Enzyme Inhibition

Some of the compounds or elements can reduce the rate of enzyme action. These substances may be of physiological or unphysiological nature. This phenomenon is known as enzyme inhibition and such enzyme are known as negative inhibitors inhibition or negative modifiers as enzyme inhibitors or negative modifiers. Enzyme may be broadly classified under three group.

1. Purely competitive,
2. Purely non-competitive,
3. Allosteric.

Purely competitive Inhibition

Competitive inhibition may be defined as that one in which the substrate as well as the inhibitor compete for the same site (S) on the enzyme molecule. In such inhibitions, both **enzyme-substrate (E.S.)** and **enzyme-inhibitor (E.I.)** complexes are formed during the course of reaction. If both the substances are added to the enzyme at the same time, the quantity of E.S. or E.S. formed will depend upon the relative affinity of the enzyme for the substrate and inhibitor. If the affinity of the enzyme towards the substrate is greater, the amount of E.I. formed will be more. On the other hand, if the affinity of the enzyme towards the inhibitor is greater, the amount of E.I. formed will be more. Secondly formation of E.S. and E.I. also depends on the relative concentration of the substrate and inhibitor. If the inhibitor concentration is kept constant and substrate concentration is increased gradually, it is observed that at low substrate concentrations, the amount of E.S. formed is smaller and E.I. is greater. At higher substrate concentration, E.S. formed is greater but E.I. formed is smaller. Thirdly, the amount of E.S.

or E.I. formed is also dependent upon the time of preincubation of the enzyme with the substrate or inhibitor. The same amount of inhibitor will show greater degree of inhibition if incubated with the enzyme prior to substrate. Competitive inhibitions are reversible in nature.

Studies of the chemical structures of competitive inhibitors have revealed that the inhibitor resembles very closely to the substrate structurally. Various examples of inhibitors may be cited.

Table 5.1

Competitive inhibitors of some Enzyme Substrates

Substrate	**Competitive inhibitor**
Succinate	Malonate
p-Aminobenzoic acid	Sulphanilamide
Dihydrofolate	Amethopterin
Thiamine	Pyrithiamine
Pantothenic acid	Pantoyltaurine
Biotin	Desthio-biotin
Pyridoxine	Deoxypyridoxine
Vitamin K	Dicumarol
Hypoxanthine	6-Mercapto-purine
Histidine	D-Histidine
Acelylcholine	Physostigmine
NAD, NADP	ADP, ATP

Kinetic studies of the competitive inhibitions have revealed that the apparent K_m value on addition of inhibitor is changed and becomes greater than the K_m value obtained without inhibitor. Higher concentration of the substrate is needed to attan 50% of the maximum activity of the inhibitor intercept the Y-axiz of inhibitor (1/v line) at the same point if extrapolated

towards Y-axis where 1/S is equal to zero, or in other words, the substrate concentration is infinity high. If the substrate concentration is increased to infinity, the velocity of the reaction will be the same in the presence or absence of the inhibitor. Various lines drownin the graph (Fig 5.7) at different substrate levels for each concentration

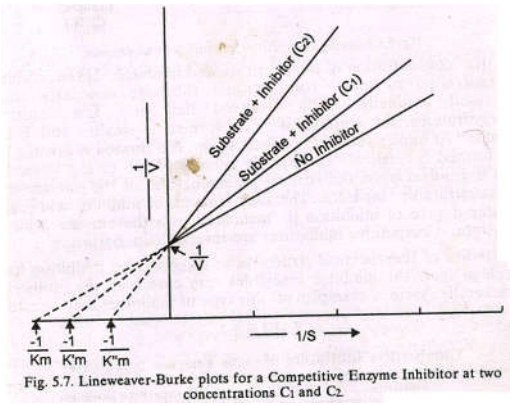


Fig. 5.7. Lineweaver-Burke plots for a Competitive Enzyme Inhibitor at two concentrations C_1 and C_2.

Purely Non-competitive Inhibition

Non-competitive inhibition is defined as that one in which substrate and inhibitor do not exhibit mutual competition. The sites of attachment of substrate and noncompetitive inhibitor are different on the enzyme molecules.

Non-competitive inhibitors are mostly structurally dissimilar to the substrate. The K_m value in the presence of inhibitor is not changed from the value obtained in the absence of inhibitor. However, the maximum velocity with a given amount of the enzyme at infinitely high substrate concentration is lowered in the presence of such an inhibitor. In competitive inhibition, on the other hand, it remains the same.

The possible mechanism of non-competitive inhibition, has been represented in

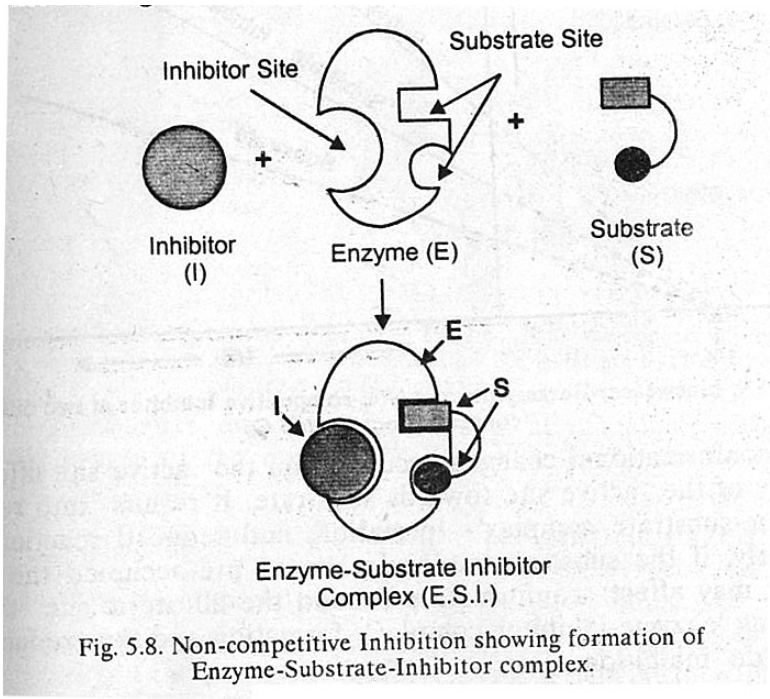


Fig. 5.8. Non-competitive Inhibition showing formation of Enzyme-Substrate-Inhibitor complex.

If the inhibitor can be removed from its site of attachment preserving the activity of the enzyme, it is known as **reversible non-competitive inhibition.** On the other hand, if it cannot be known as irreversible noncompetitive inhibition. Both reversible **and irreversible non-competitive inhibition.** Both reversible and **irreversible non-competitive inhibitions** have been found to exhibit similar reaction kinetics. In such inhibitions, the rate of combination of the substrate with the enzyme may not be altered but the rate of its dissociation is definitely reduced.

Allosteric inhibitions

In mixed inhibitions, the inhibitor molecule attached to the enzyme prevents the breakdown of the enzyme-substrate complex (E.S.), and also at

the same time, it interferes with the binding of the enzyme affinity of the active site towards substrate enzyme. Thus, such inhibitors exhibit partially competitive and partially non-competitive kinetics. The sites other than the active sites, lying on the different region in the enzyme molecule where an inhibitor molecule may get attached is known as **allosteric site**. An inhibitor molecule on this site affects the conformation around the active site.

The conformational changes occurring on the active site affect the affinity of the active site towards substrate. It result into reduced 'enzyme-substrate complex 'formation, and reduced reaction rate. Similarly, if the substrate molecules have pre-occupied the active site, it may affect conformation around the **allosteric site** thereby reducing 'enzyme-inhibitor complex' formation and thus reduces the extent do inhibition.

The shape of line weaver - burke plots of the non-competitive inhibitors in shown in

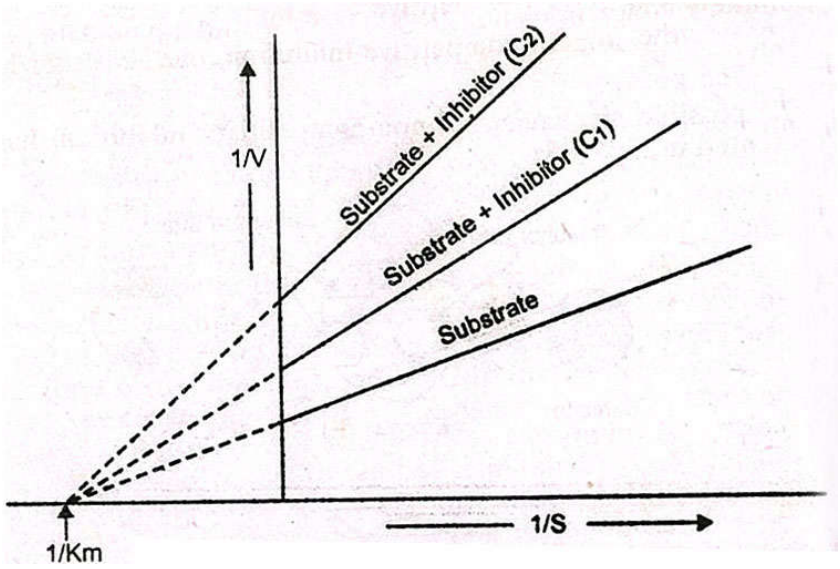


Fig. 5.9 Lineweaver-Burke Plots of a Non-Competitive Inhibitor at two different concentrations C_1 and C_2.