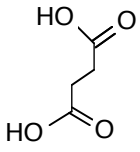
Lecture 19: Enzyme Inhibition & Analysis of Inhibition

Reading in Campbell: Chapter 6.7

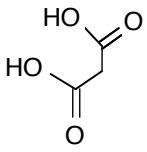
19.1 Molecular Mechanisms of Inhibition:

A: Competitive Inhibition

1. Inhibitor binds to the same site on the enzyme as the substrate.
2. Inhibitor binds ONLY the free enzyme.
3. Inhibitor is usually structurally very similar to the substrate.



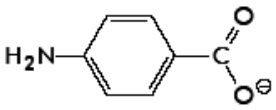
Succinate



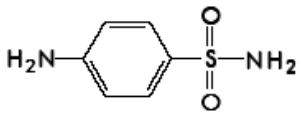
Malonate

- For example, succinate is the normal substrate for the enzyme succinate dehydrogenase. Malonate is an effective competitive inhibitor of this enzyme.

In another example, p-aminobenzoate (PABA) is the normal substrate for the bacterial enzyme dihydrofolate synthetase. The sulfa drug sulfanilamide is an effective competitive inhibitor of this enzyme. Because the conversion of p-aminobenzoate to folic acid by dihydrofolate synthetase is essential for the survival of certain bacteria, the sulfa drug is an effective antibiotic.

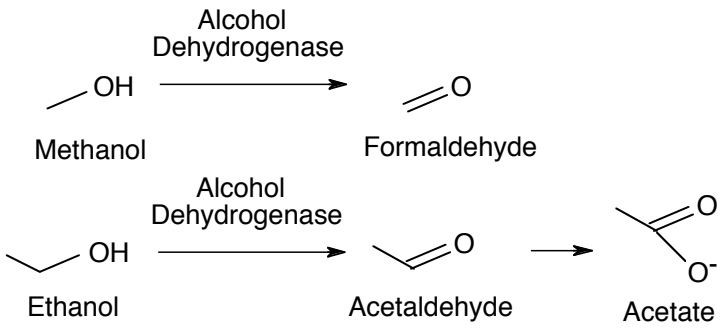


p-Aminobenzoate



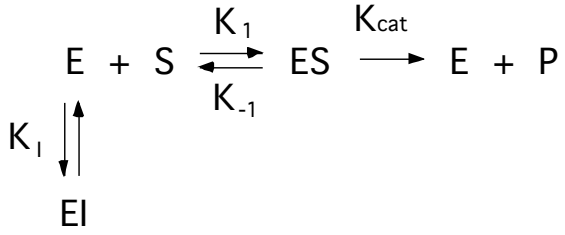
Sulfanilamide

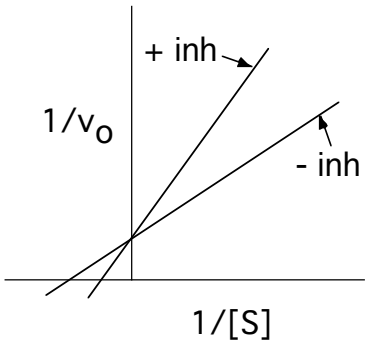
In yet another example, methanol and ethanol compete for the same binding site in alcohol dehydrogenase:



[Note: Ethanol is not a competitive inhibitor in the *classical* sense because it is converted to acetaldehyde by the enzyme.]

A competitive inhibitor reduces the amount of [E] by the formation of an [EI] complex. The inhibitor cannot affect the [ES] complex after it has formed since the inhibitor can no longer bind. There are two anticipated consequences of this binding mode on the steady-state kinetics:

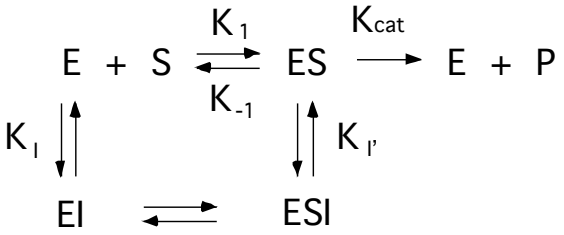


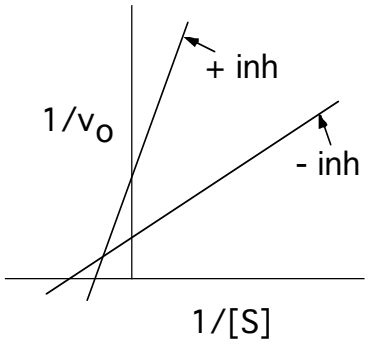


1. **V_{max} is unchanged:** At high levels of substrate all of the inhibitor can be displaced by substrate.

2. **The *apparent* K_m is increased:** It requires more substrate to reach 1/2 maximal velocity because some of the enzyme is complexed with inhibitor.

B: Non-competitive Inhibition:





In this case the inhibitor binds to **both** [E] and [ES]. The binding site of the inhibitor is **not** at the active site. However, the inhibitor binding causes a change in the conformation of the protein that affects either substrate binding (K_m), the chemical step (K_{cat}), or both. *Both* V_{max} and K_m can be altered by non-competitive inhibitors since the precise geometry of the active site is altered when the inhibitor is bound.

19.2 Analysis of Inhibition: Obtaining K_I and $K_{I'}$

The *apparent,* or observed, change in K_m and/or V_{max} depends on the inhibitor concentration. The dissociation constants (K_D) for binding of the inhibitor to the free enzyme [E], and the enzyme substrate complex [ES], are:

$$K_I = \frac{[E][I]}{[EI]} \qquad\qquad K_{I'} = \frac{[ES][I]}{[ESI]}$$

K_I applies to both a competitive and non-competitive inhibitor.
$K_{I'}$ only applies to the non-competitive since [ESI] cannot be formed by a competitive inhibitor.

Define a 'degree of inhibition':

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

Steady-state analysis of either competitive or non-competitive inhibition gives the following general formula:

$$v = \frac{V_{MAX}[S]}{K_m + [S]} \qquad v = \frac{V_{MAX}[S]}{\alpha K_m + [S]} \qquad v = \frac{\frac{V_{MAX}}{\alpha'}[S]}{\frac{\alpha}{\alpha'}K_m + [S]}$$

Several points:

1. The plot of v versus [S] is hyperbolic in *all* cases.
2. Note that the steady-state equation that describes non-competitive inhibition is the most general model. If α' is 1 (i.e. the inhibitor does not bind to the ES form), then the above formula for non-competitive inhibition is the same as that that was obtained for competitive inhibition. However, keep in mind that the *chemical mechanism* of inhibition is different.
3. For the case of non-competitive inhibitors, Campbell assumes that $\alpha=\alpha'$, or that the binding of the inhibitor to [E] is the same as to [ES]. Consequently there is no observed change in K_m ($\alpha/\alpha'=1$). This *almost never* occurs in real enzymes: *both* changes in V_{max} and K_m occur for non-competitive inhibitors.

The double reciprocals of the above equations are:

$$\frac{1}{v} = \frac{K_m}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}} \qquad \text{(no inhibitor)}$$

$$\frac{1}{v} = \frac{\alpha K_m}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}} \qquad \text{(competitive inhibitor)}$$

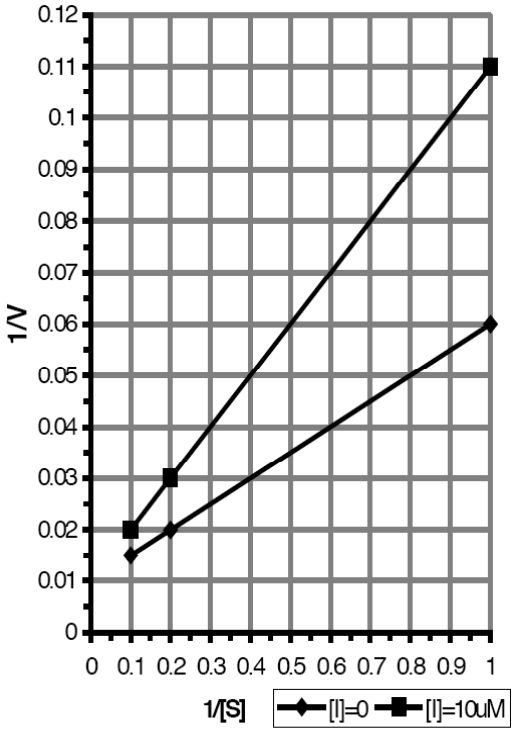
$$\frac{1}{v} = \frac{\alpha K_m}{V_{MAX}}\frac{1}{[S]} + \frac{\alpha'}{V_{MAX}} \qquad \text{(non-competitive inhibitor)}$$

Obtaining K_I and K_I'

The values of α and α' can be easily found from the slope and intercept of double reciprocal plots.

The five easy steps are:

1. Obtain v versus [S] in the *absence* of the inhibitor.
2. Obtain v versus [S] in the *presence* of a fixed and known concentration of inhibitor.
3. Plot both data sets on a double reciprocal plot.
4. α = ratio of the slopes.
5. α' = ratio of y-intercepts.



Example:

[S] mM	v ([I]=0)	v ([I]=10	1/[S]	1/v ([I]=0)	1/v ([I=10])
1	16.67	6.25	1.00	0.0600	0.1600
5	50.00	25.00	0.20	0.0200	0.0400
20	80.00	57.14	0.05	0.0125	0.0175

[I]=10 μM

Obtain α = slope (+inh) / slope (-inh):

Slope (+inh):

Slope (-inh):

α =

Obtain K_I: $$K_I = \frac{[I]}{(\alpha - 1)}$$

Obtain α' = y-int (+inh) / y-int (-inh):

Summary of Kinetic Parameters:

Parameter	Information content
V_{max}	Information on Kcat – fundamental mechanism
K_m	Information on how tight the substrate binds (but not quite its K_D)
K_I	Information on how tight the inhibitor binds to the free enzyme [E] (this **is** a K_D)
K_I'	Information on how tight the inhibitor binds to the [ES] complex (this **is** also a K_D)