A revised nomenclature and symbolism for metabolic control analysis

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1 General considerations

Since the previous recommendations for a metabolic control analysis terminology and symbolism [1] were made, the field has developed considerably, creating an urgent need for reconsideration and revision. In these recommendations we try to cope with the new complexities brought to light by the analysis of 'non-ideal' pathways, aiming to retain both the simplicity and the flexibility needed for use in both mathematical and biological contexts, i.e., allowing reference to both numbered and named conversion steps, variables and parameters. Our goal is to use a minimum number of terms and symbols, but yet to produce a system that allows application of metabolic control analysis to any system at any level of aggregation.

2 Distinguishing between response and control coefficients

In the beginning, things were relatively simple: one could get by with only two types of coefficient and, therefore, two symbols: (global) control coefficients C and (local) elasticity coefficients ε. Nevertheless, right from the start, the response coefficient with its own symbol, R, continued to be used, be it mostly for responses to changes in external metabolites, not to other parameters such as enzyme concentrations. As the theory of metabolic control analysis became more refined the need arose for a clear conceptual distinction between a response to a change in enzyme concentration (or any other parameter that affects a step in the system) and a response to a change in the local rate of a step (which should in principle be independent of how that change is brought about). The main reason is that, as more complex systems were analysed with metabolic control analysis, many situations were discovered where the use of response coefficients with respect to enzyme concentrations (called enzyme control coefficients up to now) appeared to violate the summation and connectivity theorems of control analysis. In addition, the concept of an internal response to a variable metabolite was developed, thereby broadening the scope of what should be classified as a response coefficient.

The entities that make up any system of coupled reactions can be divided cleanly into two classes: (i) reactions characterised by a rate, and (ii) molecular species characterised by a concentration (more correctly, an activity or chemical potential). Some of the molecular species (the external species) must be constant for a steady state to exist, while the other molecular species are the variable intermediates that couple the reactions (internal species). The external species form a part of the parameter set of a system.

The most far-reaching terminology change advocated here is to distinguish formally between *response coefficients* and *control coefficients* on the basis of the above classification. Control coefficients quantify systemic responses to activity changes of independent steps of the system or of aggregates of independent steps (which correspond to independent steps at a higher level of aggregation), while response coefficients quantify systemic responses to changes in the concentrations of molecular species or to changes in any other parameter of the system. In practice this means that response coefficients can be determined directly by experiment, while control coefficients have to be calculated using the framework of metabolic control analysis. However, in 'ideal' pathways where aggregated groups of independent steps form independent reaction units, the relationship between response and control coefficients is particularly simple, often leading to an equality such as that between the control coefficient of an enzyme-catalysed step and the response coefficient with respect to the enzyme concentration.

2.1 Response coefficients

An external response coefficient quantifies the steady-state response to a change in any system parameter. An internal response coefficient quantifies the steady-state response to a change in the concentration of any variable molecular species or any functions thereof (e.g., concentration ratios). In general, for steady-state variable y, the response coefficient with respect to an entity q (variable concentration or parameter) is defined as

$$R_q^y = \left(\frac{\partial \ln y}{\partial \ln q}\right)_{ss} \tag{1}$$

The subscript ss indicates that the whole system is allowed to relax to a new steady state after a change in q. If the entity q affects more than one system step, the partial response coefficient referring to the route of interaction *via* step i is symbolised by a pre-superscript ${}^{i}R_q^y$, and the response coefficient is the sum of partial response coefficients [2]:

$$R_q^y = \sum_i {}^{i}R_q^y \tag{2}$$

2.2 Control coefficients

We propose the adoption of a general definition of a control coefficient based on that given by Heinrich *et al.* [3, 4]. It follows from the partitioned response relationship [5] and incorporates directly the definitions of response and elasticity coefficients:

$$C_{v_i}^y = \frac{(\partial \ln y / \partial \ln p)_{ss}}{(\partial \ln v_i / \partial \ln p)_{step\ i}} \tag{3}$$

where p is any parameter that acts only on step i (we explain further on why this step must be 'independent'). The subscript ss indicates, as above, that the entire system relaxes to a new steady state after a change in p, and subscript *step i* indicates that only the change in local rate v_i of step i is considered at constant reactant, product and effector concentrations. This definition is in effect parameter-independent and can be conceptualised as the steady-state response in y to a change in the local rate of step i. Using the definitions of response (Eq. 1) and elasticity (Eq. 8) coefficients, Definition 3 can be written as

$$C^y_{v_i} = \frac{R^y_p}{\varepsilon^{v_i}_p} \tag{4}$$

In practice, the subscript of a control coefficient can be just the number of the step (e.g., C^J_i) or the name of a process, e.g., an enzyme name, the name of a pathway or part of a pathway. The rate symbol v can be left out. For example, the control coefficient of the enzyme hexokinase on the steady-state concentration of pyruvate should be written as $C^{pyruvate}_{hexokinase}$ (it is important that the enzyme name here identifies the step in question and refers to its local activity, and not to the enzyme concentration).

A great advantage of this definition is that it can be applied at any level of aggregation of the reaction network under consideration, step i in Definition 3 referring to anything from an elementary step in a reaction mechanism to an enzyme-catalysed reaction to a group of linked reactions, provided that it is independent [6]. Another advantage is that, when p is an enzyme concentration that has a linear effect on v_i, it reduces to the often-used definition of control coefficients in terms of modulation of enzyme concentration [1].

However, it has been shown [6] that this definition must not be applied uncritically at any level of aggregation; its use depends on the 'ideality' of the reaction system. Fig. 1 illustrates the difference between ideal and non-ideal pathways, a difference which becomes clear only when the mechanistic details of each reaction are considered. The pathway under consideration is the 2-step enzyme-catalysed pathway in Fig. 1A. In the 'ideal' form depicted in Fig. 1B the two enzyme-catalysed steps are independent because they are only linked by an unconstrained variable metabolite S_1. In the dynamically channelled mechanism depicted in Fig. 1C the existence of the intermediate complex $E_1S_1E_2$ destroys the independence of steps 1 and 2. This invalidates the use of Definition 3 at the level of aggregation used in Fig. 1A, i.e., defining control coefficients for the enzymes by Eq. 3 alone. To select a parameter p for Definition 3 in this case, we must descend to the level of elementary steps of the enzyme mechanism [6] (elementary steps are either binding, dissociation, or isomerisation processes that have well-defined forward rate constants, k_i, and reverse rate constants, k_{-i}, which are independent of other rate constants in the system). The rate equation of an elementary step i can be written as $v_i = p \cdot (k_i \cdot [\text{reactant(s)}] - k_{-i} \cdot [\text{product(s)}])$, where the parameter p is introduced to modulate the activity of the elementary step without changing its equilibrium constant; increasing p has the same effect as increasing both k_i and k_{-i} by the same factor.

It is possible to calculate the control coefficient of any aggregated group of independent steps in the system as the sum of the control coefficients of the independent steps. For example, in the system of coupled enzyme-catalysed reactions depicted in Fig. 1A, the unit steps 1 and 2 can be decomposed into the elementary steps of the catalytic mechanisms (Fig. 1B). A y-control coefficient of, say, step 1 will then be

$$C^y_{v_1} = C^y_{v_{1a}} + C^y_{v_{1b}} \tag{5}$$

In the ideal system the summation properties of metabolic control analysis hold equally

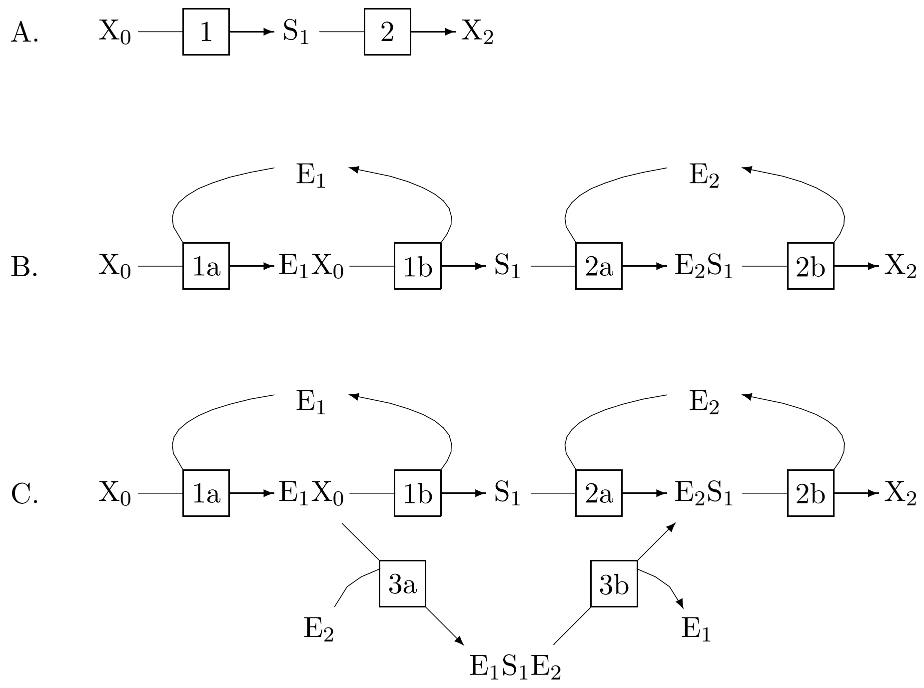

Fig. 1. A simple system of two coupled enzyme-catalysed reactions.
A. Each enzyme-catalysed reaction is aggregated into a unit step. B. An ideal form of the system in (A) where each enzyme-catalysed reaction is decomposed into elementary reactions. C. A non-ideal dynamically channelled form of the system in (A) decomposed into elementary reactions.

for control coefficients of the four elementary steps (Fig. 1B) and for the two aggregated steps (Fig. 1A).

In the non-ideal pathway of Fig. 1C one would write a control coefficient of enzyme 1 as

$$C_1^y = C_{1a}^y + C_{1b}^y + C_{3a}^y + C_{3b}^y, \tag{6}$$

summing the control coefficients of all steps in which E_1 is involved[1]. Similarly,

$$C_2^y = C_{2a}^y + C_{2b}^y + C_{3a}^y + C_{3b}^y. \tag{7}$$

While the summation theorems still hold for the six elementary steps, this is no longer true for summation of C_1^y and C_2^y, as they share elementary steps [8].

2.3 Elasticity coefficients

An elasticity coefficient quantifies the effect of any molecular species or parameter that affects a unit step directly on the local rate through that step in isolation. The original definition remains unchanged:

$$\varepsilon_s^v = \frac{\partial \ln v}{\partial \ln s} \tag{8}$$

[1] This entity has also been called the 'impact control coefficient' of enzyme 1 [7].

where v is the local rate of any unit step in the system and s the concentration of any molecular species or parameter that affects the unit step directly.

A symbolic distinction has sometimes been made between elasticity coefficients with respect to internal variable metabolites (ε) and with respect to constant concentrations of external metabolites (${}^{\kappa}\varepsilon$) or enzymes (π). However, there is no conceptual difference between these elasticity coefficients; because the subscript uniquely specifies the molecular species or parameter to which an elasticity coefficient refers, the use of different symbols is unnecessary (matrices of the different types of elasticity coefficients can be distinguished by a subscript, e.g. $\boldsymbol{\varepsilon}_{\mathbf{s}}$ or $\boldsymbol{\varepsilon}_{\mathbf{p}}$; see also Section 6).

2.4 Relationship between response and control coefficients

In practice, control coefficients can be calculated from the partitioned response relationship (Eq. 4). As stated before, this relationship must never be applied uncritically; it is valid in general only if i is an independent step. This is illustrated by comparing the ideal pathway in Fig. 1B and the non-ideal channelled system in Fig. 1C. In the ideal case any parameter p that acts only on, say, step 1 can be used to determine its control coefficients using Eq. 4, assuming that $\varepsilon_p^{v_i}$ is known. If p is the concentration of enzyme 1, $\varepsilon_{e_1}^{v_1} = 1$ when v_1 is a linear function of e_1. Then, $R_{e_1}^y = C_1^y$.

If Fig. 1A were unjustly considered a valid aggregation of the channelled pathway in Fig. 1C, then one would expect that $R_{e_1}^y$ and $R_{e_2}^y$ equal the control coefficients C_1^y and C_2^y, and would therefore obey the classical summation theorems. However, in reality (i) these control coefficients do not obey the summation theorems (see above), and (ii) the direct interdependence of the two reactions caused by the intermediate complex $E_1S_1E_2$ invalidates the equivalence between the response coefficients with respect to enzyme concentration and the respective control coefficients of those enzymes as defined in Eqs. 6 and 7 [8].

3 Co-response coefficients

The concept of a co-response coefficient has been developed as part of co-response analysis [9, 10]. A co-response coefficient quantifies the relative steady-state responses of two variables to a change in a system parameter. For steady-state variables y_1 and y_2, the co-response coefficient with respect to parameter p is defined as the ratio of the response coefficients of the two variables with respect to p:

$$\Omega_p^{y_1:y_2} = \frac{R_p^{y_1}}{R_p^{y_2}} \tag{9}$$

4 Co-control coefficients

As for response and control coefficients, there is a need for distinguishing between ratios of response coefficients and ratios of control coefficients. A co-control coefficient quantifies the relative steady-state responses of two variables to a change in the local rate of an independent step i in the system. For steady-state variables y_1 and y_2, the co-control coefficient with respect to rate i is defined as the ratio of the control coefficients of the two variables:

$$O_i^{y_1:y_2} = \frac{C_i^{y_1}}{C_i^{y_2}} \tag{10}$$

5 Scaled and unscaled coefficients

In metabolic control analysis both scaled and unscaled coefficients are used. The definitions of the unscaled coefficients are basically the same as those of the scaled coefficients, except that the derivatives are taken in linear space and not in double-logarithmic space. An unscaled elasticity coefficient, for example, is defined as

$$\tilde{\varepsilon}_s^v = \frac{\partial v}{\partial s} \tag{11}$$

with v and s as in Eq. 8.

Scaled coefficients are most helpful in physiological interpretations of control and regulation, while unscaled coefficients are sometimes more practical in mathematical contexts. We propose that the same symbols be used for both scaled and unscaled coefficients, and that the unscaled form be distinguished by a tilde above the symbol. For example, if C_i^y is a particular scaled control coefficient, then $\tilde{C}_i^y$ is the corresponding unscaled control coefficient.

6 Summary of proposed metabolic control analysis symbols and notation

Symbol	Description
Scalars	
R^y_q	response coefficient of steady-state variable y with respect to variable concentration or parameter q
${}^iR^y_q$	partial response coefficient of steady-state variable y with respect to variable concentration or parameter q referring to interaction only *via* step i
C^y_i	control coefficient of step i on steady-state variable y
$\varepsilon^{v_i}_s$	elasticity coefficient of step i with respect to molecular species s
$\Omega^{y_1:y_2}_p$	co-response coefficient of steady-state variables y_1 and y_2 with respect to parameter p
$O^{y_1:y_2}_i$	co-control coefficient of steady-state variables y_1 and y_2 with respect to a change in the local rate of step i
Vectors	
$\mathbf{s}$	vector of concentration variables
$\mathbf{x}$	vector of fixed external concentrations
$\mathbf{p}$	vector of parameters
$\mathbf{v}$	vector of reaction rates
$\mathbf{J}$	vector of steady-state fluxes
$\mathbf{T}$	vector of moiety-conserved sums
Matrices	
$\mathbf{N}$	stoichiometric matrix as in $d\mathbf{s}/dt = \mathbf{Nv}$ [11]
$\mathcal{N}$	scaled stoichiometric matrix
$\mathbf{L}$	link matrix relating metabolite concentrations to independent metabolite concentrations [11]
$\mathcal{L}$	scaled link matrix
$\mathbf{N_R}$	reduced stoichiometric matrix in $\mathbf{N} = \mathbf{LN_R}$ [11]
$\mathbf{K}$	nullspace or kernel matrix relating steady-state fluxes to independent fluxes [11]
$\mathcal{K}$	scaled kernel matrix
$\mathbf{I}_n$	identity matrix of dimension n
$\boldsymbol{\varepsilon}_\mathbf{s}$, $\boldsymbol{\varepsilon}_\mathbf{p}$	matrix of elasticity coefficients; the subscript indicates whether the coefficients refer to variable concentrations (subscript $\mathbf{s}$) or to parameters (subscript $\mathbf{p}$); if $\boldsymbol{\varepsilon}_\mathbf{p}$ contains elasticity coefficients with respect to only one type of parameter, this may be denoted by another subscript (e.g., $\mathbf{x}$ for external concentrations or $\mathbf{e}$ for enzyme concentrations)

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Symbol	Description
Matrices	(continued)
$\mathbf{M}$	Jacobian matrix ($\mathbf{N_R}\tilde{\boldsymbol{\varepsilon}}_\mathbf{s}\ \mathbf{L}$)
$\mathbf{C}$	matrix of control coefficients; if a distinction is required, the matrix of flux-control coefficients can be indicated by a superscript $\mathbf{J}$ and the matrix of concentration-control coefficients by a superscript $\mathbf{s}$
$\mathbf{E}$	matrix of structural and local properties $[\mathcal{K} \quad - \boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L}]$ that occurs in the matrix equation $\mathbf{CE} = \mathbf{I}$ [10]
$\mathbf{R}$	matrix of response coefficients; as for the elasticity matrix, a subscript can indicate whether the coefficients refer to parameters (subscript $\mathbf{p}$) or to variable concentrations (subscript $\mathbf{s}$)
$\mathbf{O}$	matrix of co-control coefficients
$\boldsymbol{\Omega}$	matrix of co-response coefficients

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