Understanding the Control of Metabolism

C1net Workshop 2; Day 2

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Outline

- Elements of Metabolic Control Analysis (MCA)
  - The flux control coefficient
  - Control coefficients and enzyme kinetics
  - Flux control coefficients in context

The context: manipulating metabolism

Two different problems:

- Easy: stopping flux to a product through a pathway. (Pick an essential enzyme; knock out by mutation or inhibition.)

- Hard: increasing flux to a product through a pathway.

Why isn't the solution to the hard problem:

1. find the rate–limiting enzyme, and

2. increase the amount of this enzyme?

The rate–limiting step concept

When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor. *Blackman (1905).*

Quotes

- The first committed step being irreversible . . . most metabolic pathways are controlled by regulating . . . their first committed steps (*Voet & Voet, 1990*).
- . . . an entire pathway can be controlled by regulating only the enzyme that catalyzes the first step in the pathway (*Zubay et al, 1995*).
- The first enzyme of a pathway is usually a strategic place for control (*Elliott & Elliott, 1997)*.
- In a multistep pathway the first enzyme is ususally regulated and the others are not (*Zubay, 1998*).

Critique of ‘rate–limiting’ steps

- It is not possible for one step in a pathway to be ‘slower’ than the others.
- The flux through multistep pathways, even with simple kinetics, has long been known to depend in principle on all the steps.
- The experimental evidence is that the genuinely rate–limiting step is rare.

Control Coefficients

Metabolic Control Analysis

originated with:

- Henrik Kacser & Jim Burns (Edinburgh) and

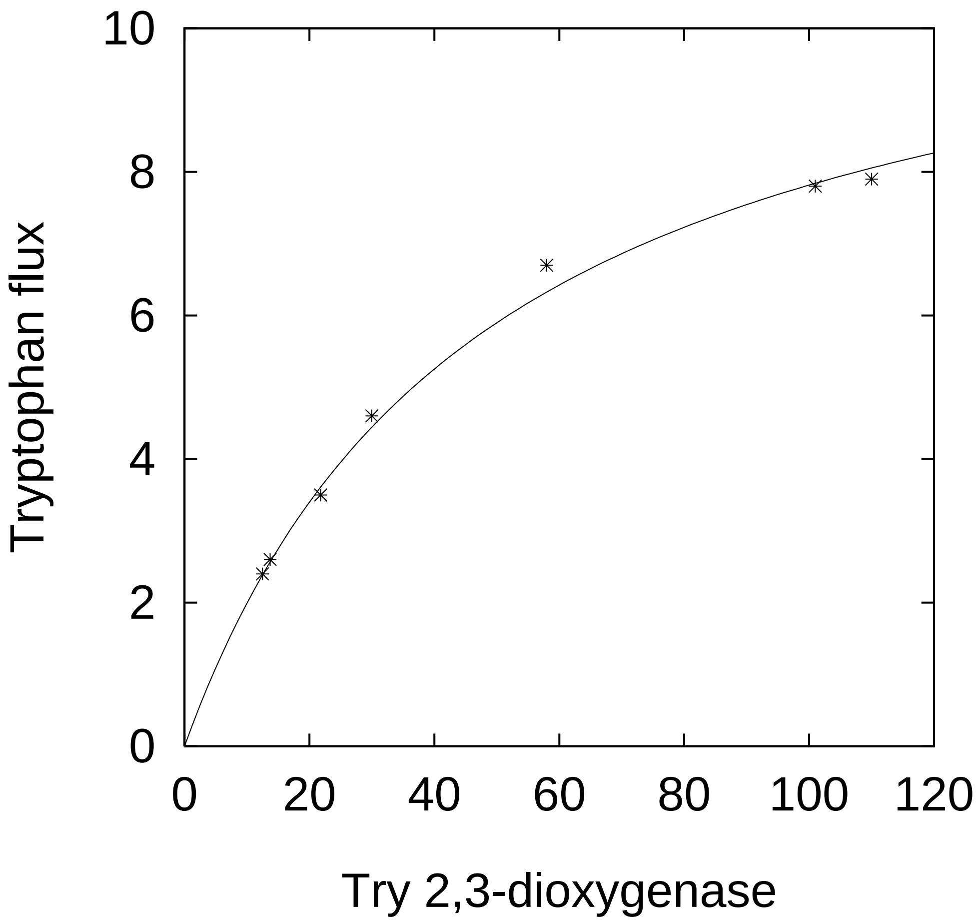
- Reinhart Heinrich & Tom Rapoport (Berlin)

independently in 1973 (based in part on earlier work by Joe Higgins).

Kacser, H. and Burns, J. A. (1973) *Symp. Soc. Exp. Biol. 27*, 65–104. Reprinted in *Biochem. Soc. Trans. 23,* 341–366, (1995).

Heinrich, R. and Rapoport, T. A. (1974) *Eur. J. Biochem. 42*, 89–95, 97–105.

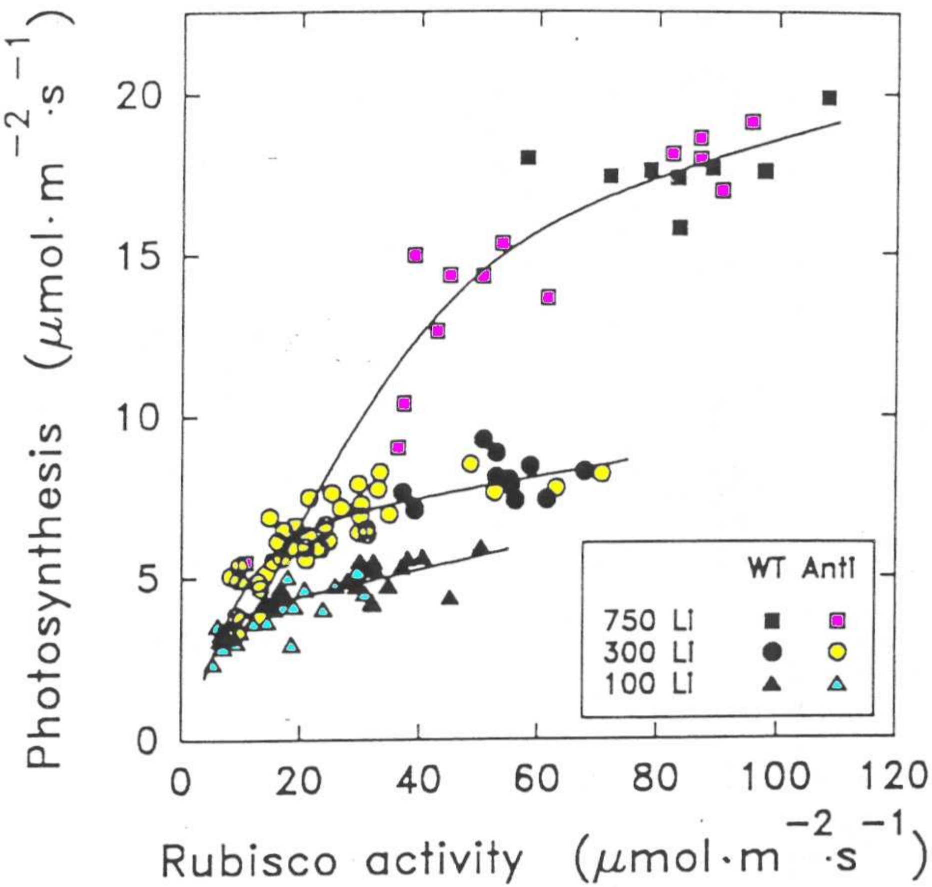
The flux–enzyme relationship



A typical example: Tryptophan 2,3–dioxygenase was adjusted by various dietary and hormonal treatments

Results of Salter et al (1986).

The flux–enzyme relationship



A typical example: dependence of carbon assimilation flux on rubisco levels in transgenic tobacco plants.

Results of Laurer et al, Planta **190** 332-345 (1993).

A specimen pathway

$$X_0 \overset{\textit{xase}}{\longrightarrow} Y \overset{\textit{ydh}}{\longrightarrow} Z \overset{\textit{zase}}{\longrightarrow} X_1$$

X_0 is termed the *source*

X_1 is the *sink*

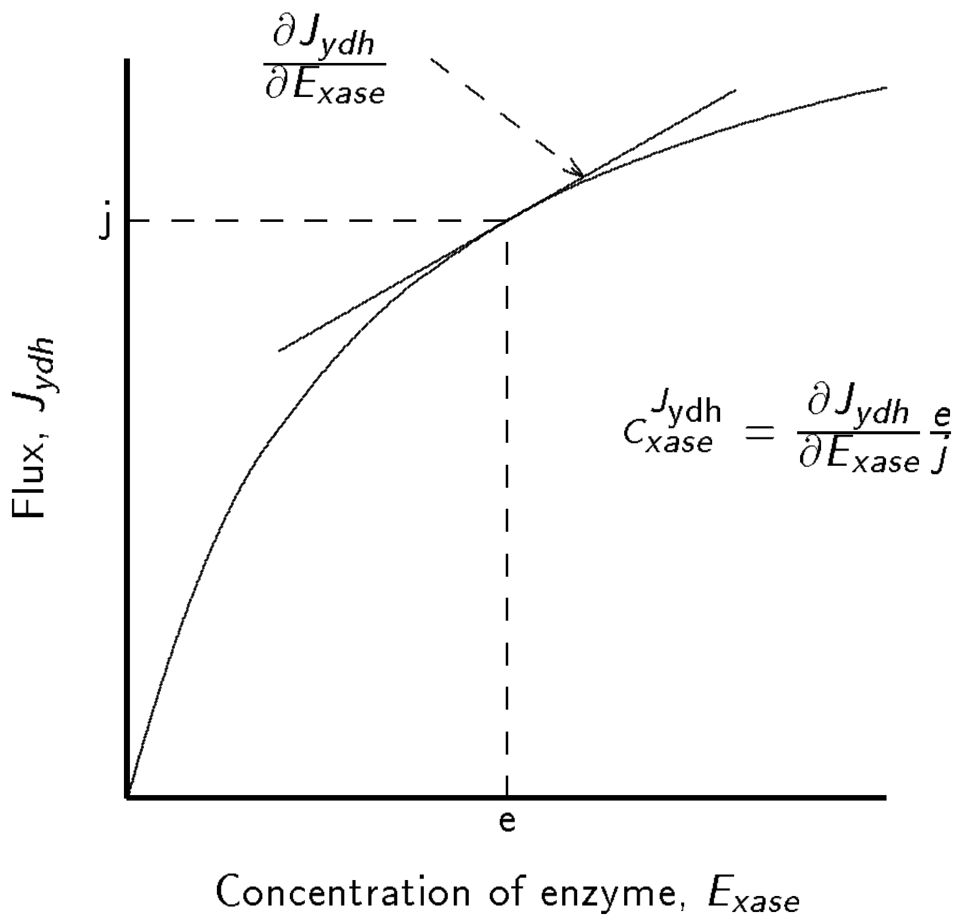
Y and Z are the variable metabolites that reach constant levels at steady state, when their rates of formation equal their rates of utilization.

Definition of the flux control coefficient

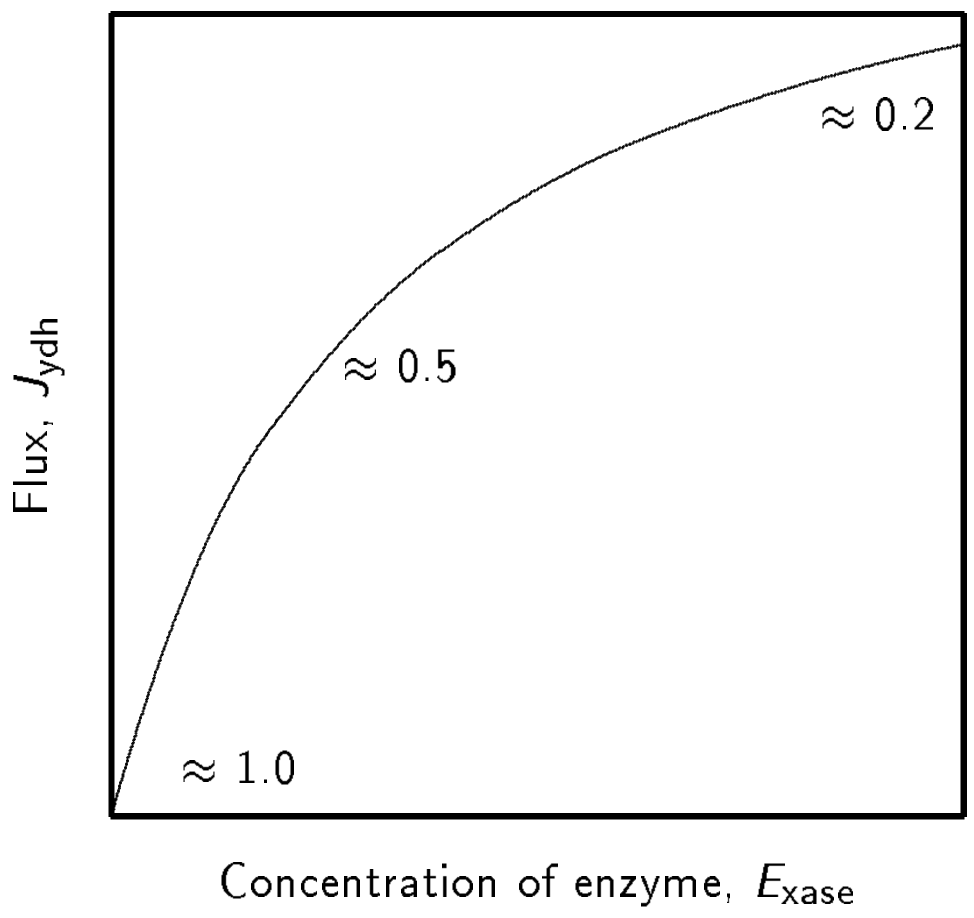
Suppose a small change, δE_{xase}, is made in the amount of enzyme E_{xase}, and that this produces a small change in the flux through the step catalyzed by *ydh*.

The flux control coefficient $C^{J_{ydh}}_{xase}$ is approximately the % change in J_{ydh} produced by a 1% change in E_{xase}.

Definition of the flux control coefficient

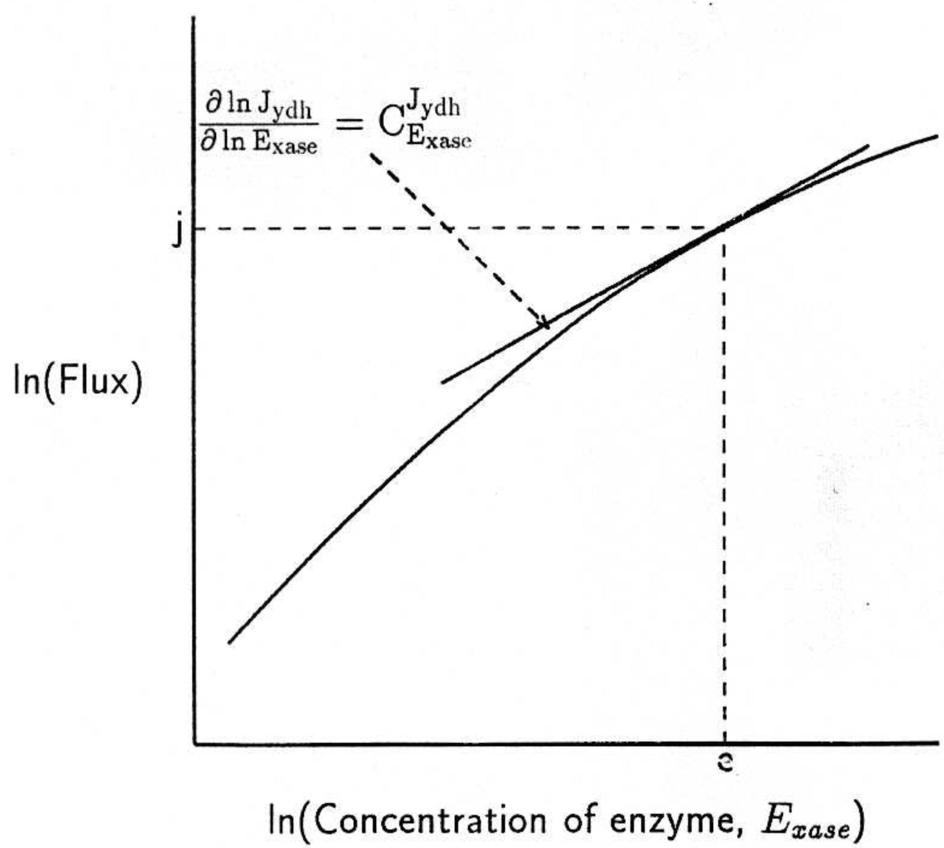


Values of the flux control coefficient

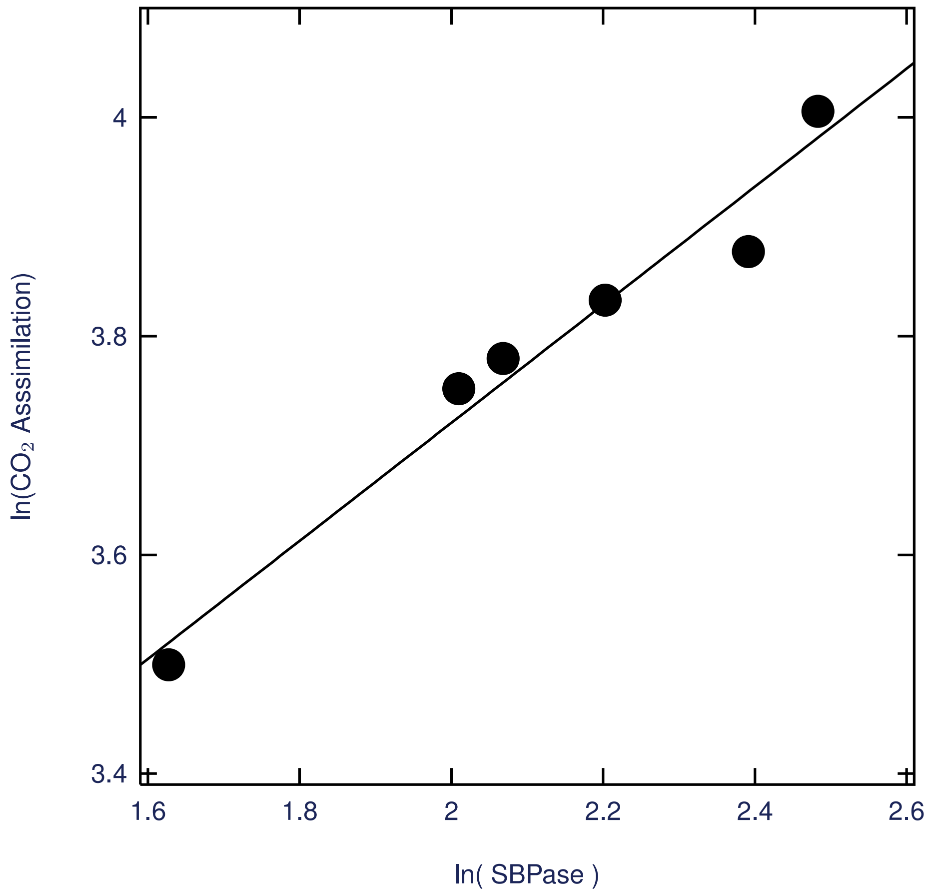


Definition of the flux control coefficient

On a logarithmic plot of the curve, the flux control coefficient is the tangent to the curve.



Experimental effect of reduced SBPase.



Photosynthetic rate in *N. tabacum* (tobacco) with reduced levels of SBPase. The slope of this line, and hence C^{Assim}_{SBPase}, is ≈ 0.5.

The flux summation theorem

Flux J_{ydh} is potentially affected by all enzymes in the system, but the sum of the flux control coefficients of them all on any flux is 1:

$$\sum_{All E} C_E^{J_{ydh}} = 1$$

If a large number of enzymes affect the flux, the average value will be small

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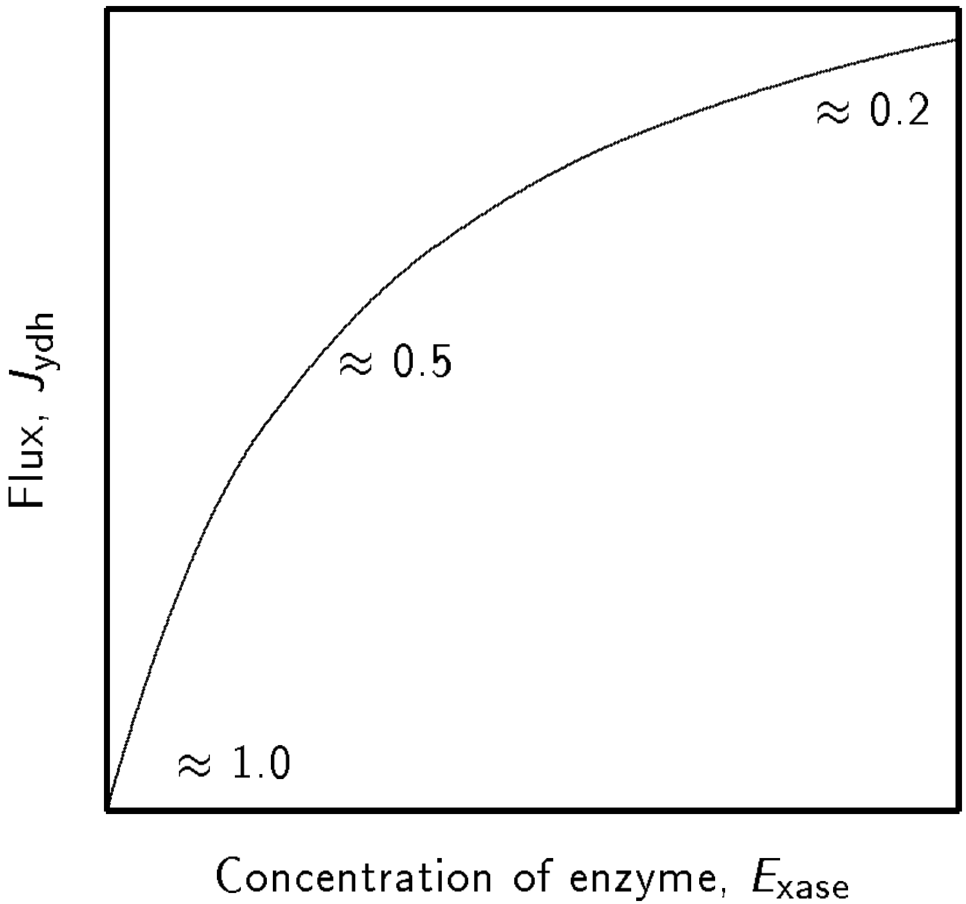
Flux control is a system property



The Concentration Control Coefficent

Suppose a small change, δE_{xase}, is made in the amount of enzyme E_{xase}, and that this produces a small change in the concentration of the metabolite, Y. The fractional changes are $\delta E_{xase}/E_{xase}$ and $\delta Y/Y$.
As δE_{xase} tends to zero, the concentration control coefficient C^Y_{xase} is given by the ratio:

$$C^Y_{xase} = \frac{\delta Y}{Y} \Big/ \frac{\delta E_{xase}}{E_{xase}}$$

Alternatively:

$$C^Y_{xase} = \frac{\partial Y}{\partial E_{xase}} . \frac{E_{xase}}{Y} = \frac{\partial \ln Y}{\partial \ln E_{xase}}$$

Concentration Control Summation

Metabolite Y is potentially affected by all enzymes in the system, but the sum of the concentration control coefficients of them all on any metabolite is 0:

$$\sum_{All E} C_E^Y = 0$$

- It follows that there are necessarily both +ve and -ve control coefficients on any metabolite.
- Even in a linear pathway, there are no bounds on the value of concentration control coefficients.

Control coefficients and enzyme kinetics

Response to a change in enzyme activity

Consider the pathway:

$$X_0 \xrightarrow{\textit{xase}} Y \xrightarrow{\textit{ydh}} Z \xrightarrow{\textit{zase}} X_1$$

Suppose that an extra amount of *ydh* is added, to increase the rate of the second step. What is the effect on the pathway?

Response to a change in enzyme activity

$$X_0 \xrightarrow{\textit{xase}} Y \xrightarrow{\textit{ydh}} Z \xrightarrow{\textit{zase}} X_1$$

The increased amount of *ydh* tends to lower the concentration of Y. The lower Y will:

- Increase the rate of *xase* because of reduced product inhibition
- Decrease the rate of *ydh* because of lower substrate concentration

Response to a change in enzyme activity

$$X_0 \overset{xase}{\longrightarrow} Y \overset{ydh}{\longrightarrow} Z \overset{zase}{\longrightarrow} X_1$$

The increased amount of *ydh* tends to raise the concentration of Z. The increased Z will:

- Decrease the rate of *ydh* because of increased product inhibition
- Increase the rate of *zase* because of higher substrate concentration

Conclusion:

- The effects of the increased amount of *ydh* involve the relative sizes of the responses of the enzymes to the pathway metabolites.
- The effects on the metabolites could tend to counteract the change in the amount of enzyme
- The effects on the metabolites could tend to change the rates of neighbouring enzymes to match the change in *ydh*

(This linkage was shown mathematically by Heinrich & Rapoport, 1974.)

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Elasticities

Definition of the elasticity coefficent

Suppose a small change, δS, is made in the amount of a metabolite S that affects the rate of the reaction, v_{ydh} catalysed by the enzyme *ydh*, producing a change δv_{ydh}. All other metabolites affecting *ydh* are kept constant at the values they have in the metabolic pathway at steady state. The fractional changes are $\delta S/S$ and $\delta v_{ydh}/v_{ydh}$.

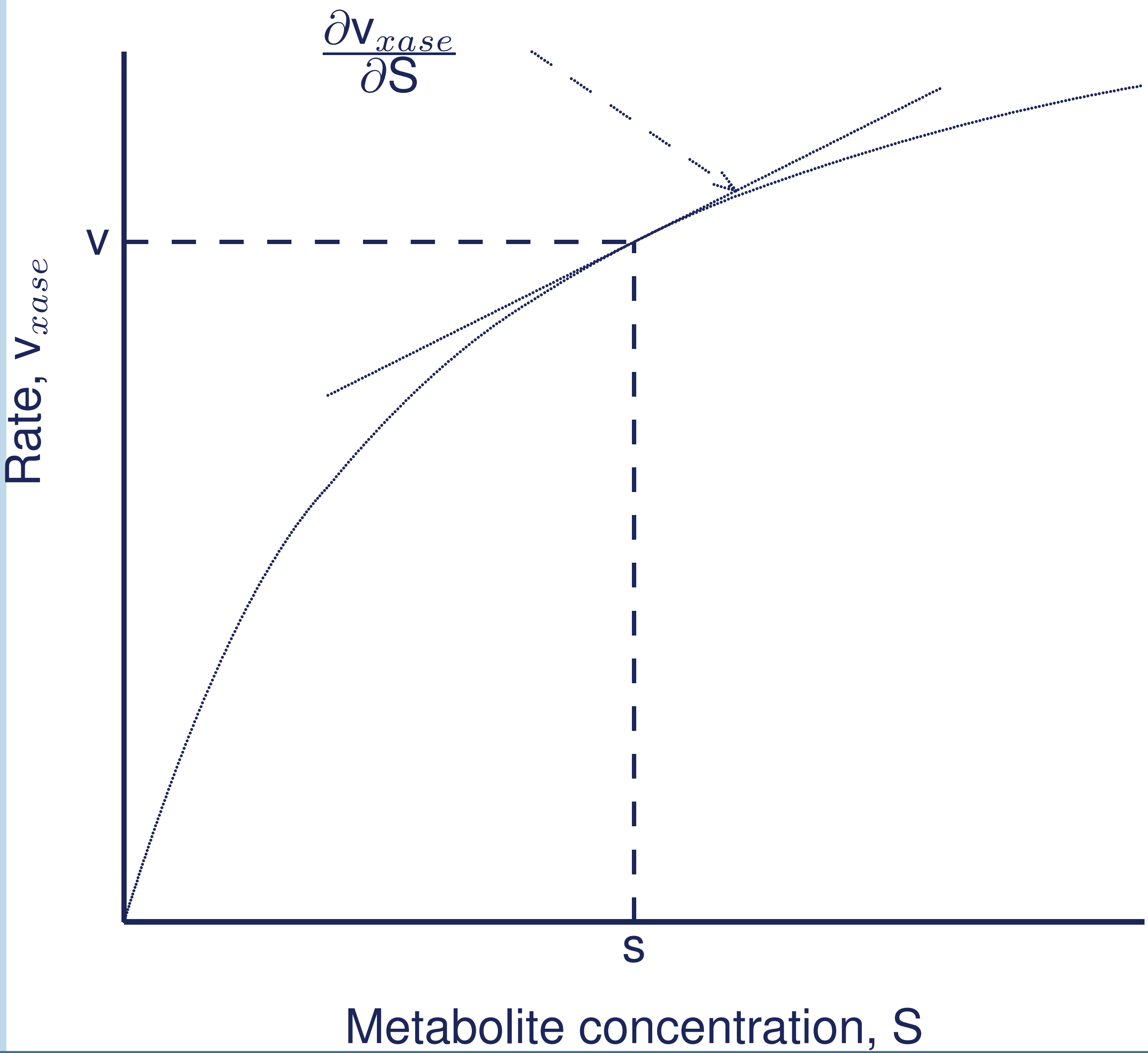
As δS tends to zero, the elasticity coefficient ε_S^{ydh} is given by the ratio:

$$\varepsilon_S^{ydh} = \frac{\delta v_{ydh}}{v_{ydh}} \bigg/ \frac{\delta S}{S}$$

Alternatively,

$$\varepsilon_S^{ydh} = \frac{\partial v_{ydh}}{\partial S} . \frac{S}{v_{ydh}} = \frac{\partial \ln v_{ydh}}{\partial lnS}$$

Definition of the elasticity



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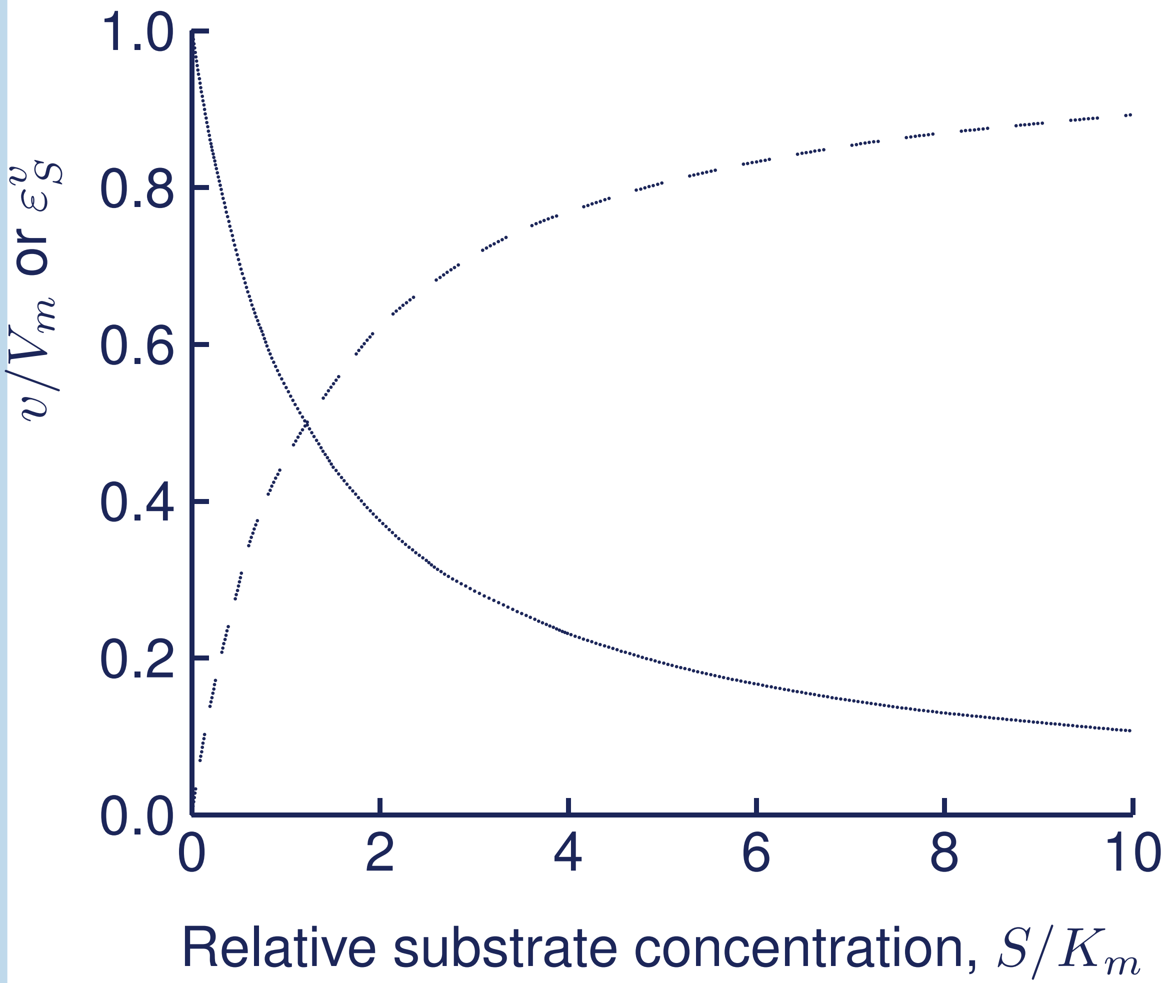
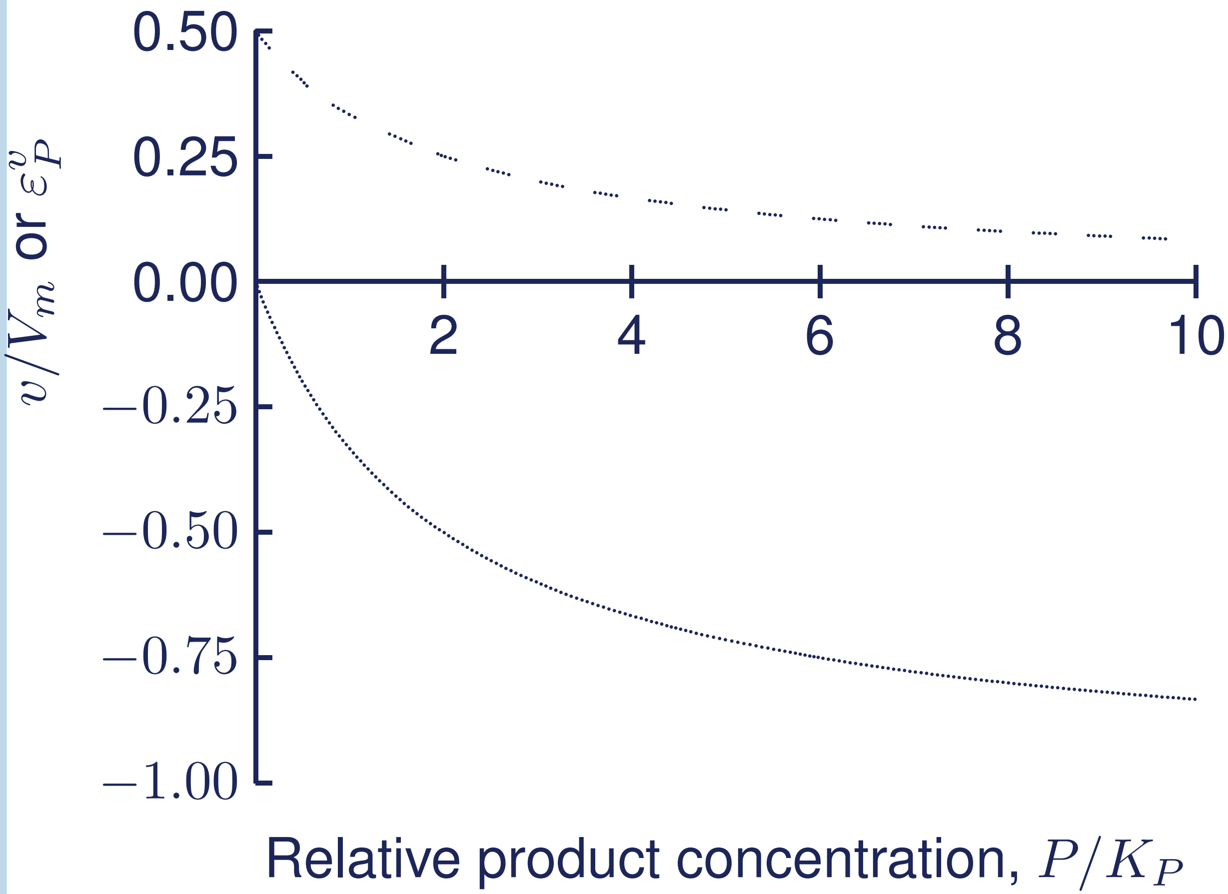
Values of the substrate elasticity



Elasticity with respect to substrate: dependence on substrate concentration for a single-substrate Michaelis–Menten enzyme.

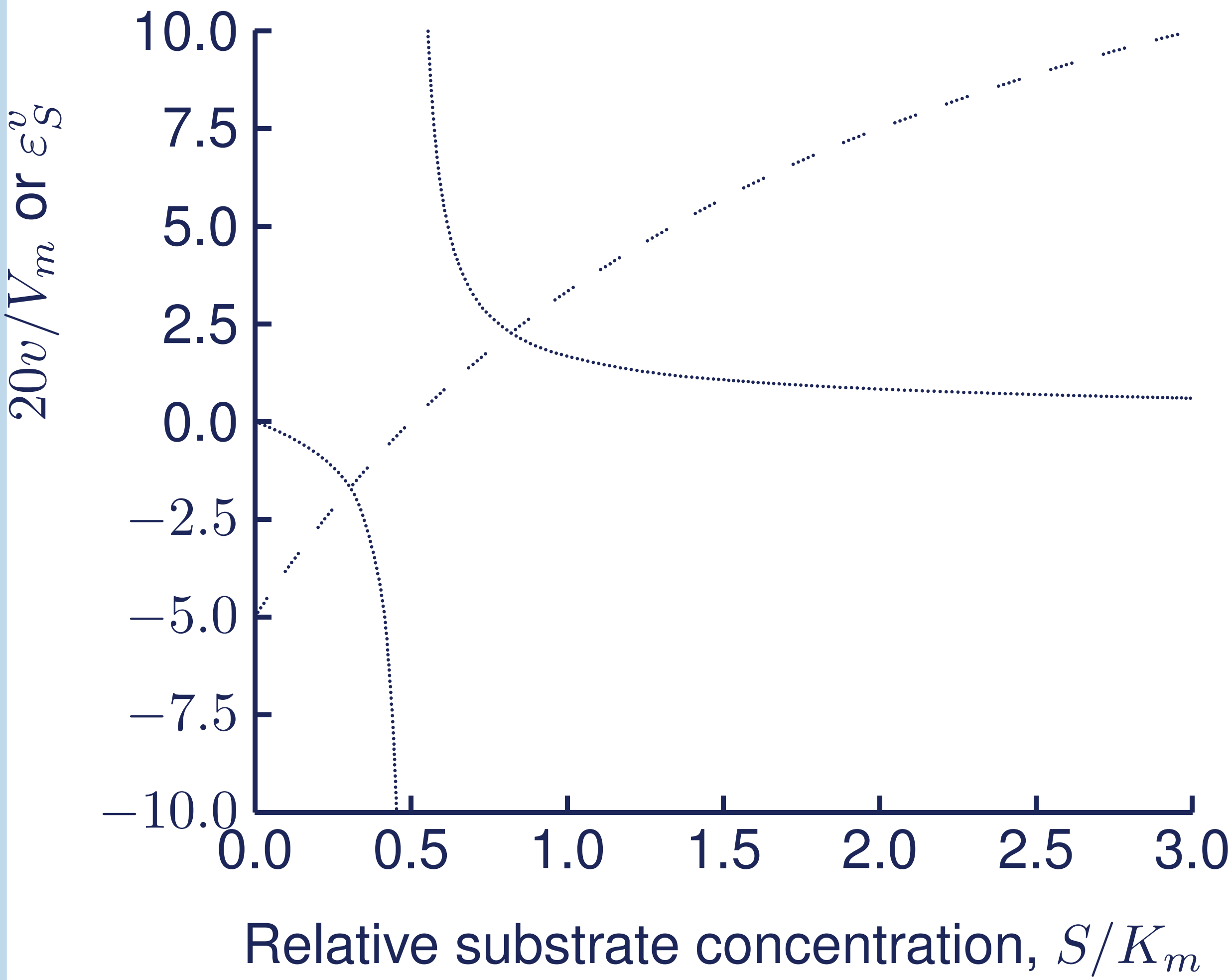
Line, ε^v_S; dashes, fractional velocity, v/V_{max}.

Values of the product elasticity



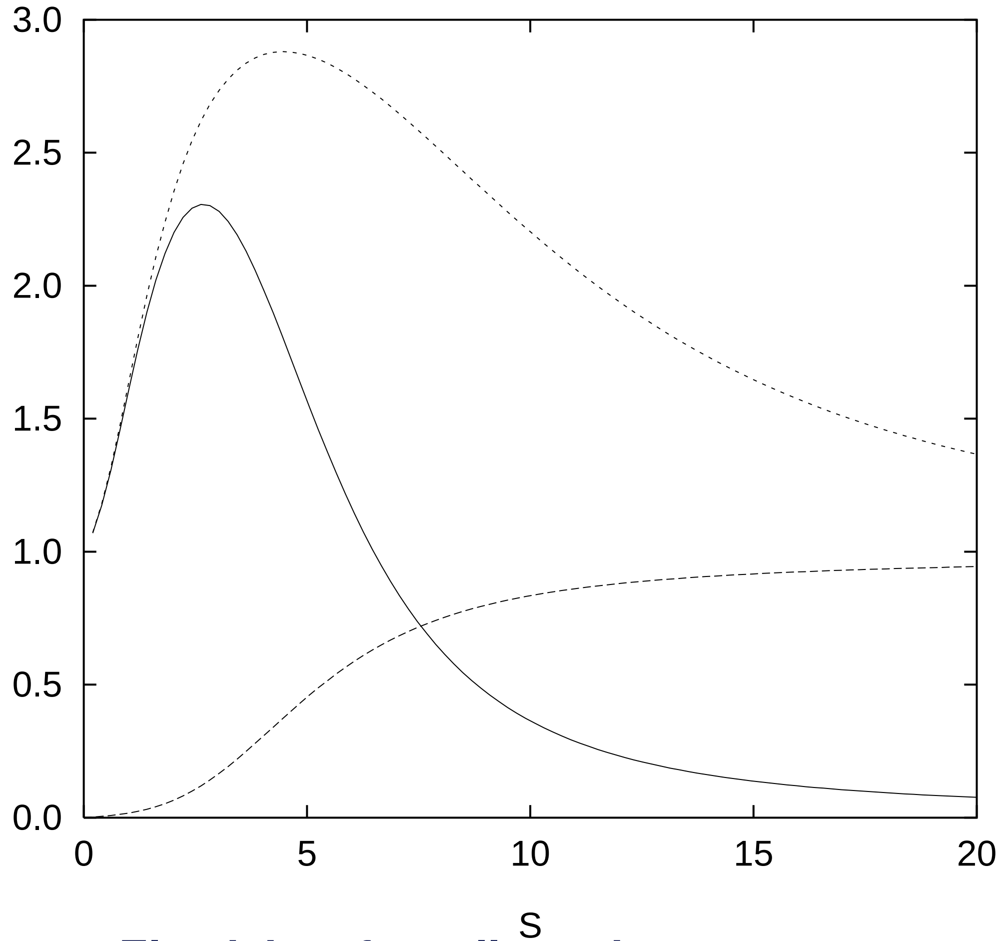
Elasticity with respect to product: dependence on product concentration for a Michaelis–Menten enzyme. — elasticity, ε^v_P; – – – fractional velocity, v/V_{max}.

Values of the substrate elasticity



Elasticity with respect to substrate: dependence on substrate concentration for a reversible Michaelis–Menten enzyme near equilibrium. ▬ elasticity, ε^v_S; – – fractional velocity, $20 \times v/V_{max}$.

Values of the elasticity



Elasticity of an allosteric enzyme

The curves show the Hill coefficient, the elasticity and the fractional saturation.

Elasticities from enzyme kinetics

$$\varepsilon^v_S = \frac{1}{1-\rho} - \frac{S/K_{m,S}}{1 + S/K_{m,S} + P/K_{m,P}}$$

$$= \frac{1}{1-\rho} - \frac{v_f}{V_{m,f}}$$

where $\rho = \Gamma/K_{eq}$, and for the reaction:

$$S \rightleftharpoons P$$

Γ, the mass action ratio, is defined as:

$$\Gamma = \frac{[P]}{[S]}$$

Connectivity theorem

The connectivity theorem

Consider the pathway:

$$X_0 \overset{\textit{xase}}{\longrightarrow} Y \overset{\textit{ydh}}{\longrightarrow} X_1$$

The *connectivity theorem* (Kacser & Burns, 1973) states the following relationships between the flux control coefficients and elasticities for this pathway:

$$C^J_{xase}\varepsilon^{xase}_Y + C^J_{ydh}\varepsilon^{ydh}_Y = 0$$

or

$$\frac{C^J_{xase}}{C^J_{ydh}} = -\frac{\varepsilon^{ydh}_Y}{\varepsilon^{xase}_Y}$$

The connectivity theorem

For a larger pathway, where Y affects more than two enzymes (*in any manner whatsoever*), the complete form of the connectivity relationship is:

$$\sum_{All E} C_E^J \varepsilon_Y^E = 0$$

Furthermore, there is a connectivity relationship for every metabolite in the pathway.

Summation and connectivity

$$X_0 \xrightarrow{\textit{xase}} Y \xrightarrow{\textit{ydh}} X_1$$

$$\begin{aligned} C^J_{xase} &+ C^J_{ydh} &= 1 \\ C^J_{xase}\varepsilon^{xase}_Y &+ C^J_{ydh}\varepsilon^{ydh}_Y &= 0 \end{aligned}$$

From this it follows that, if the elasticities are known:

$$C^J_{xase} = \frac{\varepsilon^{ydh}_Y}{\varepsilon^{ydh}_Y - \varepsilon^{xase}_Y} \; ; \; C^J_{ydh} = \frac{-\varepsilon^{xase}_Y}{\varepsilon^{ydh}_Y - \varepsilon^{xase}_Y}$$

Summation and connectivity

If the elasticities of all the enzymes in a pathway to all the metabolites in a pathway are known, it is possible to calculate the flux control coefficients.

The concentration connectivity theorem

Consider the pathway:

$$X_0 \xrightarrow{\textit{xase}} Y \xrightarrow{\textit{ydh}} X_1$$

The *concentration connectivity theorem* states the following relationships between the flux control coefficients and elasticities for this pathway:

$$C^{Y}_{xase}\varepsilon^{xase}_{Y} + C^{Y}_{ydh}\varepsilon^{ydh}_{Y} = -1$$

HOWEVER for the control coefficients on a *different* metabolite Z:

$$C^{Z}_{xase}\varepsilon^{xase}_{Y} + C^{Z}_{ydh}\varepsilon^{ydh}_{Y} = 0$$

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The concentration connectivity theorem

For a larger pathway, where Y affects more than two enzymes (*in any manner whatsoever*), the complete forms of the concentration connectivity relationships are:

$$\sum_{All E} C^{Y}_{E} \varepsilon^{E}_{Y} = -1$$

and

$$\sum_{All E} C^{Z}_{E} \varepsilon^{E}_{Y} = 0$$

Concentration summation and connectivity

$$X_0 \overset{xase}{\longrightarrow} Y \overset{ydh}{\longrightarrow} X_1$$

$$\begin{array}{lclcr} C^{Y}_{xase} & + & C^{Y}_{ydh} & = & 0 \\ C^{Y}_{xase}\varepsilon^{xase}_{Y} & + & C^{Y}_{ydh}\varepsilon^{ydh}_{Y} & = & -1 \end{array}$$

From this it follows that, if the elasticities are known:

$$C^{Y}_{xase} = \frac{1}{\varepsilon^{ydh}_{Y} - \varepsilon^{xase}_{Y}} \; ; \; C^{Y}_{ydh} = \frac{-1}{\varepsilon^{ydh}_{Y} - \varepsilon^{xase}_{Y}}$$

Relevance of flux control coefficients

Flux control coefficients: meaning

Metabolic control can involve alteration of the *amount* of active enzyme (selective induction/repression, selective proteolysis, covalent modification). The flux control coefficient indicates the impact of these changes on the metabolic flux, and direct manipulation (e.g. over–expression) of enzyme production.

Prediction of large changes is inexact because the flux control coefficient changes throughout the range.

Flux control coefficient: meaning 2

Another mechanism is the action of an external metabolite, or a second messenger, or a drug on the activity of the enzyyme (whether via V_{max} or K_m). The *response* of the pathway flux to such an effector, is the product of the flux control coefficient of the affected enzyme an d the elasticity of the effector on the enzyme.

The response coefficient

If an effector A that is not a metabolite of the pathway alters the flux via its action on an enzyme ydh, then the *response coefficient* (defined like control coefficients and elasticities) can be shown to be:

$$R_A^J = C_{ydh}^J \varepsilon_A^{ydh}$$

Problems 1

1. Suppose an enzyme in a pathway follows Michaelis-Menten kinetics with V_m = 100 units and K_m = 0.05mM:

$$v = \frac{[S]V_m}{[S] + K_m}$$

   What is the elasticity of the enzyme with respect to its substrate (a) at a substrate concentration of 0.025mM; (b) at a substrate concentration of 0.3mM? (Hint: a non-mathematical way of doing this is to determine the slope of the $\ln v$ against $\ln[S]$ curve at the two concentrations Calculate v at 90%, 95%, 100%, 105% and 110% of the required substrate concentration; plot these values as $\ln v$ against $\ln[S]$ and determine the slope at 100%.) (Mathematical answers, eg via differentiation of the rate law, also accepted.)
2. In the serine biosynthesis pathway:

$$\text{3-phosphoglycerate} \xrightarrow{1} \text{phosphoserine} \xrightarrow{2} \text{serine}$$

   the elasticity of the first step, ε^1_{pser}, is -1.43 in the liver of rabbits on a normal low protein diet. (The first step is actually catalysed by two enzymes, but the elasticity is the 'combined' elasticity for them both, so they can be treated as a single step.) The elasticity of the second step, ε^2_{pser}, is 0.041. What are the flux control coefficients, C^J_1 and C^J_2, of the two steps?

Problems 2

1. The enzyme fumarase catalyzes the reaction:

   fumarate $\rightleftharpoons$ malate

   Its rate of reaction is decsribed by the reveresible Michaelis–Menten equation:

$$v = \frac{V_m \left([fum] - \frac{[mal]}{K_{eq}} \right)}{K_{fum} + [fum] + \frac{K_{fum}[mal]}{K_{mal}}}$$

   where $V_m = 20\mu$mol.min^{-1}, K_{fum} = 0.9mM, K_{mal} = 1.2mM and K_{eq} = 11. What are the elasticities of the enzyme with respect to fumarate and malate at [fum] = 0.4mM and [mal] = 0.5mM? (Hint: a non-mathematical way of doing this is to determine the slope of the $\ln v$ against $\ln[fum]$ curve at the concentrations specified. Calculate v at 90%, 95%, 100%, 105% and 110% of the fumarate substrate concentration; plot these values as $\ln v$ against $\ln[fumarate]$ and determine the slope at 100%. Repeat for malate.) (Mathematical answers, eg via differentiation of the rate law, also accepted.)

2. Consider the glycolytic pathway, particularly the successive enzymes phosphofructokinase and aldolase:

   $\cdots$ Fru-6-P $\stackrel{PFK}{\longrightarrow}$ Fru-1,6bisP $\stackrel{Ald}{\longrightarrow}$ DHAP + GAP $\cdots$

   The elasticity of phosphofructokinase (PFK) with respect to fru-1,6-bisP, ε^{PFK}_{FBP}, is -0.01, whilst that of aldolase to the same metabolite ε^{ald}_{FBP}, is 2.5 in a particular cell. What is the ratio of the flux control coefficients of these two enzymes on glycolysis? What is the flux control coefficient of aldolase if ε^{PFK}_{FBP} is 0?