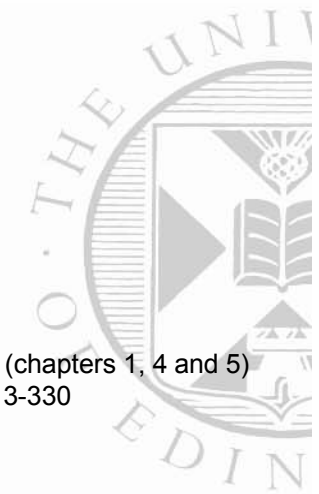
Computational Systems Biology

Lecture 6: Metabolic Control Analysis



Images and equations from:
D. Fell, Understanding the Control of Metabolism (1997) Portland Press (chapters 1, 4 and 5)
D. Fell, Metabolic Control Analysis: a survey, Biochem. J (1992) 286, 313-330

Summary:

- Reductionist vs. Systems theory
  - Regulation and control
  - Time scales
  - Metabolic Steady states

- Metabolic Control Analysis
  - Flux Control Coefficients
  - The Summation theorem
  - Metabolites effects and Elasticities
  - The Connectivity theorem
  - Response Coefficients
  - Conclusion: MCA vs. traditional approaches

Reductionist vs. System theory

Reductionist vs. System theory

- Metabolic Control Analysis (MCA) is usually discussed by its authors in the context of the theories and concepts that preceded it
- The former reductionist approach (the study of a whole system by detailed examination of the properties of its constituent parts) is criticised:
  - Because it led to an understanding of what determines the material flows in different pathways but not of how the production/utilisation of metabolites are kept in balance
  - Because the lack of understanding of metabolic regulation obtained by reductionist approaches has been revealed by poor results in increasing the rates of selected metabolic pathways
  - Because of the lack of ambition in moving from qualitative description to more rigorous specifications that could be compared with quantitative experimental observations
- A metabolic map given by reductionist approaches is compared to a town map, that is not useful for revealing how much traffic can flow through the streets. Even if the map showed all the traffic signals and the width of all streets, the actual measure of the traffic flux would still remain quantitatively elusive.

For additional material: D. Fell, Understanding the Control of Metabolism (1997) Portland Press (chapters 1, 4 and 5)

Regulation and control

- Certain terms are particularly important for discussion:
- *Flux:* is a term used in metabolic analysis to indicate the rate of a multi-component system (metabolic pathway), while “rate” is reserved for individual components (enzyme).

- The distinction between regulation and control:
- *Regulation:* is occurring when the system maintains some variable (e.g. temperature or concentration) constant over time, despite fluctuations in external conditions (a concept linked to homeostasis)
- *Control:* is used in technology to refer to adjusting the output of a system with time. Control as a verb implies the ability to start/stop/direct something.

- *Metabolic Control* is thus defined as the power to change the state of metabolism in response to an external signal, and it is measurable in terms of the strength of the metabolic response to external factors, without any assumption about the function/purpose/mechanism of the response

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Time scales

- Regulation and control exist in different time scales, from fractions of milliseconds for substrate binding to circadian/seasonal rhythms
- Is it legitimate to concentrate on just one time scale?
- Yes, if we consider living organism as being in a dynamic equilibrium (steady state)

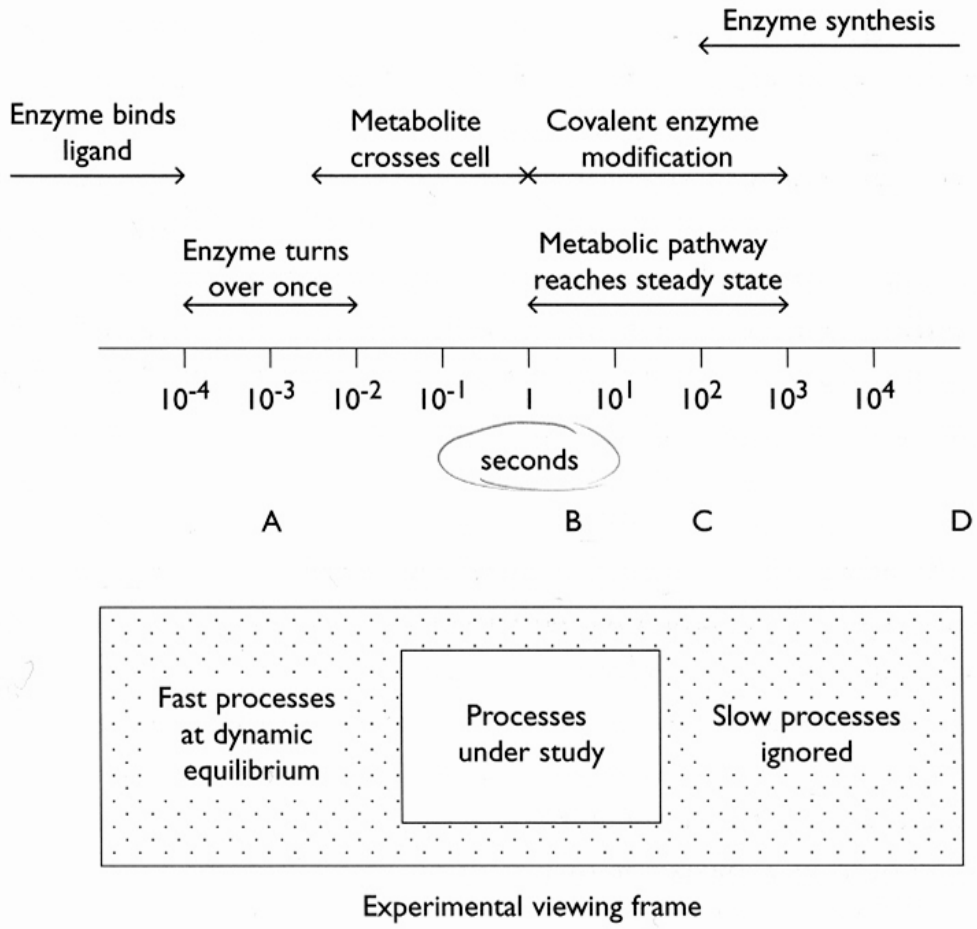

Figure 1.2 Metabolic time scales and the quasi-steady state
For different types of experiment, the experimental viewing frame is placed over the time axis in different positions, masking off the regions that are not being observed. For example, a fast reaction enzyme kineticist would centre the window of the frame above the letter A, a metabolic biochemist working on isolated cells or tissues might centre it in the range B to C, and a biochemist working on whole animals, or a nutritionist, might centre it above D. The time scales of various biochemical events are indicated as very approximate ranges.

Images from: D. Fell, Understanding the Control of Metabolism (1997)

Metabolic Steady States

- If a metabolic pathway starts with a *source* of material (here Glucose) derived at constant concentration from the environment, and ends with a *sink* (here Ethanol and CO_2), that is kept at constant concentration…
- This leads to the development of a steady state where the concentrations of the intermediates remains constant because their rates of formation have come to be in exact balance with their rate of degradation
- The consequence of ***not*** reaching a steady state would be that the metabolites would continue to accumulate in ever increasing amounts
- Nevertheless, a perfect steady state is a mathematical abstraction, but if the relative changes in metabolite concentrations are small, we can still consider it to be a *"quasi steady state"*

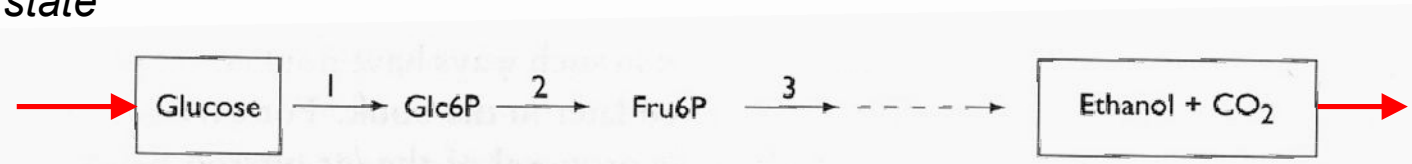

Figure 1.1 A metabolic pathway
This is part of the catabolic pathway from the source, glucose, available from the medium to the sink, the ethanol plus CO_2 which are released to the surroundings. At steady state, the rate of formation of glucose 6-phosphate (Glc6P) in the cell by reaction 1 is equal to its consumption in reaction 2 (assuming there are no other significant uses of Glc6P). Thus the rates of reactions 1 and 2 are the same. Similarly, since the rate of formation of fructose 6-phosphate (Fru6P) by reaction 2 is the same as its consumption by reaction 3, the latter also works at the same rate as reaction 1. The steady state in metabolite concentrations is therefore equivalent to a constant rate through the whole pathway.

Images from: D. Fell, Understanding the Control of Metabolism (1997)

Metabolic Control Analysis

The problems of traditional approaches…

- … there is agreement on the fact that there should be only one rate-limiting step, but no agreement on the actual step
- The rate-limiting step is defined as the slowest step in the pathway, but in a metabolic steady state all the steps along a linear pathway are going at the same rate
- If “slowest step” is interpreted as the step least able to go faster, then classical biochemical observations do not measure this “inability”
- Evidence from the 1930s onward show that even the rate of a sequence of simple chemical reactions could depend on the rate constants of *all* the reactions
- If a unique rate-limiting step exists in a pathway then varying the activity of that step alone will change the flux in the pathway, but there are few experimental observation of such phenomenon
- We need an alternative to the concept of unique rate-limiting step that takes into consideration the evidence of pathways affected by *several steps*…

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Flux Control Coefficients (1)

- From the *qualitative*: is this step rate-limiting? Yes/No,
- to the *quantitative*: how much does the metabolic flux vary as the enzyme activity is changed?

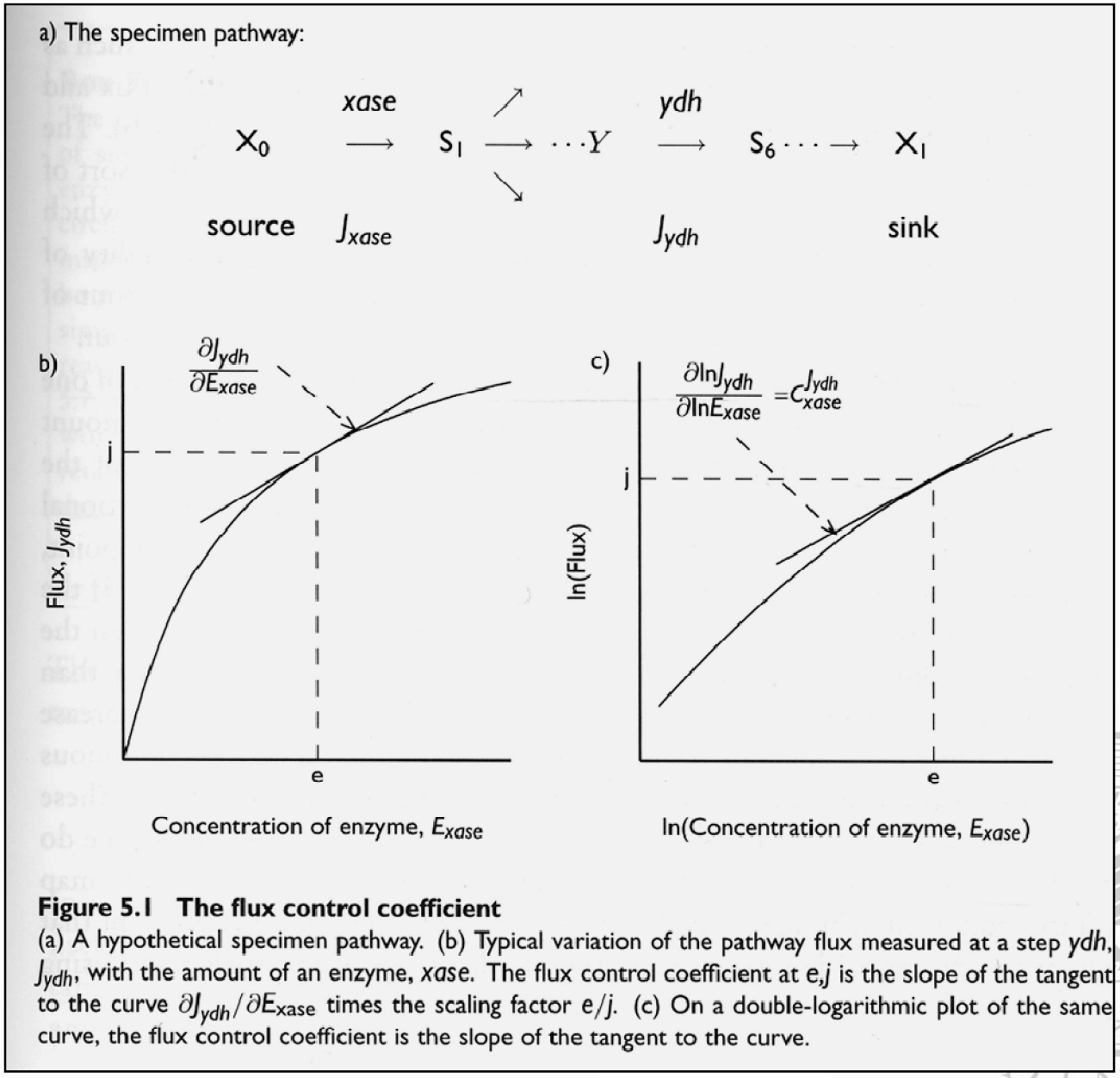


Figure 5.1 The flux control coefficient
(a) A hypothetical specimen pathway. (b) Typical variation of the pathway flux measured at a step *ydh*, J_{ydh}, with the amount of an enzyme, *xase*. The flux control coefficient at e,*j* is the slope of the tangent to the curve $\partial J_{ydh}/\partial E_{xase}$ times the scaling factor e/*j*. (c) On a double-logarithmic plot of the same curve, the flux control coefficient is the slope of the tangent to the curve.

Images from: D. Fell, Understanding the Control of Metabolism (1997) Portland Press

Flux Control Coefficients (2)

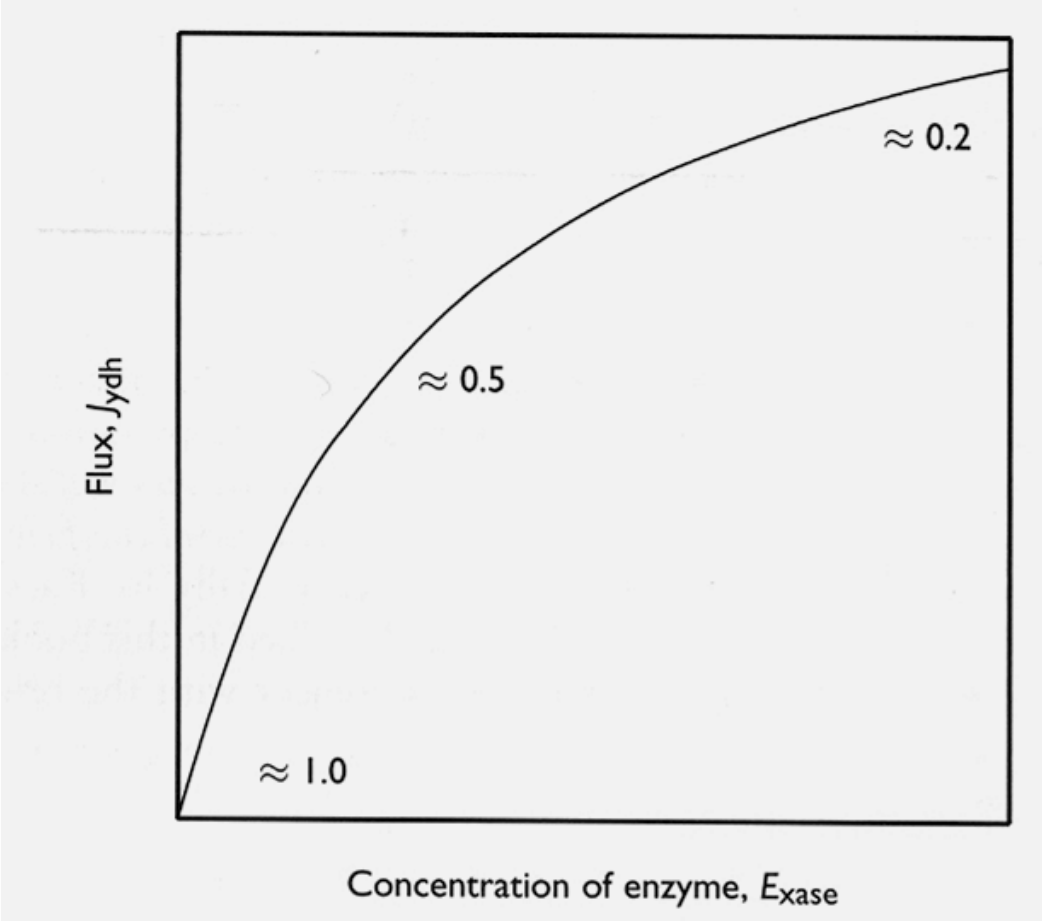
- Definition: the flux control coefficient *C* is the slope of the tangent (rate of change) on the flux/enzyme concentration diagram
- It is better to use the fractional change (e.g. $\delta E_{xase}/E_{xase}$) to obtain a dimensionless value

$$C_{xase}^{Jydh} = \frac{\partial Jydh}{\partial Exase} \cdot \frac{Exase}{Jydh} = \frac{\partial \ln Jydh}{\partial \ln Exase}$$

- The response of the flux to the concentration of enzyme depends on the position along the x-axis
- But until in vivo measurements have been made we do not know the enzyme concentration in the cell (or subcellular compartment), and hence where the enzyme maps on this diagram

Flux Control Coefficients (3)

- Flux control coefficients are commonly low
- This implies that biotechnological engineering on a single enzyme will rarely have the effect of significantly increasing the flux in a pathway



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The Summation Theorem

- Definition: if all the enzymes that can affect a particular metabolism in a cell are taken and their control coefficients are added up, the sum comes to 1

$$\sum_{i=1}^{n} C_i^J = 1$$

- Interpretation: in most cases several enzymes will share control over the flux. *To have a step that could be called "rate-limiting" one enzyme should have a control coefficient = 1 (virtually no examples of this) and all the other enzymes should have coefficients = 0*
- This also shows that the flux control coefficient of *each enzyme* is a *system property*:
- Since the flux control coefficient decreases if we increase the amount of one enzyme, the summation theorem states that the coefficients of some other enzymes must be increasing at the same time to maintain the sum = 1

Metabolites effect

- The flux control coefficient of an enzyme is a system property that cannot be related to the enzyme in isolation, but there must be links between the enzyme kinetic properties and its potential for flux control
- If we add an extra amount of *ydh* to the pathway below, what happens?

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

1. More Y is used up, and so its concentration is reduced, this will:
   - increase the rate of xase because of reduced product inhibition
   - **decrease** the rate of ydh because of lower substrate concentration
2. More Z is produced, and this will:
   - **decrease** the rate of ydh because of increased product inhibition
   - Increase the rate of zase because of higher substrate concentration
- In practice, the effect of metabolites tends to counteract the change in the amount of enzyme

Elasticities (1)

- The measure of the metabolite effect on an enzyme is given by the Elasticity Coefficient

$$\varepsilon_S^{xase} = \frac{\partial v_{xase}}{\partial S} \cdot \frac{S}{v_{xase}} = \frac{\partial \ln |v_{xase}|}{\partial \ln S}$$

- Each enzyme can have more than one elasticity: elasticities have positive values for metabolites that stimulate the rate of reactions of the enzyme (substrates, activators) and negative values for metabolites that slow the reaction (product, inhibitors)
- Compared to the classic Michaelis-Menten kinetic (a rectangular hyperbola of a single-substrate enzyme in the absence of product) the set of elasticities of an enzyme capture a more realistic in vivo situation where most enzymes have more than one substrate and work in the presence of appreciable concentrations (and hence inhibition) of products

Elasticities (2)

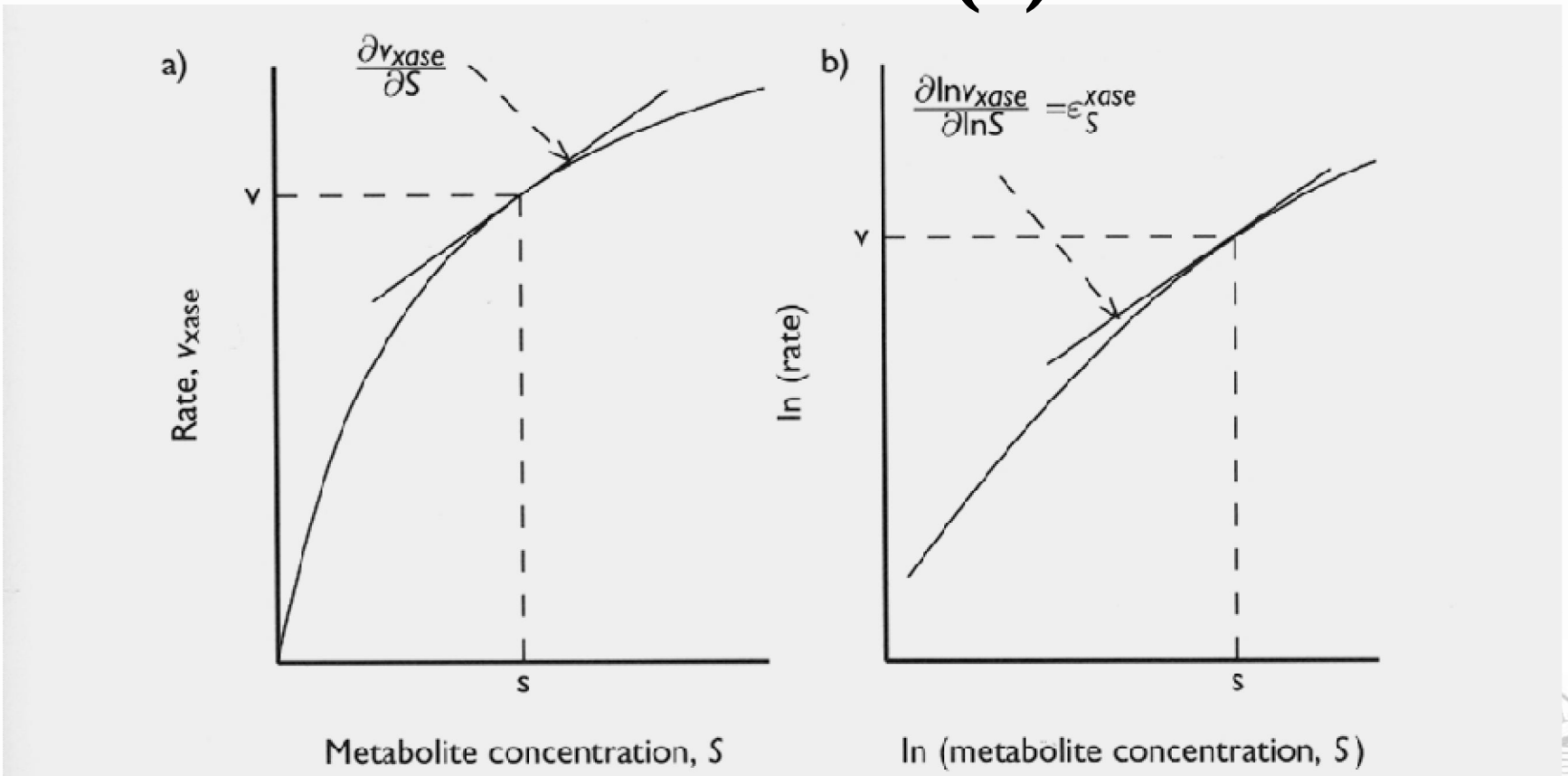

Figure 5.4 The elasticity coefficient

(a) Typical variation of the rate of enzyme *xase*, v_{xase}, with the concentration of metabolite S. The elasticity coefficient, ϵ_S^{xase}, at s,v is the slope of the tangent to the curve $\partial v_{xase}/\partial S$ times the scaling factor s/v. (b) On a double-logarithmic plot of the same curve, the elasticity coefficient is the slope of the tangent to the curve.

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The Connectivity Theorem

- How are kinetic properties of enzymes connected to flux control coefficients?
- Suppose we take a pathway metabolite S and we find all enzymes whose rates respond to its concentration (enzymes *i*, *j* and *k*). The connectivity theorem states that the coefficients for the action on a flux *J* times the elasticities sum up to zero:

$$C_i^J \varepsilon_S^i + C_j^J \varepsilon_S^j + C_k^J \varepsilon_S^k = 0$$

- In a more general form we can include all *n* enzymes in our system since enzymes not affected by the metabolite S will have elasticities = 0

$$\sum_{i=1}^{n} C_i^J \varepsilon_S^i = 0$$

Response Coefficients (1)

- Some control mechanisms of pathways operate on the catalytically active amount of enzyme, like induction/repression of enzyme synthesis, activation/inactivation by covalent modification. Other controls do not affect the amount of enzyme, but change the kinetic characteristics of the enzyme instead, like allosteric effectors changing the enzyme affinity for its substrate.
- Flux control coefficients seem to relate only to the first class of control mechanisms
- To consider also the second category, the effect of a *constant* parameter *P* affecting the flux is defined in the same way as a control coefficient but it is referred to as a *Response Coefficient, R:*

$$R_P^{Jydh} = \frac{\partial J_{ydh}}{\partial P} \cdot \frac{P}{J_{ydh}} = \frac{\partial \ln J_{ydh}}{\partial \ln P}$$

Response Coefficients (2)

- If an external constant metabolite P acts on the flux J_{ydh} through being an effector of the pathway enzyme *xase*, the response coefficient for the effect of P is composed by the flux control coefficient with respect to *xase* and the elasticity of *xase* with respect to P:

$$R_P^{J_{ydh}} = C_{xase}^{J_{ydh}} \varepsilon_P^{xase}$$

- So the response of a pathway to an effector depends on 2 factors that have to be not-null:
  1. The sensitivity of the pathway to the activity of the enzyme that is target for the effector (flux control coefficient)
  2. The strength of the effect of P on the enzyme (elasticity)

- If a number of effectors act on a pathway at a particular enzyme, their response coefficient would all contain the same flux control coefficient which indicates whether an enzyme has the *potential* to be a site at which the pathway is controlled

- Another useful property is that the equation is true regardless of the specific molecular mechanism that P uses to affect *xase*

Conclusion: MCA vs. traditional approaches

- In MCA the qualitative categories “rate-limiting” and “not rate-limiting” are replaced by a quantitative scale for the influence of and enzyme on a metabolic flux: the *flux control coefficients*
- The flux control coefficient of an enzyme is a system property
- MCA shows that the degree of displacement of a reaction from equilibrium is not a reliable guide to the degree of control an enzyme can exert on a flux, despite having been widely used in the past