Chapter 11

Web Text Box 2

Analyzing enzyme kinetics: finding out how drugs work

Many drugs work by inhibiting enzymes. There are a number of different ways by which a drug can inhibit an enzyme's operation. By studying the kinetics of the reaction (book pages 182-185) in the absence and presence of drug we can learn more about the way in which the drug impedes the enzyme's function.

Competitive Reversible Inhibition

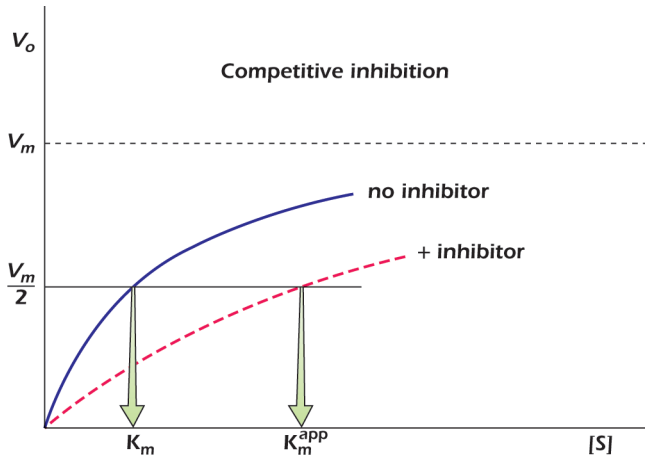
As mentioned in Medical Relevance Box 11.3 (book page 187) a reversible inhibitor is a molecule capable of inhibiting an enzyme by binding to it reversibly so the free enzyme, inhibitor and enzyme inhibitor complex are in equilibrium.

$$E + I \rightleftarrows EI$$

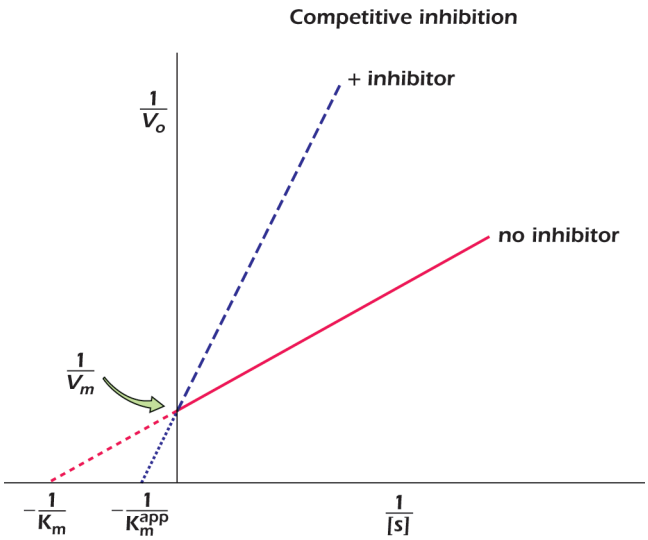
Reversible inhibitors can be removed by dialysis. In the body a drug that is a reversible inhibitor will dissociate from the target enzyme, diffuse away and eventually be excreted by the kidneys.

If a reversible inhibitor binds at the active site of an enzyme so that the substrate cannot bind it is said to be a competitive inhibitor. If the substrate concentration [S] is increased sufficiently then the substrate will displace the inhibitor and the enzyme will work at its maximum rate; that is, a competitive inhibitor will not change V_m: no matter how strongly the inhibitor binds or how high its concentration it is always going to be swamped out by the substrate eventually. In the plot of v_o against [S] the initial velocity v_o will be lower in the presence of the inhibitor, but both lines tend towards the same asymptote, V_m. In the presence of the inhibitor the apparent K_M, K_M^{app} will be greater than K_M in the absence of the inhibitor (remember that K_M is the substrate concentration that half saturates the

enzyme so the stronger the binding the lower the K_M) as it takes more substrate to half saturate the enzyme.



A Lineweaver-Burke plot (In Depth 11.3 on book page p185) for a competitive inhibitor is shown below. The intercept on the y axis ($1/V_m$) does not change, but the slope of the straight line increases in the presence of the inhibitor, so that the intercept on the x axis ($-1/K_M$) is closer to the origin, reflecting the larger apparent value of K_M.



One can define a dissociation constant for the EI complex to give a quantitative measure of how tightly the inhibitor binds to the enzyme:

$E + I \rightleftarrows EI$ where $K_I = \frac{[E][I]}{[EI]}$

Some algebra allows us to derive the apparent K_M when the inhibitor is present:

$$K_M^{app} = K_M\left(1 + \frac{[I]}{K_I}\right)$$

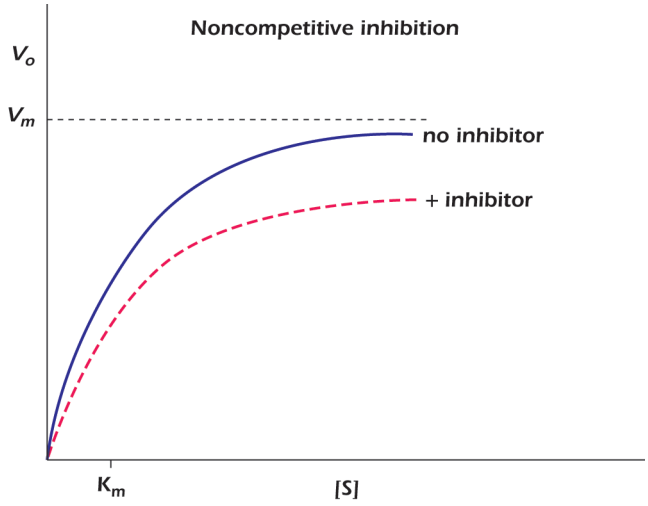
so that by measuring K_M^{app} at different [I] we can estimate K_I. This is critical information for a drug, since it tells allows us to calculate the concentration of drug that must be present in the body to achieve the desired degree of enzyme inhibition.

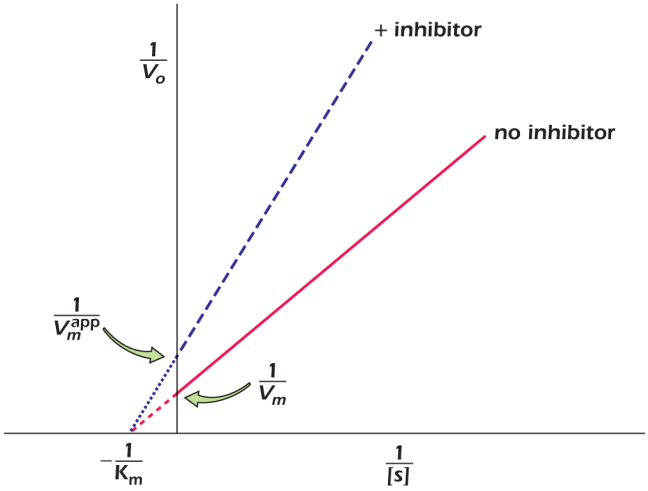
Competitive inhibitors are not ideal as drugs – not only are they reversible (and therefore lost in the urine) but their effect will diminish if, as is likely, inhibition of the enzyme causes the concentration of the no longer metabolized substrate to increase. A competitive inhibitor that binds very strongly (that is, K_I is very much less than K_M) may be viable as a drug, and determination of its K_I helps determine this. For example the drug methotrexate is a useful competitive inhibitor of an enzyme called dihydrofolate reductase that is critical in the synthesis of purine nucleotides. Methotrexate binds to dihydrofolate reductase 1000 times more strongly than the normal substrate, dihydrofolate, and is used in cancer chemotherapy. Cancer cells divide rapidly and therefore require lots of purines in order to replicate their DNA. Other body cells divide less frequently and so are less affected by the drug.

Competitive inhibition occurs naturally in the cell. There are many molecules in metabolism that are sufficiently similar to one another to bind to several enzymes. Cells have evolved to live with this and it is rarely significant except in some disease states (or sometimes as the side effect of a drug treatment).

Noncompetitive Reversible Inhibition

We have seen that competitive inhibitors increase K_M but do not affect V_m. The exact opposite case is called noncompetitive reversible inhibition: K_M stays the same and V_m diminishes. This is shown on the v_o against [S] and Lineweaver-Burke plots shown below.

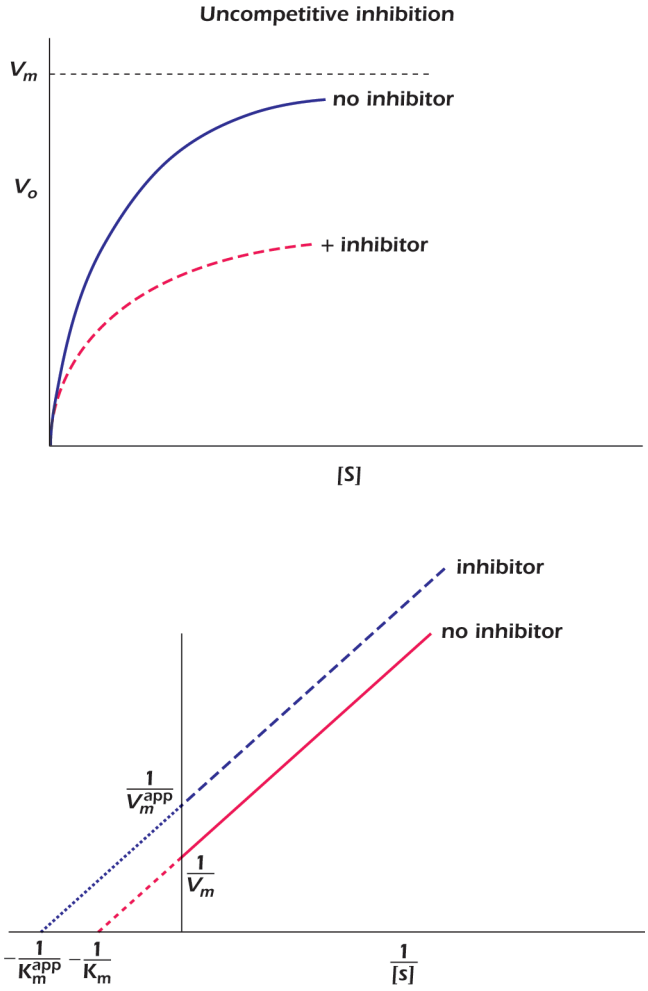




Noncompetitive inhibition is really very rare but is a nice textbook case. It is thought that the antibiotic deoxycycline, used to treat gum disease, inhibits a collagen destroying enzyme in a noncompetitive fashion at low concentrations.

Uncompetitive Reversible Inhibition

Rather more common is uncompetitive inhibition. An uncompetitive inhibitor alters both V_m and K_M but keeps the ratio V_m/K_M constant. It can arise when the inhibitor binds to the ES complex only. The v_o against [S] plot will vary in appearance depending upon the effects on K_M and V_m. Lineweaver-Burke plots give parallel lines, as shown below.



Algebraic methods exist for measuring the dissociation constants for the inhibitors in these types of inhibition. A general treatment shows that these are all special cases of what is called mixed inhibition.

Our discussion is somewhat simplified and conditions in the cell require much more complex treatments but this gives an idea of the basics. We have only discussed the effects of inhibitors on enzymes that bind their substrate(s) following a simple hyperbola, that is, enzymes that obey Michaelis-Menten kinetics. Allosteric enzymes (book page 186) can be inhibited too. As they do not usually give hyperbolic kinetics they will not give straight lines in Lineweaver-Burke plots.