UNIT 6

ENZYME INHIBITION

Structure

6.1 INTRODUCTION

In the previous unit, you have learnt about the kinetics of enzyme catalyzed reactions. In this unit we will introduce you to important class of molecules called “enzyme inhibitors”. Enzyme inhibitors are the molecules that regulate the activity of enzymes in the cell. Inhibitors alter the catalytic action of the enzyme; as a result slows down the enzyme activity, or in some cases, close the catalysis. The study of these inhibitors provides wealth of information on the working of enzymes and their mechanism. The blockage of enzyme activity can lead to several changes such as correction of metabolic imbalance or killing of a bacteria or pathogen. Therefore, many of the drug molecules are enzyme inhibitors and their discovery as well as improvement has been a major area of research for biochemistry and pharmacology.

Objectives

This unit will give you an overview about different types of enzyme inhibitors. After studying this unit, you will be able to:

- determine types of enzyme inhibitor;
- find out how inhibitor interacts with the enzyme;
- state the role of inhibitor affecting enzyme kinetic parameters;
- distinguish between reversible and irreversible inhibitors; and
- explain the role of enzyme inhibitors and their classification according to nature and function.

6.2 ENZYME INHIBITION-REVERSIBLE AND IRREVERSIBLE

Molecules that bind to the enzymes and cause a decrease in their activity are called enzyme inhibitors. These molecules either bind at the active site of enzyme thereby preventing the substrate molecule to bind to the enzyme or they may inhibit the catalytic activity of enzyme. You should know that many of these molecules perform several regulatory roles in the metabolism. Some of them are used as herbicides or pesticides. Most of the drug molecules also act as enzyme inhibitors. Natural enzymes inhibitors e.g. poison are a part of the defense mechanisms in wild life animals.

Enzyme inhibitions are mainly classified into two types:

a) Reversible Inhibition

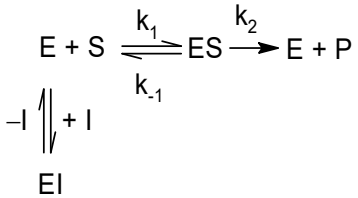
b) Irreversible Inhibition

6.3 REVERSIBLE INHIBITION

In this unit, we will discuss about the inhibition of simple single substrate enzyme catalyzed reactions. In reversible enzyme inhibition, loss of the enzyme activity due to inhibitory molecule is reversible. Enzyme activity gets restored on the removal of inhibitor. These inhibitors bind non-covalently and give rise to different kinds of inhibition. Multiple weak bonds between the inhibitor and the enzyme combine to give strong binding which prevents the formation of product. They can be easily removed by dilution or dialysis to restore full enzyme activity. Reversible inhibitors tend to form equilibrium with an enzyme leading to certain level of inhibition.

6.3.1 Competitive Inhibition

There is a direct competition between the substrate and the inhibitor for the binding site of enzyme in a competitive inhibition. If inhibitory molecule is bound to the enzyme then substrate will not be able to bind it and vice versa. In such kind of scenario, inhibition can be overcome by increasing substrate concentration in comparison to inhibitor. Therefore, the maximum velocity (V_{max}) of the reaction will remain unchanged while K_m will be decreased in a competitive inhibition. Most of the competitive inhibitors are structurally similar to the substrate molecules.



The dissociation constant (K_i) or inhibitor constant for the reaction is

$$\frac{[E][I]}{[EI]} = K_i \; or \; \frac{[E][I]}{[K_i]} = [EI] \qquad \text{..... equation 1}$$

I = Inhibitor

EI = Enzyme bound to Inhibitor

E = Free Enzyme

E_o = Total enzyme

ES = Enzyme bound to substrate

As you know in the previous unit, using steady state assumption, Michaelis-Menten constant for the rate of reaction is given by the following equation.

$$\frac{[E][S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m \qquad \text{..... equation 2}$$

$$E_o = [E] + [ES] + [EI] \qquad \text{..... equation 3}$$

Substituting the value of [*EI*] as given in equation 1

$$E_o = [E] + [ES] + \frac{[E][I]}{K_i}$$

$$E_o = [E](1 + \frac{[I]}{K_i}) + [ES]$$

$$E = \frac{[E_o] - [ES]}{(1 + \frac{[I]}{K_i})}$$

Substituting the value of [*E*] in equation 2:

$$\frac{([E_o] - [ES])[S]}{(1 + [\frac{I}{K_i}])[ES]} = K_m$$

$$\frac{([E_o][S])}{[ES]} - [S] = K_m(1 + [\frac{I}{K_i}])$$

$$\frac{([E_o][S])}{[ES]} = K_m(1 + [\frac{I}{K_i}]) + [S]$$

$$\frac{([E_o][S])}{K_m(1 + [\frac{I}{K_i}]) + [S]} = [ES]$$

$$v_o = k_2[ES] \qquad \text{..... equation 4}$$

(Ref: Michaelis Menten equation in the previous Unit-4, Block-2)

Putting the value of [*ES*] in equation 4

$$v_o = \frac{k_2[E_o][S]}{K_m(1 + [\frac{I}{K_i}]) + [S]}$$

$$V_{max} = k_2 [E_0]$$ ..... equation 5

(Ref: Michaelis Menten equation in the previous Unit-4, Block-2)

$$v_o = \frac{V_{max}[S]}{K_m(1+[\frac{I}{K_i}])+[S]}$$

Since inhibitor concentration is generally of the same order of magnitude as the substrate concentration and much higher than the enzyme concentration [*I*] ~ [I_o] just as [*S*] ~ [S_o]. The above equation can be expressed as

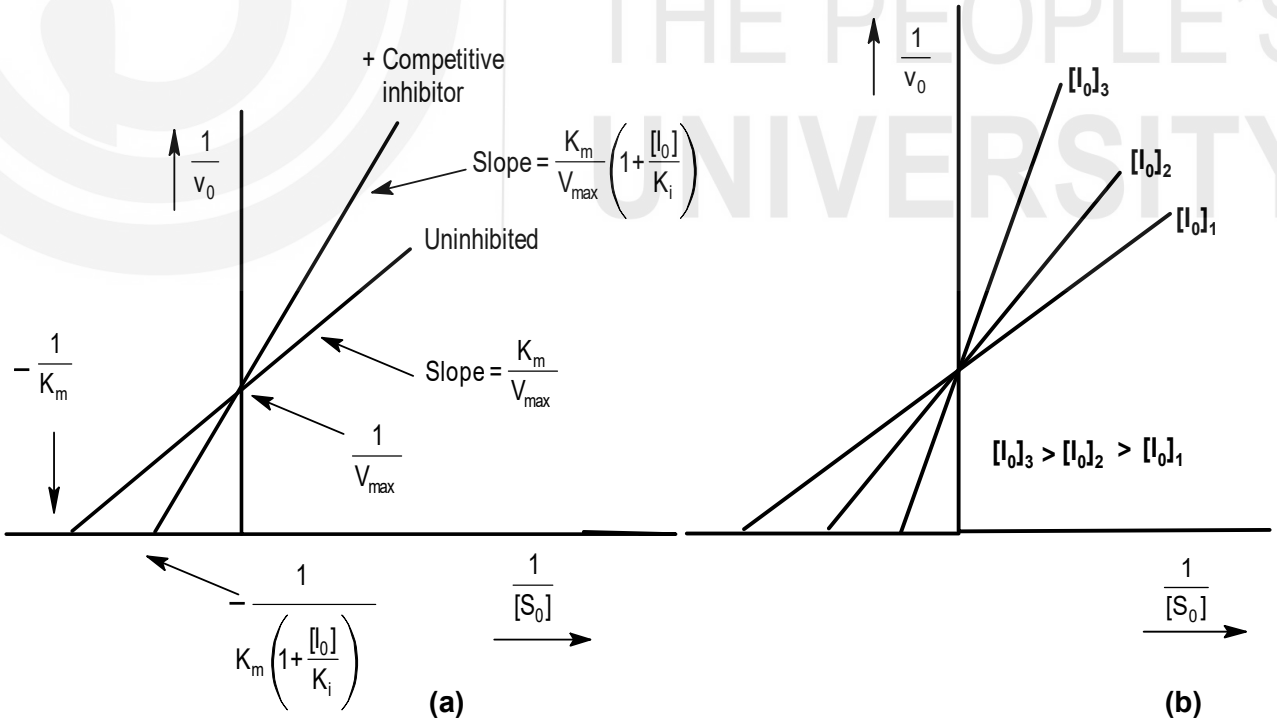
$$v_o = \frac{V_{max}[S_o]}{K_m(1+[\frac{I_o}{K_i}])+[S_o]}$$ ..... equation 6

This equation is similar to Michaelis- Menten equation and the K_m is increased by a factor

$$K_m(1+[\frac{I_o}{K_i}]) \text{ or } \quad K'_m = K_m(1+[\frac{I_o}{K_i}])$$

The Lineweaver- Burk equation and plot (Fig. 6.1) of competitive inhibition will be:

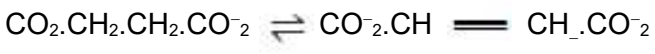
$$\frac{1}{v_o} = \frac{K'_m}{V_{max}[S_o]} + \frac{1}{V_{max}}$$



Fig,6.1(a): Lineweaver-Burk plot for competitive inhibitor (b) Plot at fixed enzyme concentration but different inhibitor concentrations

Examples of Competitive Inhibition: Succinate dehydrogenase (SDH) enzyme converts succinate to fumarate. Succinate is competitively inhibited by

malonate. Malonate ($CO^{-}_{2}.CH_2.CO^{-}_{2}$) is structurally similar to fumarate having two carboxyl groups. Similarly, inhibition of xanthine oxidase enzyme by allopurinol leads to decreased formation of uric acid and act as a remedy for gout.



Succinate **Fumarate**

SAQ 1

Draw Lineweaver- Burk plot for different inhibitory concentrations of a competitive inhibitor at the fixed enzyme concentrations.

.....

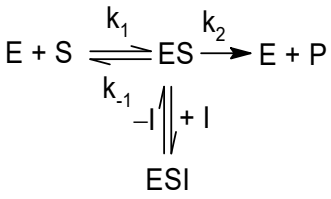
.....

.....

.....

6.3.2 Un-Competitive Inhibition

Uncompetitive inhibitors are those inhibitors who do not compete with the substrate and do not bind to the free enzyme. They bind to the enzyme substrate complex thereby forming a dead end complex. It is quite likely that binding of substrate to the enzyme may cause some conformational changes and reveals an inhibitor binding site where inhibitor can bind. This type of inhibition cannot be overcome by excess of substrate. Both K_m and V_{max} gets changed in this type of inhibition pattern.



The inhibitor constant K_i is as given below:

$$\frac{[ES][I]}{[ESI]} = K_i \quad \text{or} \quad \frac{[ES][I]}{Ki} = [ESI] \qquad \text{.....equation 7}$$

I = Inhibitor

ESI = Enzyme bound to Inhibitor and substrate

E = Free Enzyme

ES = Enzyme bound to substrate

E_o = Total enzyme

Using steady state assumption, Michaelis- Menten constant for the rate of reaction is given by

$$\frac{[E][S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m \qquad \text{..... equation 2}$$

$$E_o = [E] + [ES] + [ESI] \qquad \text{..... equation 8}$$

Substituting the value of [*ESI*] as given in equation 7

$$E_o = [E] + [ES] + \frac{[ES][I]}{K_i}$$

$$E_o = [E] + [ES](1 + \frac{[I]}{K_i})$$

$$E = [E_o] - [ES](1 + \frac{[I]}{K_i})$$

Substituting the value of *E* in equation 2

$$\frac{[E_o] - [ES](1 + \frac{[I]}{K_i})][S]}{[ES]} = K_m$$

$$\frac{[E_o][S]}{[ES]} - (1 + \frac{I}{K_i})[S] = K_m$$

$$\frac{[E_o][S]}{[ES]} = K_m + (1 + \frac{I}{K_i})[S]$$

$$\frac{[E_o][S]}{K_m + (1 + \frac{I}{K_i})[S]} = [ES]$$

$$v_o = k_2[ES] \qquad \text{..... equation 4}$$

(Ref: Michaelis Menten equation section in the previous Unit 4, Block 2)

Putting the value of [*ES*] in equation 4

$$v_o = k_2 \frac{[E_o][S]}{K_m + (1 + \frac{I}{K_i})[S]}$$

$$V_{max} = k_2[E_0]$$ …… equation 5

(Ref: Michaelis Menten equation section in the previous Unit-4, Block-2)

Since inhibitor concentration is generally of the same order of magnitude as the substrate concentration and much higher than the enzyme concentration [*I*] ~ [I_o] just as [*S*] ~ [S_o]. Thus continuing similarly, above equation can be expressed as

$$v_o = \frac{V_{max}[S_o]}{K_m + (1 + \frac{I_o}{K_i})[S_o]}$$ …… equation 9

Dividing the numerator and denominator by $(1 + \frac{[I_o]}{K_i})$ gives

$$v_o = \frac{\frac{V_{max}[S_o]}{(1 + \frac{I_o}{K_i})}}{\frac{Km}{(1 + \frac{I_o}{K_i})} + [S_o]}$$

The above equation is similar to Michaelis-Menten equation. However, the constants K_m and V_{max} are changed as following:

$$V'_{max} = \frac{V_{max}}{(1 + \frac{I_o}{K_i})}$$

and

$$K'_m = \frac{K_m}{(1 + \frac{I_o}{K_i})}$$

The Lineweaver-Burk equation in the presence of an uncompetitive inhibitor is:

$$\frac{1}{v_o} = \frac{K'_m}{V'_{max}[S_o]} + \frac{1}{V_{max}}$$

The slope of a Lineweaver-Burk plot will remain unchanged as given by the following equation:

$$\frac{K'_m}{V'_{max}} = \frac{\frac{K_m}{(1 + \frac{[I_o]}{K_i})}}{\frac{V_{max}}{(1 + \frac{[I_o]}{K_i})}} = \frac{K_m}{V_{max}}$$

The x-intercept as well as y-intercept of the Lineweaver-Burk plot in the presence of an uncompetitive inhibitor (Fig. 6.2) will change but the slope will remain unaltered.

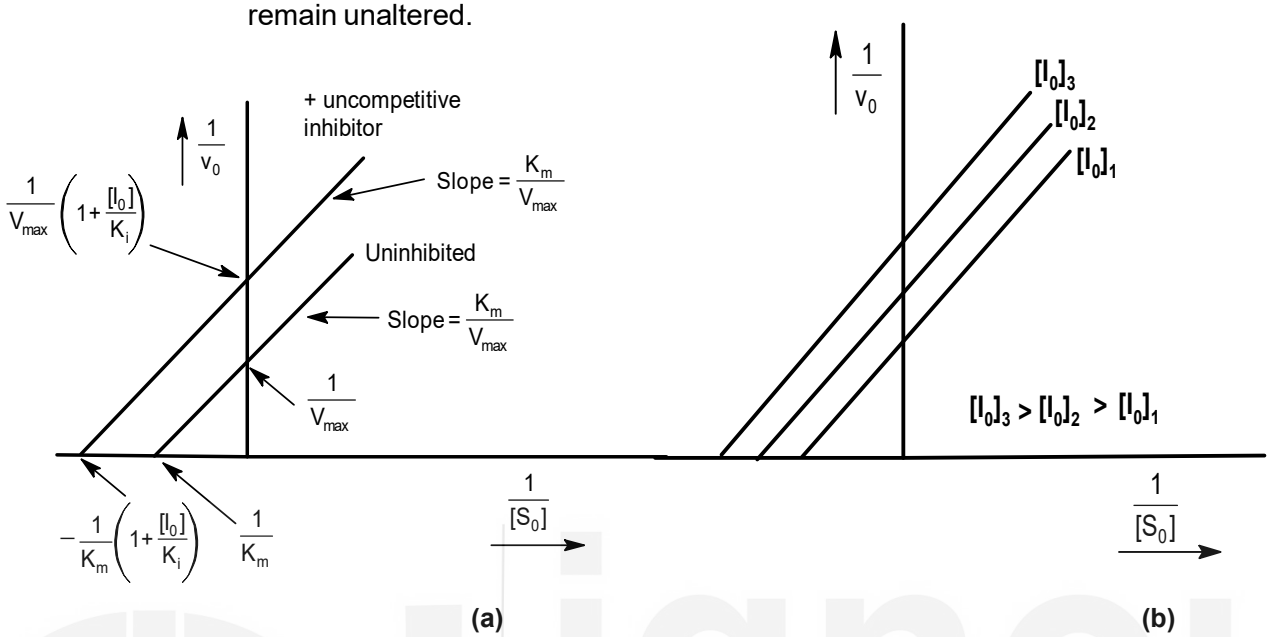


Fig.6.2 (a): Lineweaver-Burk plot of enzyme inhibited by an uncompetitive inhibitor (b) Plot at fixed enzyme concentration but different inhibitor concentrations.

Example of Uncompetitive Inhibitor: Enzyme aryl sulphatase is uncompetitively inhibited by hydrazine. Generally uncompetitive inhibition pattern is not seen in single substrate reactions but several bi-substrate reactions shows this kind of pattern.

SAQ 2

Draw Lineweaver- Burk plot for different inhibitory concentrations of an uncompetitive inhibitor at the fixed enzyme concentrations.

.....

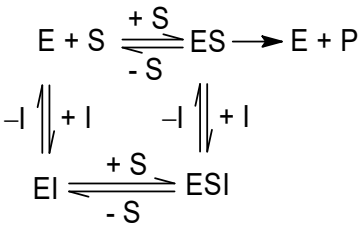
.....

.....

.....

6.3.3 Non-Competitive Inhibition

Non-competitive inhibitory molecules react with the enzyme at a site other than the active site. The inhibitor can bind to the free enzyme or when it is bounded to the substrate molecule. It destroys the catalytic activity of the enzyme. The impact of inhibitor can not be overcome by increasing the substrate concentration. Apparently, K_m value will not be changed but V_{max} will change. Let us consider a simple situation involving single substrate reaction



Since *ES* can be formed via two alternative routes and considering the fact that inhibitory rates could vary in binding the free enzyme or when it is bound to the substrate the situation will become quite complex. In order to simplify the above model, we assume that substrate binding will have no effect on the binding of inhibitor. The dissociation constant (inhibitor constant) for both the reactions $E + I \rightleftharpoons EI$ and $ES + I \rightleftharpoons ESI$ will then be the same. The total enzyme concentration will be reduced in the presence of inhibitor and it will lead to decrease in V_{max}. The value of K_m will not change because binding of inhibitor does not affect the binding of substrate to the enzyme or vice versa.

In the presence of non-competitive inhibitor

$$\frac{[E][I]}{[EI]} = K_i = \frac{[ES][I]}{[ESI]} \qquad \text{..... equation 10}$$

$$E_o = [E] + [ES] + [EI] + [ESI] \qquad \text{..... equation 11}$$

Substituting the value of [*EI*] and [*ESI*] as given in equation 10

$$E_o = [E] + [ES] + \frac{[E][I]}{K_i} + \frac{[ES][I]}{K_i}$$

$$E_o = [E] + \frac{[E][I]}{K_i} + [ES] + \frac{[ES][I]}{K_i}$$

$$E_o = [E](1 + \frac{[I]}{K_i}) + [ES](1 + \frac{[I]}{K_i})$$

$$E_o = ([E] + [ES])(1 + \frac{[I]}{K_i})$$

$$E = \frac{[E_o]}{(1 + \frac{[I]}{K_i})} - [ES]$$

Substituting the value of *E* in equation 2

$$\frac{(\frac{[E_o]}{(1 + \frac{[I]}{K_i})} - [ES])[S]}{[ES]} = K_m$$

$$\frac{(\frac{[E_o][S]}{(1+\frac{[I]}{K_i})} - [ES][S])}{[ES]} = K_m$$

$$\frac{\frac{[E_o][S]}{(1+\frac{[I]}{K_i})} - [ES][S](1+\frac{[I]}{K_i})}{[ES]} = K_m$$

$$\frac{[E_o][S]}{[ES]} - [S](1+\frac{[I]}{K_i}) = K_m(1+\frac{[I]}{K_i})$$

$$\frac{[E_o][S]}{[ES]} = K_m(1+\frac{[I]}{K_i}) + [S](I+\frac{[I]}{K_i})$$

$$\frac{[E_o][S]}{[ES]} = (K_m + S)(I+\frac{[I]}{K_i})$$

$$\frac{[E_o][S]}{(K_m+[S])(1+\frac{[I]}{K_i})} = [ES]$$

$v_o = k_2[ES]$ ……. equation 4

(Ref: Michaelis Menten equation section in the previous Unit-4, Block-2)

Putting the value of [*ES*] in equation 4

$$v_o = k_2 \frac{[E_o][S]}{(K_m+[S])(1+\frac{[I]}{K_i})}$$

$V_{max} = k_2[E_o]$ ……. equation 5

(Ref: Michaelis Menten equation section in the previous Unit-4, Block-2)

Continuing similarly as in previous sections,

$$v_o = \frac{V_{max}[S_o]}{(K_m+[S_o])(1+\frac{[I_o]}{K_i})}$$ ……. equation 12

This is similar to the Michaelis-Menten equation with V_{max} divided by a factor

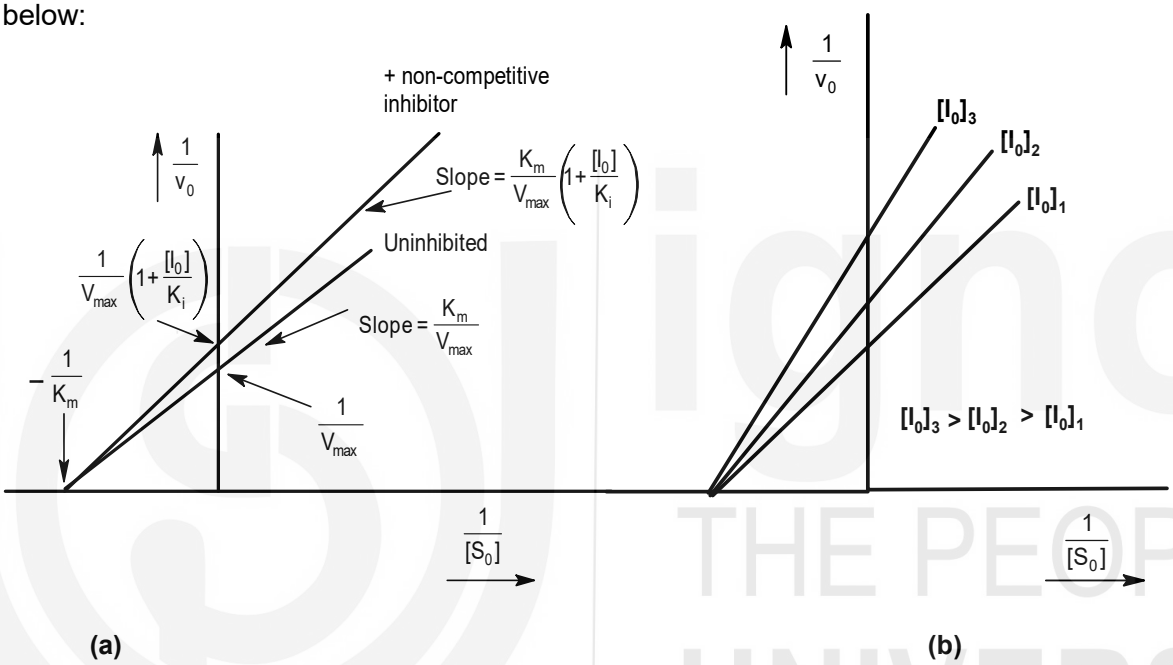
$$(1+\frac{[I_o]}{K_i})$$

$$V'_{max} = \frac{V_{max}}{\left(1+\frac{I_o}{K_i}\right)}$$

The Lineweaver-Burk equation in the presence of a noncompetitive inhibitor is:

$$\frac{1}{v_o} = \frac{K_m}{V'_{max}[S_o]} + \frac{1}{V'_{max}}$$

The Lineweaver-Burk plot (Fig. 6.3) of a non-competitive inhibitor is as given below:



Fig, 6.3 (a): Lineweaver-Burk plot for non-competitive inhibitor (b) Plot at fixed enzyme concentration but different inhibitor concentrations.

Example of Noncompetitive Inhibition: Chymotrypsin is noncompetitively inhibited by H^+. Heavy metal ions and small organic molecules bound to the –SH groups of the cysteine moiety in an enzyme can also lead to the noncompetitive inhibition.

SAQ 3

Draw Lineweaver- Burk plot for different inhibitory concentrations of a noncompetitive inhibitor at the fixed enzyme concentrations.

.....

.....

.....

.....

.....

6.3.4 Mixed Inhibition

Mixed inhibition occurs when an inhibitor may binds to the enzyme as well as enzyme substrate complex. However, the affinity with which inhibitor binds to two different states may varies. It may bind with greater affinity when the enzyme is free than the enzyme substrate complex or vice versa. The inhibition is a mixture of competitive inhibition and uncompetitive inhibition. If the inhibitor has equal affinity for both the states of enzyme (free as well as bound to the substrate), mixed inhibition will become non-competitive inhibition. In mixed inhibition, inhibitor binds to the enzyme at a site different from the active site, the site where substrate binds. As you know, there are two processes by which inhibitor can bind to the enzyme.

$E + I \rightleftharpoons EI$ (inhibitor constant K_i)

and

$ES + I \rightleftharpoons ESI$ (inhibitor constant K_1)

Therefore

$$\frac{[E][I]}{[EI]} = K_i$$

and

$$K_1 = \frac{[ES][I]}{[ESI]}$$

As you know in a single substrate reaction,

$$K_m = \frac{[E][S]}{[ES]}$$

$$E_o = [E] + [ES] + [EI] + [ESI] \quad \text{.....equation 13}$$

Since K_i and K_1 are not similar, they are different. So

$$E_o = [E] + [ES] + \frac{[E][I]}{K_i} + \frac{[ES][I]}{K_1}$$

$$E_o = [E] + \frac{[E][I]}{K_i} + [ES] + \frac{[ES][I]}{K_1}$$

$$E_o = [E](1 + \frac{[I]}{K_i}) + [ES](1 + \frac{[I]}{K_1})$$

$$\frac{\left([E_0] - [ES]\left(1 + \frac{[I]}{K_1}\right)\right.}{\left(1 + \frac{[I]}{K_i}\right)} = [E]$$

Substituting for [*E*] in equation 2, the expression for K_m :

$$\frac{\left([\mathrm{E}_0]-[\mathrm{ES}]\left(1+\frac{[\mathrm{I}]}{K_\mathrm{I}}\right)\right)[S]}{\left(1+\frac{[\mathrm{I}]}{K_\mathrm{i}}\right)[\mathrm{ES}]} = K_m$$

$$\therefore [\mathrm{E}_0][\mathrm{S}]\text{-}[\mathrm{S}]\,[\mathrm{ES}]\left(1+\frac{[\mathrm{I}]}{K_\mathrm{I}}\right) = K_\mathrm{m}[\mathrm{ES}]\left(1+\frac{[\mathrm{I}]}{K_\mathrm{i}}\right)$$

$$\therefore [\mathrm{ES}]\left([\mathrm{S}]\left(1+\frac{[\mathrm{I}]}{K_\mathrm{I}}\right)+K_\mathrm{m}\left(1+\frac{[\mathrm{I}]}{K_\mathrm{i}}\right)\right)=[\mathrm{E}_0][\mathrm{S}]$$

$$\therefore [\mathrm{ES}]=\frac{[\mathrm{E}_0][\mathrm{S}]}{[\mathrm{S}]\left(1+\frac{[\mathrm{I}]}{K_\mathrm{I}}\right)+K_m\left(1+\frac{[I]}{K_\mathrm{i}}\right)}$$

$v_o = k_2[ES]$ ……. equation 4

(Ref: Michaelis Menten equation section in the previous Unit-4, Block-2 of BBCCT-107)

Putting the value of [*ES*] in equation 4

$$v_0 = \frac{k_2[\mathrm{E}_0][S]}{[\mathrm{S}]\left(1+\frac{[\mathrm{I}]}{K_\mathrm{I}}\right)+K_m\left(1+\frac{[I]}{K_\mathrm{i}}\right)}$$

$V_{\max} = k_2[E_o]$ ……. equation 5

(Ref: Michaelis Menten equation section in the previous Unit-4, Block-2 of BBCCT-107) Continuing similarly as in previous sections:

$$v_0 = \frac{V_{\max}[S_o]}{[\mathrm{S}]\left(1+\frac{[\mathrm{I}_0]}{K_\mathrm{I}}\right)+K_m\left(1+\frac{[I_0]}{K_\mathrm{i}}\right)}$$ ……. equation 14

Dividing the numerator and denominator by $(1 + ([I_0]/K_i))$,

$$v_0 = \frac{\frac{V_{\max}}{\left(1+\frac{[\mathrm{I}_0]}{K_\mathrm{I}}\right)}[S_o]}{[\mathrm{S}_o]+\frac{K_m\left(1+\frac{[I_0]}{K_\mathrm{i}}\right)}{\left(1+\frac{[\mathrm{I}_0]}{K_\mathrm{I}}\right)}}$$

The above equation assumes the same form as the Michaelis-Menten equation and can be written as:

$$v_0 = \frac{V'_{max}\ [S_o]}{[S_o] + K'_m}$$

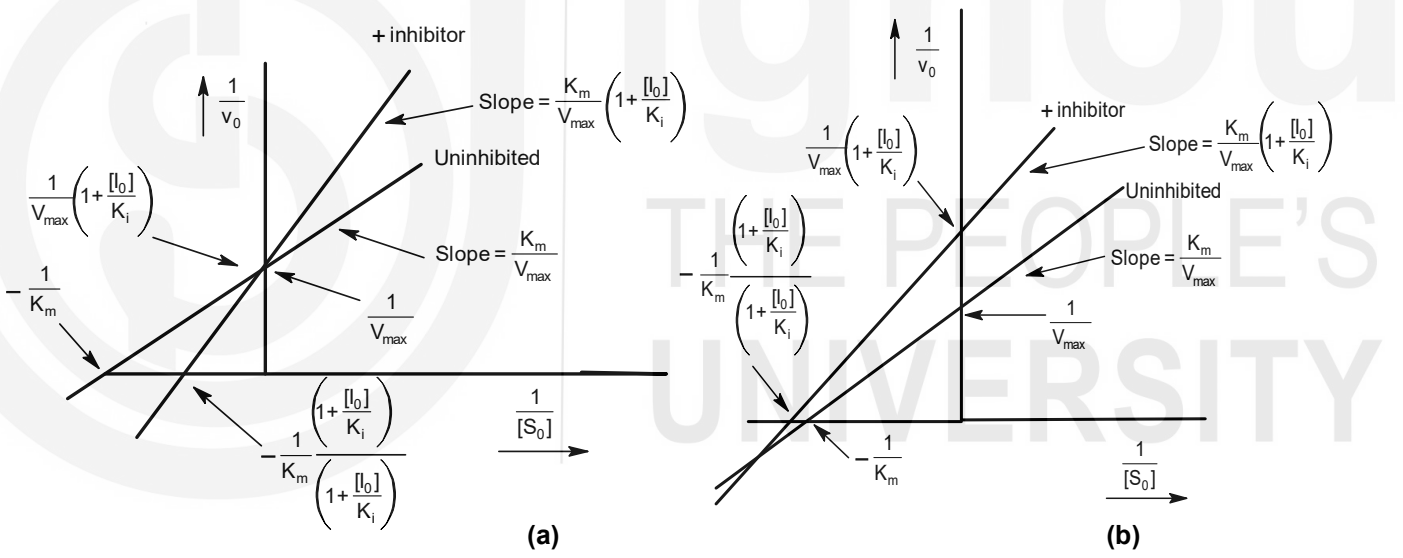
where

$$V'_{max} = \frac{V_{max}}{\left(1 + \frac{[I_0]}{K_I}\right)} \text{ and } K'_m = K_m \frac{\left(1 + \frac{[I_0]}{K_i}\right)}{\left(1 + \frac{[I_0]}{K_I}\right)}$$

The Lineweaver-Burk equation will be:

$$\frac{1}{v_0} = \frac{K'_m}{V'_{max}} \frac{1}{[S_o]} + \frac{1}{V'_{max}}$$

From the equation, it appears that the Lineweaver-Burk plot will be linear. However, K_m, V_{max} and slope will be affected by the inhibitor. The Lineweaver-Burk plot will be represented as shown in the Fig. 6.4.

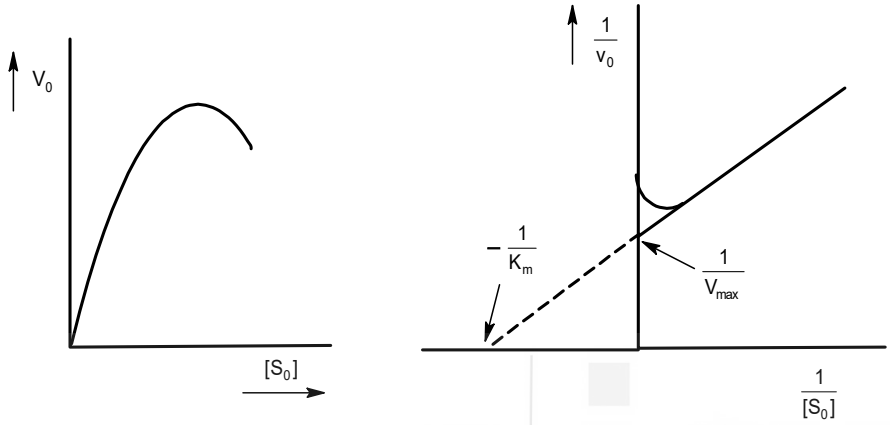


Fig, 6.4 : Lineweaver-Burk plots showing the effect of mixed inhibition: (a) $K_1 > K_i$; (b) $K_1 < K_i$.

6.3.5 Substrate Inhibition

In enzyme catalyzed reactions, when the amount of enzyme is kept constant and substrate concentration is increased gradually the rate of reaction will increase and the velocity curve will reach a maximum. A further increase in substrate concentration does not increase the rate of reaction and the velocity curve falls. This event occurs on account of substrate inhibition whenever a dead-end-enzyme substrate complex is formed thereby inhibiting its own conversion to the product. Several enzymes are inhibited by their own substrates. For example, enzyme succinate dehydrogenase catalyzes dehydrogenation of substrate succinate. If you will look at the structure of succinate, you will see two carboxyl groups at the two ends of the molecule.

The enzyme reaction takes place when both the carboxyl groups of the substrate bind to the enzyme. Excess concentration of succinate will lead to an increased possibility of carboxyl groups from two different molecules of succinate binding to the same enzyme, thereby stopping the enzyme reaction due to the formation of a dead-end complex. The graphs of characteristic substrate inhibition are shown in the Fig. 6.5a and 6.5b.



a) Michaelis-Menten plot **b) Lineweaver-Burk plot**

Fig. 6.5: Graphs on Substrate inhibition

The biological significance of substrate inhibition of phosphofructokinase ensures resources are not dedicated to the formation of ATP when it is in plenty. Another enzyme DNA metyltransferases catalyze transfer of methyl group to DNA. Their substrate inhibition leads to faithfully copy of DNA methylation patterns when cells divide while preventing de novo methylation of methyl-free promoter regions.

Regarding the enzyme kinetics, if you recall from the uncompetitive inhibition

$$v_o = \frac{\frac{V_{max}[S_o]}{(1+\frac{I_o}{K_i})}}{\frac{K_m}{(1+\frac{I_o}{K_i})}+[S_o]}$$

If the inhibitor is identical to the substrate, the equation will become:

$$v_0 = \frac{\frac{V_{max}}{\left(1+\frac{[S_o]}{K_i}\right)}\ [S_o]}{[S_o]+\frac{K_m}{\left(1+\frac{[S_o]}{K_i}\right)}} = \frac{V_{max}[S_o]}{[S_o]\left(1+\frac{[S_o]}{K_i}\right)+K_m}$$

When the substrate concentration is low [*S*] the term [*S*]/K_i becomes negligible and the equation will be the normal Michaelis-Menten equation. However, when the substrate concentration is high, then

$$[S_o]\left(1+\frac{[S_o]}{K_i}\right) + K_m \simeq [S_o]\left(1+\frac{[S_o\ ]}{K_i}\right)$$

The rate of equation will be

$$v_0 = \frac{V_{max}}{1+\frac{[S_o]}{K_i}} \qquad \text{.....equation 15}$$

As shown in the above equation, v_o will decrease on an increase of substrate concentration due to substrate inhibition.

SAQ 4

Do as Directed:

a) Mixed inhibition is a mixture of competitive inhibition and uncompetitive inhibition. (True/False)

b) If the inhibitor has equal affinity for both the states of enzyme (free as well as bound to the substrate), mixed inhibition will become non-competitive inhibition. (True/False)

c) The substrate inhibition of phosphofructokinase ensures resources are not dedicated to the formation of ATP when it is in less/excess. (Pick one option)

d) Succinate has one/two carboxyl groups. (Pick one option)

6.4 MECHANISM BASED INHIBITORS- ANTIBIOTICS AS INHIBITORS

Irreversible Enzyme Inhibition

Irreversible inhibition is different from temporary enzyme inactivation by the reversible inhibitor. The enzyme activity is lost on the binding of inhibitor molecule to enzyme and its activity cannot be recovered afterwards. These inhibitory molecules are highly specific and can modify enzyme 3D structure. The enzyme gets inactive or there is time dependent loss of enzyme concentration. Inhibition cannot be removed by dilution or dialysis without losing enzyme activity. Inhibitors generally form or break covalent bonds with the amino acid residues essential for substrate binding, catalysis or maintenance of enzyme conformation.

Examples: Heavy metal ions such as mercury, lead, aldehydes and haloalkanes. Alkylating agents such as iodoacetate and iodoacetamide forms covalent linkages with –*SH* groups of the enzyme.

$$E\text{-}SH + ICH_2.CO^-_2 \longrightarrow E\text{-}S\text{-}CH_2.CO^-_2 + HI$$

The **mechanism-based inhibitors** are modified substrates that are also known as suicide inhibitors or suicide inactivators. They modify the active site of enzyme irreversibly. These suicide inactivators or inhibitors are generally inactive unless they bound to the enzyme at its active site. The inhibitor binds to the enzyme as substrate and is catalyzed in a similar manner. The mechanism of catalysis generates a chemically reactive intermediate that leads to the inactivation of enzyme by covalent modification. These inactivators are used to unravel the mechanism of enzyme reaction and substrate reactivity. This mechanism has proved to be a boon for *rational drug* designing by pharmaceutical companies to determine the molecules which could be synthesized by the chemists as pharmaceutical agents.

Several important drugs are well known examples of irreversible inhibitors. Widely used drugs such as penicillin act by covalently inhibiting the enzyme transpeptidase, thereby preventing the synthesis of bacterial cell walls and thus killing the bacteria. You must have heard of tablet aspirin. The drug aspirin inhibits the enzyme cyclooxygenase thereby reducing the synthesis of inflammatory signals. Enzyme monoamine oxidase deaminates neurotransmitters such as dopamine and serotonin, and lowers the levels of these hormones in the brain. A neurodegenerative disease such as Parkinson's disease is linked with low levels of dopamine, while depression is related with low levels of serotonin. Suicide inhibitor such as drug (-)deprenyl, is widely used to treat Parkinson disease and depression.

6.5 SUMMARY

1) Enzyme inhibition can be either reversible or irreversible. The different modes of reversible enzyme inhibition can be distinguished by their effects on the kinetic behavior of enzymes: competitive, uncompetitive or non-competitive.
2) Competitive inhibitors compete with the substrates for the same binding site available on the enzyme. Therefore the graphs of these inhibitors show that K_m is increased but V_{max} remain unchanged in the presence of an inhibitor.
3) The uncompetitive inhibitor does not compete with the substrate. However, it binds at a site other than the substrate binding site on the enzyme-substrate complex. The graphs of these inhibitors show that K_m and V_{max} is altered in the presence of an inhibitor but the slope remains unchanged.
4) Non-competitive inhibitors also bind at a different site other than the substrate binding site on the enzyme and enzyme substrate complex. In the presence of such inhibitors, K_m remains unchanged, however V_{max} is decreased.
5) Mixed inhibition is a mixture of competitive inhibition and uncompetitive inhibition.
6) Substrate inhibition seems to be a type of uncompetitive inhibition, the extra substrate molecule acts as an inhibitor.

7) Irreversible inhibitors can be used to map the active site of enzyme. They bind to the active site of enzyme and modify it covalently and hence cannot dissociate from the enzyme. They reduce the concentration of enzyme present.

8) Mechanism based inhibitors or suicide inhibitors are processed by the enzyme in a catalytic mechanism resulting in the formation of a reactive compound that inactivates or inhibits the enzyme. These inhibitors are widely used for rational drug designing.

6.6 TERMINAL QUESTIONS

1) Discuss uncompetitive inhibition and derive the equation for an uncompetitive inhibitor.
2) Distinguish between reversible and irreversible inhibition?
3) Explain the substrate inhibition.
4) What are the mechanism-based inhibitors? How they help in drug designing?

6.7 ANSWERS

Self-Assessment Questions

1) Refer to Fig. 6.1 b
2) Refer to Fig. 6.2 b
3) Refer to Fig. 6.3 b
4) a) True, b) True, c) Excess, d) Two

Terminal Questions

1. Refer to section 6.3.2
2. Refer to section 6.2
3. Refer to section 6.3.5
4. Refer to section 6.4

6.8 SUGGESTED READINGS

1) David L. Nelson and Michael M. Cox: Lehninger Principles of Biochemistry 6th Ed., W.H. Freeman.
2) Robert K. Murray, Daryl K. Granner, Victor W. Rodwell Harper's Illustrated Biochemistry, 27th edition. 2006, McGraw-Hill.
3) Donald J Voet and Judith G. Voet:Principles of Biochemistry4th ed., John Wiley and Sons, Inc, USA.
4) Eric E Conn, Paul K Stumpf: Outlines of Biochemistry, John Wiley and Sons, Inc, USA.
5) S. Shanmugan and T. Sathish Kumar: Enzyme Technology, I K International Publishing House Pvt Ltd, New Delhi.
6) Nicholas C Price and Lewis Stevens: Fundamentals of Enzymology, Oxford University Press, Oxford, New York, USA.