

Inhibition kinetics

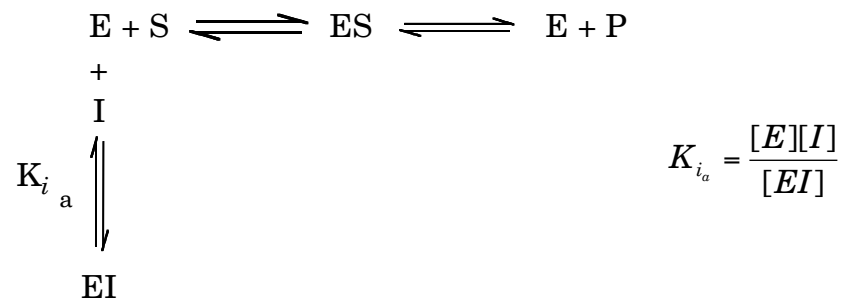
Structure-based enzyme mechanism studies are extremely useful for understanding the mechanism used by an inhibitor. However, for many proteins, structural information is not available. In addition, even if structural information is available for the enzyme, information may not be available for the inhibitor/enzyme complex. Finally, enzyme-inhibitor binding affinity is very difficult to measure using the structural information alone. In many cases, therefore, full understanding of the inhibition of a given enzyme requires performing kinetic analyses of the system.

Kinetic studies will yield information regarding the mechanism; however, kinetics cannot prove a given mechanism. Kinetic studies can only either refute the proposed mechanism, or provide supporting evidence for it.

Competitive inhibition

In one common type of inhibition, the inhibitor acts as though it competes with the substrate for binding to the enzyme. This is called competitive inhibition: kinetically, the enzyme behaves as though the binding of the substrate and inhibitor are mutually exclusive.

This adds an additional term to the reaction scheme that we have already discussed (shown below). Note that the K_i (the equilibrium dissociation constant for the inhibitor) is an equilibrium constant (*i.e.* a *ratio* of rate constants). The K_i can be expressed as a function of the inhibitor concentration, free enzyme concentration, and enzyme-inhibitor complex concentration as shown in the equation below:



This reaction scheme requires the addition of a new term to the possible states of the enzyme:

$$[\text{E}]_{\text{total}} = [\text{E}]_{\text{free}} + [\text{ES}] + [\text{EI}]$$

Taking the additional term in the reaction into account results in a modification to the Michaelis-Menten equation:

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

This additional term also appears in the slope of the Lineweaver-Burk equation:

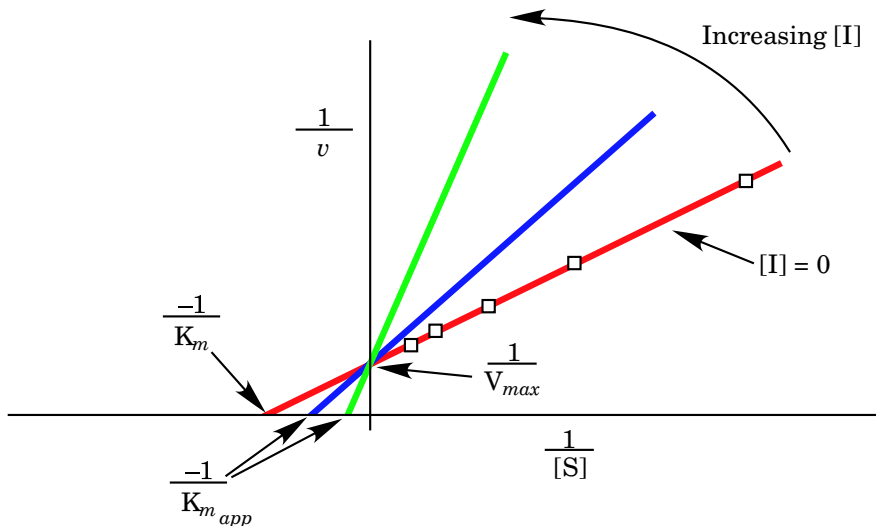
$$\frac{1}{v} = \left(1 + \frac{[I]}{K_{i_a}}\right) \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

$$y = m x + b$$

The usual method of determining the K_i is to perform K_m determinations at several different inhibitor concentrations. (Look at the equation. What do you expect to happen to the apparent K_m and V_{max} as the result of a competitive inhibitor?)

In competitive inhibition, the apparent K_m **increases** as a result of the inhibitor. The effect of the inhibitor is to raise the concentration of substrate required for a given velocity. In contrast, the apparent V_{max} is unaffected by a competitive inhibitor. This is because once substrate binds, the reaction proceeds normally, and therefore V_{max} depends only on the maximum possible ES complex concentration (and on the k_{cat} , but k_{cat} is unaffected by a competitive inhibitor), and the maximum ES concentration depends only on the total amount of enzyme present.

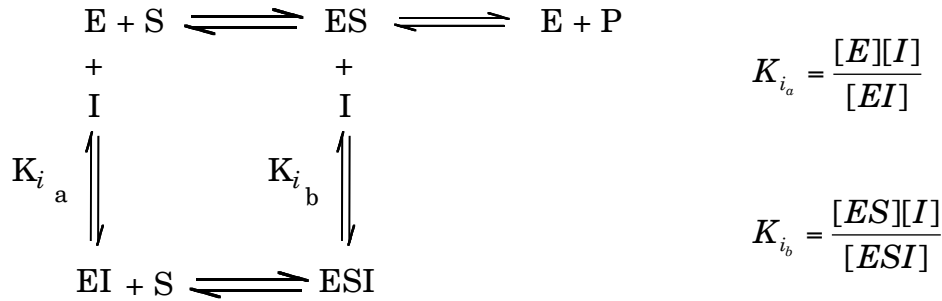
Each line in the Lineweaver-Burk plot corresponds to a set of data obtained at a single inhibitor concentration. The fact that the V_{max} does not change is immediately obvious, because the lines all intersect at the same point on the y-axis.



Note that, in competitive inhibition the true values of the K_m and V_{max} do not change. The enzyme either binds the inhibitor, and does nothing, or binds the substrate and behaves normally. The measured values of K_m in the presence of the inhibitor are altered, and are called the **apparent K_m** . The apparent K_m varies depending on the inhibitor concentration involved.

Mixed Inhibition

The other major type of inhibition occurs when the inhibitor is capable of binding to both the free enzyme and to the enzyme-substrate complex. This adds an additional term to the reaction scheme. Note that the dissociation constant for binding the free enzyme may differ from the dissociation constant for binding the ES complex.



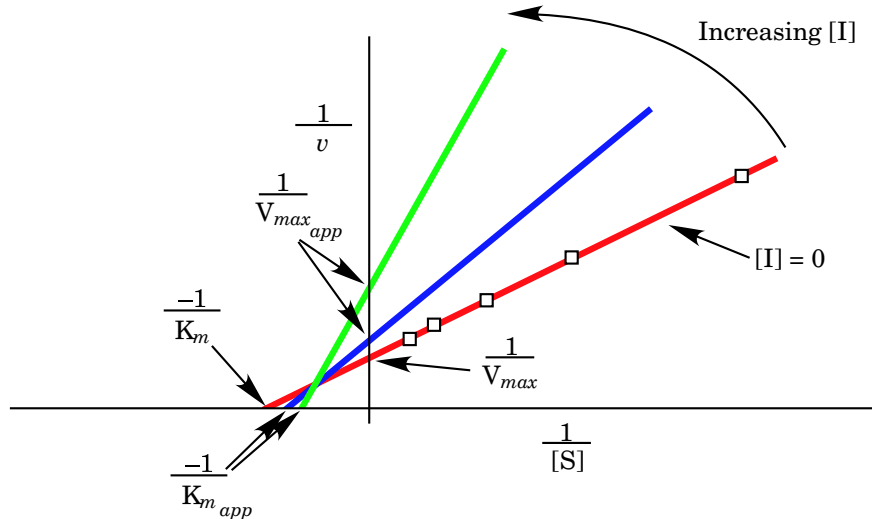
The result of the inhibitor binding is a somewhat different alteration to the Michaelis-Menten equation.

$$v = \frac{V_{max} [\text{S}]}{K_m \left(1 + \frac{[\text{I}]}{K_{i_a}}\right) + [\text{S}] \left(1 + \frac{[\text{I}]}{K_{i_b}}\right)}$$

This results in an altered Lineweaver-Burk equation.

$$\frac{1}{v} = \left(1 + \frac{[\text{I}]}{K_{i_a}}\right) \frac{K_m}{V_{max}} \frac{1}{[\text{S}]} + \frac{\left(1 + \frac{[\text{I}]}{K_{i_b}}\right)}{V_{max}}$$

$$y = m x + b$$



In the case of mixed inhibition, the apparent V_{max} changes, because the inhibitor is capable of preventing catalysis *regardless* of whether the substrate is bound to the enzyme. With mixed inhibitors, the change in apparent K_m varies, depending on the relative values of K_{i_a} (the K_i for binding to the free enzyme) and K_{i_b} (the K_i for binding to the ES complex). Note that, depending on the relative values of the K_i , the apparent K_m may decrease.

In the special case where the two K_i values are equal, the apparent K_m will be unchanged. This special case is called **non-competitive inhibition**. In non-competitive inhibition, all of the lines of a Lineweaver-Burk plot intersect at the same point on the x -axis.

Uncompetitive Inhibition

One more special case for inhibitor kinetics is occasionally observed, primarily with multisubstrate enzymes. This final type of inhibition, which is really another special case for mixed inhibition, occurs when the inhibitor only binds to the ES complex.

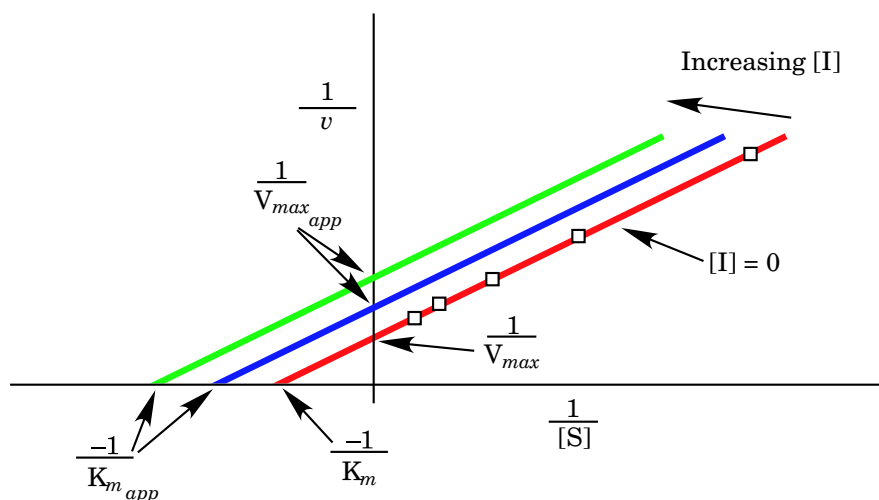
For this type of inhibition, the altered Michaelis-Menten and Lineweaver-Burk equations are:

$$v = \frac{V_{max}[S]}{K_m + [S] \left(1 + \frac{[I]}{K_{i_b}} \right)}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{\left(1 + \frac{[I]}{K_{i_b}} \right)}{V_{max}}$$

$$y = m \quad x + b$$

Uncompetitive inhibition results in parallel lines on a Lineweaver-Burk plot:



Summary

The binding of molecules to enzymes may alter the activity of the enzyme. If this alteration is a decrease in activity, the compound is called an inhibitor.

Inhibitors that bind only the free enzyme are called competitive inhibitors. Competitive inhibitors increase the apparent K_m of the enzyme, but have no effect on the V_{max} .

Inhibitors that bind to both the free enzyme and the ES complex are called mixed inhibitors. In the special case where the inhibitor has the same affinity for the free enzyme and the ES complex (non-competitive inhibition), the inhibitor has no effect on apparent K_m . Most mixed inhibitors, however, alter the apparent K_m (either increasing or decreasing the value). All mixed inhibitors decrease the apparent V_{max} .

All biochemists need to be able to interpret Lineweaver-Burk plots for experiments performed using inhibitors.

Type of Inhibition	$K_{m_{app}}$	$V_{max_{app}}$
None	K_m	V_{max}
Competitive (Inhibitor only binds to free enzyme)	$K_m \left(1 + \frac{[I]}{K_{i_a}} \right)$	V_{max}
Mixed (Inhibitor binds E and ES)	$K_m \frac{\left(1 + \frac{[I]}{K_{i_a}} \right)}{\left(1 + \frac{[I]}{K_{i_b}} \right)}$	$\frac{V_{max}}{\left(1 + \frac{[I]}{K_{i_b}} \right)}$
Non-competitive (Inhibitor binds E and ES with equal affinity)	K_m	$\frac{V_{max}}{\left(1 + \frac{[I]}{K_{i_b}} \right)}$
Uncompetitive (Inhibitor only binds to ES complex)	$\frac{K_m}{\left(1 + \frac{[I]}{K_{i_b}} \right)}$	$\frac{V_{max}}{\left(1 + \frac{[I]}{K_{i_b}} \right)}$