ENZYME INHIBITIONS

Enzymes Are Subject to Reversible or Irreversible Inhibition

Enzyme inhibitors are molecular agents that interfere with catalysis, slowing or halting enzymatic reactions.

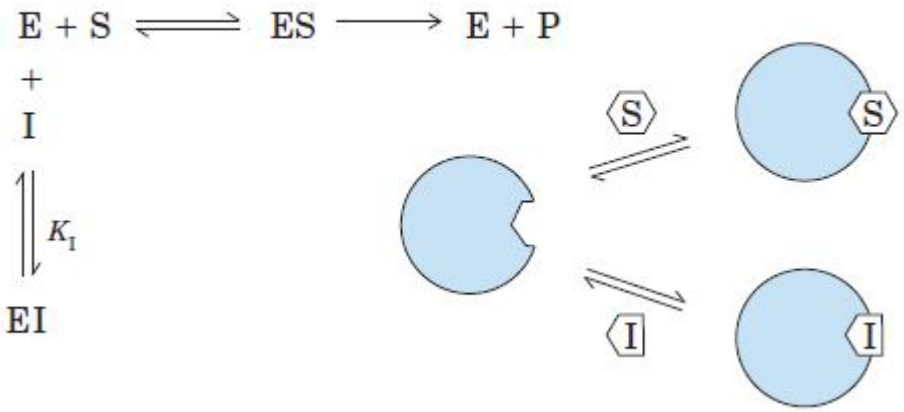
Enzymes catalyze virtually all cellular processes, so it should not be surprising that enzyme inhibitors are among the most important pharmaceutical agents known.

For example, aspirin (acetylsalicylate) inhibits the enzyme that catalyzes the first step in the synthesis of prostaglandins, compounds involved in many processes, including some that produce pain.

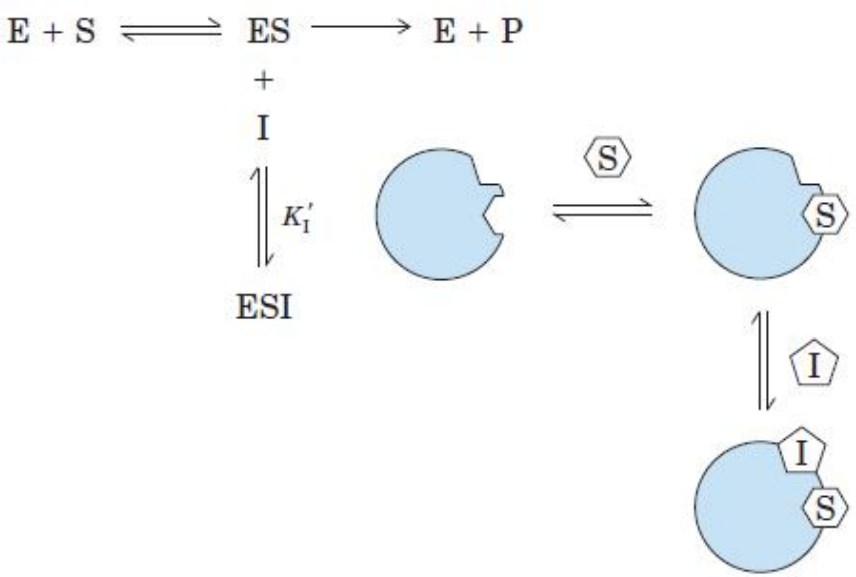
The study of enzyme inhibitors also has provided valuable information about enzyme mechanisms and has helped define some metabolic pathways.

There are two broad classes of enzyme inhibitors: reversible and irreversible.

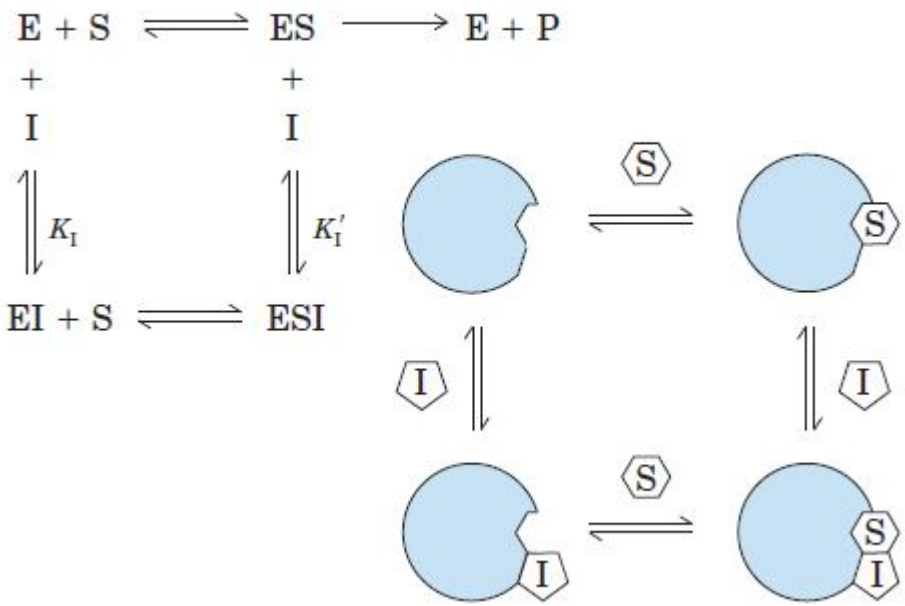
(a) Competitive inhibition



(b) Uncompetitive inhibition



(c) Mixed inhibition



***Reversible Inhibition One common type of reversible* inhibition is called competitive**

A competitive inhibitor competes with the substrate for the active site of an enzyme. While the inhibitor (I) occupies the active site it prevents binding of the substrate to the enzyme.

Many competitive inhibitors are compounds that resemble the substrate and combine with the enzyme to form an EI complex, but without leading to catalysis. Even fleeting combinations of this type will reduce the efficiency of the enzyme.

By taking into account the molecular geometry of inhibitors that resemble the substrate, we can reach conclusions about which parts of the normal substrate bind to the enzyme.

Competitive inhibition can be analyzed quantitatively by steady-state kinetics. In the presence of a competitive inhibitor, the Michaelis-Menten equation becomes

$$V_0 = \frac{V_{\max}[\mathrm{S}]}{\alpha K_\mathrm{m} + [\mathrm{S}]}$$

where

$$\alpha = 1 + \frac{[\mathrm{I}]}{K_\mathrm{I}} \quad \text{and} \quad K_\mathrm{I} = \frac{[\mathrm{E}][\mathrm{I}]}{[\mathrm{EI}]}$$

The experimentally determined variable αK_m, the K_m observed in the presence of the inhibitor, is often called the “apparent” K_m.

Because the inhibitor binds reversibly to the enzyme, the competition can be biased to favor the substrate simply by adding more substrate.

When [S] far exceeds [I], the probability that an inhibitor molecule will bind to the enzyme is minimized and the reaction exhibits a normal V_{max}. However, the [S] at which $V_0 = ½ V_{max}$, the apparent K_m, increases in the presence of inhibitor by the factor .

This effect on apparent Km, combined with the absence of an effect on V_{max}, is diagnostic of competitive inhibition and is readily revealed in a doublereciprocal plot.

The equilibrium constant for inhibitor binding, KI, can be obtained from the same plot.

Two other types of reversible inhibition, uncompetitive and mixed, though often defined in terms of one substrate enzymes, are in practice observed only with enzymes having two or more substrates.

An **uncompetitive inhibitor binds at a site distinct** from the substrate active site and, unlike a competitive inhibitor, binds only to the ES complex. In the presence of an uncompetitive inhibitor, the Michaelis-Menten equation is altered to

$$V_0 = \frac{V_{max}[S]}{K_m + \alpha'[S]}$$

where

$$\alpha' = 1 + \frac{[I]}{K'_I} \quad \text{and} \quad K'_I = \frac{[ES][I]}{[ESI]}$$

As described by Equation, at high concentrations of substrate, V_0 approaches Vmax/α'. Thus, an uncompetitive inhibitor lowers the measured V_{max}. Apparent K_m also decreases, because the [S] required to reach one-half V_{max} decreases by the factor α' .

A **mixed inhibitor also binds at a site** distinct from the substrate active site, but it binds to either E or ES. The rate equation describing mixed inhibition is

$$V_0 = \frac{V_{\max}[\mathrm{S}]}{\alpha K_{\mathrm{m}} + \alpha'[\mathrm{S}]}$$

A mixed inhibitor usually affects both K_m and V_{max}. The special case of $\alpha = \alpha'$, rarely encountered in experiments, classically has been defined as **noncompetitive inhibition.**

Kinetic Tests for Determining Inhibition Mechanisms

The double-reciprocal plot (see Box 6–1) offers an easy way of determining whether an enzyme inhibitor is competitive, uncompetitive, or mixed. Two sets of rate experiments are carried out, with the enzyme concentration held constant in each set. In the first set, [S] is also held constant, permitting measurement of the effect of increasing inhibitor concentration [I] on the initial rate V_0 (not shown). In the second set, [I] is held constant but [S] is varied. The results are plotted as $1/V_0$ versus $1/[S]$.

Figure 1 shows a set of double-reciprocal plots, one obtained in the absence of inhibitor and two at different concentrations of a competitive inhibitor. Increasing [I] results in a family of lines with a common intercept on the $1/V_0$ axis but with different slopes. Because the intercept on the $1/V_0$ axis equals $1/V_{max}$, we know that V_{max} is unchanged by the presence of a competitive inhibitor. That is, regardless of the concentration of a competitive inhibitor, a sufficiently high substrate concentration will always displace the inhibitor from the enzyme's active site. Above the graph is the rearrangement of Equation 6–28 on which the plot is based. The value of α can be calculated from the change in slope at any given [I]. Knowing [I] and α, we can calculate K_I from the expression

$$\alpha = 1 + \frac{[I]}{K_I}$$

For uncompetitive and mixed inhibition, similar plots of rate data give the families of lines shown in Figures 2 and 3. Changes in axis intercepts signal changes in V_{max} and K_m.

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

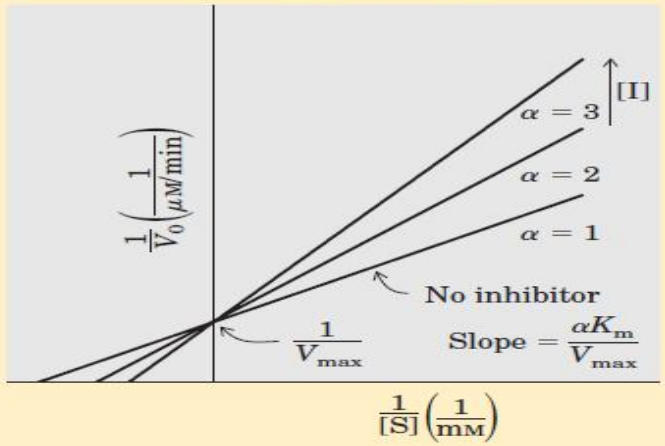


FIGURE 1 Competitive inhibition.

$$\frac{1}{V_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

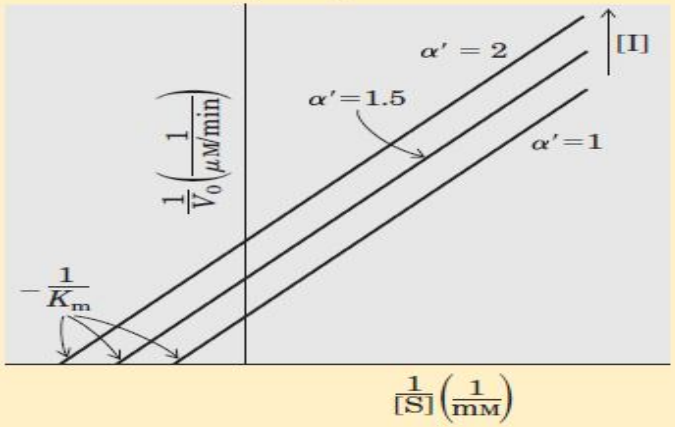


FIGURE 2 Uncompetitive inhibition.

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

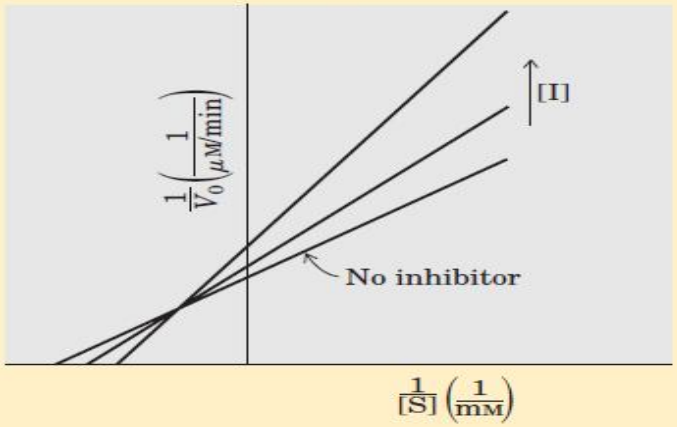


FIGURE 3 Mixed inhibition.

Irreversible Inhibition: The irreversible inhibitors are those that bind covalently with or destroy a functional group on an enzyme that is essential for the enzyme's activity, or those that form a particularly stable noncovalent association.

Formation of a covalent link between an irreversible inhibitor and an enzyme is common. Irreversible inhibitors are another useful tool for studying reaction mechanisms.

Amino acids with key catalytic functions in the active site can sometimes be identified by determining which residue is covalently linked to an inhibitor after the enzyme is inactivated.

A special class of irreversible inhibitors is the ***suicide inactivators***. These compounds are ***relatively unreactive*** until they bind to the active site of a specific enzyme. A suicide inactivator undergoes the first few chemical steps of the normal enzymatic reaction, but instead of being transformed into the normal product, the