

Chem*2580 Lecture 16: Enzyme Inhibition

Synopsis: Inhibitors control enzyme activity by reversibly decreasing the enzyme activity. Different mechanisms of inhibition depend on the relationship between inhibitor and substrate, and can be distinguished by observing how inhibitor affects K_M or V_{max} of the enzyme.

Competitive inhibition increases K_M with no effect on V_{max} .

Non-competitive inhibition decreases V_{max} with no effect on K_M .

Reading: Lehninger, (4th Ed) p.209-212 ; (3rd Ed) p.265-269; Horton p142-147.

Enzymes are subject to various substances that act to reduce their activity.

Inactivation results from a reactive molecule that may form covalent bonds with key amino acids, preventing the enzyme from completing its reaction cycle. Inactivation tends to be **irreversible**. Essentially, an inactivator reduces the quantity of available enzyme irreversibly and in a **stoichiometric** manner:

3 μmol enzyme + 2 μmol inactivator leaves 1 μmol enzyme to continue working.

e.g. **Diisopropylfluorophosphate** reacts with *acetylcholinesterase* irreversibly, blocking transmission of nerve impulses. Many so-called nerve gases act this way and many are halogen-phosphorus compounds.

Reversible Inhibition results from a substance which binds to an enzyme and limits its capacity to catalyze reaction. The binding is **non-covalent and reversible**, and if inhibitor is removed, normal activity is restored. The concentration of inhibitor, like substrate, is typically much higher than enzyme concentration.

Enzymes need to be **regulated** in the course of normal metabolism, i.e. an enzyme that is temporarily not needed is **turned off**. Reversible inhibition can contribute to regulation, since activity can be restored by removing the inhibitor without having to make new enzyme. Enzyme regulation and its consequences are major themes of Chem*3560.

Also, many of the substances which we use as **drugs** act by inhibiting a key enzyme in the body. For example, **acetylsalicylic acid** (ASA or aspirin) inhibits an enzyme called *cyclooxygenase*, responsible for making prostaglandins which stimulate the inflammatory response. When ASA inhibits cyclooxygenase, less prostaglandin is made and inflammation is kept under control. Finding and analyzing properties of enzyme inhibitors is an important aspect of pharmaceutical research.

There are several reversible **inhibition mechanisms**, distinguished by the relationship between inhibitor and the substrate of the enzyme.

Competitive inhibition: the enzyme either binds substrate or binds inhibitor, but not both. In other words, the substrate and inhibitor **compete** for occupation of the enzyme molecule.

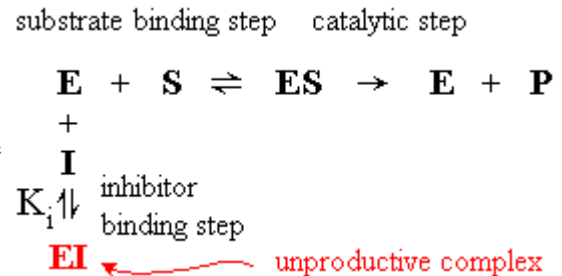
Non-competitive inhibition: inhibitor can bind to enzyme whether substrate is also bound or not, i.e. substrate binding has no effect on inhibition.

Uncompetitive inhibition: opposite to competitive, the inhibitor can **only** bind to the ES complex, and substrate must bind first. This mode may occur with two-substrate enzymes.

Mixed inhibition: is some combination of non-competitive with either of the other mechanisms. True non-competitive inhibition is rare, and most cases are actually mixed inhibition that closely approximates the non-competitive case.

Competitive inhibition:

A competitive inhibitor I can only bind to the unoccupied enzyme E, not to the ES complex. The quantity of complex EI that forms is governed by the equilibrium constant K_i , known as the inhibition constant. If more EI forms, less enzyme is available to form productive ES complex.

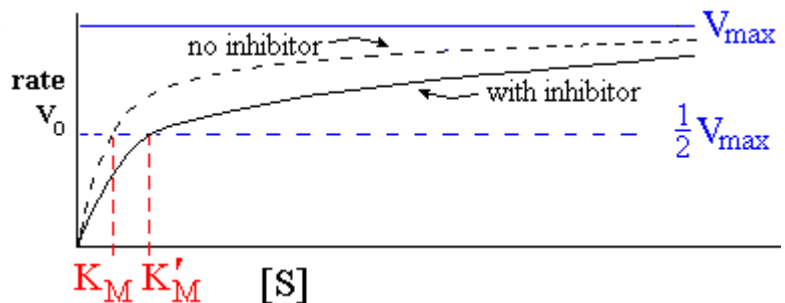


However, since inhibitor I can't bind to ES, very high substrate concentrations can overcome the inhibitor by forcing the substrate binding equilibrium in the direction of ES, and this brings the enzyme up to its normal V_{\max} . Hence the term **competitive** to describe this inhibition.

Michaelis Menten hyperbolic plot: shows the rate v_0 of the enzyme reaction **with a constant concentration [I]** of inhibitor as [S] is varied.

The rate rises more gradually when inhibitor is present, but eventually reaches normal V_{\max} when [S] is very high.

If inhibitor concentration [I] is set equal to K_i , this causes the K_M' observed to be **doubled** relative to uninhibited enzyme.



Characteristics of a competitive inhibitor: V_{\max} is **unchanged**, but **observed K_M' increases**.

In presence of inhibitor at concentration $[I]$, $K_M' = K_M \left(1 + \frac{[I]}{K_i}\right)$

Remember that **higher K_M** implies that the enzyme binds its substrate with **less affinity**.

i.e. a higher concentration of $[S]$ is needed before the enzyme can reach 50% of V_{\max} .

K_i is equal to the concentration of inhibitor which **doubles the observed K_M of the enzyme**.

If we set the inhibitor concentration $[I] = K_i$, then $K_M' = K_M \left(1 + \frac{K_i}{K_i}\right) = K_M (1 + 1)$

The term $\left(1 + \frac{[I]}{K_i}\right)$ is the **inhibition factor**, and appears in all inhibition equations.

The change in K_M is detected by plotting the data in one of the linear forms,

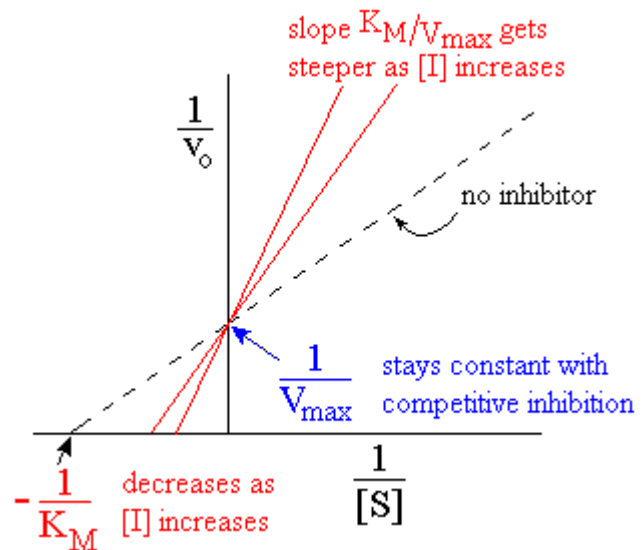
e.g. in the **Lineweaver Burk Plot**

The dashed line represents the activity of the normal uninhibited enzyme, and then the experiment is repeated with a series of different concentrations of I . The other two lines show the effect increasing $[I]$.

Each line shows the behaviour of the enzyme for a **given value of $[I]$** . The higher concentration of inhibitor gives a steeper slope to the line.

The series of lines pivot on the y intercept, since V_{\max} is **not changed for competitive inhibition**.

The X-intercept becomes smaller as $[I]$ increases, since K_M **increases for competitive inhibition**.



To measure K_i , one finds the inhibitor concentration $[I]$ that just **doubles the observed K_M'** . This is represented by the middle line in the figure (x-intercept halved means K_M' was doubled.).

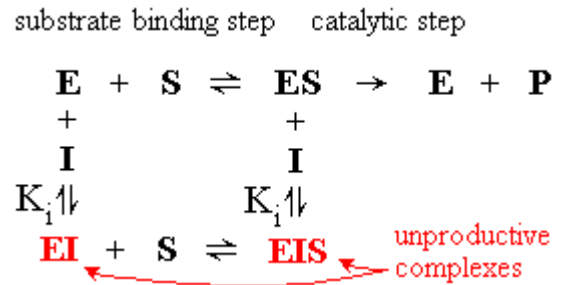
To see how this would appear in Woolf-Hanes or Eadie Hofstee plots, see the supplementary web page “More enzyme inhibition plots”

Non-Competitive inhibition:

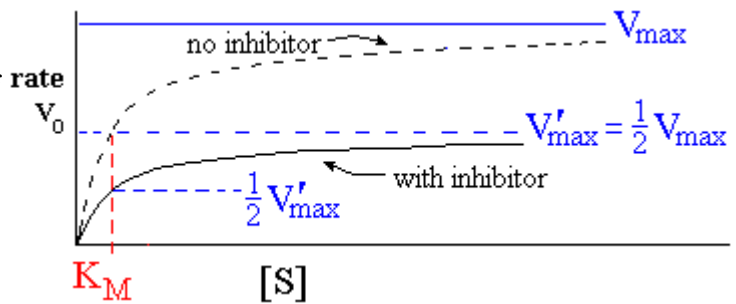
A non-competitive inhibitor I can bind both to unoccupied enzyme E, and to ES complex. The EI complex can bind S, but EIS is unable to proceed to give products.

The quantity of complexes EI and EIS that form are governed by the equilibrium constant K_i . If more EI or EIS forms, less enzyme is available to form

productive ES complex. Since I can also bind to ES, high substrate concentrations do not overcome the inhibitor. Hence the term **noncompetitive** to describe this inhibition. True non-competitive inhibition requires K_i to be the same at both stages. If the K_i for I binding to empty E is not the same as for I binding to occupied ES, **mixed inhibition** will be observed.



Michaelis Menten hyperbolic plot: shows the rate v_o of the enzyme reaction **with a constant concentration [I]** of inhibitor as [S] is varied. The rate rises more gradually when inhibitor is present, and levels off at a **lower V'_{max}** .



If inhibitor concentration [I] is set equal to K_i , this causes the V'_{max} observed to be **halved** relative to uninhibited enzyme.

Characteristics of a noncompetitive inhibitor: V'_{max} decreases, but K_M is unchanged.

$$V'_{max} = \frac{V_{max}}{(1 + [I]/K_i)}$$

Lineweaver-Burk Plot:

The dashed line represents the activity of the uninhibited enzyme. The other two lines show the effect of added inhibitor I. The series of lines pivot on the **-ve x intercept**, since **K_M is unchanged for non-competitive inhibition**.

Y-intercept and slope increase due to the reciprocal dependence on V_{max} , which decreases.

To measure K_i , one finds the inhibitor concentration [I] that just **halves the observed V'_{max}** .

