enzymes do not interact among themselves, meaning thereby that if the enzyme is multimeric then binding of a S molecule to one binding site does not affect the activity of the neighbouring sites. On the other hand, there are certain enzymes involved in regulation of activity of a metabolic pathway exhibiting a different behaviour. When one studies e.g., the effect of substrate concentration on the velocity of the catalyzed reaction then in this case the shape of graph obtained is given in Fig. 14 (compare with hyperbolic binding given in broken line). This shows that at very low concentration of S the rate of reaction is not proportional to its concentration, unlike that of hyperbolic binding, but after a certain [S] there is a quantum jump in the rate of reaction. In this case the S acts as activator and its binding on the enzyme molecule makes the other binding sites to interact among themselves. Initially, when the binding takes place at a site other than the active site, called allosteric site, then this binding initiates such conformational changes at the active site that the subsequent binding of S molecules at active site becomes more favourable resulting the higher rate of reaction.

So, one important behavioural difference between two types of enzymes is that in case of allosteric enzymes binding sites interact thereby, increase the ability of the other active sites to bind more substrate. This is called positive cooperativity. In case of enzymes giving normal Michaelis-Menten kinetics this does not take place. Besides, substrate, there are substances which on binding to enzymes show activation and is called positive cooperativity or allosteric activation (Fig. 15). Substances resulting in positive cooperativity are called allosteric activators. Similarly, there are substances which on binding to enzyme decreas its ability to bind substrate (Fig.16). This is called negative cooperativity or allosteric inhibition. These substances are called allosteric inhibitors.

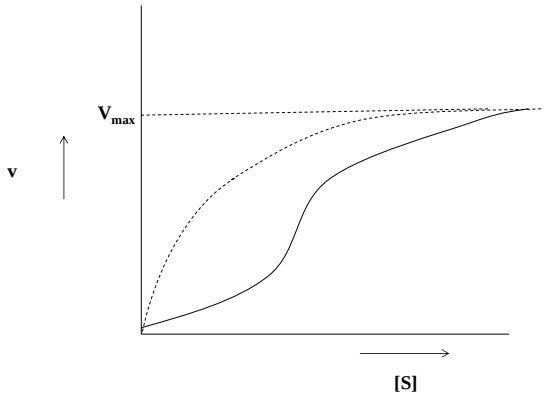



**Fig. 14: Substrate saturation curve for an allosteric enzyme catalyzed reaction. The broken curve indicates the course of hyperbolic binding**

The enzyme binding molecules such as substrate, activator and inhibitor are called ligands. Besides substrate, the other molecules affecting enzyme activity are also called 'Effector molecules'. If the binding of one ligand to the enzyme affects subsequent binding of its own type then it is called homotrophic effect or homoallostery, but when the binding of different ligand is affected then it is called heterotropic effect or heteroallostery.

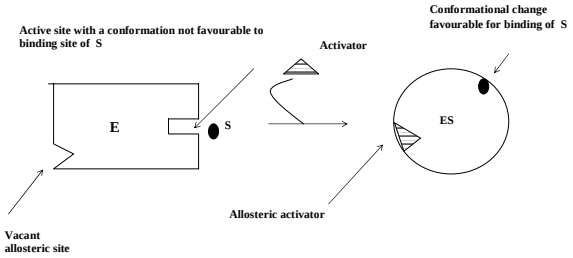



**Fig. 15: Diagramatic depiction of transitional conformational change at the active site caused by an allosteric activator**

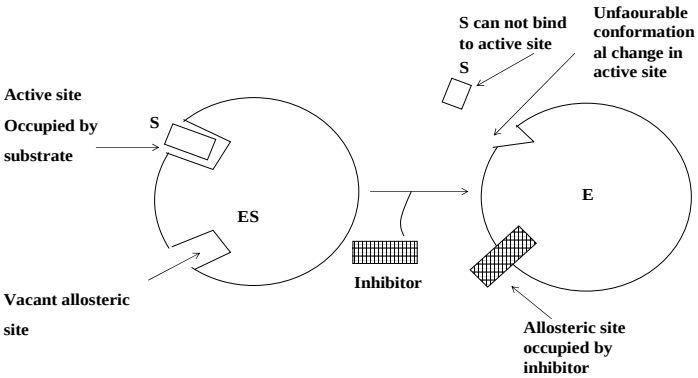



**Fig. 16: Diagramatic depiction of transitional conformational change at the active site caused by an allosteric inhibitor**

As you have seen that allosteric effects (kinetics) are more complex than Michaelis-Menten kinetics therefore it is to be mentioned that there exists complexity in structure of these proteins. All allosteric enzymes have a quaternary structure and these proteins are made up of catalytically active smaller subunits called protomers. These protomers are linked to each other by weak interactions such as hydrogen bonds and hydrophobic interactions forming a multimeric structure which is essential for the phenomenon of allostery. As these subunits are attached by weak interactions so they can easily detach and reattach themselves. It is therefore to be expected that in solution there exists an equilibrium between individual units and a complete enzyme.

In general the allosteric enzymes show following features:

1. The graph between v and [S] is sigmoidal (S-shaped).
2. The enzyme is sensitive to 'effector molecules'.
3. The enzyme protein has 'quaternary structure'.
4. The phenomenon of allostery is sensitive to mild denaturation.

## Suggested Readings


1. Dixon, M. & Webb, E.C., Enzymes 3rd ed., Longman Group, 1979.
2. Marangoni, A.G., Enzyme Kinetics, Wiley Interscience, 2003.
3. Mathews, C.K. & Van Holde, H.E., Biochemistry, The Benjamin/ Cummings, 1990.
4. Metzler, D.E., Biochemistry: A Chemical Reaction of Living Cells, Vol.I & II, 2nd ed., Harcourt Academic Press, 2001.
5. Palmer, T., Enzymes: Biochemistry, Biotechnology, Clinical Chemistry, EWP, 2004.
6. Voet, D. & Voet, J.G. , Biochemistry 3rd ed., John-Wiley & Sons, 2004.