Further Enzyme Kinetics for Model Building

C1net Workshop 2; Day 2

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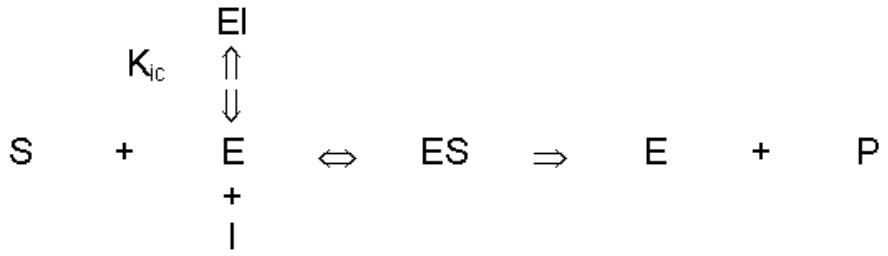
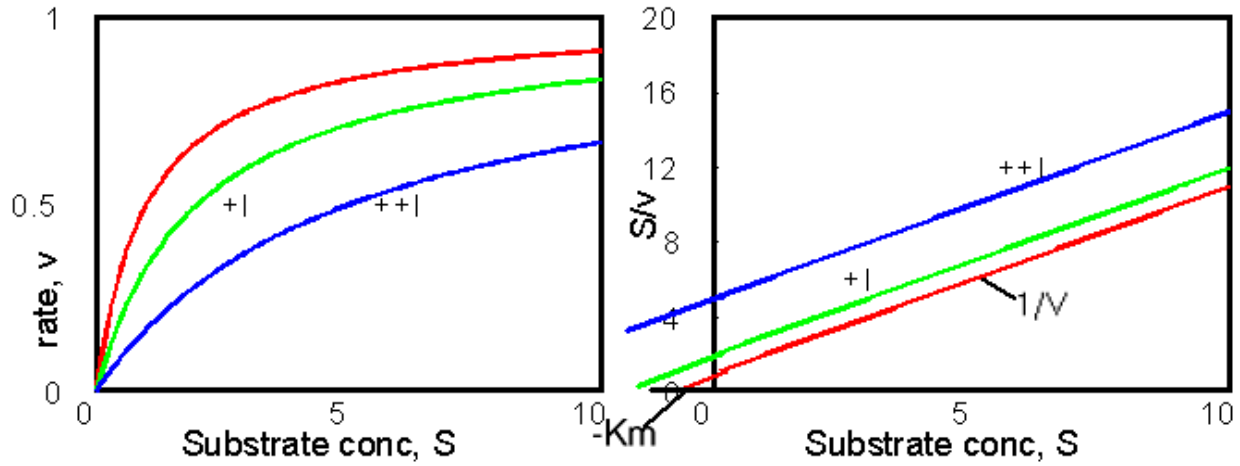
Enzyme Inhibition

Competitive Inhibition

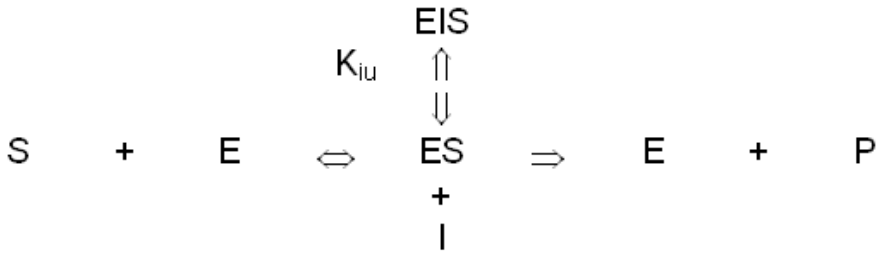


In competitive inhibition, K_{m} is increased but V remains unchanged:

$$v = \frac{V.S}{K_{\mathrm{m}}\left(1 + \frac{I}{K_{\mathrm{ic}}}\right) + S}$$

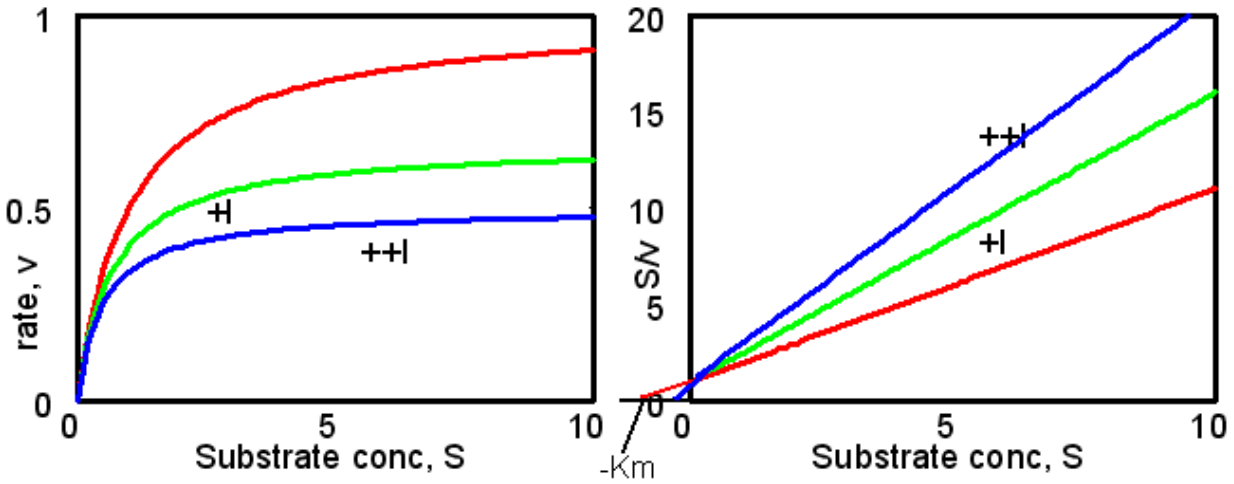


Uncompetitive Inhibition

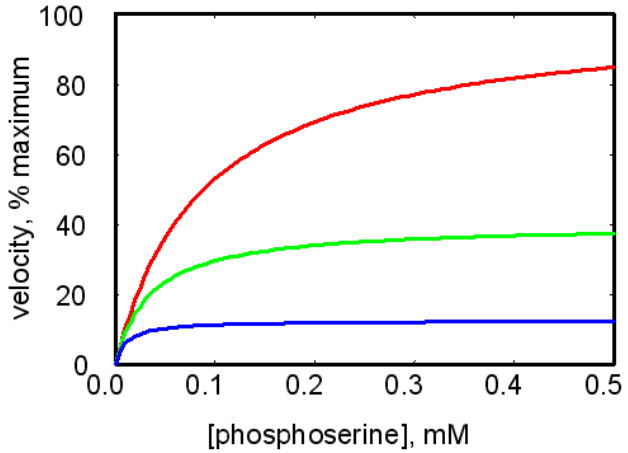


I binds only to ES and both K_{m} and V are reduced. The inhibition is stronger at higher substrate concentrations.

$$v = \frac{\frac{V.S}{1+\frac{I}{K_{\mathrm{iu}}}}}{\frac{K_{\mathrm{m}}}{1+\frac{I}{K_{\mathrm{iu}}}} + S}$$



Uncompetitive Inhibition Example

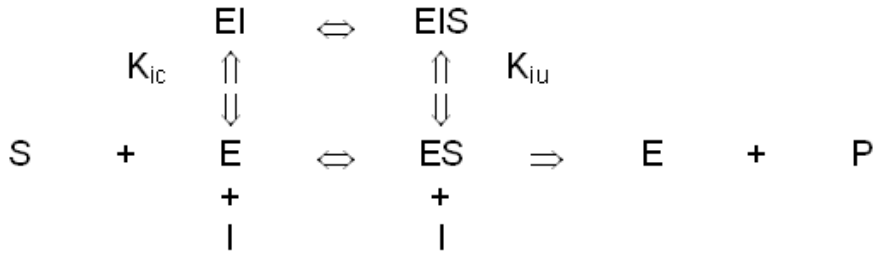


Inhibition of mammalian phosphoserine phosphatase at 0, 1 and 6 mM serine. The enzyme is the last step of the 3–step serine synthesis pathway.

Glyphosate — the herbicide ‘Roundup’ — is an uncompetitive inhibitor of 3–phosphoshikimate–1–carboxyvinyl transferase, a step in the synthesis of aromatic amino acids. Plants die of the accumulation of toxic shikimate.

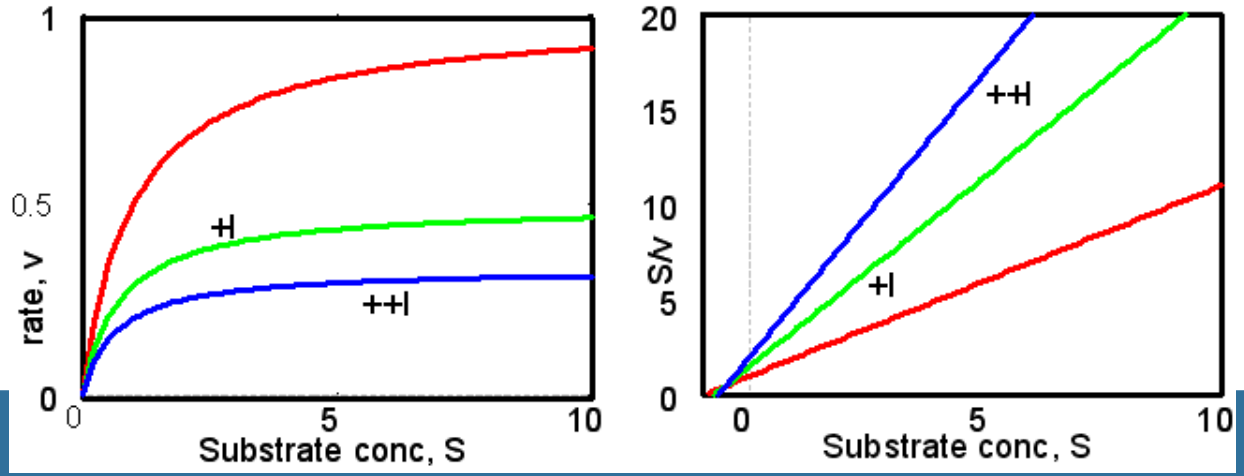
Triclosan is a bacteriocide that is an uncompetitive inhibitor of enoyl reductase in fatty acid biosynthesis.

Mixed Inhibition



I binds to E and ES, and V is reduced; K_{m} may increase or decrease depending whether the competitve or uncompetitive component is dominant.

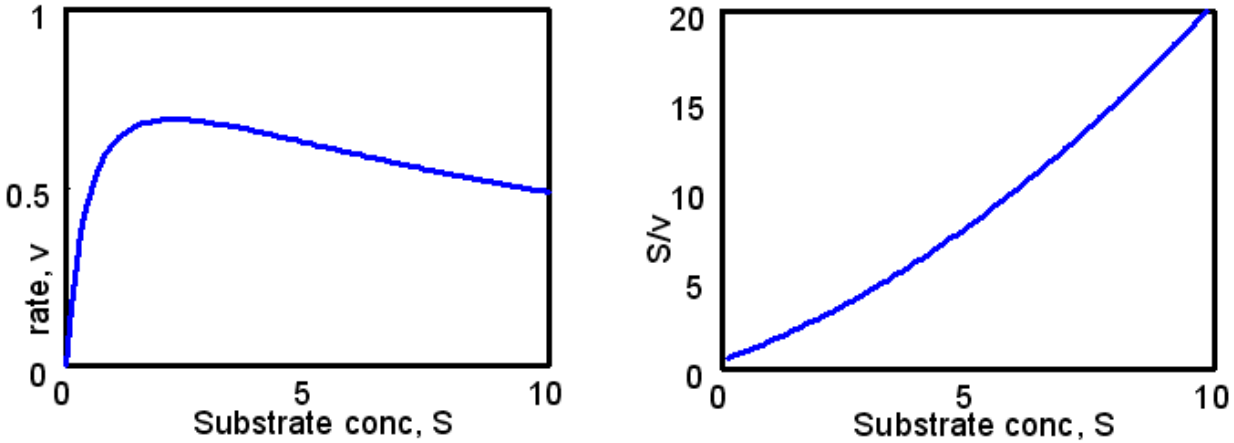
$$v = \frac{\frac{V.S}{1+\frac{I}{K_{\mathrm{iu}}}}}{\frac{K_{\mathrm{m}}\left(1+\frac{I}{K_{\mathrm{ic}}}\right)}{1+\frac{I}{K_{\mathrm{iu}}}} + S}$$



Substrate Inhibition

Occurs as a result of a second substrate molecule binding more weakly and inhibiting the enzyme. The usual reason is that in a two substrate enzyme, one of the substrates also acts as a partial analogue of the second.

$$v = \frac{V.S}{K_{\mathrm{m}} + S + \frac{S^2}{K_{\mathrm{is}}}}$$



e.g. Inhibition of heart muscle lactate dehydrogenase by pyruvate.

Inhibition - the main messages

1. Inhibition terms in enzyme rate equations have the same hyperbolic form as the substrate saturation, and are characterised by a K_{i}.

2. The different types are marked by different effects on V and K_{m} or both.

3. Uncompetitive and mixed may seem unusual, but they often arise as forms of product inhibtion in two–substrate enzymes, which leads to . . .

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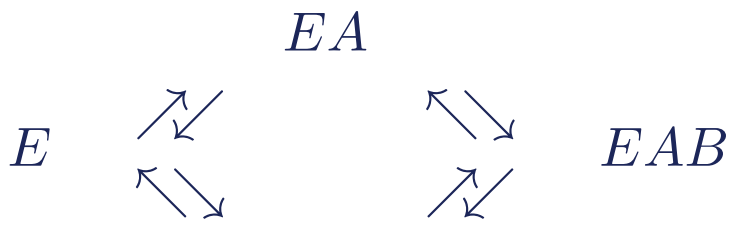
Two Substrate Enzymes

1. Random order

$$A + B \rightleftharpoons P + Q$$

Mechanism:



$$EAB \rightleftharpoons EPQ$$

$$EPQ \longrightarrow E + P + Q$$

2. Compulsory order

$$A + B \rightleftharpoons P + Q$$

Mechanism:

$$E + A \rightleftharpoons EA$$

$$EA + B \rightleftharpoons EAB$$

$$EAB \rightleftharpoons EPQ$$

$$EPQ \longrightarrow E + P + Q$$

3. Double displacement (ping–pong):

$$A + B \rightleftharpoons P + Q$$

Mechanism:

$$E + A \rightleftharpoons EA$$

$$EA \rightleftharpoons EX + P$$

$$EX + B \rightleftharpoons EXB$$

$$EXB \longrightarrow E + Q$$

Rate equation for two substrate enzymes

With slight variations for different mechanisms, a general equation in the absence of products is:

$$v = \frac{VAB}{K_{\mathrm{iA}}K_{\mathrm{B}} + K_{\mathrm{B}}A + K_{\mathrm{A}}B + AB}$$

For high B, with A varied, both top and bottom lines of the right hand side can be divided by AB to give:

$$v = \frac{V}{\frac{K_{\mathrm{iA}}K_{\mathrm{B}}}{AB} + \frac{K_{\mathrm{B}}}{B} + \frac{K_{\mathrm{A}}}{A} + 1}$$

We can ignore terms divided by B to give:

$$v \approx \frac{V}{\frac{K_{\mathrm{A}}}{A} + 1} = \frac{VA}{K_{\mathrm{A}} + A}$$

This is the Michaelis–Menten equation, and when B is fixed high and A varied, the enzyme behaves like a single substrate enzyme, and K_{A} is the K_{m} for A.

Generic reversible two substrate equation

The following equation is an approximation for a reaction A + B $\Leftrightarrow$ P + Q, where P is competitive with substrate A and Q with substrate B. It is more important to get the product inhibition type correct than to worry too much about the differences between K_{A} and K_{iA}.

$$v = \frac{V_{\mathrm{f}}\left(AB - \frac{PQ}{K_{\mathrm{eq}}}\right)}{\left(K_{\mathrm{A}}(1 + \frac{P}{K_{\mathrm{P}}} + \frac{I}{K_{\mathrm{iI}}}) + A\right)\left(K_{\mathrm{B}}(1 + \frac{Q}{K_{\mathrm{Q}}}) + B\right)}$$

Haldane relationship

The derivation assumes the existence of a Haldane relationship:

$$K_{\mathrm{eq}} = \frac{V_{\mathrm{f}} K_{\mathrm{P}} K_{\mathrm{Q}}}{V_{\mathrm{r}} K_{\mathrm{A}} K_{\mathrm{B}}}$$

Haldane relationships vary with the two–substrate mechanism, but most of the mechanisms have the above relationship, except with one term in the numerator and one in the denominator equal to a K_{iS} term. This is not critical here because the term $K_{\mathrm{A}} K_{\mathrm{B}}$ in is taking the place of the term $K_{\mathrm{iA}} K_{\mathrm{B}}$.

Allosteric Enzymes

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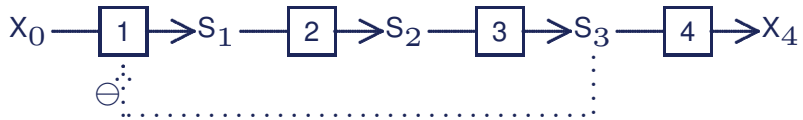
Feedback Inhibition: Discovery

1. Umbarger, 1956: the first enzyme in the pathway to isoleucine, threonine deaminase, is specifically and strongly inhibited by isoleucine. This *feedback inhibition* is highly specific since the closely similar leucine and valine are not effective.

2. Yates and Pardee, 1956: aspartate transcarbamylase in *E. coli*, the first step in the synthesis of pyrimidine nucleotides from aspartate, is inhibited by the pathway end product CTP.

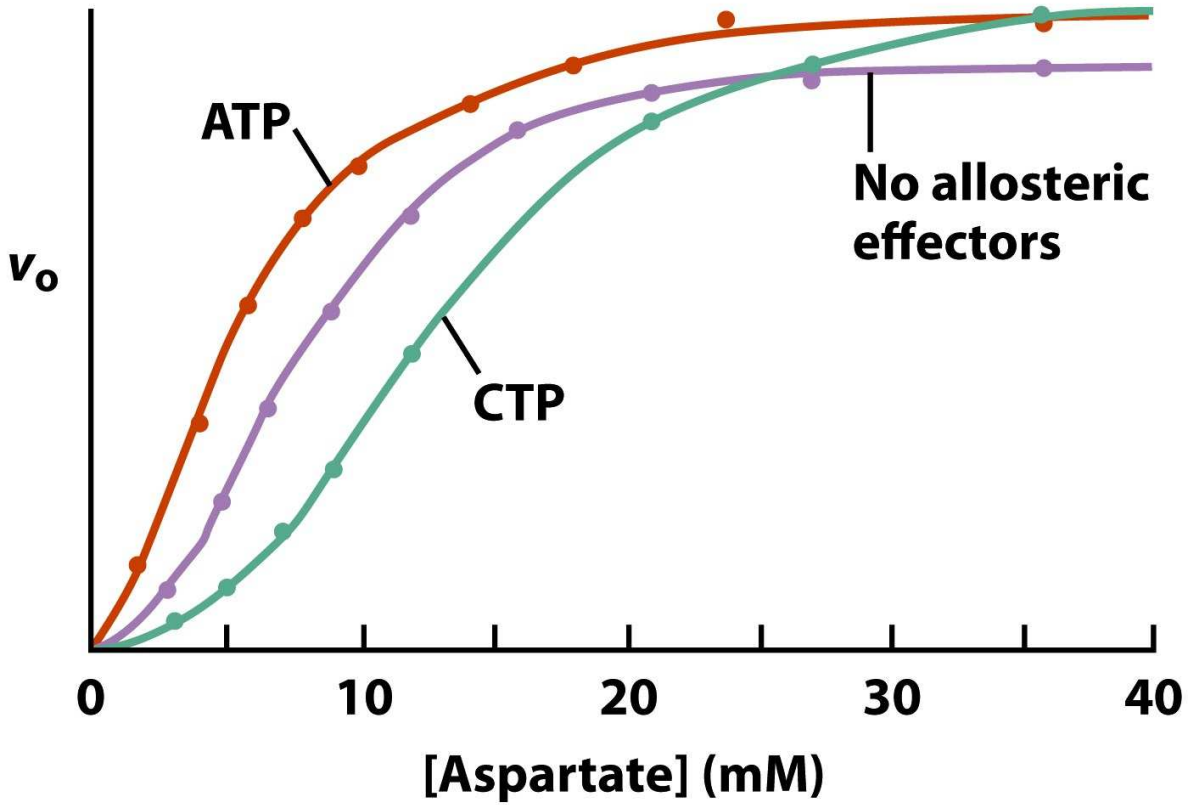
Feedback Inhibition: Schematic & Role



This is a generalized feedback inhibition loop, with the enzymes represented by numbered boxes. Metabolite S_3 inhibits enzyme 1.

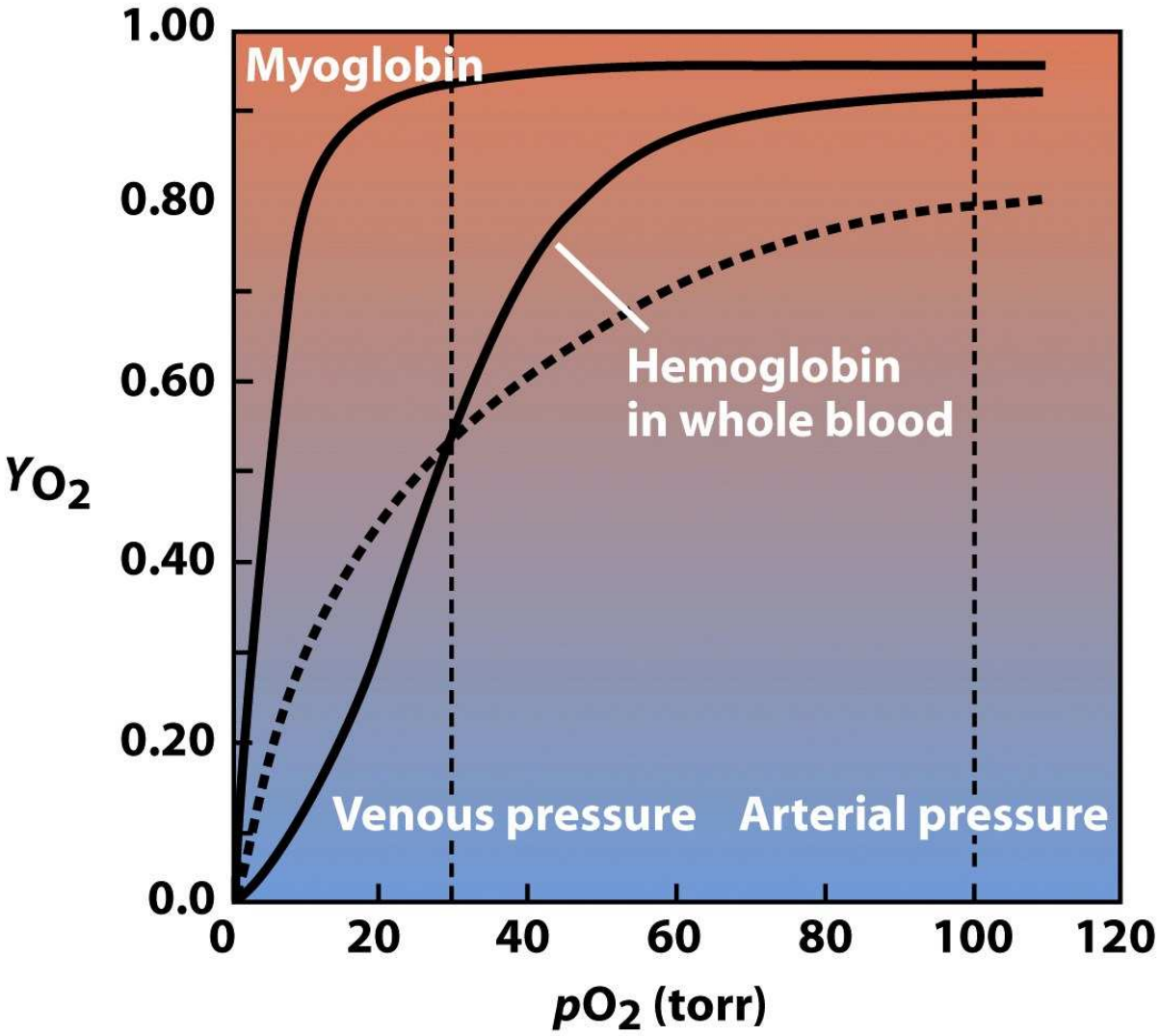
The role of feedback inhibition in metabolism has been widely misunderstood right from the first. We'll be looking at how it really works later in the course.

ATCase



The kinetics of the allosteric enzyme aspartate transcarbamylase (ATCase). The rate of ATCase is shown with respect to one of its substrates, aspartate (centre curve/purple). The enzyme is inhibited in a feedback manner by the pathway end–product CTP (lower/turquoise). ATP acts as an activator (upper/red).

Haemoglobin — an Honorary Enzyme



The Hill Equation

Fractional saturation, $\bar{Y}$:

$$\bar{Y} = \frac{\text{No. of } O_2 \text{ sites occupied}}{\text{Total no. of } O_2 \text{ sites on Hb}}$$

The Hill equation for the reaction:

$$\text{Hb} + n\text{O}_2 \overset{K}{\rightleftharpoons} \text{Hb}(\text{O}_2)_n$$

is:

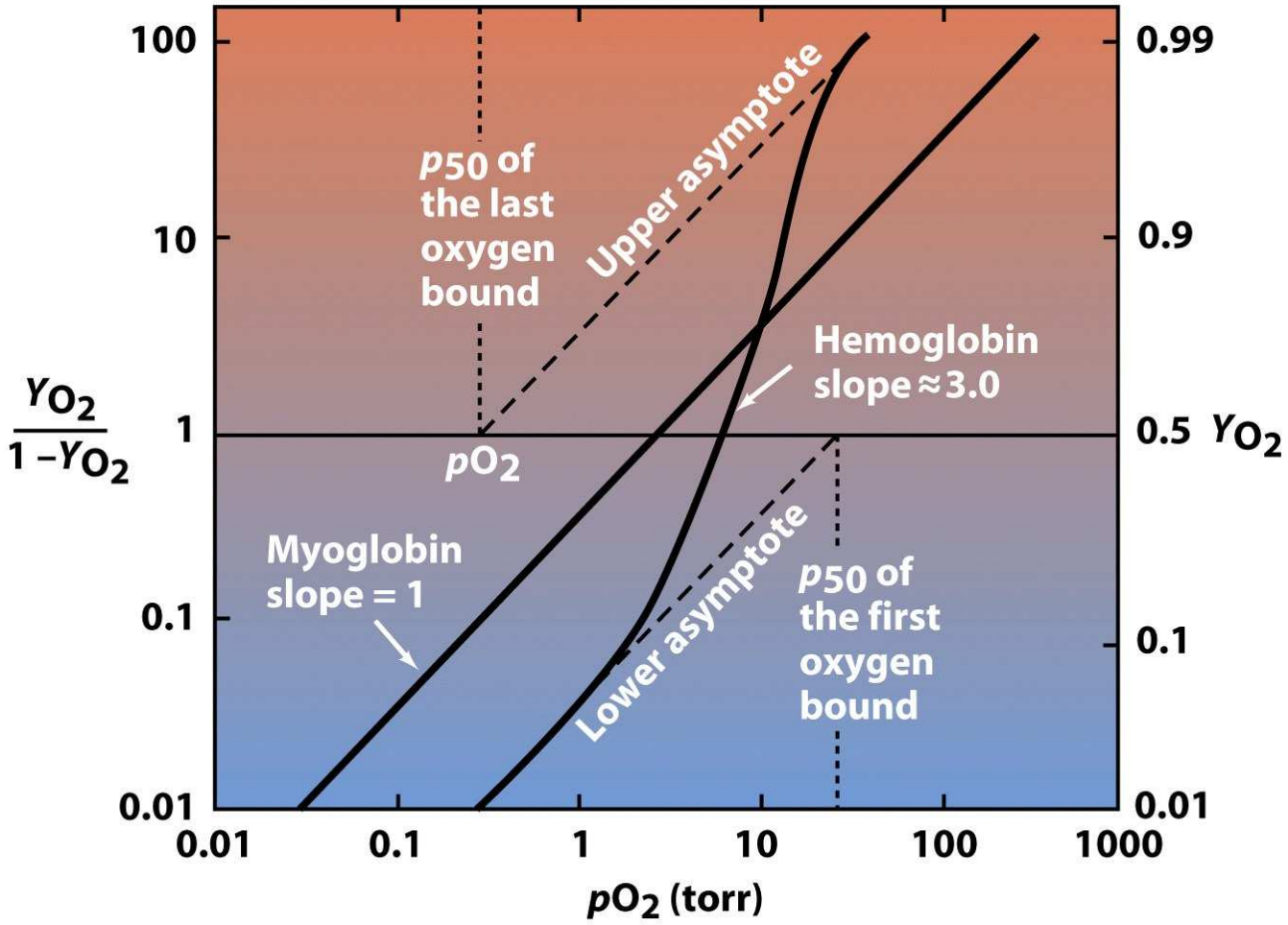
$$\bar{Y} = \frac{[O_2]^{n_H}}{K^n + [O_2]^{n_H}}$$

which has the linear form:

$$\log\left\{\frac{\bar{Y}}{1-\bar{Y}}\right\} = n_H \log[O_2] - n_H \log K$$

Positive cooperativity: $n_H > 1$; non-cooperative (Michaelis-Menten): $n_H = 1$; negative cooperativity: $n_H < 1$. K is also expressed as p_{50}.

The Hill Plot



$\log\left\{\frac{\bar{Y}}{1-\bar{Y}}\right\} = 0$ and $[O_2] = K = p_{50}$ when $\bar{Y} = 0.5$.

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Allosteric Enzymes: Characteristics

Monod, Wyman & Changeux (1963) defined allosteric enzymes as a class, and devised the name:

- The enzymes are composed of a number of subunits, i.e. they are *multimeric*.
- The feedback inhibitor binds at a site distinct from the active site of the enzyme, named an *allosteric site*, hence *allosteric enzymes*. In some cases, active site and inhibitor site are on different subunits.
- In some cases, there are activators as well as inhibitors of the enzymes: i.e. effectors
- Sigmoid rate curves are common.

See my book for more details of equations. For many modelling purposes, a version of the Hill equation will suffice.

K Systems and V Systems