

BL/CH401 Lecture 13 -- Enzyme Kinetics Part II. Enzyme Inhibition

I. Basic Enzyme Kinetics

For an enzyme obeying the Michaelis-Menten equation:

$$v_0 = \frac{V_{\max} [S]}{K_M + [S]}$$

Figure 1.

V_{\max} and K_M describe the catalytic properties of the enzyme.

The meaning of K_M is related to the affinity of the substrate for the enzyme. If K_M has a very low concentration - substrate has a high affinity for the enzyme.

$v_0 = V_{\max}$ at very high $[S]$. V_{\max} has units same as v_0 (mmoles product/min)

K_M is $[S]$ when $v_0 = V_{\max}/2$. K_M has units same as $[S]$ (mM)

V_{\max} is relative to total enzyme used.

$$V_{\max} = k_{\text{cat}} [E]$$

k_{cat} = catalytic rate constant

k_{cat} is called the turnover number

k_{cat} = substrate moles reacted/mole of enzyme/second

Since $[E]$ is difficult to determine, V_{\max}/K_M is useful.

V_{\max}/K_M measures enzyme efficiency and all enzymes can be compared by using it.

II. Enzyme Inhibitors. A. Competitive Inhibition

Inhibitors of enzymes: Two types are considered - Competitive and Non-Competitive.

A *Competitive Inhibitor* has a chemical similarity to the substrate and competes with the substrate for binding to the active site of the enzyme. A good example to describe competitive inhibition is the mitochondrial enzyme, succinate dehydrogenase:

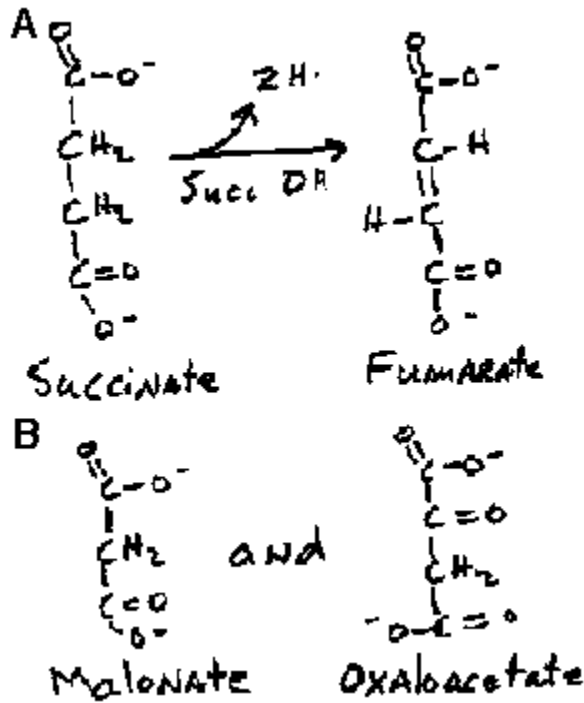


Figure 2. (A) The reaction catalyzed by succinate dehydrogenase is the oxidation of succinate to fumarate. (B) Malonate and oxaloacetate are competitive inhibitors of succinate dehydrogenase.

Both these competitive inhibitors, malonate and oxaloacetate, look like succinate in their chemical character. Both inhibitors are dicarboxylic acids like the substrate succinate so they have groups which can bind in the same places in the active site of succinate dehydrogenase as the substrate. However, neither inhibitor has the capacity to undergo the reaction and so the enzyme is inhibited. Since these inhibitors simply bind to the enzyme, when the succinate concentration is high, they will be pushed out of the site by the substrate and the enzyme will catalyze the reaction as if no inhibitor were present.

An enzyme mechanism model of the action of a competitive inhibitor (I_c) based on the standard model of a Michaelis-Menten enzyme where E + S leads to the E-S complex, which leads to product P:

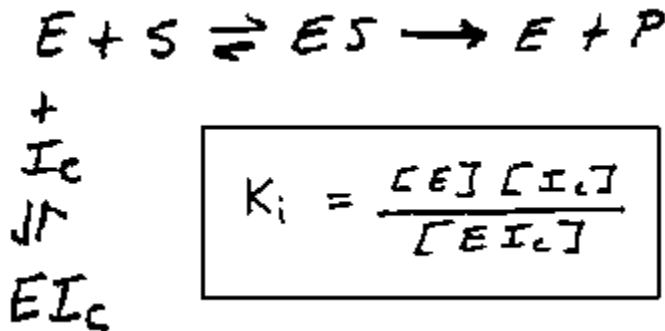


Figure 3A. Model of a Competitive Inhibitor (I_c) Interacting with the Enzyme (E) and an equation for the equilibrium formed between the I_c and E, which is governed by the inhibitor

binding constant, K_i .

This model is the same as the one described in the previous lecture where enzyme (E) and substrate (S) bind to form the ES complex, which will go forward during catalysis to form product (P) and the free enzyme. In the presence of the competitive inhibitor, I_c , a complex forms with enzyme when the inhibitor binds, the E- I_c complex. This is a dead-end complex and can not go on to form product. However, the I_c is bound reversibly to the enzyme and when more substrate is added, the inhibition is overcome by pulling the enzyme free via the breakdown of the E- I_c complex, which is in equilibrium with free enzyme and free I_c . Another way to think about this is - when lots of substrate is added, the concentration of free enzyme (E) falls to such a low level, that some of the E- I_c complex must breakdown to replenish the free E demanded by the equilibrium between E and I_c . This can also be demonstrated by comparing the V_o versus $[S]$ plots for uninhibited enzyme and enzyme in the presence of a competitive inhibitor:

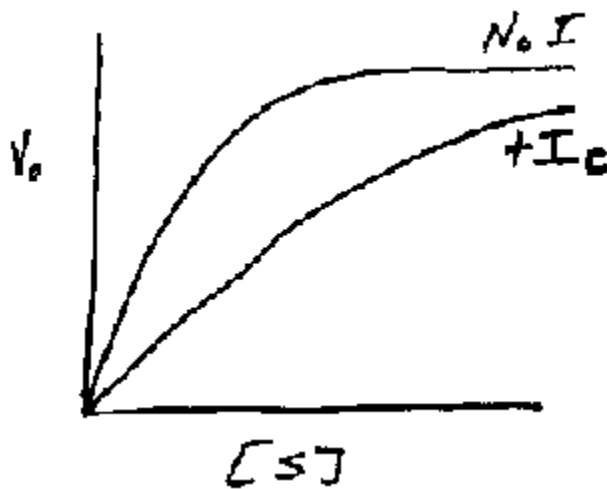


Figure 3B. V_o versus $[S]$ plot comparing the kinetics of the reaction in the absence of inhibitor and in the presence of the competitive inhibitor (I_c). At high $[S]$, the initial velocity in the presence of I_c will be about the same as it is in the absence of the inhibitor. The concentration of S which will be required to overcome the effect of the competitive inhibitor will depend on the $[I_c]$ (ie. concentration of the competitive inhibitor) and the K_i (ie. the binding constant of the inhibitor to enzyme).

In competitive inhibition, addition of more substrate will out compete the inhibitor and overcome the inhibition of the enzyme's catalytic rate - thus, the V_{max} will be the same and only K_m will be altered. This is most clearly illustrated with the double reciprocal plot comparing the uninhibited reaction to that in the presence of I_c .

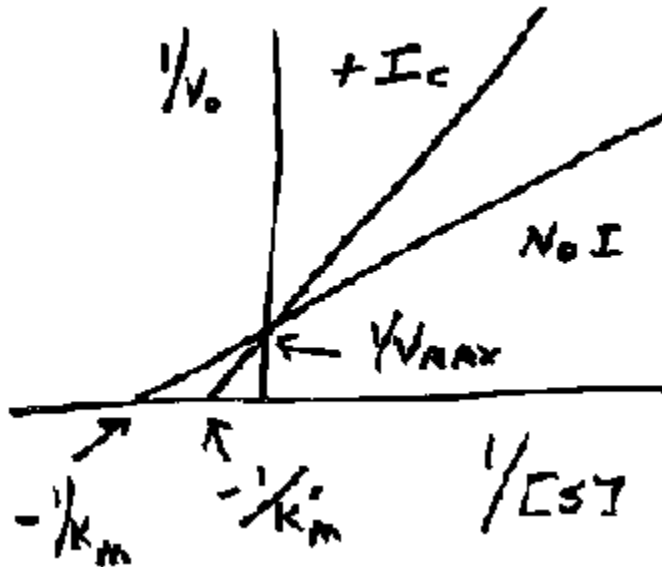


Figure 4A. Double reciprocal plot for competitive inhibitor (I_c).

Here the uninhibited reaction gives the standard double reciprocal plot from which K_m and V_{max} can be calculated. The reaction in the presence of the competitive inhibitor yields apparent constants for the enzyme which are called the K_m' and V_{max}' . For the true competitive inhibitor, the V_{max}' (apparent V_{max} for inhibited enzyme) will be the same as the real V_{max} , while the K_m' (apparent K_m for the inhibited enzyme) will be greater than the real K_m . Thus, the $-1/K_m'$ will be smaller than $-1/K_m$. After finding K_m and K_m' , the K_i for the I_c can be calculated using the equation shown using the given concentration of the competitive inhibitor ($[I]$).

$$K_m' = K_m \left(1 + \frac{[I_c]}{K_i} \right)$$

Figure 4B. Quantitative relationship between the K_m' (apparent K_m) and the real K_m in the presence of a competitive inhibitor. This equation is used to calculate the K_i for the competitive inhibitor at known $[I_c]$.

III. Enzyme Inhibitors B. Non-competitive Inhibition.

A *Non-Competitive Inhibitor* does not compete with substrate and the $[S]$ has no influence on the degree of inhibition of the enzyme's catalytic rate. For example, enzymes with a thiol ($-SH$) not at the active site can be inhibited:

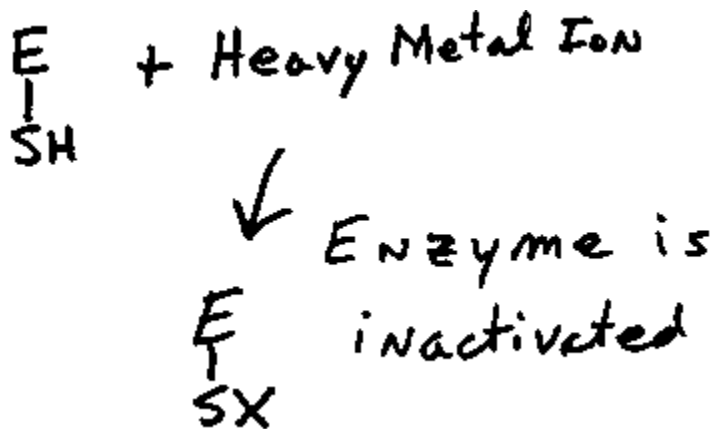
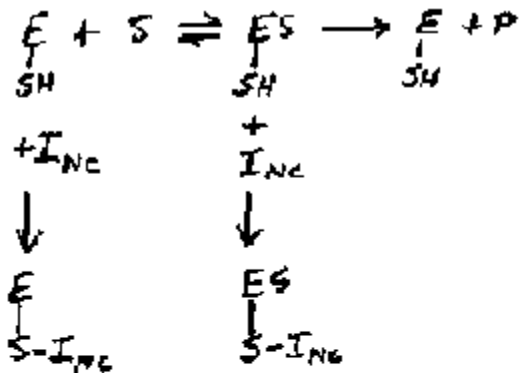


Figure 5. Example of a heavy metal inhibiting an enzyme by binding to a thiol group not at the active site and inactivating the enzyme.

Non-Competitive Inhibition can be model using the standard model for the Michaelis-Menten enzyme where $E + S$ form the ES complex which leads to formation of product P . In this case where the non-competitive inhibitor (I_{nc}) reacts with the enzyme at a site other than the active site, both the free enzyme (E) and the enzyme-substrate complex ($E-S$) react with I_{nc} . Clearly, in this case the reaction of the non-competitive inhibitor is irreversible and the substrate can not overcome the inhibitors impact on the enzyme:



$$K_i = \frac{[E_T] [I_{nc}]}{[E-I_{nc}]}$$

$E_T = \text{All Forms of ENZYME}$

Figure 6. Model of the Non-Competitive Inhibitor (I_{nc}). The equilibrium between enzyme and I_{nc} now depends on the total concentration of enzyme in all forms present (ie. both the free E and the $E-S$ complex) and defines the K_i .

A V_o versus $[S]$ plot for the Non-competitive Inhibitor looks very different than that for a

competitive inhibitor since increasing the [S] has no impact:

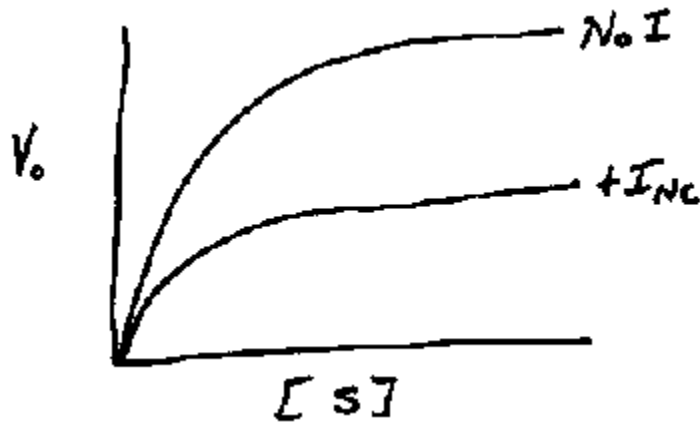


Figure 7. V_o versus [S] plot for enzyme in the absence and presence of Inc.

The double reciprocal plot for this same model shows that Inc decreases V_{max} , as if some of the enzyme had been removed from the system. In classic example of pure non-competitive inhibition, the uninhibited reaction and the enzyme in the presence of Inc will yield the same K_m value.

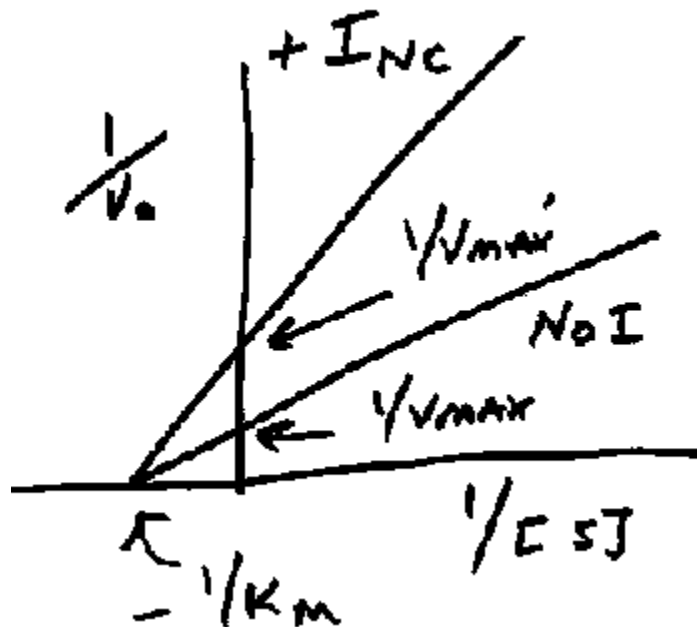


Figure 8A. Double Reciprocal plot for the Non-Competitive Inhibitor (Inc).

Non competitive inhibitors decrease V_{max} but have no effect on K_m .

The apparent V_{max}' is smaller than the real V_{max} and the K_i for the Non-Competitive Inhibitor can be calculated using the following equation and the known [I]:

$$V_{MAX}' = \frac{V_{MAX}}{\left(1 + \frac{[I_{NC}]}{K_i}\right)}$$

Figure 8B. Equation showing the relationship between V_{max}' (apparent V_{max}) and real V_{max} in the presence of a Non-Competitive Inhibitor. Use this equation for calculating the K_i of the Non-Competitive Inhibitor at known $[I_{NC}]$.

IV. Evaluating Enzyme Inhibitors to determine type and their K_i .

To determine what type an inhibitor is:

1. Find K_m and V_{max} for uninhibited from $1/V_o$ vs $1/[S]$ plot.
2. On same graph find K_m' and V_{max}' for inhibited reaction.

A. If $V_{max} = V_{max}'$ then inhibitor is competitive type.

(V_{max} and V_{max}' should not be more than 10% different)

B. If V_{max} does not equal V_{max}' , then if $K_m = K_m'$, inhibitor is non competitive type.

After finding inhibitor type, then use equations to calculate K_i . K_i is a binding constant for inhibitor to the enzyme. K_i has same units as the $[I]$. If $[I] = \text{mM}$, then $K_i = \text{mM}$.

Equations used for calculating K_i values:

Competitive Inhibitor

$$K_M' = K_M \left(1 + \frac{[I_c]}{K_i}\right)$$

Figure 9A. Equation for Competitive Inhibitor.

Non competitive Inhibitor

$$V_{MAX}' = \frac{V_{MAX}}{\left(1 + \frac{[I_{nc}]}{K_i}\right)}$$

Figure 9B. Equation for Non-Competitive Inhibitor.

Rearrange these equations to solve for K_i .

Summary:

Enzyme inhibitors - two types

I. Competitive

II. Non competitive

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