

Control in channelled pathways. A matrix method calculating the enzyme control coefficients

Boris N. Kholodenko^{a,b,*}, Hans V. Westerhoff^{c,d}, Joaquim Puigjaner^b,
Marta Cascante^{b,*}

^a A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119899 Moscow, Russian Federation

^b Departament de Bioquímica i Fisiologia, Universitat de Barcelona, Martí i Franques 1, Barcelona, Spain

^c E.C. Slater Institute, Biocentrum, University of Amsterdam, Plantage Muidergracht 12, Amsterdam, The Netherlands

^d Department of Molecular Biology, H5, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

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Abstract

The usual equations expressing the enzyme control coefficients (quantitative indicators of ‘global’ control properties of a pathway) via the elasticity coefficients (reflecting local kinetic properties of an enzyme reaction), cannot be applied to a variety of ‘non-ideal’ pathways, in particular to pathways with metabolic channelling. Here we show that the relationship between the control and elasticity coefficients can be obtained by considering such a metabolic pathway as a network of elemental chemical conversions (steps). To calculate the control coefficients of enzymes one should first determine the elasticity coefficients of such elemental steps and then take their appropriate combinations. Although the method is illustrated for a channelled pathway it can be used for any non-ideal pathway including those with high enzyme concentrations where the sequestration of metabolites by enzymes cannot be neglected.

Keywords: Metabolic channelling; Matrix method; Enzyme control coefficients

1. Introduction

The degree to which an enzyme controls a flux or metabolite concentration can be quantified in the frameworks of metabolic control analysis and biochemical system theory [1–4]. In metabolic control analysis such quantitative indicators of the regulation are called the control coefficients of the enzymes [5]. They are defined mathematically as the log–log derivative of the steady-state flux (J) or the concentration of a metabolite (x) with respect to the enzyme concentration (e_i) [2,3,5],

$$c_{e_i}^J = d \ln |J| / d \ln e_i |_{st.st.}, \quad C_{e_i}^x = d \ln x / d \ln e_i |_{st.st.} \quad (1)$$

Here the concentrations of all other enzymes (e_j , $j \neq i$) are kept constant. Although the control coefficients are defined for small, in the limit, infinitesimal changes in the enzyme concentration, in practice a series of finite

* Corresponding author.

changes can be made and the response curve can be defined by interpolation. Therefore, Eq. (1) acquires the clear operational meaning of measuring the effect which an enzyme exerts on the steady-state flux or concentration by addition of that enzyme to the system *in vitro* [6], by using heterokaryons [7] or by manipulating the expression of the corresponding gene in the intact system [8,9].

Definition (1) of the control coefficients implies that the pathway as a whole attains a new steady state (for every new value of the concentration of an enzyme). Hence, the control coefficients (as well as the fluxes and metabolite concentrations in a new steady-state) depend on the kinetic properties of all pathway enzymes and on pathway structure. Therefore, the control coefficients are the ‘global’ coefficients.

Local regulatory properties of functional units of a system, i.e., enzyme reactions in metabolic control analysis, are quantified by the ‘local’ coefficients which are called the elasticity coefficients. These local coefficients describe the response of a single enzyme rate to a change in the concentration of a metabolite or a parameter affecting that rate.

One of the goals of quantitative approaches to metabolic regulation is to relate control properties of a pathway as a whole to kinetic properties of individual enzyme reactions and the structure of the links between the latter. In the framework of metabolic control analysis the summation and connectivity theorems [2] play the role of such relations. Together they allow one to determine the control coefficients (global control properties) in terms of the elasticity coefficients (local properties) [10–12].

To summarize the relationships between control and elasticity coefficients matrix methods have been applied [3,10,12–18]. However, these matrix approaches addressed only the case of so called ‘simple’ or ‘ideal’ [19] metabolic pathways. In such pathways the enzymes are independent catalysts and the concentrations of enzyme-bound metabolites can be neglected as compared to their free concentrations. Only with this oversimplification of real metabolic pathways the classical summation and connectivity hold true [20–23]. The existence of this limitation was well understood; Sauro and Kacser [22] have indicated a principal solution of it for pathways with direct enzyme–enzyme interactions (cf. [44]). Assuming thermodynamic equilibrium between the separate enzymes and the complex they calculated the elasticities of partial reactions (i.e. proceeding in the bulk phase and in the channelled phase) with respect to the total enzyme concentrations. However, only under special conditions these elasticities which are referred to as protein (π) elasticities [22,24] depend only on the equilibrium constant and the total amounts of the enzymes. In the more general case π -elasticities of a given partial reaction (step) may depend on the rate constants of other partial reactions and, therefore can not be considered as local properties.

A method for determining the enzyme control coefficients for a general case of (partially) channelled [25] pathways has been proposed recently [19,26]. The enzyme control coefficients were expressed in terms of the control coefficients of the elemental steps of the reaction network and the relative concentrations of enzyme–enzyme complexes (the ratios of mean life times of the monomeric and complexed enzyme forms). For non-ideal pathways (e.g., channelled pathways) we here express the control coefficients of the enzymes directly into the elasticity coefficients of the elemental steps and derive a simple algorithm for such calculations. A concise matrix formulation for determining the control coefficients of enzymes in non-ideal pathways of any complexity (involving enzyme–enzyme interactions) is obtained.

2. Results

2.1. Theoretical background

2.1.1. Microdescription: a metabolic pathway as a network of elemental chemical steps

In ordinary (‘ideal’) metabolic pathways there is a one-to-one correspondence between enzymes and reactions. Moreover, the regulatory properties of ideal pathways allow analysis at the ‘macroscopic’ level of a set of enzyme catalyzed reactions without descending to the ‘microlevel’ of detailed mechanisms of those

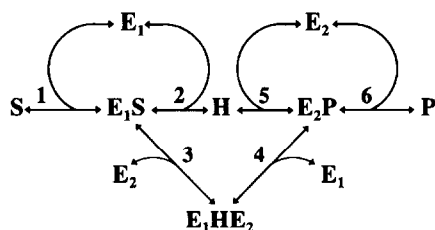


Fig. 1. The 'dynamic' channel. The enzyme–enzyme complex E_1HE_2 is formed after binding the substrate S to E_1 . The upper route represents the usual reaction pathway through the bulk phase intermediate H , catalyzed by free enzymes, and the lower route represents the "channeling". The numbering of the elemental steps is shown. The positive direction of the flux J is from the substrate S to the product P .

reactions. However, studies of the control structure of non-ideal pathways require consideration of a more elemental level than the level of enzymes and reactions [19,21–23,27–29].

We shall consider an arbitrary metabolic pathway which includes r enzyme reactions as a network of many more elemental processes (steps). The latter correspond to the transitions between different states of enzymes [27–30], or to sequences of such transitions that are not interrupted by branches. Usually the number of n of the elemental processes in the pathway greatly exceeds the number (r) of enzymes. Let m be the number of variables (variable concentrations) which include concentrations both of different enzyme forms and of free metabolites. We shall designate by x a vector of variable concentrations, $x = (x_1, x_2, \dots, x_m)^t$ and by v a vector of the rates of the elemental steps, $v = (v_1, v_2, \dots, v_n)^t$. Superscript t specifies a transposed vector-row, i.e., vector-column. For example, in the simple 'dynamic' channel [31–35] of Fig. 1, $r = 2$, $n = 6$, $m = 6$, and $x_1 = E_1S$, $x_2 = E_1HE_2$, $x_3 = H$, $x_4 = E_2P$, $x_5 = E_1$, $x_6 = E_2$.

Local kinetic properties of the elemental steps are determined by the sensitivities of their rates (v_i) to a change in the concentration of a metabolite (x_k):

$$\epsilon_{x_k}^{v_i} = \partial \ln |v_i| / \partial \ln x_k, \quad (2)$$

In this definition the partial derivative specifies that changes in the rate v_i caused by a change in x_k are considered at constant concentrations of all other metabolites and parameters that may influence the rate. In contrast to the definition of the control coefficients (Eq. (1)) no evolution of the entire metabolic pathway is permitted in Eq. (2).

Most often the rate of an elemental step depends linearly on any concentration x_k participating in that step (usually no more than one molecule of the given species is involved into an elemental chemical process, e.g., the collision and subsequent conversion). In this case the elasticity coefficients, $\epsilon_{x_k}^{v_i}$, of an elemental step (i) can be readily expressed into the ratios of the forward (v_i^+) and reverse (v_i^-) rates to the net rate ($v_i = v_i^+ - v_i^-$) of that step. More definitely, the elasticity coefficients of any elemental step i assume values (for different values of j) of either 0, or v_i^+/v_i , or $-v_i^-/v_i$. For example, for the dynamic channel of Fig. 1, the rate and the elasticity coefficients of step 3 (a 'channelled' step) with the rate

$$v_3 = k_3^+ x_1 x_6 - k_3^- \cdot x_2,$$

read:

$$\epsilon_{x_1}^{v_3} = \frac{k_3^+ x_1 x_6}{v_3} = \epsilon_{x_6}^{v_3} = \frac{v_3^+}{v_3},$$

$$\epsilon_{x_2}^{v_3} = -\frac{k_3^- x_2}{v_3} = -\frac{v_3^-}{v_3},$$

$$\epsilon_{x_j}^{v_3} = 0, \quad \text{if } j \neq 1, 2, 6.$$

(3)

Similar expressions can be written for the elasticity coefficients of each step of the dynamic channel shown in Fig. 1. Then, the n by m matrix (ϵ) of the elemental elasticity coefficients reads for this pathway:

$$\epsilon = \begin{pmatrix} -v_1^-/v_1 & 0 & 0 & 0 & v_1^+/v_1 & 0 \\ v_2^+/v_2 & 0 & -v_2^-/v_2 & 0 & -v_2^-/v_2 & 0 \\ v_3^+/v_3 & -v_3^-/v_3 & 0 & 0 & 0 & v_3^+/v_3 \\ 0 & v_4^+/v_4 & 0 & -v_4^-/v_4 & -v_4^-/v_4 & 0 \\ 0 & 0 & v_5^+/v_5 & -v_5^-/v_5 & 0 & v_5^+/v_5 \\ 0 & 0 & 0 & v_6^+/v_6 & 0 & -v_6^-/v_6 \end{pmatrix} \quad (4)$$

The ratios v_i^+/v_i and $-v_i^-/v_i$ and, therefore, the elasticity coefficients can be expressed in terms of the Gibbs energy (free-energy) difference (ΔG_i) across the elemental step i :

$$\begin{aligned} \frac{v_i^+}{v_i} &= \frac{1}{1 - e^{-\Delta G_i/RT}} = \rho_i^+, \\ \frac{v_i^-}{v_i} &= \frac{1}{e^{\Delta G_i/RT} - 1} = \rho_i^-. \end{aligned} \quad (5)$$

Here the ratios of the unidirectional rates to the net rate of the step (i) are designated by ρ_i^+ and ρ_i^- . The sign of ΔG_i corresponds to taking the chemical potentials of the substrates minus the chemical potentials of the products. For step 3 of the example above (Fig. 1), $\Delta G_3 = \mu_{x_1} + \mu_{x_6} - \mu_{x_2} = \mu_{E_1S} + \mu_{E_2} - \mu_{E_1HE_2}$.

2.1.2. Moiety-conserved cycles of enzymes and metabolites

Structural properties of the network of the elemental processes are described by the stoichiometric matrix \mathbf{N} of m rows (the number of the variable concentrations) and n columns (the number of the processes), see e.g., Ref. [12]. An element (N_{ij}) at row i and column j of the matrix \mathbf{N} is the stoichiometry by which x_i participates in the j th elemental process. For any network under consideration the rank (m_0) of \mathbf{N} is *always* less than m , and not all the concentrations (x_k) are independent. At least, there are r constraints, each of which corresponds to the moiety conservation of an enzyme (e_i),

$$e_i = E_i + E_iS_k + \dots + E_iE_j + E_iS_lE_j + \dots, \quad i = 1, 2, \dots, r \quad (6)$$

(for clarity, here instead of x_k the usual symbols are used for the concentrations of different enzyme forms: E_i designates free enzyme, E_iS designates the enzyme–substrate form, E_iE_j and $E_iS_lE_j$ designate enzyme–enzyme and enzyme–substrate–enzyme complexes, respectively). In addition, substrate (metabolite) moiety-conserved cycles can be present in a pathway [36],

$$T_i = \gamma_{i1}x_1 + \dots + \gamma_{im}x_m = \sum_{k=1}^m \gamma_{ik}x_k, \quad i = 1, 2, \dots, s. \quad (7)$$

Here T_i designates the total concentration of i th conserved substrate (not enzyme) moiety, s is the number of independent conservation relationships for substrates, γ_{ik} are constant coefficients. Note, that x_k entering Eq. (7) correspond (for a different value of k) to the concentrations of both free and enzyme-bound metabolites.

Due to the $(r+s)$ conservation constraints, Eqs. (6) and (7), the number of the linearity independent concentrations is equal to: $m_0 = m - (r+s)$. Along with a vector (\mathbf{x}) of m variable concentrations, $\mathbf{x} = (x_1, x_2, \dots, x_m)^t$, we shall consider a reduced vector (\mathbf{x}^R) of the linearly independent concentrations only (marked by the superscript R), $\mathbf{x}^R = (x_1, x_2, \dots, x_{m_0})^t$. Without loss of generality it is assumed here that the concentrations x_k are renumbered such the first m_0 concentrations are independent. In view of Eqs. (6) and (7),

all the concentrations (\mathbf{x}) can then be expressed as linear functions of these independent concentrations (\mathbf{x}^R), parameters $\mathbf{e} = (e_1, e_2, \dots, e_r)^t$ and $\mathbf{T} = (T_1, T_2, \dots, T_s)^t$:

$$\mathbf{x} = \mathbf{L} \cdot \mathbf{x}^R + \mathbf{M}. \quad (8)$$

The m by m_0 matrix \mathbf{L} is the link matrix [12] determined by the stoichiometric matrix \mathbf{N} . The m -dimensional vector \mathbf{M} is a linear function of parameters \mathbf{e} and \mathbf{T} , $\mathbf{M} = \mathbf{M}(\mathbf{e}, \mathbf{T})$.

Let us designate by \mathbf{x}^D the vector of the remaining $m - m_0 = r + s$ 'dependent' concentrations, $\mathbf{x}^D = (x_{m_0+1}, x_{m_0+2}, \dots, x_{m_0+r}, x_{m_0+r+1}, \dots, x_{m_0+r+s})^t$. Representing the vector \mathbf{x} as $\mathbf{x} = (\mathbf{x}^R, \mathbf{x}^D)^t$, one can see from Eq. (8), that the first m_0 rows of the link matrix (\mathbf{L}) coincide with the m_0 -dimensional identity matrix (\mathbf{I}_{m_0}) [12], and the first m_0 components of the vector \mathbf{M} are equal to 0. In what follows it is convenient to present Eq. (8) in the form:

$$\begin{pmatrix} \mathbf{x}^R \\ \mathbf{x}^D \end{pmatrix} = \mathbf{L} \cdot \mathbf{x}^R + \begin{pmatrix} \mathbf{0} \\ \mathbf{M}^D \end{pmatrix} = \begin{pmatrix} \mathbf{I}_{m_0} \\ \mathbf{L}^0 \end{pmatrix} \cdot \mathbf{x}^R + \begin{pmatrix} \mathbf{0} \\ \mathbf{M}^D \end{pmatrix} \quad (9)$$

Here $\mathbf{M}^D = \mathbf{M}^D(\mathbf{e}, \mathbf{T})$ is the $(r + s)$ -dimensional vector composed of the last $r + s$ elements of the vector \mathbf{M} , and \mathbf{L}^0 is an $(r + s)$ -row and m_0 -column matrix. Note, that both \mathbf{L}^0 and \mathbf{M}^D depend on a particular choice of the linearly independent concentrations. Only after such a choice they are determined in a unique manner (see below).

2.1.3. Properties of the elemental fluxes determined by the network structure

The system steady state is determined by the equation:

$$\mathbf{N} \cdot \mathbf{v} = 0, \quad (10)$$

which should be considered together with the conservation constraints, Eqs. (6) and (7). A steady-state rate ($v_i^{\text{st.}}$) of an elemental step will be referred to as a (steady-state) flux (J_i) through that step (i). It follows from Eq. (10), that any flux vector $\mathbf{J} = (J_1, J_2, \dots, J_n)^t$ belongs to the kernel ($\text{Ker}(\mathbf{N})$) of the stoichiometric matrix \mathbf{N} , and, therefore, for any reaction network not all fluxes (J_i) are independent. Since a basis of $\text{Ker}(\mathbf{N})$ consists of $n - m_0$ linearly independent vectors, there are $n - m_0$ linearly independent fluxes (see, e.g., Ref. [18]). A vector (\mathbf{J}) of all n elemental fluxes can be expressed in terms of a reduced vector (\mathbf{J}^R) of the independent fluxes, $\mathbf{J}^R = (J_1, J_2, \dots, J_{n-m_0})^t$, as follows (without loss of generality it is assumed that the elemental processes are renumbered such that the first $n - m_0$ fluxes are linearly independent ones):

$$\mathbf{J} = \Phi \cdot \mathbf{J}^R. \quad (11)$$

Here Φ is the n by $n - m_0$ matrix the first $(n - m_0)$ rows of which coincide with the $(n - m_0)$ -dimensional identity matrix, \mathbf{I}_{n-m_0} ,

$$\Phi = \begin{pmatrix} \mathbf{I}_{n-m_0} \\ \Phi^0 \end{pmatrix}$$

Φ^0 is the m_0 by $n - m_0$ matrix. Note, that the columns of the matrix Φ constitute a basis of $\text{Ker}(\mathbf{N})$. For instance, in the channelled pathway of Fig. 1 the fluxes through all six elemental steps can be expressed into the fluxes through the steps 1 and 2:

$$\begin{pmatrix} J_1 \\ J_2 \\ J_3 \\ J_4 \\ J_5 \\ J_6 \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ 1 & -1 \\ 1 & -1 \\ 0 & 1 \\ 1 & 0 \end{pmatrix} \begin{pmatrix} J_1 \\ J_2 \end{pmatrix}. \quad (12)$$

2.2. Expressing the enzyme control coefficients into the elasticity coefficients

2.2.1. Derivation of a general matrix equation for determining the control coefficients of the enzymes

According to Eqs. (1) and (11) a set of control coefficients of any enzyme with respect to n elemental fluxes, $\mathbf{J} = (J_1, J_2, \dots, J_n)^t$, can be expressed into control coefficients of that enzyme with respect to $n - m_0$ linearly independent fluxes, $\mathbf{J}^R = (J_1, J_2, \dots, J_{n-m_0})^t$. To write these relationships in the matrix form we designate by \mathbf{C}_e^J the n by r matrix of the enzyme control coefficients over all the elemental fluxes; the coefficient at row i and column j of the matrix \mathbf{C}_e^J is the control coefficient of the enzyme j with respect to the flux J_i . Similarly, we designate by $\mathbf{C}_e^{J^R}$ the reduced $n - m_0$ by r matrix composed of the enzyme control coefficients with respect to the linearly independent fluxes only. Note, that linearly independent fluxes are renumbering as the first $n - m_0$ fluxes, and the reduced matrix $(\mathbf{C}_e^{J^R})$ is the submatrix formed by the first $n - m_0$ rows of the matrix \mathbf{C}_e^J . Also we shall use a simple (conventional) designation $(d \ln a / d \ln b)$ for matrices of log–log derivatives $(d \ln a_i / d \ln b_j)$, e.g. the matrix \mathbf{C}_e^J is written as

$$\mathbf{C}_e^J = \left(\frac{d \ln \mathbf{J}}{d \ln \mathbf{e}} \right) = (\text{diag } \mathbf{J})_n^{-1} \cdot (d\mathbf{J}/d\mathbf{e}) \cdot (\text{diag } \mathbf{e})_r, \quad (13)$$

where $(d\mathbf{J}/d\mathbf{e})$ is the n by r matrix of the (non-scaled) derivatives dJ_i/de_j . Here and below $(\text{diag } \mathcal{F})_h^{-1}$ and $(\text{diag } \mathcal{F})_h$ designate (for any vector \mathcal{F} of dimension h) the square h by h matrices with diagonal elements equal to \mathcal{F}_i^{-1} and \mathcal{F}_i , respectively, and all off-diagonal elements equal to zero.

With this nomenclature the expression of the matrix \mathbf{C}_e^J (of the control coefficients over all elemental fluxes) in terms of the reduced matrix $\mathbf{C}_e^{J^R}$ (of the control coefficients over independent fluxes only) reads:

$$\mathbf{C}_e^J = \left(\frac{d \ln \mathbf{J}}{d \ln \mathbf{e}} \right) = \left(\frac{\partial \ln \mathbf{J}}{\partial \ln \mathbf{J}^R} \right) \cdot \left(\frac{d \ln \mathbf{J}^R}{d \ln \mathbf{e}} \right) = \mathbf{W} \cdot \mathbf{C}_e^{J^R}, \quad (14)$$

where the n by $n - m_0$ matrix \mathbf{W} relates to the matrix Φ of Eq. (11) as:

$$\mathbf{W} = \left(\frac{\partial \ln \mathbf{J}}{\partial \ln \mathbf{J}^R} \right) = (\text{diag } \mathbf{J})_n^{-1} \cdot \Phi \cdot (\text{diag } \mathbf{J}^R)_{n-m_0}. \quad (15)$$

On the other hand the flux control coefficients can be determined by direct differentiation of the steady-state rates (v_i^{st}) with respect to the enzyme concentrations (e_j). In view of Eqs. (6)–(10) these rates (fluxes J_i) can be represented as functions of the parameters $\mathbf{e} = (e_1, e_2, \dots, e_r)^t$ (for the moment the parameters T are assumed to be constant, since it is always possible to change the total concentrations, \mathbf{e} , of enzymes only, leaving the total concentrations of substrate moiety-conserved cycles unchanged):

$$J_i(\mathbf{e}) = v_i^{\text{st}}(\mathbf{x}(\mathbf{x}^R(\mathbf{e}), \mathbf{M}(\mathbf{e}))), \quad i = 1, 2, \dots, n. \quad (16)$$

Differentiating Eq. (16) with respect to the total enzyme concentrations, one obtains

$$\mathbf{C}_e^J = \left(\frac{\partial \ln \mathbf{v}}{\partial \ln \mathbf{x}} \right) \cdot \left(\frac{\partial \ln \mathbf{x}}{\partial \ln \mathbf{x}^R} \right) \cdot \left(\frac{d \ln \mathbf{x}^R}{d \ln \mathbf{e}} \right) + \left(\frac{\partial \ln \mathbf{v}}{\partial \ln \mathbf{e}} \right) = \boldsymbol{\epsilon}^* \cdot \mathbf{C}_e^{x^R} + (\partial \ln \mathbf{v} / \partial \ln \mathbf{e}) \quad (17)$$

Here $\boldsymbol{\epsilon}^*$ is the n by m_0 matrix of the ‘reduced’ elasticity coefficients which is expressed in terms of n by m matrix $(\boldsymbol{\epsilon})$ of the elasticity coefficients, $(\boldsymbol{\epsilon} = (\partial \ln \mathbf{v} / \partial \ln \mathbf{x})$, see Eq. (2)) and the link matrix (\mathbf{L}) (see Eq. (8)):

$$\boldsymbol{\epsilon}^* = \left(\frac{\partial \ln \mathbf{v}}{\partial \ln \mathbf{x}} \right) \cdot \left(\frac{\partial \ln \mathbf{x}}{\partial \ln \mathbf{x}^R} \right) = \boldsymbol{\epsilon} \cdot (\text{diag } \mathbf{x})_m^{-1} \cdot \mathbf{L} \cdot (\text{diag } \mathbf{x}^R)_{m_0}, \quad (18)$$

$\mathbf{C}_e^{x^R}$ is the m_0 by r matrix of the control coefficients of the enzymes over the linearly independent concentrations only,

$$\mathbf{C}_e^{x^R} = \left(\frac{d \ln \mathbf{x}^R}{d \ln \mathbf{e}} \right) = (\text{diag } \mathbf{x}^R)_{m_0}^{-1} \cdot (d\mathbf{x}/d\mathbf{e}) \cdot (\text{diag } \mathbf{e})_r. \quad (19)$$

The n by r matrix, $\partial \ln v / \partial \ln e$, of the partial derivatives reflects the explicit dependence of the elemental rates on the total enzyme concentrations arising from the dependence of \mathbf{M}^D on e (after substitution of Eq. (9) into the rate laws). Equating the right-hand sides of Eqs. (14) and (17), and rearranging one has:

$$\mathbf{W} \cdot \mathbf{C}_e^{J^R} - \boldsymbol{\epsilon}^* \cdot \mathbf{C}_e^{x^R} = \partial \ln v / \partial \ln e. \quad (20)$$

According to the rules of the matrix algebra, Eq. (20) can be rewritten as

$$(\mathbf{W} \mid -\boldsymbol{\epsilon}^*) \cdot \begin{pmatrix} \mathbf{C}_e^{J^R} \\ \mathbf{C}_e^{x^R} \end{pmatrix} = \mathbf{E} \cdot \mathbf{C}_e = \left(\frac{\partial \ln v}{\partial \ln e} \right). \quad (21)$$

Here \mathbf{E} is the n by n square matrix the first $(n - m_0)$ columns of which coincide with the matrix \mathbf{W} and the remaining m_0 columns coincide with the matrix $-\boldsymbol{\epsilon}^*$. The first $(n - m_0)$ rows of the n by r matrix, \mathbf{C}_e , are formed by the control coefficients of the enzymes with respect to the linearly independent fluxes ($\mathbf{C}_e^{J^R}$) and the remaining m_0 rows are formed by the control coefficients of the enzymes with respect to the linearly independent concentrations ($\mathbf{C}_e^{x^R}$).

For non-pathological cases the square matrix \mathbf{E} is invertible. Therefore, the matrix \mathbf{C}_e of the control coefficients of the enzymes is determined as:

$$\mathbf{C}_e = \mathbf{E}^{-1} \cdot (\partial \ln v / \partial \ln e) \quad (22)$$

2.2.2. A simple algorithm for calculating the matrix $(\partial \ln v / \partial \ln e)$

As was mentioned above an explicit dependence of the elemental rates (v) on the total enzyme concentrations (e) appears after the substitution of the dependent concentrations (\mathbf{x}^D) as functions of the independent concentrations (\mathbf{x}^R) and conservation totals (\mathbf{M}^D) into the corresponding rate expressions, i.e. via the dependence of the vector \mathbf{M}^D on e (see Eq. (9)):

$$v_i = v_i(\mathbf{x}^R, \mathbf{x}^D(\mathbf{x}^R, \mathbf{M}^D(e))), \quad i = 1, 2, \dots, n. \quad (23)$$

Here, according to Eq. (9)

$$\mathbf{x}^D(\mathbf{x}^R, \mathbf{M}^D) = \mathbf{L}^0 \cdot \mathbf{x}^R + \mathbf{M}^D. \quad (24)$$

It should be noted that the choice of the dependent concentrations is not unique. Any particular form of the matrix $(\partial \ln v / \partial \ln e)$ will depend significantly on the concentrations that are chosen as the dependent concentrations.

Using Eqs. (23) and (24) the matrix, $(\partial \ln v / \partial \ln e)$, of the partial derivatives of the elemental rates with respect to the enzyme concentrations can be expressed in terms of the elasticities and the corresponding derivatives of the vector \mathbf{M}^D :

$$\begin{aligned} (\partial \ln v / \partial \ln e) &= (\text{diag } \mathbf{J})_n^{-1} \cdot (\partial v / \partial e) \cdot (\text{diag } e)_r \\ &= (\text{diag } \mathbf{J})_n^{-1} \cdot (\partial v / \partial \mathbf{x}^D) \cdot (\partial \mathbf{x}^D / \partial \mathbf{M}^D) \cdot (\partial \mathbf{M}^D / \partial e) \cdot (\text{diag } e)_r \\ &= \boldsymbol{\epsilon}_D \cdot (\text{diag } \mathbf{x}^D)_{r+s}^{-1} \cdot (\partial \mathbf{M}^D / \partial e) \cdot (\text{diag } e)_r. \end{aligned} \quad (25)$$

Here it was taken into account, that in view of Eq. (24) the matrix $(\partial \mathbf{x}^D / \partial \mathbf{M}^D)$ is the $r + s$ by $r + s$ identity matrix (\mathbf{I}_{r+s}). The matrix $\boldsymbol{\epsilon}_D$ is n by $r + s$ matrix of the elasticity coefficients of the elemental steps with respect to the dependent concentrations ($\boldsymbol{\epsilon}_D = \partial \ln v / \partial \ln \mathbf{x}^D$). It is formed by the last $r + s$ columns of the n by m elasticity matrix ($\boldsymbol{\epsilon} = \partial \ln v / \partial \ln \mathbf{x}$).

The vector \mathbf{M}^D (and as a consequence the matrices $\partial \mathbf{M}^D / \partial e$ and $\partial \ln v / \partial \ln e$) depends on a particular choice of the concentrations \mathbf{x}^D . Taking into account that the concentrations of free enzyme forms, E_i , enter

enzyme moiety-conserved cycles only and do not enter substrate moiety-conserved cycles, T_j (see Eqs. (6) and 7), we choose these concentrations, E_1, E_2, \dots, E_r , as the r dependent concentrations. Then, for an arbitrary choice of the remaining (s) dependent concentrations (chosen from the concentrations entering Eqs. (7)), the vector \mathbf{M}^D takes the form

$$\mathbf{M}^D = (e_1, e_2, \dots, e_r, f_1, f_2, \dots, f_s)^t. \quad (26)$$

Here f_i are linear functions of the substrate totals (\mathbf{T}) only. Then, the $(r+s)$ by r matrix $(\partial \mathbf{M}^D / \partial \mathbf{e})$ reads:

$$(\partial \mathbf{M}^D / \partial \mathbf{e}) = \begin{pmatrix} \mathbf{I}_r \\ 0 \end{pmatrix} \quad (27)$$

where \mathbf{I}_r is r by r identity matrix. Choosing r dependent concentrations as the concentrations of *free* enzymes (E_i) one obtains from Eqs. (25) and (27)

$$\begin{aligned} (\partial \ln v / \partial \ln \mathbf{e}) &= \boldsymbol{\epsilon}_E \cdot (\text{diag } \mathbf{E})_r^{-1} \cdot (\text{diag } \mathbf{e})_r \\ &= \boldsymbol{\epsilon}_E \cdot (\text{diag } \mathbf{e} / \mathbf{E})_r, \end{aligned} \quad (28)$$

where $\boldsymbol{\epsilon}_E$ designates n by r matrix of the elasticity coefficients of the elemental steps with respect to the concentrations of free enzyme, $(\boldsymbol{\epsilon}_E)_{ij} = \partial \ln v_i / \partial \ln E_j$.

2.3. Example

Now we shall calculate the control coefficients of the enzymes in the simple dynamic channel [31–35] shown in Fig. 1. The first step is the construction of the matrix \mathbf{E} (see Eq. (21)). The part of this matrix that reflects the structural properties of the channel, i.e., the matrix \mathbf{W} , is obtained from Eqs. (12) and (15):

$$\mathbf{W} = \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ J_1 & J_2 \\ \frac{J_1}{J_1 - J_2} & -\frac{J_2}{J_1 - J_2} \\ J_1 & J_2 \\ \frac{J_1}{J_1 - J_2} & -\frac{J_2}{J_1 - J_2} \\ 0 & 1 \\ 1 & 0 \end{pmatrix} \quad (29)$$

here all the fluxes J_i are expressed into the independent fluxes, J_1 and J_2 .

To construct the remaining part of \mathbf{E} formed by the matrix of the ‘reduced’ elasticities ($\boldsymbol{\epsilon}^*$) we choose the concentrations of the free enzymes, E_1 and E_2 , as the dependent concentrations. They are expressed in terms of the independent concentrations by (see Eq. (6)):

$$\begin{aligned} E_1 &= e_1 - x_1 - x_2 = e_1 - E_1 S - E_1 H E_2, \\ E_2 &= e_2 - x_2 - x_4 = e_2 - E_1 H E_2 - E_2 P. \end{aligned} \quad (30)$$

From Eq. (30) it follows that the link matrix (\mathbf{L}) has a form (see Eq. (9))

$$\mathbf{L} = \begin{pmatrix} \mathbf{I}_4 \\ -1 & -1 & 0 & 0 \\ 0 & -1 & 0 & -1 \end{pmatrix}, \quad (31)$$

where \mathbf{I}_4 is the 4 by 4 identity matrix. Using Eqs. (18) and (4), (31) one obtains for the ‘reduced’ matrix (ϵ^*) of the elasticities:

$$\epsilon^* = \begin{pmatrix} -\rho_1^+ \frac{x_1}{E_1} - \rho_1^- & -\rho_1^+ \frac{x_2}{E_1} & 0 & 0 \\ \rho_2^+ + \rho_2^- \frac{x_1}{E_1} & \rho_2^- \frac{x_2}{E_1} & -\rho_2^- & 0 \\ \rho_3^+ & -\rho_3^+ \frac{x_2}{E_2} - \rho_3^- & 0 & -\rho_3^+ \frac{x_4}{E_2} \\ \rho_4^- \frac{x_1}{E_1} & \rho_4^+ + \rho_4^- \frac{x_2}{E_1} & 0 & -\rho_4^- \\ 0 & -\rho_5^+ \frac{x_2}{E_2} & \rho_5^+ & -\rho_5^+ \frac{x_4}{E_2} - \rho_5^- \\ 0 & \rho_6^- \frac{x_2}{E_2} & 0 & \rho_6^+ + \rho_6^- \frac{x_4}{E_2} \end{pmatrix}, \quad (32)$$

ρ_i^+ and ρ_i^- are the ratios v_i^+/v_i and v_i^-/v_i , see Eq. (5). Especially when steps are quasi-irreversible, measurement of the enzyme intermediate levels (E_1 , E_2 , $x_1 = E_1S$, $x_2 = E_1HE_2$ and $x_4 = E_2P$) allow one to calculate ϵ^* .

Now the matrix \mathbf{E} is obtained by combining the matrices \mathbf{W} and $-\epsilon^*$ given by Eqs. (29) and (32) (see Eq. (21)). Note, that an equivalent way to derive the matrix (ϵ^*) is to substitute the dependent concentrations from Eq. (30) into the elemental rates and, then, differentiate these rate expressions with respect to the independent variables. Consequently, ϵ^* can be calculated once the rate equations are known.

The second step is determining of the matrix $(\partial \ln v / \partial \ln e)$. Using Eq. (28) one obtains:

$$\left(\frac{\partial \ln v}{\partial \ln e} \right) = \begin{pmatrix} \epsilon_{E_1}^{v_1} e_1 / E_1 & 0 \\ \epsilon_{E_1}^{v_2} e_1 / E_1 & 0 \\ 0 & \epsilon_{E_2}^{v_3} e_2 / E_2 \\ \epsilon_{E_1}^{v_4} e_1 / E_1 & 0 \\ 0 & \epsilon_{E_2}^{v_5} e_2 / E_2 \\ 0 & \epsilon_{E_2}^{v_6} e_2 / E_2 \end{pmatrix} = \begin{pmatrix} \rho_1^+ e_1 / E_1 & 0 \\ -\rho_2^- e_1 / E_1 & 0 \\ 0 & \rho_3^+ e_2 / E_2 \\ -\rho_4^- e_1 / E_1 & 0 \\ 0 & \rho_5^+ e_2 / E_2 \\ 0 & -\rho_6^- e_2 / E_2 \end{pmatrix}. \quad (33)$$

The last step is to employ Eq. (22) (in which matrices \mathbf{E} and $(\partial \ln v / \partial \ln e)$ must be substituted) to determine the control coefficients of the enzymes in the channelled pathway.

As a numerical elaboration of this example, Fig. 2 shows the dependence of the enzyme control coefficients (Fig. 2a) and their sum (Fig. 2b) on the total concentration of the enzymes for the dynamic channel of Fig. 1 (for simplicity the concentrations of the enzymes were taken to be equal). The corresponding curves were calculated from Eq. (22) by inverting matrix \mathbf{E} . One can see that the sum of the enzyme control coefficients coincides with the classical value 1 at very low concentrations of the enzymes where the channelled flux fraction is negligible. With an increase in the enzyme concentrations the channelled flux fraction increases because more enzyme–enzyme complex is formed (Fig. 2b). The initial increase in the channelled flux is accompanied by an increase in the control exerted by ‘channelled’ steps. As a result the sum of the control coefficients goes up, attaining (maximal) values much greater than unity [19,37–42]. With a further increase in the concentrations of the enzymes this sum goes down again. The latter effect reflects ‘enzyme sequestration’ in channelled pathways [26]. At such high enzyme concentrations the sum of the enzyme control coefficients

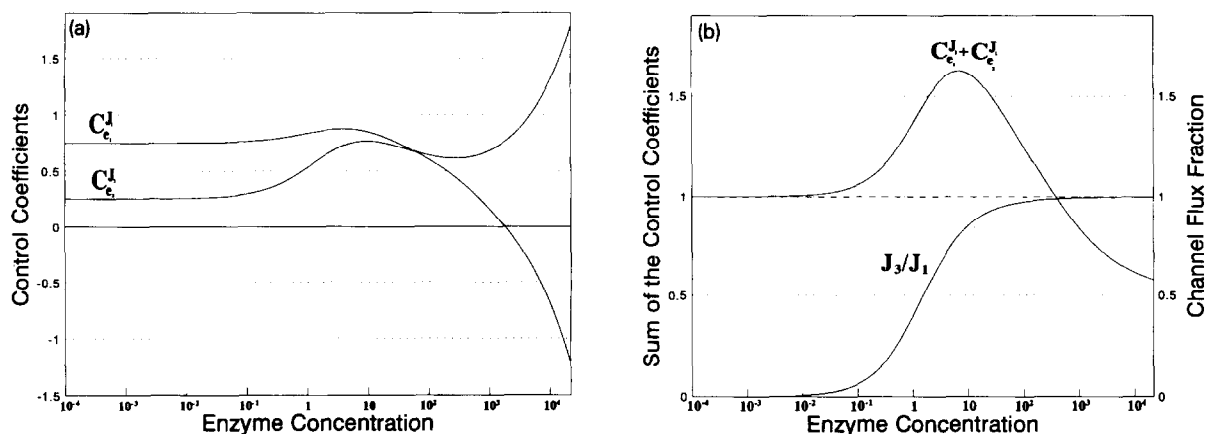


Fig. 2. The dependence of (a): the control coefficients of the enzymes 1 ($C_{e_1}^{J_1}$) and 2 ($C_{e_2}^{J_1}$) and (b): their sum ($C_{e_1}^{J_1} + C_{e_2}^{J_1}$) and the fraction of the flux going through the channel (J_3/J_1) on the total concentration of the enzymes of the pathway of Fig. 1. The control coefficients and their sum were calculated over the input (output) flux (J_1) which is equal to the total flux through the channel and the bulk phase. The concentrations of the enzymes were taken to be equal. The parameter values were (dimensionless units); $S = 1$, $P = 0$, $k_1^+ = 100$, $k_1^- = 50$, $k_2^+ = 1$, $k_2^- = 1$, $k_3^+ = 1$, $k_3^- = 20$, $k_4^+ = 20$, $k_4^- = 1$, $k_5^+ = 1$, $k_5^- = 1$, $k_6^+ = 200$, $k_6^- = 100$. Steady-state values of the concentrations and fluxes were obtained by numerical integration and the control coefficients were calculated using Eq. (22).

control coefficient drops below unity (Fig. 2b), and the control coefficient of the first enzyme even becomes negative (Fig. 2a). These entirely new phenomena cannot occur in ideal pathways lacking the channel; there proportional increases in enzyme concentrations always result in the same proportional increase of the flux [2].

In the pathway considered there were no substrate moiety-conserved cycles. Regardless whether such substrate cycles are present, the same routine can be used for calculating the enzyme control coefficients. Moreover, the expression of the matrix $(\partial \ln v / \partial \ln e)$ via the elemental elasticities with respect to free enzyme concentrations (E_i) is not affected by the presence of substrate moiety-conserved cycles (see Eq. (28)) when the concentrations of free enzymes (E_i) are chosen as the dependent variables.

3. Discussion

Elegant matrix equations allowing one to calculate the control coefficients of the enzymes from their elasticity coefficients have been proposed for usual (ideal) metabolic pathways [3,10,12–18]. However, for channelled pathways there is no one-to-one correspondence between enzymes and reactions and the elasticity coefficients of enzymes ‘in isolation’ do not describe the kinetic properties of reactions in situ [43]. As has been shown recently [19] to treat such pathways one should use a ‘microdescription’, i.e. analyze them at the level of elemental processes (see also [34,35]).

In this paper we showed that to calculate the control coefficients of the enzymes one should determine the matrix of the elemental elasticity coefficients and the matrix of the partial derivatives of elemental rates with respect to the total enzyme concentrations. A simple routine for deriving the latter matrix via the elasticity coefficients with respect to the concentrations of free (empty) enzymes is proposed.

Matrix **E** involving the elasticities of the elemental steps depends also on the structural properties of the network of the elemental steps in the enzyme reactions of a pathway. These structural properties are involved in terms of both, the basis vectors of the kernel of the stoichiometry matrix (**N**), (matrix **W**), and the link matrix (**L**) [12] (see Eqs. (21) and (18)).

We illustrated our results for the pathway of two enzymes forming a complex capable of channelling the intermediate (see Fig. 1). Such a channelled pathway where at each catalytic cycle the enzyme-enzyme complex has to be formed and broken-down, is referred to as a ‘dynamic’ channel [31–35]. In ideal (unchannelled) pathways equal relative increases in the concentrations of the all enzymes leads to the same relative increase in the metabolic flux; the sum of the enzyme control coefficients equals 1. Here we showed that in the channelled pathway under conditions considered the flux first grows much faster than the enzyme concentrations (the sum of the enzyme control coefficients exceeded 1). Then, with further increase in the concentrations of the enzymes, flux fell behind the increase in the concentrations. This phenomenon is a hitherto unrecognized distinctive feature of the channelled pathways [26].

In an arbitrary system the steady-state rates of some elemental processes can be equal to zero (e.g., in some cases of so called static channelling [25] a step of the formation of ‘static’ complex is at thermodynamic equilibrium while the fluxes through the other steps are not equal to zero. In such cases one should use the usual derivatives rather than the log–log derivatives (non-normalized coefficients, cf., Ref. [12]). The matrix equations of the present work can be easily transformed into the corresponding equations for non-normalized coefficients. For instance, the non-normalized analogue of Eq. (21) reads

$$\left(\Phi - \frac{\partial v}{\partial x} \cdot L \right) \cdot \begin{pmatrix} G_e^{J^R} \\ G_e^{x^R} \end{pmatrix} = \begin{pmatrix} \frac{\partial v}{\partial e} \end{pmatrix} \quad (34)$$

Here $G_e^{J^R}$ and $G_e^{x^R}$ are the matrices of the non-normalized coefficients,

$$G_e^{J^R} = \begin{pmatrix} \frac{dJ^R}{de} \end{pmatrix}, \quad G_e^{x^R} = \begin{pmatrix} \frac{dx^R}{de} \end{pmatrix}.$$

The results obtained in this paper are applicable both to channelled pathways and to other non-ideal pathways, for instance, with high enzyme concentrations and (substrate) moiety conserved cycles [21,23]. Because the usual methods and theorems of control analysis break down when applied to such systems this may be an important advantage.

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