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# Dramatic changes in control properties that accompany channelling and metabolite sequestration

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A simple summation theorem describes the control of fluxes in 'ideal' metabolic pathways. This paper shows how this theorem and the control properties of a pathway change when direct transfer of intermediates and/or sequestration of metabolites involved in moiety conservations (by enzymes present at high concentrations) take place. The derived generalized summation theorem quantifies the extent to which metabolite sequestration decreases and direct metabolite transfer can increase the control exerted by enzymes on the flux. The implications of metabolite channelling for the control of fluxes are discussed quantitatively.

Metabolic channelling; High enzyme concentrations; Moiety conservation; Metabolic control theory

## 1. INTRODUCTION

A quantitative indicator of the control of the flux ($J$) through a metabolic pathway by an enzyme is the flux control coefficient ($C^J_{e_i}$) of that enzyme ($i$) [1]. This coefficient is defined as the ratio of relative changes in the steady-state flux ($\Delta J/J$) and in the enzyme concentration ($\Delta e_i/e_i$) which, in the limit to infinitesimal changes, is equal to the log–log derivative of the flux with respect to the enzyme concentration [1]. With such a definition significant advances were achieved in the quantitative description of the control of 'simple' or 'ideal' [2] multienzyme pathways, in which enzyme reactions can be treated as independent 'block' reactions inside the pathway (recently reviewed in [3]).

There are cellular metabolic pathways in which enzyme concentrations are comparable to or even exceed the concentrations of substrates. Glycolysis may serve as an example [4,5]. In such a case enzymes may sequester appreciable amounts of metabolites. If moiety conservations apply to such metabolites, this may have implications for the control [6,7]. Moreover, in highly organized cellular structures enzyme–enzyme interactions and even direct transfer of intermediates may take place [8,9]. Describing the control of such pathways may require consideration of some properties of the mechanism of enzyme reactions [6,7,10]. Moreover, in non-ideal pathways unequivocal definitions of the control and regulatory features are possible only at a more elemental level than the level of enzyme reactions, i.e. at the level of the elemental chemical transformations (catalytic steps) in the reaction cycles of the enzymes.

This paper shows how, for non-ideal pathways, the control coefficients of enzymes are related to the elemental control coefficients. We derive a general expression for the sum of the enzyme control coefficients which suggests experiments allowing one to elucidate the control properties of real cellular pathways.

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## 2. RESULTS

### 2.1. *Definitions and theoretical background*

The conceptual shift underlying this paper is to consider an arbitrary metabolic pathway which includes $r$ enzyme reactions as a network of the elemental processes (steps) [11]. These processes correspond to the transitions between different states of enzymes [12], or to sequences of such transitions that are not interrupted by branches. Such a consideration can be called a 'microdescription' contrary to the usual description treating a metabolic pathway at the 'macroscopic' level of 'block' (enzyme) reactions.

Let $n$ be the number of the elemental processes in a pathway (typically $n > r$). In the corresponding chemical equations the different enzyme forms (e.g. enzyme–metabolite, enzyme–enzyme or enzyme–metabolite–enzyme complexes) and free metabolites are present. These participants of the metabolic network will be called 'substances'. Let $m$ be the number of these substances. Obviously, there are some constraints on the

variation of the concentrations $(x_1,x_2,...,x_m)$ of these substances, and not all concentrations $(x_k)$ are independent. At least, there are $r$ constraints, corresponding to the moiety conservations of every enzyme $(i)$, which may have the following form:

$$e_i = E_i + E_iS + ... + E_iE_j + E_iSE_j + ..., \; i = 1,2,...,r \quad (1)$$

Here instead of $x_k$ the apparent symbols are used for the concentrations: $E_i$ designates free enzyme, $E_iS$ designates the enzyme–substrate form, $E_iE_j$ and $E_iSE_j$ designate enzyme–enzyme and enzyme–substrate–enzyme complexes, respectively. Such participants of the enzyme $i$ moiety-conserved cycle will be called the enzyme $i$ forms (or states, cf. [12]). Obviously, complexes involving two enzymes $i$ and $j$ (e.g. $E_iE_j$ and $E_iSE_j$) enter both enzyme $i$ and enzyme $j$ moiety-conserved cycles, as they are enzyme $i$ and enzyme $j$ forms simultaneously.

In what follows it is convenient to present the total enzyme concentration $e_i$ as the sum of the concentrations of the monomeric enzyme forms $(e_i^{mon})$ (which may be complexed only with metabolites), and complexes $(e_{ij}^{comp})$ of the enzyme $i$ with other enzymes $(j)$,

$$e_i = e_i^{mon} + \sum_{j \neq i} e_{ij}^{comp} = e_i^{mon} + e_i^{comp} \quad (2)$$

where, $e_i^{comp}$ is the part of the enzyme $i$ concentration complexed with all the other enzymes. Here, for the sake of simplicity, we consider the case when only one molecule of any particular enzyme enters enzyme–enzyme complexes (in particular, here we do not consider homodimers $e_ie_i$). The generalization for the case of several enzyme molecules is straightforward.

A common feature of metabolic pathways is the presence of substrate moiety-conserved cycles, the interconversion of $NAD^+$ and NADH may serve as an example. In an arbitrary metabolic pathway $s$ such substrate moiety-conserved cycles may be present,

$$T_i = \sum_{k=1}^{m} \gamma_{ik} x_k \qquad i = 1,2,...,s \quad (3)$$

Here $T_i$ designates the total concentration of the $i^{th}$ conserved substrate (not enzyme) moiety. Note, that the $x_k$ entering. Eqn. **(3)** correspond to the concentrations of both free and (for a different value of $k$) enzyme-bound metabolites.

The rate $(v_i)$ of the $i^{th}$ elemental process is a homogeneous function of zero- or first-order with respect to the concentrations of forms of any of the enzymes. Indeed, any elemental process (step) in the network under consideration is a mono- or bimolecular reaction with respect to enzyme forms. Moreover, if any of the enzyme $j$ forms enters the left-hand side of the chemical equation of the elemental process then some other form of the same enzyme $j$ will enter the right-hand side of that chemical equation. All the elemental processes with rates depending on the enzyme $j$ forms will be called $E_j$-dependent processes [11].

Now we define $n$ parameters $\xi_i$, $i = 1,2,...,n$ each modulating the activity only of the $i^{th}$ process,

$$v_i(x,\xi_i) = \xi_i \cdot v_i(x,1) \quad (4)$$

The elemental flux control coefficient of any process can be defined as following:

$$C_{v_i}^J = \mathrm{dln}|J|/\mathrm{dln}\,\xi_i\Big|_{\text{all } \xi_k = 1} \qquad i = 1,2,...,n \quad (5)$$

Note, that for the classical 'macrodescription' of a pathway the parameter $\xi_i$ plays the role of the activity or the concentration of the enzyme and the control coefficient defined by Eqn. **(5)** coincides with the 'classical' control coefficient.

### 2.2. *Relating control by enzymes to control by elementary steps*

To elucidate the particular features of the control in pathways which may involve channelling and moiety sequestrating, we apply a method of perturbation of the steady state [7,13]. Let in the initial steady state the concentrations and parameters be perturbed as follows: (i) every concentration involved in the enzyme $i$ moiety-conserved cycle (see Eqn. **(1)**) is increased by a factor $\lambda_i$:

$$E_i(\lambda_i) = \lambda_i \cdot E_i,\; E_iS(\lambda_i) = \lambda_i \cdot E_iS,\; E_iE_j(\lambda_i) = \lambda_i \cdot E_iE_j$$

$$E_iSE_j(\lambda_i) = \lambda_i \cdot E_iSE_j,..., \qquad i = 1,2,...,r \quad (6)$$

(ii) parameters, $\xi_k$, which correspond to the rates of $E_i$-dependent elemental processes (in which any of the forms of the enzyme $i$ participates) are decreased by the same factor $\lambda_i$:

$$\xi_k(\lambda_i) = \xi_k/\lambda_i, \quad (7)$$

if $\xi_k$ corresponds to an $E_i$-dependent process.

Since the rates of $v_k$ of $E_i$-dependent processes are homogeneous first-order functions of the concentrations of the enzyme $i$ forms (see above), all the rates in the new steady state will be equal to the initial non-perturbed rates. However, the parameters $e$, $T$, $\xi$ do differ between the old and the new steady state (see Eqns. **(6)**, **(7)**). The new values of $e_j$ and $T_l$ are:

$$e_i(\lambda_i) = \lambda_i \cdot e_i$$

$$\text{for } j \neq i,\; e_j(\lambda_i) = e_j^{mon} + \sum_{k \neq j,i} e_{jk}^{comp} + \lambda_i \cdot e_{ji}^{comp} = e_j + (\lambda_i - 1) \cdot e_{ji}^{comp}$$

$$T_l(\lambda_i) = T_l + (\lambda_i - 1) \cdot T_l^{e_i} \qquad l = 1,2,...,s \quad (8)$$

Here $T_l^{e_i}$ designates the part of $l^{th}$ conserved moiety

bound to all forms of the enzyme $i$. Since the steady-state fluxes ($J$) are functions of ($e$, $T$, $\xi$) one can write:

$$0 = \mathrm{dln}|J|/\mathrm{dln}\,\lambda_i = \sum_{j=1}^{r} C^J_{e_j} \cdot \mathrm{dln}\, e_j/\mathrm{dln}\,\lambda_i + \sum_{l=1}^{s} R^J_{T_l} \cdot \mathrm{dln}\, T_l/\mathrm{dln}\,\lambda_i + \sum_k C^J_{v_k} \cdot \mathrm{dln}\,\xi_k/\mathrm{dln}\,\lambda_i$$

$$R^J_{T_l} = \mathrm{dln}|J|/\mathrm{dln}\, T_l \qquad (9)$$

Here $R^J_{T_l}$ is the flux response coefficient to a change in $l^{\text{th}}$ substrate total $T_l$, and the third sum in Eqn. **(9)** includes only $E_i$-dependent elemental processes (where the enzyme $i$ forms participate). Substituting into Eqn. **(9)** the derivatives with respect to ln $\lambda_i$ calculated (using Eqns. **(6–8)**) at $\lambda_i = 1$, we obtain:

$$C^J_{e_i} + \sum_{j \neq i} C^J_{e_j} \cdot e^{\mathrm{comp}}_{ji}/e_j + \sum_{l=1}^{s} R^J_{T_l} \cdot T^{e_i}_l / T_l = \sum_{\substack{\text{all } E_i\text{-dependent} \\ \text{processes } k}} C^J_{v_k} = {}^{\mathrm{imp}}C^J_{e_i} \qquad i = 1,2,\ldots,r \qquad (10)$$

The right-hand side of this equation represents *the impact control coefficient* of the enzyme $i$ which has been defined [11] to quantify the effect of simultaneous equal relative changes in the rates of all the elemental processes in which enzyme $i$ is involved. In this sense it evaluates the total impact the enzyme $i$ has on the flux. Earlier we have shown that in some cases the impact control coefficient coincides with a normalized response of the flux to effector molecules for which the enzyme $i$ is a receptor [14].

### 2.3. *General summation theorem for the flux control coefficients*

Summing Eqn. **(10)** over all enzymes one obtains:

$$\sum_{i=1}^{r} C^J_{e_i} \cdot \left(1 + \frac{e^{\mathrm{comp}}_i}{e_i}\right) = 1 + \sum_{\substack{\text{protein} \\ \text{interaction} \\ \text{steps } i}} C^J_{v_i} - \sum_{l=1}^{s} R^J_{T_l} \cdot \left(\frac{T^{\mathrm{mon}}_l + 2T^{\mathrm{comp}}_l}{T_l}\right) \qquad (11)$$

here the sum of $T^{e_i}_l$ over all enzymes ($i$) is subdivided into two parts: $T^{\mathrm{mon}}_l$ bound to the monomeric enzyme forms and $T^{\mathrm{comp}}_l$ bound to the enzyme–enzyme complexes (note that $T^{\mathrm{comp}}_l$ enter twice the sum of $T^{e_i}_l$ over all enzymes). The additional to unity sum in the right-hand side of Eqn. **(11)** is taken over all 'protein interaction' steps, i.e. steps in which two different enzyme moieties participate (either as monomeric or complexed enzymes).

## 3. DISCUSSION

Eqns. **(10)** and **(11)** reveal the dramatic changes in the control properties of the enzymes in real cell pathways in comparison with 'ideal' pathways considered by the classical metabolic control theory. In ideal pathways both sums on the left-hand side of Eqn. **(10)** vanish. In this case it states that the control coefficient of an enzyme can be expressed as the sum of the elemental control coefficients over all steps in the reaction cycle of this enzyme. In pathways with high enzyme concentrations and moiety conservations but without direct enzyme–enzyme interactions the first sum on the left-hand side of Eqn. **(10)** equals zero, and the right-hand side of Eqn. **(10)** represents the control coefficient with respect to the enzyme rate or activity [6,7,10]. In this case Eqn. **(10)** shows that the control coefficient with respect to the enzyme concentration can be significantly less than the control coefficient with respect to the enzyme activity due to sequestration of metabolites. Indeed, the former can even take negative values when its 'classical' analog is positive [7].

Eqn. **(11)** presents the summation theorem valid now for an arbitrary pathway. In ideal pathways the sum of the enzyme control coefficients equals 1 [1]. Eqn. **(11)** shows that sequestrating of metabolites from moiety-conserved cycles by binding them to the enzymes present in high concentrations, can significantly decrease this sum. On the other hand, channelling of metabolites can affect the sum of the enzyme control coefficients in two different modes depending on the average complexed fraction of enzymes and on the control exerted by 'channelled' steps. The latter may reach as much as 1, so in a pathway without substrate moiety-conservations and low mean life time of enzyme–enzyme complexes the sum of the flux control coefficients of the enzymes can reach 2 [15].

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