## Lecture # 5, 6 – Enzyme Inhibition and Toxicity

Handout: Derivation of Inhibition Kinetics

Now that we've considered enzyme kinetics, let's talk about the phenomenon of enzyme inhibition. In this situation, either the substrate itself or a different molecule affects the ability of the enzyme to convert. In a "clean" system where the substrate is pure and only one product is formed, the inhibitor will be the substrate or the product. This is the case that we'll consider; however, keep in mind that if the substrate or the reaction environment is mixed, other compounds may function as inhibitors.

There are three types of inhibition – *competitive, uncompetitive,* and *noncompetitive*. Each kind of inhibition leads to a different form of the rate equation. It's the impact on the kinetics that leads one to identify inhibition in an enzyme reaction. Let's look at each of the three cases and how the rate equations are altered from the standard Michaelis-Menten form. In each case, we'll assume that inhibition is reversible. We'll consider the case of irreversible inhibition to be *toxicity*, which will be discussed later.

1. Competitive Inhibition

In this case, the inhibitor binds to the active site and prevents binding of the substrate. The reaction equations are as follows:

(1.1) $[E]+[S] \xleftrightarrow{K_S} [E \cdot S] \xrightarrow{k_2} [E]+[P]$

(1.2) $[E]+[I] \xleftrightarrow{K_I} [E \cdot I]$
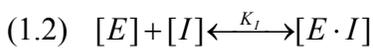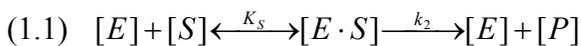
Note that we are assuming that formation of both enzyme complexes is in equilibrium with the respective substrate/inhibitor, and that K_S and K_I are *dissociation* constants. The final form of the equation will be the same if we don't assume equilibrium and instead use the quasi-steady state assumption, except that $K_M=K_S$ in this case. The rate equation is then obtained from a mass balance on product formation and the enzyme species:

(1.3) $\dfrac{d[P]}{dt} = k_2[E \cdot S]$

(1.4) $K_M = \dfrac{[E][S]}{[E \cdot S]} \Rightarrow [E \cdot S] = \dfrac{[E][S]}{K_M}$

(1.5) $K_I = \dfrac{[E][I]}{[E \cdot I]} \Rightarrow [E \cdot I] = \dfrac{[E][I]}{K_I}$

(1.6) $[E_0] = [E] + [E \cdot S] + [E \cdot I] = [E]\left(1 + \frac{[S]}{K_M} + \frac{[I]}{K_I}\right)$

(1.7) $[E \cdot S] = \frac{[S]}{K_M}\left(\frac{[E_0]}{1 + \frac{[S]}{K_M} + \frac{[I]}{K_I}}\right)$ Plug this back into Eq (1.3):

(1.8) $\frac{dP}{dt} = v = \frac{k_2[E_0][S]}{K_M\left(1 + \frac{[I]}{K_I}\right) + [S]} = \frac{v_{max}[S]}{K_M^{app} + [S]}$

So from the final rate expression, you can see that the impact of a competitive inhibitor is to alter the Michaelis constant K_M such that the enzyme would appear to have a lower affinity for the substrate (higher K_M = lower affinity). This makes sense, since the inhibitor is binding to the same site as the substrate. So, as is the case with high K_M, it is necessary to have more substrate to achieve a higher reaction rate, since the substrate can outcompete for the binding sites.

2. Uncompetitive Inhibition

In the case of *uncompetitive* inhibition, the inhibitor binds to the E-S complex and prevents conversion to product.

(2.1) $[E] + [S] \xleftrightarrow{K_S} [E \cdot S] \xrightarrow{k_2} [E] + [P]$
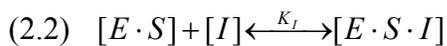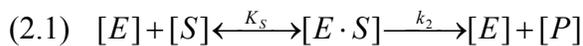
(2.2) $[E \cdot S] + [I] \xleftrightarrow{K_I} [E \cdot S \cdot I]$

We also assume that this binding is in equilibrium and can be represented with a dissociation constant. So, the rate equation can be derived as follows:

(2.3) $\frac{dP}{dt} = k_2[E \cdot S]$

(2.4) $K_S = K_M = \frac{[E][S]}{[E \cdot S]} \Rightarrow [E \cdot S] = \frac{[E][S]}{K_M}$

(2.5) $K_I = \frac{[E \cdot S][I]}{[E \cdot S \cdot I]} \Rightarrow [E \cdot S \cdot I] = \frac{[E \cdot S][I]}{K_I} = \frac{[E][S][I]}{K_M K_I}$

(2.6) $[E_0] = [E] + [E \cdot S] + [E \cdot S \cdot I] = [E]\left(1 + \frac{[S]}{K_M} + \frac{[S][I]}{K_M K_I}\right)$

(2.7) $\quad [E] = \dfrac{[E_0]}{1 + \dfrac{[S]}{K_M}\left(1 + \dfrac{[I]}{[K_I]}\right)}$ $\quad$ Plug this back into Eq (2.4), then into (2.3)
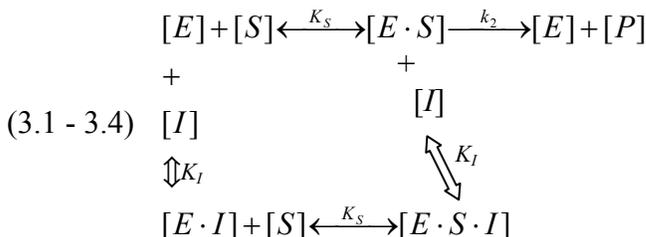
(2.8) $\quad \dfrac{dP}{dt} = v = \dfrac{k_2[E_0][S]}{K_M + [S]\left(1 + \dfrac{[I]}{K_I}\right)} = \dfrac{\dfrac{v_{max}}{\left(1 + \dfrac{[I]}{K_I}\right)}[S]}{\dfrac{K_M}{\left(1 + \dfrac{[I]}{K_I}\right)} + [S]} = \dfrac{v_{max}^{app}[S]}{K_M^{app} + [S]}$

From this expression, you can see that both V_{max} and K_M are altered by a term that includes the inhibitor concentration and dissociation constant. For both parameters, the values *decrease* as the inhibitor concentration increases. This means the maximum velocity decreases, but the affinity for substrate appears to increase (K_M is decreasing). This is NOT intuitive. It doesn't make much sense for the presence of an inhibitor to increase the affinity for the substrate, and in fact, it is important that you remember that the inhibitor does not change the intrinsic properties of the enzyme with respect to a particular substrate. However, this makes a little more sense if we remember what a rapid equilibrium assumption implies *and* how one would drive an equilibrium reaction towards product. [Ask the class how you do this.] To drive an equilibrium reaction, you'd want to remove the product. In effect, an uncompetitive inhibitor does this, by removing the E-S complex (to an E-S-I complex), so it would appear that the equilibrium constant would be increasing, *ie,* the dissociation constant is decreasing.

Note, though, that the impact of this $(1+[I]/K_I)$ term is greater for the numerator (which contains V_{max}) than for the denominator (which contains K_M), so the net result is always a decrease in reaction rate with increasing inhibitor concentration. Mathematically, we want to express this in the Michaelis-Menten form because we know how to find the parameters this way, but it is better to look at the pre-MM form in Eq 2.8 to see the impact of an uncompetitive inhibitor on the reaction rate.

3. Noncompetitive Inhibition

The third case of inhibition is *noncompetitive* inhibition. In this case, the inhibitor can bind to either free enzyme or enzyme-substrate complex, and likewise, the substrate can bind to free enzyme or the enzyme-inhibitor complex. Binding of one does not prohibit binding of the other; however, the E-I and E-S-I complexes are both dead-end (meaning product cannot be formed if I is bound). The reaction equations are then as follows:

$$[E] + [S] \xleftrightarrow{\;K_S\;} [E \cdot S] \xrightarrow{\;k_2\;} [E] + [P]$$
$$+ \qquad\qquad\qquad +$$
(3.1 - 3.4) $\quad [I] \qquad\qquad\qquad [I]$
$$\updownarrow K_I \qquad\qquad\qquad \Downarrow K_I$$
$$[E \cdot I] + [S] \xleftrightarrow{\;K_S\;} [E \cdot S \cdot I]$$

Here, we've made the assumption that binding of substrate doesn't affect the equilibrium of binding to inhibitor and vice versa. Derivation of the rate equation then follows:

(3.5) $\quad \dfrac{dP}{dt} = v = k_2[E \cdot S]$

(3.6) $\quad K_S = K_M = \dfrac{[E][S]}{[E \cdot S]} = \dfrac{[E \cdot I][S]}{[E \cdot S \cdot I]} \Rightarrow [E \cdot S] = \dfrac{[E][S]}{K_M}, [E \cdot S \cdot I] = \dfrac{[E \cdot I][S]}{K_M}$

(3.7) $\quad K_I = \dfrac{[E][I]}{[E \cdot I]} = \dfrac{[E \cdot S][I]}{[E \cdot S \cdot I]} \Rightarrow [E \cdot I] = \dfrac{[E][I]}{K_I}, [E \cdot S \cdot I] = \dfrac{[E \cdot S][I]}{K_I}$

Let's now get everything in terms of [E], [I], and [S] –

(3.8) $\quad [E \cdot S \cdot I] = \dfrac{[E][I][S]}{K_I K_M}$

And write our mass balance for enzyme concentration –

(3.9) $\quad [E_0] = [E] + [E \cdot S] + [E \cdot I] + [E \cdot S \cdot I] = [E]\left(1 + \dfrac{[S]}{K_M} + \dfrac{[I]}{K_I} + \dfrac{[S][I]}{K_M K_I}\right)$

And, as usual, plug this back into Eq (3.6) to get an expression for [E-S], then into Eq (3.5) for the final rate equation –

(3.10) $\quad v = \dfrac{k_2[E_0][S]}{K_M\left(1 + \dfrac{[S]}{K_M} + \dfrac{[I]}{K_I} + \dfrac{[S][I]}{K_M K_I}\right)} = \dfrac{v_{\max}[S]}{K_M\left(1 + \dfrac{[I]}{K_I}\right) + [S]\left(1 + \dfrac{[I]}{K_I}\right)} = \dfrac{\dfrac{v_{\max}}{\left(1 + \dfrac{[I]}{K_I}\right)}[S]}{K_M + [S]} = \dfrac{v_{\max}^{app}[S]}{K_M + [S]}$

So, in this case, we again get $K_S = K_M$, and see that the $V_{\max}$ term is altered by a term that includes the inhibitor concentration. So the affinity of the substrate appears unaltered, while the maximum reaction rate is changed. Note that as in the case of uncompetitive inhibition, you have E-S-I being formed from E-S, but you also have E-I being formed from free E. So the effect that was apparent in the case of uncompetitive inhibition is not present here because of the balanced drain on free enzyme. Instead, consider that the circular nature of the binding of enzyme, substrate and inhibitor has the net effect of reducing the amount of free enzyme available. So, you can think of this as reducing $V_{\max}$ by reducing E_0. Note that the assumption of binding of substrate not affecting the dissociation for binding of inhibitor and vice versa is usually not true. In this case, the

rate expression becomes more complicated since there are now four different dissociation constants. The inhibition is then considered *mixed*.

4. Taking a look at all three cases…

In looking at all three cases, consider the following:

(1) Note that even in the case of inhibition (albeit *ideal* inhibition), the rate expressions take the Michaelis-Menten form when one considers the reaction rate as a function of the substrate concentration. The way in which you determine the kinetic parameters is hence the same as for no inhibition, but you do have to remember that the parameter values you obtain may be *apparent* and not *intrinsic*. Determining how you obtain those parameters on Lineweaver-Burke and Eadie-Hofstee plots is left as a homework exercise!

(2) Let's look at the extremes of each case. For very low substrate concentrations, S will be eliminated from the denominator, and for very high substrate concentrations, S will disappear from the equation all together. The impact is then as follows:

- Competitive inhibition – at low S, the reaction rate is reduced by the $(1+[I]/K_I)$ term, but at high S, the *intrinsic* maximum velocity can be reached. Therefore, competitive inhibition can be overcome by assuring that $S \gg I$, which makes sense.

- Uncompetitive inhibition – at low S, the effect of the inhibitor cancels out. The way to think about this is that if S is present in very small amounts, there's not enough of E-S around to form the E-S-I complex, so the effect of the inhibitor is not seen. At high S, then the effect is seen with the maximum velocity, V_{max}.

- Noncompetitive inhibition – at low S or high S, the effect is on V_{max}, so the net effect will always be a reduction in the reaction rate.

Note then that only in the case of competitive inhibition can you minimize the effect of the inhibitor by increasing the substrate concentration, and only in the case of uncompetitive inhibition can you minimize the effect by operating at low substrate concentration (where your reaction rate will already be low.)

(3) In the case of a conversion with a purified enzyme, you generally only have substrate and product, so one (or both) of these compounds also acts as the inhibitor. For *substrate inhibition*, you can see that it must be uncompetitive. This is because there is no such thing as an enzyme-inhibitor [E-I] complex, and the uncompetitive case is the only one without such a complex.

The rate equation then becomes as follows:

$$(4.1) \quad v = \frac{v_{max}[S]}{K_M + [S]\left(1 + \frac{[S]}{K_{S2}}\right)} = \frac{v_{max}[S]}{K_M + [S] + [S]^2 / K_{S2}}$$

It is clear from this equation that the reaction rate will decrease as the substrate concentration increases [example kinetics shown below]. Therefore, it is to your advantage to operate a reactor with substrate inhibition such that you minimize the concentration of substrate. [Homework question: *Traditional CSTR is recommended to keep substrate concentration low; however, this is not advisable in free enzymes? Why? What reactor configuration would you suggest in this case?* Using a traditional CSTR with free enzyme is not advisable, since you'd lose your catalyst in the outflow! The other options are to use a fed-batch reactor (either substrate shots or continuous), or a continuous system with an immobilized catalyst that is retained in the reactor.]
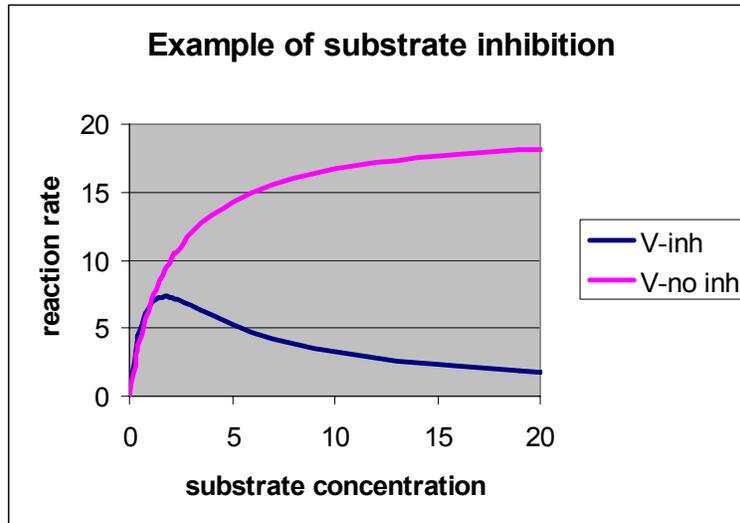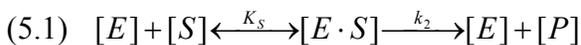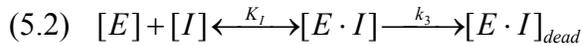

(3) If there is *product* inhibition, it can take any of these forms, and you must use the characteristic rate expressions to determine the exact nature of the inhibition.

5. The special case of toxicity

Recall that in each of the cases discussed, we've assumed that the binding of the inhibitor is reversible, and that the ratio of [enzyme-inhibitor] to [enzyme-no inhibitor] complexes can be described using the dissociation constants. We've also assumed that once bound inhibitor has been released from the enzyme, the enzyme has not been negatively affected by the experience. If the enzyme has indeed been rendered inactive by the binding *or* if the bound inhibitor does not release, then this is a case of *toxicity* and not inhibition. Think of this as the difference between being a fatal and non-fatal injury. If there is just inhibition, then if the inhibitor can be removed, the enzyme will function fine. If the inhibitor is actually toxic, then removing the compound will not help.

The design equations also become much more complicated. The reactions are (for competitive inhibition):

(5.1) $[E]+[S] \xleftrightarrow{K_S} [E \cdot S] \xrightarrow{k_2} [E]+[P]$

(5.2) $[E]+[I]\xleftrightarrow{K_I}[E\cdot I]\xrightarrow{k_3}[E\cdot I]_{dead}$

There is now no quasi-steady state or equilibrium assumption that can be made with regard to enzyme-substrate or enzyme-inhibitor complexes, because the [E-I] complex is reversible and the amount of enzyme available will diminish over time. Note, however, that a toxic or irreversible inhibitor will not affect the substrate parameters, only the amount of enzyme available for reaction. We will not derive the rate expressions here because the math is a bit too complex. You would want to use a numerical solutions software package to solve two sets of differential equations, one for dP/dt, and the other for dE/dt.

We will talk about how to design around substrate and product inhibition and toxicity later in the course. In the meantime, think about what you already know of kinetics and how you may change your design to account for a substrate or product that causes inhibition.