Volume 320, number 1, 71–74 FEBS 12171 March 1993
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Metabolic channelling and control of the flux

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Received 11 January 1993

Metabolic control theory is extended to include channelled metabolism in general. A simple relationship between the flux control by the enzymes and the degree of metabolite channelling is derived. This relationship suggests experiments in which modulation of gene expression allows one to quantify channelling.

Metabolic control theory; Control coefficient; Direct transfer; Enzyme–enzyme interaction and kinetics

1. INTRODUCTION

Metabolic control analyses have led to significant advances in the understanding of the control of cellular metabolism (for review see [1,2]). In this approach the contribution of any enzyme to the control of the metabolic flux (J) is quantified by the enzyme control coefficient, $C^J_{E_i}$. It relates a fractional change dJ/J in the steady-state flux to the fractional modulation de_i/e_i of the total enzyme concentration [3]:

$$C^J_{E_i} = (dJ/J)/(de_i/e_i) = d\ln|J|/d\ln e_i \quad \textbf{(1)}$$

In view of the difficulty to influence the enzyme concentrations directly in the native system, an alternative definition of the control coefficient was proposed [4]. It compares a variation (dJ/J) of the flux, caused by an effector of the enzyme E_i, with a variation (dv_i/v_i) in the enzyme rate, the effector would cause if the enzyme were 'isolated' from the system. The effector should affect only the rate v_i. These control coefficients can be designated as $C^J_{v_i}$ or C^J_i in order to emphasize that they refer to a change in the i-th reaction (v_i).

In ordinary metabolic pathways there is a one-to-one correspondence between the enzymes and reactions. Moreover, in what we shall call 'simple' pathways [5], any reaction rate v_i is a linear function of the enzyme concentration e_i [6]. In such pathways the 'true' control coefficient, $C^J_{E_i}$, with respect to the concentration (e_i) of any enzyme is identical to the control coefficient with respect to the corresponding process, C^J_i [2,4].

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In highly organized cellular metabolic pathways direct enzyme–enzyme interactions and enzyme associations take place [7,8]. Metabolic control theory does not address all cases of organization of cellular metabolism (see also [9]). A number of authors have attempted to extend control analysis to such systems with metabolic channelling (see e.g. [10–12]). However, no control theory dealing with the general case of partial channelling, dynamic and/or static, has been developed, partly because it was unclear how to define the appropriate control coefficients. In this paper we develop the essentials of such a control theory for a sample pathway with metabolic channelling. We show, in particular, how the sum of the enzymes' flux control coefficients depends on the degree of metabolic channelling.

2. RESULTS AND DISCUSSION

Fig. 1 shows a metabolic pathway where channelling is absent. Traditionally this pathway is treated in terms of two consecutive, enzyme catalyzed reactions. Each of them, e.g. reaction 1, has a control coefficient with respect to the flux defined, as indicated above, by considering a small increase in the total concentration of enzyme E_1. Here we note that this corresponds to a simultaneous proportional increase in all the forward and reverse (pseudo-) first order rate constants of enzyme 1. To be able to make this statement exact, we need to define the control coefficients of the elemental processes of enzyme 1 with respect to the overall steady-state flux through the system of Fig. 1:

$$C^J_{11} = d\ln|J|/d\ln k_{11}, \quad k_{-11}/k_{11} = \text{constant} \quad \textbf{(2)}$$

where the differentiation conditions are such that the forward elemental (k_{11}) and reverse (k_{-11}) rate constants of the process v_{11} are changed by the same factor, all

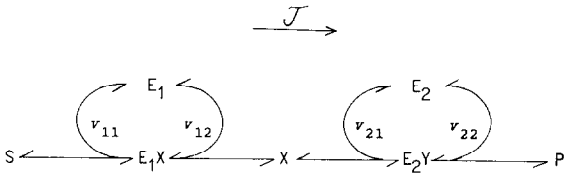

Fig. 1. 'Simple' pathway of two enzymes E_1 and E_2. The concentrations of the initial substrate, S, and the end product, P, are constants. X is the intermediate in the bulk phase. v_{11}, v_{12} and v_{21}, v_{22} are the rates of E_1- and E_2-dependent elemental processes, respectively.

other parameters being kept constant. It should be noted that this definition does not affect microscopic reversibility (cf. [13]). Now the above statement can be written as:

$$C^J_{E_j} = C^J_{1j} + C^J_{2j} \quad (3)$$

and the summation theorem [3] for the pathway of Fig. 1 can be written as:

$$C^J_{E_1} + C^J_{E_2} = C^J_{11} + C^J_{12} + C^J_{21} + C^J_{22} = 1 \quad (4)$$

This reformulation of the summation theorem for simple pathways may be useful for the cases where one is attempting to understand what the implication is of a regulation of one of the transitions in an enzyme-catalyzed reaction on the flux through the metabolic pathway. However, here we merely use it as a prelude to the control theoretical treatment for the channelled systems in Fig. 2. Fig. 2a represents the case of static channelling, where the enzymes E_1 and E_2 form a complex $Q{=}E_1E_2$ (which catalyzes the direct conversion of S to P) independently of their interactions with metabolite molecules. In this case, the number of enzyme molecules that participate in the channel does not vary with the metabolic flux. Fig. 2b denotes the general case of dynamic channelling, where the extent of channelling depends on the relative rates of collision of E_1X and E_2 compared to the rate of dissociation of E_1X into E_1 plus X and also on the other rate constants. Fig. 2 may illustrate the problem of defining control coefficients of the participating enzymes in the case of channelling. For instance, enzyme E_1 participates in two rather than one reaction (the channelled reaction from S to P as well as the reaction from S to X). Indeed, this is where control theory broke down. For the case of static channelling (Fig. 2a) Sauro and Kacser [10] have indicated a solution. They calculated the elasticities of partial reactions (v_{11}, v_{12}, v_Q) with respect to total enzyme concentrations, assuming thermodynamic equilibrium between the enzyme monomers and the complex. Only under such conditions do the elasticities not depend on the steady-state flux and concentrations of metabolites. However, this approximated method fails for the more general case of Fig. 2b.

Our consideration of Fig. 1 (above) now suggests a solution to this dilemma: one should recognize that Fig. 2a and b are still networks of chemical conversions and that they may be treated in terms of control coefficients with respect to the elemental processes (these processes correspond to transitions between states, or to sequences of such transitions that are not interrupted by branches). Consequently, there are six elemental flux control coefficients for the system in Fig. 2b, for example:

$$C^J_{Q1} = \mathrm{d}\ln|J|/\mathrm{d}\ln k_{Q1}, \text{ where } k_{-Q1}/k_{Q1} = \text{constant} \quad (5)$$

Because the flux through the system is a homogeneous function of all the elemental rate constants the following summation theorem holds [1,2]:

$$C^J_{11} + C^J_{12} + C^J_{21} + C^J_{22} + C^J_{Q1} + C^J_{Q2} = 1 \quad (6)$$

i.c. the sum of the flux control coefficients over all the elemental processes continues to equal 1. Elsewhere we shall show how the connectivity theorems can be reformulated in terms of the elemental control coefficients.

The above formalism suffices for a complete control analysis of systems that are partly or completely channelled. However, it is possible also to express part of the control properties via the control coefficients related to enzyme activities. Suppose, we simultaneously change the elemental rate constants of *all processes* in which any subform of the enzyme E_i is involved, by the same factor. Considering the corresponding change in the steady state flux J we define the 'impact' control coefficient, ${}^{\mathrm{imp}}C^J_{E_i}$, as:

$${}^{\mathrm{imp}}C^J_{E_i} = \sum_{\substack{\text{all} \\ E_i\text{-dependent} \\ \text{processes}}} C^J_{ik} \quad (7)$$

This coefficient evaluates the total impact enzyme E_i has on the flux J via all E_i-dependent processes. In the accompanying paper [14] we explain this terminology more thoroughly by considering the experimental methods of measuring the enzyme control coefficients (see also [5,15]). For the scheme of Fig. 2a:

$${}^{\mathrm{imp}}C^J_{E_1} = C^J_{11} + C^J_{12} + C^J_{Q}, \quad {}^{\mathrm{imp}}C^J_{E_2} = C^J_{21} + C^J_{22} + C^J_{Q} \quad (8)$$

and for the scheme of Fig. 2b:

$${}^{\mathrm{imp}}C^J_{E_1} = C^J_{11} + C^J_{12} + C^J_{Q1} + C^J_{Q2}, \quad (9)$$

$${}^{\mathrm{imp}}C^J_{E_2} = C^J_{21} + C^J_{22} + C^J_{Q1} + C^J_{Q2}$$

We note that the definition of the impact control coefficient ${}^{\mathrm{imp}}C^J_{E_1}$ (by modulation of activities of all E_1-dependent processes) does not correspond to just a change in the total concentration of the enzyme E_1 at constant total distribution of E_1 over all its subforms and at a constant concentration of the enzyme E_2. Indeed, the

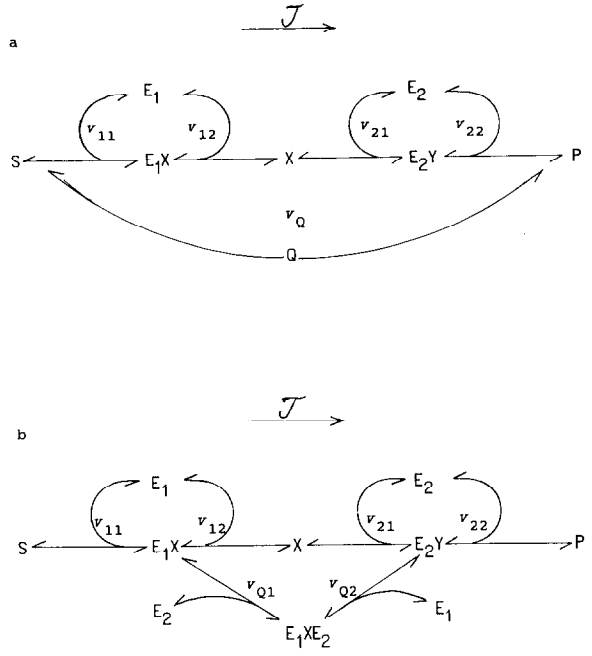

Fig. 2. 'Static' (a) and 'dynamic' (b) channels. The dynamic complex E_1XE_2 is formed after binding X to E_1, while the static complex Q is formed independently of the presence of a common intermediate. In both systems the upper route represents the usual reaction pathways through the bulk phase intermediate X, catalyzed by free enzymes, and the lower routes represent the 'channelling'. The rates of E_1- and E_2-dependent processes are: (a) v_{11}, v_{12}, v_Q and v_{21}, v_{22}, v_Q, respectively. (b) v_{11}, v_{12}, v_{Q1}, v_{Q2} and v_{21}, v_{22}, v_{Q1}, v_{Q2}, respectively.

concomitant change in the form E_1XE_2 violates the conservation constraint imposed on the total concentration of the enzyme E_2. So, in cases of enzyme–enzyme interactions as well as in other 'non-simple' pathways [9] there is a difference between the control coefficients defined in terms of modulations of activity and those defined in terms of modulations of the enzyme concentration (Eqns. **7** and **1**). Both definitions are important since they refer to different experimental methods of determining the control coefficients [14,15]. Definition **1** has the operational meaning of measuring the ('true') control coefficient, $C^J_{E_i}$, by manipulating the expression of the gene encoding the enzyme E_i.

Noting that the scheme of Fig. 2 can be viewed as any chemical network to which metabolic control theory can be applied (transitions taking the role of enzymes), one can relate the elemental control coefficients to the enzyme control coefficients (Kholodenko and Westerhoff, in preparation):

$$C^J_{E_1} + C^J_{E_2} \cdot Q/e^t_2 = {}^{\mathrm{imp}}C^J_{E_1} \qquad \textbf{(10)}$$

$$C^J_{E_1} \cdot Q/e^t_1 + C^J_{E_2} = {}^{\mathrm{imp}}C^J_{E_2}$$

here $Q = [E_1E_2]$ or $[E_1XE_2]$ for a static or a dynamic channel, respectively. e^t_1 and e^t_2 represent the total concentrations of the enzymes, ${}^{\mathrm{imp}}C^J_{E_1}$ and ${}^{\mathrm{imp}}C^J_{E_2}$ are given in Eqns. **8** and **9**. Inversely, Eqn. **10** can be used to express the control coefficients with respect to enzyme concentrations into those with respect to the elemental processes. This then allows one also to evaluate the expected magnitude of the sum of the enzymes control coefficients. For the dynamic channel the result reads:

$$C^J_{E_1}+C^J_{E_2}=\{1+(J_{\mathrm{chan}}/J)\cdot(1-(C^J_{11}+C^J_{22}))\}/(1+Q/e_t) \qquad \textbf{(11)}$$

For simplicity we here considered the case where the total concentrations of the two enzymes are equal. In the case of static channelling C^J_{11} and C^J_{22} disappear from this expression.

Eqn. **11** shows that the sum of the flux control coefficients can vary from less than unity to two depending on the ratio of the channelled and bulk-phase fluxes and the kinetic properties of the enzymes involved.

The analysis presented here may remove an important limitation of metabolic control theory. Moreover, it provides new definitions that should facilitate the quantitative characterization of metabolic channelling, without taking the system apart. After modulating enzyme concentrations [16] and measuring changes in pathway flux, one should be able to measure the sum of the flux control coefficients. Determination of how much of the enzymes is complexed in situ ('Q') then allows for the calculation of how much of the flux proceeds through the channel (i.e., J_{chan}/J in Eqn. **11**). The theory developed here also allows one to analyze implications of channelling for the regulation of cellular metabolism [14].

Acknowledgement: This work was supported by the Netherlands Organization for Scientific Research (NWO).

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