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DOI
10.1016/0301-4622(95)00039-Z

Publication date
1995

Published in
Biophysical Chemistry

ELSEVIER

Biophysical Chemistry 56 (1995) 215–226

Biophysical Chemistry

Defining control coefficients in non-ideal metabolic pathways

Boris N. Kholodenko [a,b], Douwe Molenaar [c], Stefan Schuster [b,d], Reinhart Heinrich [d], Hans V. Westerhoff [b,c,e,*]

[a] *A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119899 Moscow, Russia*
[b] *E.C. Slater Institute, BioCentrum, University of Amsterdam, NL-1018 TV Amsterdam, The Netherlands*
[c] *Division of Molecular Biology, Netherlands Cancer Institute, NL-1066 CX Amsterdam, The Netherlands*
[d] *Institut für Biophysik der Humboldt-Universität zu Berlin, Berlin, Germany*
[e] *Vrije Universiteit, Faculty of Biology, Dept. of Microbial Physiology, de Boelelaan 1087, NL-1081 HV Amsterdam, The Netherlands*

Received 13 June 1994; revised 17 January 1995; accepted 8 February 1995

Abstract

The extent to which an enzyme controls a flux has been defined as the effect on that flux of a small modulation of the activity of that enzyme divided by the magnitude of the modulation. We here show that in pathways with metabolic channelling or high enzyme concentrations and conserved moieties involving both enzymic and non-enzymic species, this definition is ambiguous; the magnitude of the corresponding flux control coefficient depends on how the enzyme activity is modulated. This is illustrated with two models of biochemically relevant pathways, one in which dynamic metabolite channelling plays a role, and one with a moiety-conserved cycle.

To avoid such ambiguity, we view biochemical pathways in a more detailed manner, i.e., as a network of elemental steps. We define 'elemental control coefficients' in terms of the effect on a flux of an equal modulation of the forward and reverse rate constant of any such elemental step (which may correspond to transitions between enzyme states). This elemental control coefficient is independent of the method of modulation. We show how metabolic control analysis can proceed when formulated in terms of the elemental control coefficients and how the traditional control coefficients are related to these elemental control coefficients. An 'impact' control coefficient is defined which quantifies the effect of an activation of all elemental processes in which an enzyme is involved. It equals the sum of the corresponding elemental control coefficients. In ideal metabolic pathways this impact control coefficient reduces to the traditional flux control coefficient. Differences between the traditional control coefficients are indicative of non-ideality of a metabolic pathway, i.e. of channelling or high enzyme concentrations.

Keywords: Control coefficients; Non-ideal metabolic pathway

1. Introduction

In analyses of the regulation of cellular processes the intuitive concept of the rate-limiting step has been substituted by the more subtle definition of

* Corresponding author at: Vrije Universiteit, Faculty of Biology, Dept. of Microbial Physiology, de Boelelaan 1087, NL-1081 HV Amsterdam, The Netherlands.

SSDI 0301-4622(95)00039-9

control exerted by any enzyme on the flux. The quantitative formulation was introduced by Higgins [1] and, in the context of metabolic control theory by Kacser and Burns [2] and Heinrich and Rapoport [3,4]. It has been renamed to flux control coefficient by Burns et al. [5]. Originally control analysis dealt only with 'ideal' metabolic systems where the enzymes, present at much lower concentrations than their substrates, can be considered as independent catalysts coupled by homogeneous concentrations of the metabolites. Since then, control analysis has grown to include systems with enzyme–enzyme interactions, metabolite channelling, high enzyme concentrations and regulated gene expression [6–13]. It has been realized that the control exerted by an enzyme on a flux can be defined and measured in various ways which, in these more complex systems, may lead to different magnitudes of the flux control coefficient [7,8,13–15].

Already in early works on metabolic control analysis [4] attempts were made to formulate more fundamental definitions of the flux control coefficient of the enzyme. Recently, Schuster and Heinrich [15] revised these definitions and emphasized the advantage of definitions that do not depend on the way the activity of the enzyme is modulated. They proposed a definition that should exhibit such independence.

In this paper we address the question whether the definition proposed by Schuster and Heinrich [15] is always independent of how enzyme activity is modulated. We show that in systems with high enzyme concentrations and moiety-conservation involving both enzymic and non-enzymic species, this is not the case. The same holds for systems with direct transfer of intermediates (metabolite channelling). To show this we shall use simple models of a dynamic channel [16,17] and of a covalent modification cycle of an enzyme.

For 'non-ideal' systems we show that a fundamental definition of the control coefficient which is independent of the choice of a modulation parameter is possible only at a more elemental level than the level of complete enzyme reactions. We define the elemental (microscopic) control coefficients and show that to determine the control properties of non-ideal pathways one should descend to the level of the elemental processes, i.e. the level of the elemental chemical transformations or catalytic steps in the reaction cycle of the enzymes. We show how the traditional 'macroscopic' control coefficients determined by different ways of modulation of enzyme activity are related to the elemental control coefficients [17,18].

2. The different definitions for the flux control by an enzyme coincide in ideal pathways

Kacser and Burns [2] proposed to quantify the contribution of any enzyme to the control of the steady-state flux (J) in terms of a fractional change $\delta J/J$ in the flux, induced by an infinitely small fractional modulation $\delta e/e$ of the enzyme concentration:

$$C_e^J = \left(\frac{(\mathrm{d}J/J)}{(\mathrm{d}e/e)} \right)_{\mathrm{sys}} = \left(\frac{\mathrm{d}\ln|J|}{\mathrm{d}\ln e} \right)_{\mathrm{sys}} \qquad (1)$$

The subscript 'sys' signifies that differentiation conditions require the steady state of the system and allow the concentrations of metabolites to adjust accordingly. The dimensionless coefficient C_e^J is called the flux control coefficient of the enzyme E. Similarly, concentration control coefficients can be defined by replacing the flux J by a steady-state concentration. Definition (1) has the operational meaning of measuring the enzyme control coefficient by addition of E to a system [19] or by manipulating the expression of the corresponding gene in an intact system [20–23].

Another definition for the control coefficient [3] compares a variation ($\delta J/J$) of the flux, caused by a change (δp) in any parameter p, with the variation ($\delta v/v$) in the enzyme rate this parameter change would cause if the enzyme E was 'isolated' from the system. The necessary condition is that the parameter p should affect only the rate v, and not any other rate:

$$C_v^J = \frac{(\mathrm{d}\ln|J|/\mathrm{d}p)_{\mathrm{sys}}}{(\partial \ln|v|/\partial p)_{\mathrm{enz}}} = \frac{v}{J} \cdot \frac{(\mathrm{d}J/\mathrm{d}p)_{\mathrm{sys}}}{(\partial v/\partial p)_{\mathrm{enz}}} \qquad (2)$$

The subscript 'enz' signifies that differentiation conditions require the steady state of the enzyme reaction in isolation from the system. When taking the derivative $\partial v/\partial p$ all the concentrations of metabolites should be kept at the same values as in

the steady state of *the system*. In many cases definition (2) does not depend on the choice of parameter p [4,15,24–26].

Classical control analysis e.g., [2–7,27] focused on what we shall call 'ideal' multi-enzyme pathways, in which every reaction rate (v_i) is proportional to the corresponding enzyme concentration (e_i) and independent of the concentrations of all other enzymes, except through the concentrations of metabolites:

$$v_i = V_{\max,i} \cdot W_i(\boldsymbol{X}) = k_i^{\text{cat}} \cdot e_i \cdot W_i(\boldsymbol{X}),$$
$$\boldsymbol{X} = X_1, X_2, ..., X_n \qquad (3)$$

$W_i(\boldsymbol{X})$ is a function of metabolite concentrations $\boldsymbol{X}$. In case of reversible reactions, W_i can become negative. $V_{\max,i}$ is the maximum rate of reaction attained if all substrate concentrations were infinite and all product concentrations zero. The parameter k_i^{cat}, identical to the forward maximum rate per enzyme molecule, is proportional to the forward enzyme turnover number ($V_{\max,i}$ per enzyme molecule; a variation in k_i^{cat} is equivalent to the same relative change in the rate constants of all the elementary steps of the enzyme reaction).

In the ideal pathways, to which classical metabolic control analysis applies, the enzyme concentration e can be chosen as the parameter in Eq. (2). In view of Eq. (3) definitions (1) and (2) with $p = e_i$ are equivalent for such pathways; v_i is proportional to e_i and no other reaction rate depends on e_i.

Definition (2) allows one to measure the control coefficients using inhibitors specific to a single enzyme [28,29]. In the considered case of 'ideal' pathways, the result of such a measurement does not depend on the particular mechanism of inhibitor action, if one accounts for the elasticity ($\partial v/\partial I$) of the 'target' reaction (v) to this inhibitor (I) at the same metabolite concentrations as in the steady state of the system [15]. We conclude that in ideal pathways, the various operational definitions of the flux control coefficient coincide.

3. In non-ideal pathways, the flux control by an enzyme may depend on how it is determined

In systems with enzyme–enzyme interactions Eq. (3) may not apply, as v_i may well depend on e_j.

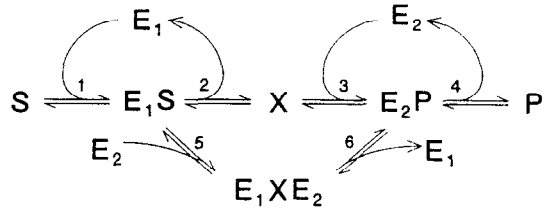

Fig. 1. A dynamically channelled pathway. Enzymes E_1 and E_2 catalyze the conversion of S to P via the intermediate X. X can either be released into a pool of free X or it can be transferred directly to E_2 in the enzyme–enzyme complex E_1XE_2.

Contrary to the classical results, the control coefficients determined by titrating such a non-ideal system with an inhibitor may depend on the peculiarities of both the inhibitor and the system. To emphasize this, we call these coefficients the 'effector-dependent' control coefficients and stress their dependence on which inhibitor (I) is used to measure them [12,30]. Thus, the control by enzyme e on flux J, measured using a specific inhibitor I is quantified by:

$$^{\text{eff}}C_{e,I}^{J} = \left(\frac{(\mathrm{d}\ln|J|/\mathrm{d}I)_{\text{sys}}}{(\partial \ln|v|/\partial I)_{\text{enz}}} \right) = \left(\frac{v}{J} \cdot \frac{(\mathrm{d}J/\mathrm{d}I)_{\text{sys}}}{(\partial v/\partial I)_{\text{enz}}} \right)_{I=0} \qquad (4)$$

We shall now show that, when the pathway is not ideal, these flux control coefficients can depend on how the enzyme activity is modulated.

Fig. 1 shows a pathway where partial channelling of metabolite X occurs through enzyme–enzyme interaction in the E_1XE_2 complex. Such a channelled pathway where at each catalytic cycle an enzyme–enzyme complex has to be formed and disintegrated, is usually referred to as a dynamic channel [16,17,31,32]. Two effector-dependent control coefficients of enzyme E_1 (Eq. (4)) on the total flux through the pathway (i.e. the net production rate of P) were calculated using either a competitive or a non-competitive inhibitor of E_1. The first effector considered, a competitive inhibitor (I^c) of the enzyme E_1, was assumed to bind to the free enzyme form (E_1) only. The other effector, a non-competitive inhibitor (I^{nc}), was assumed to bind to all forms of the enzyme, with equal dissociation equilibrium constants, i.e. irrespective of the enzyme's interac-

tion with metabolites or other enzymes. Inhibitor binding was assumed to be in rapid equilibrium. The dissociation equilibrium constants of the inhibitors were equal to 1. The steady-state flux through the pathway was calculated by solving the system of steady-state equations numerically (i.e. equal synthesis and degradation rates for each subform). The dependence of the steady-state flux on the concentrations of the different inhibitors was also calculated, and from this the partial derivatives of the steady-state flux with respect to the inhibitors were numerically estimated at inhibitor concentration equal to zero. The control coefficients were then calculated according to the following formulas [28,30]:

$$^{\mathrm{eff}}C^{J}_{e_1,I^{\mathrm{nc}}} = -\frac{K_I^{\mathrm{nc}}}{J}\cdot\left[\frac{\partial J}{\partial I^{\mathrm{nc}}}\right]_{I^{\mathrm{nc}}=0}$$

$$^{\mathrm{eff}}C^{J}_{e_1,I^{\mathrm{c}}} = -\frac{K_I^{\mathrm{c}}}{J}\cdot\left(1+\frac{S}{K_{\mathrm{M}}^{\mathrm{S}}}+\frac{X}{K_{\mathrm{M}}^{\mathrm{X}}}\right)\cdot\left[\frac{\partial J}{\partial I^{\mathrm{c}}}\right]_{I^{\mathrm{c}}=0}$$

K_I^{nc} and K_I^{c} are the inhibition constants of the inhibitors I^{nc} and I^{c}, respectively. $K_{\mathrm{M}}^{\mathrm{S}}$ and $K_{\mathrm{M}}^{\mathrm{X}}$ denote the Michaelis constants for the substrate (S) and intermediate product (X) of enzyme E_1.

Fig. 2 shows that at high stability of the E_1XE_2 complex, the two effector-dependent control coefficients differed greatly. As the stability of the complex was decreased, both control coefficients converged to the same value; in the limit of no enzyme–enzyme interaction this example reduces to the ideal, non-channelled pathway. Appendix A gives a more general analytical proof of this result.

This shows that the extent to which enzyme E_1 controls the flux through the pathway of Fig. 1 is not uniquely defined. This is understandable in that E_1 plays more than a single role in the pathway. Because of the channelling these roles cannot be summarized into a single one.

Our second example of a non-ideal pathway is that of a moiety-conserved cycle and high concentrations of enzymes relative to the coenzymes X, Y (Fig. 3). In this scheme molecules X and Y are converted into one another by enzymes E_1 and E_2. This is the general scheme for, e.g., protein modification by a protein-kinase and -phosphatase, where

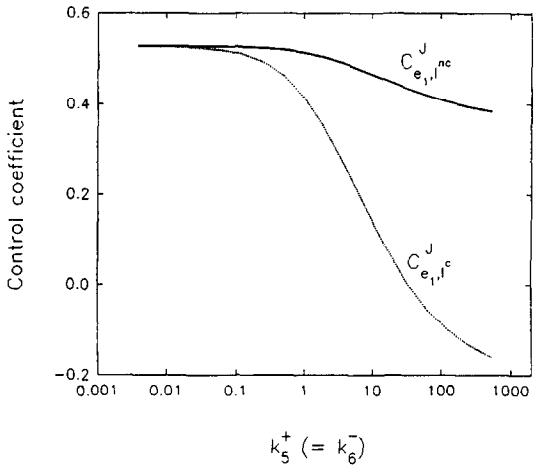

Fig. 2. The difference between two effector-dependent flux control coefficients of enzyme E_1 on the flux through the dynamically channelled pathway. Effector-dependent control coefficients of E_1 with respect to the total flux through the pathway of Fig. 1 were calculated as a function of the stability of the enzyme–enzyme complex E_1XE_2. The flux control coefficients of enzyme E_1 were calculated as if determined either with a competitive $[C^{J}_{e1,I^{c}}]$ or a non-competitive $[C^{J}_{1,I^{nc}}]$ inhibitor. All rate constants were chosen equal to 1, except k_{-1} and k_{-4} which were equal to 0.5. The subscripts refer to the reaction number in Fig. 1 and the sign to the direction: positive when going from S to P and negative when going from P to S. Notice that with these constants microscopic reversibility holds true for the cyclic part of the pathway. The stability of the E_1XE_2 complex was varied by simultaneous variation of the rate constants k_{+5} and k_{-6}. In this way microscopic reversibility was preserved (k_{+5}/k_{-6} remaining constant). The concentrations of S and P were clamped to 10 and 1, respectively, and the total concentration was 1 for either enzyme.

X and Y represent the phosphorylated and dephosphorylated forms of a protein. In case of both moiety-conservation [33] and high enzyme concentration, a variation of some of the parameters that affect the enzyme rate, results in a change in the sum of the free concentrations of the substances that contain the moiety. Such change in a moiety conserved sum will be absent at a variation in other parameters [10,11,25]. When the moiety conserved sum exerts flux control, the result of determining the control coefficient of the enzyme E according to definition (2) may depend on choice of the modulated parameter (p) that is used to modulate the enzyme rate.

The effector-dependent control coefficients of E_1 on the total (cyclic) flux through the pathway were calculated for a competitive or a non-competitive

inhibitor of E_1 in the same manner as for the channelled pathways, with X as substrate and Y as the product of the enzyme E_1 in the relevant equation. The control coefficients determined with the two inhibitors differed (Fig. 4). The difference was most pronounced when the moiety-conserved sum $X_{total} + Y_{total} = X_T$ was lower than the concentration of E_1. Also this case reduces to the ideal case, when the total concentration of E_1 is small compared to the total concentration of substrates, X and Y; then both control coefficients are equal. Appendix B gives an analytical treatment of this example.

We conclude that both in case of moiety conservation and in case of metabolite channelling, the flux control by an enzyme depends on how it is determined.

4. 'Elemental' processes as the basis for unequivocal definition of control coefficients and unequivocal metabolic control analysis

Of many metabolic pathways it is not known to what extent they are ideal. Other pathways, such as those involved in signal transduction, depend on protein–protein interactions [9,34]. Consequently the

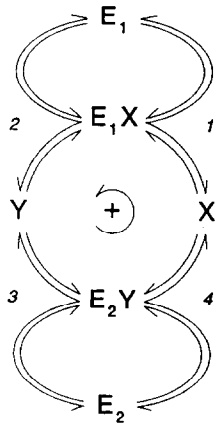

Fig. 3. Reaction scheme of a triple moiety-conserved cycle. Enzymes E_1 and E_2 convert the molecules X and Y into one another. X and Y may correspond to an unphosphorylated and a phosphorylated form of a protein, respectively. E_1 and E_2 may represent a protein kinase and a protein phosphatase, respectively. The reactions are modelled as being reversible, and are numbered for reference to the rate constants described in the section 'Methods'.

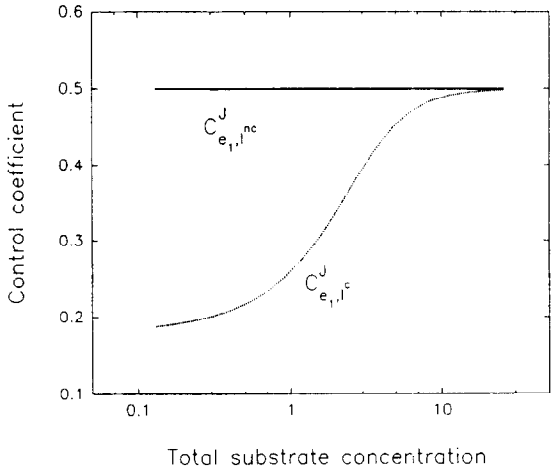

Fig. 4. The difference between the effector-dependent flux control coefficients of enzyme E_1 in Fig. 3 as a function of the total concentration of substrate. The total substrate concentration, i.e., X plus Y plus their enzyme-bound forms, was varied. The control coefficient was determined either with a competitive $[C^J_{e1,I^c}]$ or a non-competitive $[C^J_{e1,I^{nc}}]$ inhibitor of E_1. All rate constants were chosen to be equal to 1, except k_{-1} and k_{-3} which were equal to 0.1. The subscripts refer to the reaction number and their signs refer to the direction as indicated in Fig. 3. The total of the enzyme subform concentrations was 1 for either enzyme.

observation (c.f., the preceding section of this paper) that in non-ideal pathways the flux control coefficient is not unequivocal, would seem to compromise the application of classical metabolic control theory.

In the present section we shall show that a slight extension allows metabolic control theory to deal with arbitrary pathways, both ideal and non-ideal. Noting that in non-ideal pathways direct or indirect interdependence of reactions catalyzed by different enzymes causes the flux control coefficients to be dependent on the way they are measured, we retreat to the truly independent processes (c.f. [13]). The basis for the approach developed here is the presumption that any metabolic network can be viewed as consisting of a number of 'elemental' processes with (well-defined) forward and reverse rate constants. Notably, this implies that we no longer agglomerate all processes catalyzed by an enzyme into a 'separate' reaction catalyzed in 'isolation' from the rest of the system. In the general case of a 'complex' pathway we shall treat the network of the enzyme-catalyzed reactions as the network of chemical conversions where the 'metabolites' (i.e. the system

variables) are the concentrations of both free metabolites and enzyme intermediate forms (states). These include enzyme-bound metabolites and enzyme–enzyme complexes. These conversions will be called the elemental processes of the system. They correspond to transitions between different enzyme subforms (states) [12].

Now we define the control coefficients of the lth elemental process, C_l^J, with respect to any steady state flux in the system by:

$$C_l^J = \left(\frac{\mathrm{d}\ln|J|}{\mathrm{d}\ln k_l}\right)_{\mathrm{sys}}, \quad \frac{k_{-l}}{k_l} = \text{constant} \tag{5}$$

Here the differentiation conditions (referred to by the subscript 'sys') are such that the forward (k_l) and reverse (k_{-l}) rate constants of the elemental process are changed by the same factor, at constant magnitudes of all other parameters. The concentrations of all metabolites and enzyme subforms, are allowed to adjust so as to progress to a new steady state. This definition does not compromise microscopic reversibility c.f. [9].

For control by the elemental process l, the control coefficient (C_l^J) can also be defined as:

$$C_l^J = \frac{\left(\frac{\mathrm{d}\ln|J|}{\mathrm{d}\ln p_l}\right)_{\mathrm{sys}}}{\left(\frac{\partial \ln v_l}{\partial \ln p_l}\right)_{\mathrm{proc}\ l}} \tag{6}$$

The question is whether this definition (6) gives a general quantity independent of a special choice of a parameter (p_l) and identical to the quantity defined by Eq. (5). Control coefficients defined in this way can be expressed in terms of the stoichiometry matrix, the link matrix (which can, in turn, be calculated from the stoichiometry matrix) and the elasticity matrix [26,24]. Since none of these matrices depends on choice of the parameter p_l used for definition (6), neither do the control coefficients C_l^J, provided that p_l affects only the rate v_l. From this, it follows that definition (6) is equivalent to definition (5), which is based on the condition that the equilibrium constant (k_{-l}/k_l) of the elemental process remains unchanged. Thus, the control by an elemental process is defined unequivocally by Eqs. (5) and (6).

A subsequent question should be whether the theorems that made classical metabolic control theory powerful vis-à-vis ideal pathways, carry over to a control theory in terms of the elemental control coefficients. We shall now argue that the answer is yes.

Since any steady-state flux of the system is a first order homogeneous function of all the elemental rate constants, the classical summation theorem holds true for the 'elemental' control coefficients C_l^J (c.f. [9,24,27]):

$$\sum_{\text{all the elemental processes } l \text{ of the network}} C_l^J = 1 \tag{7}$$

Consequently, in the sense of the summation theorem the elemental control coefficients are a generalization of the control coefficients defined by Heinrich and Rapoport [3]. Treating the pathway as a network of elemental processes and using a general formalism [24,26] one can obtain the other summation and connectivity relations in terms of the control and elasticity coefficients of the elemental processes. This then allows one to express the elemental control coefficients into elasticity coefficients and steady-state values of some concentrations and fluxes [24]. We conclude that non-ideal systems can be addressed by metabolic control theory, provided that the analysis proceeds through the elemental control coefficients.

5. Relating various modes of control and their control coefficients to the elemental control coefficients

We shall now show that the different ways in which an enzyme controls a macroscopic flux through a non-ideal pathway arise because of the variety in enzyme actions at the microscopic level.

5.1. Flux control by enzyme concentrations

If we view a metabolic pathway as a network of chemical conversions of enzyme intermediate forms and metabolites, the enzyme concentrations acquire a different meaning than in the more usual view of total enzyme reactions. In a network of elemental chemical conversions, enzyme concentrations have

the meaning of total concentrations of enzyme moieties which are conserved in the network interconversions. Therefore, at the macrolevel the enzyme-concentration control coefficient (C_e^J) corresponds to the response coefficient to a change in enzyme-moiety conserved sum (e) [33]. This sum includes the concentrations of the free unbound enzyme, the enzyme-substrate complexes and the complexes formed by the association of two or more different enzymes.

Using the formalism developed in [24,35] one can express this response coefficient (C_e^J) into the elemental (microscopic) control and elasticity coefficients. Such an approach was employed in [12,34] where the generalized summation theorem for the enzyme control coefficients was derived. Interestingly, some special properties of the elemental elasticity coefficients [36,35] allow one to express the enzyme-concentration control coefficients for the channelled pathways in terms of the elemental control coefficients and the concentrations of enzyme–enzyme complexes ([12,34] c.f. [17]) alone.

5.2. Flux control by enzyme activities

It follows from Eq. (3) and definition (5) that in ideal pathways the control coefficient C_e^J equals the sum of the control coefficients over all the elemental steps which are dependent on E:

$$C_e^J = \sum_{\text{all E-dependent elemental steps } l} C_l^J \qquad (8)$$

An E-dependent process is a process in which any form of the enzyme E partakes as a reactant.

In 'non-ideal' pathways Eq. (8) is no longer valid, i.e., the control exerted by the enzyme concentration, C_e^J, is not directly related to the effect of a change in rate constants [7,8,10,11]. What then is the meaning of the right-hand side of Eq. (8) in non-ideal pathways? Suppose that we simultaneously change the elemental rate constants of all processes in which any subform of the enzyme E is involved, by the same factor. We define the 'impact' control coefficient, ${}^{\text{imp}}C_e^J$, so as to quantify the resulting change in steady-state flux J (c.f. [12]):

$${}^{\text{imp}}C_e^J = \sum_{\text{all E-dependent elemental steps } l} C_l^J \qquad (9)$$

In relation to various non-ideal pathways the impact control coefficient is an analogue of the rate-linked control coefficient (C_v^J, see Eq. (2)). The latter has been also referred to as the control coefficient with respect to enzyme activity or turnover number (c.f. Eq. (3)) [7,8,11].

In pathways where direct protein interactions are absent, the impact control coefficients of different enzymes involve different elemental control coefficients. For instance, for Fig. 3 the impact control coefficient of enzyme 1 is equal to the sum of the elemental control coefficients of the steps 1 and 2 and the impact control coefficient of the enzyme 2 is equal to the sum of the elemental control coefficients of the steps 3 and 4. In the absence of direct enzyme–enzyme interaction, the sum of the impact control coefficients over all pathway enzymes coincides with the sum of all the elemental control coefficients and is always equal to unity, Eq. (7).

However, the impact control coefficient of each enzyme which interacts directly with other enzymes will include the elemental control coefficients of all the corresponding 'protein-interaction' steps [12,34]. For instance, for the dynamic channel of Fig. 1 the impact control coefficients of both enzymes 1 and 2 include the elemental control coefficients of the steps 5 and 6. Therefore, in systems with direct protein interactions the sum of the impact control coefficients is usually greater than unity since some elemental processes depend on two or more enzymes.

The concept of the impact control coefficient is useful as an estimation of the total control which an enzyme may exert on the flux via all processes in which it is involved. It has been shown [12,34] that the impact control coefficient of any enzyme in the pathway can be expressed in terms of the enzyme-concentration control coefficients of all the enzymes with which it interacts directly.

5.3. Effector dependent flux control coefficients as determined by the use of inhibitors

We return to the control coefficients measured by using specific inhibitors. Except for a normalizing factor, the numerator of Eq. (4) coincides with the response coefficient (R_I^J) of the system flux (J) to a change in the inhibitor concentration (I). In the general case we can understand the response ($\mathrm{d}\ln|J|/\mathrm{d}I$) of the flux to an inhibitor specific to the

enzyme E in terms of a weighted sum of the control coefficients (C_l^J) of the E-dependent elemental processes (the response theorem; Kholodenko, [37]):

$$\frac{\mathrm{d}\ln|J|}{\mathrm{d}I} = \sum_{\text{all E-dependent processes } l} C_l^J \cdot \epsilon_I^{v_l} \qquad (10)$$

where $\epsilon_I^{v_l} = \partial \ln v_l / \partial I$ is the elasticity coefficient of the elemental processes v_l with respect to the inhibitor (similarly as above the derivatives with respect to I rather than to $\ln I$ are used here in order to avoid indefiniteness at zero inhibitor concentration). A specific inhibitor will directly affect the elemental step l (inside the catalytic cycle of the enzyme E) if and only if it binds to some of the enzyme forms preceding or following the step l. To determine these elemental elasticity coefficients with respect to inhibitor we should know the kinetic constants of inhibitor binding to enzyme forms. Then, Eq. (5) for the effector-dependent control coefficient takes the form:

$$C_{e,I}^J = \sum_{\text{all E-dependent processes } l} C_l^J \cdot \frac{\epsilon_I^{v_l}}{\epsilon_l^v} \qquad (11)$$

where $\epsilon_l^v = \partial \ln v / \partial l$ is the elasticity of the affected reaction v_l considered in 'isolation' from the pathway, with respect to the inhibitor.

In non-ideal metabolic systems the elasticity of the reaction in 'isolation' from the pathway may be the same for different inhibitors, but the response of the pathway may be different. Indeed, for the moiety-conserved cycle of Fig. 3 we have seen a difference between the control coefficients determined using inhibitors that replace metabolites at the binding site (competitive) and those that do not (e.g., purely non-competitive) when the concentration of enzymes was sufficiently high compared to the substrate concentrations (Fig. 4). In the former case we have determined the control coefficients with respect to the enzyme concentration, C_e^J, and in the latter case we have determined the impact control coefficient, ${}^{\mathrm{imp}}C_e^J$ (see also [10],[18]).

In systems with enzyme–enzyme interactions the response of the pathway flux to the inhibitor titration strongly depends on how an inhibitor affects the enzyme complexes. Indeed, we have observed a difference between the control coefficients determined using inhibitors that bind only to the free enzyme and those that bind to any form of the enzyme irrespectively of its complexation with the other enzymes (Figs. 1 and 2). Depending on the particular mechanism of the effector action the value of the effector-dependent control coefficient can cover the range from the value of the enzyme-concentration control coefficient to the value of the impact control coefficient [38].

Only in the 'ideal' pathways the response coefficient R_I^J is equal to the control coefficient C_e^J of the affected enzyme E, multiplied by the elasticity coefficient of the latter (ϵ_I^v) with respect to this inhibitor [2]. As defined by Eq. (4) this control coefficient then does not depend on the type of inhibitor used provided that the latter only affects the target enzyme.

6. Discussion

In this paper we have shown that in non-ideal pathways it is not possible to define a single control coefficient that quantifies the control exerted by an enzyme on a flux. This is at variance with simpler systems [15]. Unless the control by the elemental steps is considered, the parameter independence of control coefficients as established in [15,24,26] does not hold true in the non-ideal cases considered in this paper.

Not only classical Metabolic Control Analysis, but also much of the mainstream kinetic theory of biochemistry is based on the assumption that the enzymatic rate equations as derived from quasi-equilibrium or quasi-steady-state models are the same for the isolated enzymatic reaction and for the reaction as embedded in the biochemical system. The assumption is, however, not appropriate if there is a moiety conservation linking enzymic species and free metabolites. In derivations of enzymatic rate laws, the concentrations of enzyme–substrate complexes are eliminated by using quasi-steady-state (or quasi-equilibrium) assumptions and conservation relations between these complexes and the free enzyme (c.f. [39]). Upon determining the steady state of the whole system, only the conservation relations between the free metabolite concentrations are taken into account, because the concentrations of enzymic species are no longer available at this level of de-

scription. This simplification, however, causes inaccuracies if enzyme concentrations are large and are linked with metabolites by moiety conservation (for recent discussion see: [10,11,40,41]).

Perhaps the most important result obtained in this paper is that these complications do not affect the ability to analyze non-ideal systems in terms of metabolic control theory. If one wishes to analyze a complex, non-ideal network, one should first discern what are the truly elemental steps (these may correspond to catalytic transitions between states of enzymes). Subsequently one should define control coefficients in terms of modulations of these elemental steps without infringing upon microscopic reversibility. The various flux control coefficients of an enzyme can then always be expressed in terms of these elemental control coefficients, c.f. [36,41–45].

Using this procedure the differences between the various definitions of the flux control by an enzyme can be evaluated. In many cases the differences will not be significant. Indeed, whenever enzyme concentrations are much lower than metabolite concentrations and metabolite channelling is absent, the standard definitions of control coefficients are unique.

When the differences do exist, they are of interest because they contain mechanistic information about the system, such as how much of the flow is channelled [17]. Indeed, in non-ideal pathways an enzyme can control a flux in more than one mode. One of these reflects the effect of the change of a concentration of the enzyme on the flux, another mode corresponds to the effect of changes of the activity of one or all of the catalytic transitions within the enzyme. Future work may reveal whether nature makes use of the diverse ways in which an enzyme may control a flux in the microworld [18] of non-ideal pathways. In principle it could do so by choosing between increasing gene expression or covalent modification to regulate the activity of an enzyme. Glutamine synthetase is a well known example where either regulation exists.

Acknowledgements

We thank Jan-Hendrik S. Hofmeyr, Ronny Schuster for substantial discussions and Clas Blomberg for valuable suggestions for improving the manuscript. This study was supported by the Netherlands Organization for Scientific Research (NWO) and the Netherlands Foundation for Biophysics.

Appendix A

Analytical treatment of a channelled pathway

We analyze the channelled pathway shown in Fig. 1 and assume, for simplicity, the elemental step 2 to be irreversible ($k_{-2} = 0$). First, we consider changes of the steady state of enzyme E_1 in isolation. Let ${}^*v_1 = {}^*v_2$ be the rate catalysed by this enzyme when it is at quasi-steady state. ${}^*v_5$ is zero when enzyme 1 is studied in isolation since the complex E_1XE_2 is not formed in the absence of enzyme E_2. By standard calculations, we obtain

$$\frac{\partial {}^*v_1}{\partial k_1} = k_2 \frac{S \cdot E_{1_t}(k_{-1} + k_2)}{(k_1 S + k_{-1} + k_2)^2} \tag{A1}$$

$$\frac{\partial {}^*v_1}{\partial k_{-1}} = -k_2 \frac{k_1 S \cdot E_{1_t}}{(k_1 S + k_{-1} + k_2)^2} \tag{A2}$$

Now we study perturbations of the steady state of the whole system and, in particular, effects on the concentration X. Let $V = (v_1, v_2, \ldots, v_6)^T$, $\Xi = (E_1S, X, E_2P, E_1XE_2)^T$, and N^0 be the reduced stoichiometry matrix of the system (i.e. with the linearly dependent rows cancelled). In the concentration vector Ξ, the free enzyme concentrations E_1 and E_2 are not included since they can be eliminated by the conservation relations. The response of steady-state concentrations to changes in a parameter p can be written as

$$\frac{d\Xi}{dp} = -\left(N^0 \frac{\partial V}{\partial \Xi}\right)^{-1} \cdot \left(N^0 \frac{\partial V}{\partial p}\right) \tag{A3}$$

(c.f. [26]). Taking $p = k_1, k_{-1}$, we have

$$\frac{\partial v_1}{\partial k_1} = S \cdot E_1, \quad \frac{\partial v_1}{\partial k_{-1}} = -E_1 S \tag{A4a,b}$$

with all other components of $\partial V/\partial p$ being zero. So

we can write, for that row of the matrix equation (A3) that corresponds to the concentration X,

$$\frac{dX}{dk_1} = T \cdot S \cdot E_1, \quad \frac{dX}{dk_{-1}} = -T \cdot E_1 S \qquad \text{(A5a,b)}$$

with T being a common factor resulting from Eq. (A3). Using k_1 or k_{-1} as perturbation parameters, we can calculate the control coefficient $C^X_{1/2}$ (the index 1/2 refers to the overall reaction formed by the elemental steps 1 and 2) alternatively as

$$C^X_{1/2} = \frac{\dfrac{dX}{dk_1}}{\dfrac{\partial^* v_1}{\partial k_1}} = C\frac{S \cdot E_1}{k_{-1} + k_2} \qquad \text{(A6a)}$$

or

$$C^X_{1/2} = \frac{\dfrac{dX}{dk_{-1}}}{\dfrac{\partial^* v_1}{\partial k_{-1}}} = C\frac{E_1 S}{k_1} \qquad \text{(A6b)}$$

with C being a common factor. It should be noted that $^* v_1$ refers to the rate of the reaction catalyzed by enzyme 1 if in isolation. As such, the control coefficients defined here refer to control by enzyme 1 as a whole, not to control by the elemental step 1 in Fig. 1. These two coefficients are identical if, and only if,

$$k_1 S \cdot E_1 - (k_{-1} + k_2) E_1 S = 0 \qquad \text{(A7)}$$

This term equals $(dE_1S/dt) - v_5$. Since E_1S is assumed to be at steady state, Eq. (A7) holds true only if $v_5 = 0$, i.e. if no channelling occurs. Consequently, in case the channel is operative, the value of the concentration control coefficient $C^X_{1/2}$ depends on choice of the perturbation parameter. This is understandable from the reasoning that part of the enzyme is sequestered in the complex E_1XE_2. This effect is taken into account in the numerators of the control coefficients, but not in the denominators since the derivative $\partial^* v_i/\partial p$ is taken for the enzyme considered in isolation. At variance with the situation of moiety conservation considered in Appendix B, even parameters of one and the same step give different magnitudes for the control by the enzyme (but not for the control by the elemental step). For the cyclic system shown in Fig. 3, one can derive an equation similar to Eq. (A7). Since it holds true in that system, the coefficients calculated with the perturbation parameters k_a and k_{-a} then coincide.

Appendix B

Analytical treatment of a system in which enzymic and non-enzymic species are linked by conservation relations

We consider the reaction system shown in Fig. 3 with the simplification that the total concentration of enzyme 2 is so low that it can be neglected in comparison with the concentrations of X and Y. We refer to reactions 1 and 2 by the indices a and b, respectively, and to the reaction catalysed by enzyme 2 by the index c. Be p any parameter that directly affects the elemental steps a and/or b only. For the change of the steady state of enzyme 1 in isolation, i.e. with X and Y clamped, we have

$$\frac{\partial v_a}{\partial E_1}\frac{d^* E_1}{dp} + \frac{\partial v_a}{\partial E_1 X}\frac{d^* E_1 X}{dp} + \frac{\partial v_a}{\partial p} = \frac{\partial v_b}{\partial E_1}\frac{d^* E_1}{dp} + \frac{\partial v_b}{\partial E_1 X}\frac{d^* E_1 X}{dp} + \frac{\partial v_b}{\partial p} \qquad \text{(B1)}$$

The asterisk refers to the quasi-steady state of the enzyme with X and Y clamped. Using the conservation relation

$$E_1 + E_1 X = E_{1_T} \qquad \text{(B2)}$$

we obtain

$$\frac{d^* E_1 X}{dp} = \frac{\dfrac{\partial v_a}{\partial p} - \dfrac{\partial v_b}{\partial p}}{A + B} \qquad \text{(B3)}$$

With the abbreviations $A = (\partial v_a/\partial E_1) - (\partial v_a/\partial E_1 X)$ and $B = (\partial v_b/\partial E_1 X) - (\partial v_b/\partial E_1)$. Let $^* v_a = {}^* v_b$ be the enzyme rate when the enzyme

is in quasi-steady state. With the help of Eqs. (B2) and (B3), we derive

$$\frac{\partial^* v_b}{\partial p} = \frac{\frac{\partial v_a}{\partial p}B + \frac{\partial v_b}{\partial p}A}{A+B} \tag{B4}$$

When X and Y are allowed to attain new steady-state values after perturbation of the original state, the two sides of Eq. (B1) have to be extended by including the terms $(\partial v_a/\partial \mathrm{X}) \cdot (\mathrm{dX}/\mathrm{d}p)$ and $(\partial v_b/\partial \mathrm{Y}) \cdot (\mathrm{dY}/\mathrm{d}p)$, respectively. Inserting the conservation relation $\mathrm{X} + \mathrm{Y} + \mathrm{E_1X} = \mathrm{T} = \text{constant}$, we obtain

$$\frac{\mathrm{dE_1X}}{\mathrm{d}p} = \frac{\frac{\partial v_a}{\partial p} - \frac{\partial v_b}{\partial p} + F\frac{\mathrm{dX}}{\mathrm{d}p}}{A + B - \frac{\partial v_b}{\partial \mathrm{Y}}} \tag{B5}$$

with $F = (\partial v_a/\partial \mathrm{X}) + (\partial v_b/\partial \mathrm{Y})$. For calculating the derivative $\mathrm{dX}/\mathrm{d}p$, we use the total derivative of the steady-state condition for X with respect to p, the two conservation relations and Eq. (B5). This gives the concentration control coefficient

$$C^{\mathrm{X}}_{a/b} = \frac{(\mathrm{dX}/\mathrm{d}p)}{(\partial^* v_b/\partial p)} = \frac{\left[\frac{\partial v_a}{\partial p}\left(\frac{\partial v_b}{\partial \mathrm{Y}} - \frac{\partial v_c}{\partial \mathrm{Y}} - B\right) + \frac{\partial v_b}{\partial p}\left(\frac{\partial v_c}{\partial \mathrm{Y}} - A\right)\right]\cdot(A+B)}{\left(\frac{\partial v_a}{\partial p}B + \frac{\partial v_b}{\partial p}A\right)\left[\left(\frac{\partial v_c}{\partial \mathrm{Y}} - A\right)F + G\left(A + B - \frac{\partial v_b}{\partial \mathrm{Y}}\right)\right]} \tag{B6}$$

where $G = (\partial v_a/\partial \mathrm{X}) + (\partial v_c/\partial \mathrm{Y}) - (\partial v_c/\partial \mathrm{X})$. From Eq. (B6), we see that when p affects v_a specifically (i.e. $\partial v_b/\partial p = 0$), both the numerator and the denominator on the right-hand side become proportional to the term $\partial v_a/\partial p$, which can be cancelled. Thus, $C^{\mathrm{X}}_{a/b}$ is in this case independent of what parameter of reaction a is changed (k_a or k_{-a} or the like). If p affects v_b specifically, $C^{\mathrm{X}}_{a/b}$ does not contain derivatives with respect to p either, but it has, in general, a different value from $C^{\mathrm{X}}_{a/b}$ in the former case. If p affects both v_a and v_b, the derivatives with respect to p cannot be cancelled in Eq. (B6).

Now we compare the situation that $\mathrm{E_1}$ and $\mathrm{E_1X}$ are of the same order of magnitude as X and Y, with the case of a very low enzyme concentration, i.e. $\mathrm{E'_{1_T}} = \mathrm{E} + \mathrm{E_1X'} \ll \mathrm{X'} + \mathrm{Y'} + \mathrm{E_1X'} = \mathrm{T'}$ (the prime referring to the case of low enzyme concentration). Let $\mathrm{E'_{1_T}} = \mathrm{E_{1_T}}/\rho$ with $\rho \gg 1$. In order that the reaction rates are nearly the same in the two situations, $v_i = v'_i (i = a,b)$, some kinetic parameters have to be rescaled (for example, one may multiply all k_i^{cat} values by ρ). This implies (provided that the conservation sums T and T′ differ by an appropriate value)

$$\frac{\partial v'_i}{\partial \mathrm{E'_1}} = \rho\frac{\partial v_i}{\partial \mathrm{E_1}}, \quad \frac{\partial v'_i}{\partial \mathrm{E_1X'}} = \rho\frac{\partial v_i}{\partial \mathrm{E_1X}}, \quad i = a,b \tag{B7a,b}$$

$$\frac{\partial v'_i}{\partial \mathrm{X'}} = \frac{\partial v_i}{\partial \mathrm{X}}, \quad \frac{\partial v'_i}{\partial \mathrm{Y'}} = \frac{\partial v_i}{\partial \mathrm{Y}}, \quad i = a,b,c \tag{B8a,b}$$

Accordingly,

$$A' = \rho A, \quad B' = \rho B, \quad F' = F, \quad G' = G$$

Thus, in the limit $\mathrm{E_{1_T}} \to 0$, Eq. (B6) transforms to

$$C^{\mathrm{X'}}_{a/b} = \frac{A+B}{G(A+B) - AF} \tag{B9}$$

which no longer contains any derivative with respect to parameters. Thus the value of $C^{\mathrm{X'}}_{a/b}$ is independent of the choice of the perturbation parameter p in the case of very low enzyme concentrations.

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