Hypothesis

Composite control of cell function: metabolic pathways behaving as single control units**

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Abstract This paper shows that under some conditions the control exerted by a part of a metabolic network (a pathway) on a flux or concentration in any other part can be described through a *single* (overall) control coefficient. This has the following implications: (i) the relative contributions of a pathway enzyme to the regulation of the pathway (output) flux and of any flux or concentration outside are identical; therefore, the control analysis of the pathway 'in isolation' allows one to determine the control exerted by *any* pathway enzyme on the rest of the cell by estimation of the control efficient of just one, arbitrarily chosen enzyme; (ii) the relative control of any two metabolic variables outside the pathway (measured as the ratio of the control coefficients over these two variables outside) is the same for all pathway enzymes. These properties allow one to substitute effectively a pathway by a single (super)reaction and make it possible to consider such a pathway as a metabolic unit within the cellular enzyme network.

Key words: Metabolic control analysis; Overall control coefficient; Metabolic unit

1. Introduction

Metabolic Control Analysis and the related approach of Biochemical Systems Theory provide methods for determining special indicators, so-called control coefficients, which quantify the contribution of any enzyme to the control of pathway fluxes and concentrations [1–3]. If all the control coefficients could be measured or estimated, this should provide a complete description of the regulation and control of steady-state fluxes and metabolite concentrations in the living cell. Moreover, such information should make it possible to reveal the local kinetic properties of the enzymes in situ (the elasticity coefficients) [4,5]. However, the enormous complexity of cellular metabolic and information networks, their supramolecular organization, and direct protein–protein interactions (for recent reviews see, e.g. [6,7]) compromises any hope to measure the control coefficients of all enzymes in the intact cell.

To understand how an enormous number of interrelated biochemical processes can be regulated coordinately, it helps to analyse them in terms of functional units, i.e. subsystems or modules of cell metabolism. Development of such a modular control analysis began in the early eighties [8–10]. Formulation of the 'top-down' analysis ([11,12]; for a review see [13]) gave new impetus to experimental studies of the control exerted by a metabolic subsystem on the flux through a pathway. Hierarchical and modular analysis [14,15] relaxed the restriction that subsystems must be connected by only a single intermediate. Kholodenko [10,16] focussed on the conditions under which the control coefficients as determined within a pathway would coincide, after scaling, with the control coefficients as measured in the entire metabolic network. Such an invariance of the relative distribution of the control allowed one to determine a single ('overall' [17,18]) control coefficient of that pathway in a unique and unambiguous way. The ideas of how control exerted by pathway enzymes on the rest of the cell depends on the links between this pathway and its surroundings [10,16] and the implications of this type of modular organization of cellular metabolism were, however, not elaborated in details. In the present paper we revisit the ideas considered in [10,16] and show under what conditions a whole pathway can be regarded as a metabolic unit (super-reaction) within enzyme networks of the cell. We develop Metabolic Control Analysis further to cover the composite control of cell function.

2. Results

2.1. Dividing a metabolic network into subsystems

A metabolic system under investigation will be divided conceptually into two parts, below referred to as the pathway and its surroundings. We shall designate by v_i^{path} and v_i^{surr} the rates of reactions, and by x_i^{path} and x_i^{surr} the concentrations of metabolites inside the pathway and its surroundings, respectively.

Representing a metabolic network as a combination of its two parts one can normally find reactions in either part that produce or consume the metabolites of the other. Now we wish that at any steady state all the influences of the pathway on its surroundings act through a single mode, i.e. with a single degree of freedom [10,16]. This suggestion requires that all those pathway rates (v_i^{path}) that produce (consume) any of the outside meta-

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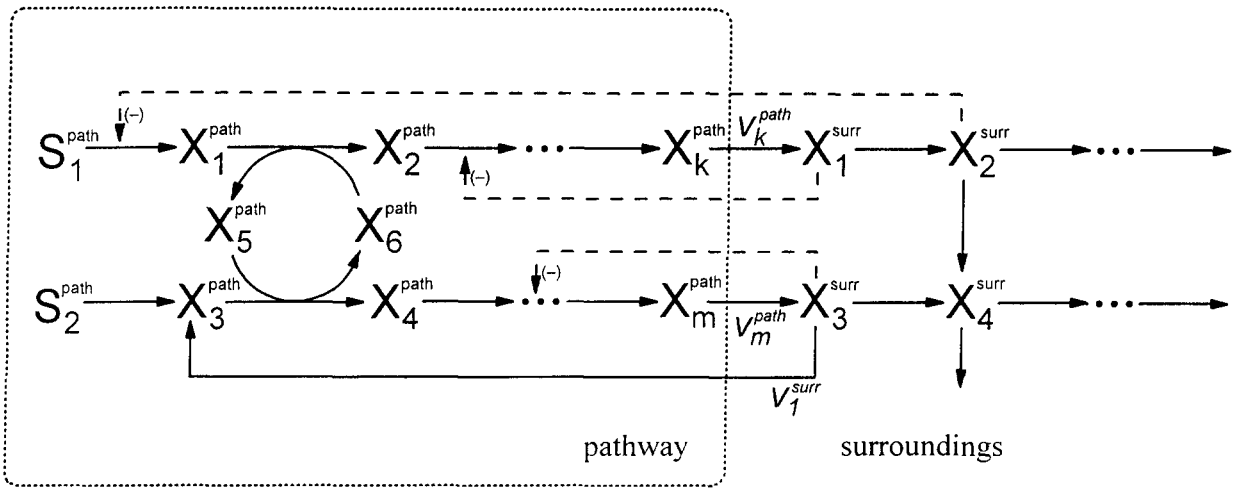

Fig. 1. A metabolic network is divided conceptually into two parts, i.e. the pathway and its surroundings, where the dotted line shows the border between these. At steady state the rates, v_k^{path} and v_m^{path}, producing outside metabolites, x_1^{surr} and x_2^{surr}, respectively, are constrained by a constant stoichiometric relation, hence, are proportional to the same output flux. The outside rate, v_1^{surr}, producing the pathway metabolite (x_3^{path}) is supposed to be independent of the concentration of the latter. Direct influences of metabolites in the surroundings on pathway reactions are shown by dashed lines.

bolites (x_i^{surr}) must be constrained by constant stoichiometric relations at steady state (see Fig. 1). In other words, any such rate (v_i^{path}) must be proportional to the same flux (J_b) which is called an output pathway flux [10,16] or a 'bridging' flux (a term coined in [15]):

$$v_i^{path}\big|_{\text{steady state}} = n_i J_b$$

if any of the outside metabolites, x_i^{surr}, is produced or consumed in the pathway reaction i **(1)**

Here n_i is a constant stoichiometry. Moreover, the rates v_i^{surr} must depend only on the concentrations (x_i^{surr}) which belong to the surroundings, whereas pathway rates (v_i^{path}) can depend on both concentrations inside the pathway (x_i^{path}) and in the surroundings (x_i^{surr}) [10,16]. As a consequence, if there existed some rates v_i^{surr} flowing into the pathway (Fig. 1), these should not depend on the metabolite concentrations (x_i^{path}) of the pathway. The suggestion of a single degree of freedom in the influence of a pathway on its surroundings implies also a more subtle constraint on the links between these two parts of the network. There should be no moiety-conserved cycles that involve metabolites of both a pathway and its surroundings. Accordingly, after substituting all linearly dependent concentrations (x_i^{surr}) via linearly independent ones, the resulting expressions for the rates, v_i^{surr} must still depend only on the concentrations of the surroundings [10,16].

2.2. *Composed regulation of cell function: the control within a pathway and its overall control of the environment*

We shall first analyse the control properties of the pathway 'in isolation', i.e. at clamped concentrations of the metabolites in the surroundings. Within the pathway one can estimate the control coefficients of its enzymes over the bridging flux (J_b):

$$C_i^{J_b}(\text{path}) = \left(\frac{d\, J_b/J_b}{d\, e_i/e_i}\right)_{x^{surr}} = \left(\frac{d \ln|J_b|}{d \ln e_i}\right)_{x^{surr}} \quad \textbf{(2)}$$

Here e_i is the total concentration of the enzyme i of the pathway, the subscript x^{surr} signifies that the steady state of the pathway is considered at clamped metabolite concentrations outside the pathway; the dimensionless coefficient $C_i^{J_b}$(path) is called the flux control coefficient of the enzyme i, the argument *path* specifies that this control coefficient is determined within the pathway.

Let us choose any metabolic variable (Y) within the surroundings, i.e. any flux or metabolite concentration outside the pathway. Considering a steady state of both the pathway and its surroundings simultaneously (i.e. a steady state of the entire metabolic network), one can estimate the control coefficient of the enzyme i, now in the entire system:

$$C_i^Y = \left(\frac{d \ln|Y|}{d \ln e_i}\right)_{sys} \quad \textbf{(3)}$$

The subscript *sys* signifies that the steady state of the entire system is required; accordingly, the absence of the argument of C_i^Y specifies that this control coefficient is determined within the original (entire) metabolic network.

Now we relate the control coefficients estimated within the entire network to those estimated within an 'isolated' subsystem. The effects of the modulation of an enzyme on any metabolic variable (Y) outside a pathway act only via a single bridging flux (J_b, which is also a pathway flux; Eqn. 1). As a consequence, at any steady state the variable Y can be represented as a function of the variable J_b and the parameters of the surroundings only. According to usual differentiation rules one can write:

$$C_i^Y = \left(\frac{d \ln|Y|}{d \ln|J_b|}\right) \cdot \left(\frac{d \ln|J_b|}{d \ln e_i}\right)_{x^{surr}} = C_{J_b}^Y \cdot C_i^{J_b}(\text{path}) \quad \textbf{(4)}$$

The meaning of the control coefficient $C_{J_b}^Y$ becomes clear when summing Eqn. 4 over all the enzymes i of the pathway and taking into consideration that for the control coefficients $C_i^{J_b}$(path) (determined inside the pathway in isolation) this sum equals unity:

$$C_{J_b}^Y = \sum_{\text{all the pathway enzymes } i} C_i^Y = C_{\text{path}}^Y \quad \textbf{(5)}$$

Hence, $C^Y_{J_b}$ is the 'overall' control coefficient of the pathway with respect to the outside variable Y and can be also designated as C^Y_{path}.

Eqn. 4 has two remarkable consequences. First, if in the entire metabolic network one measures the control coefficient of any pathway enzyme (i) over any outside variable (Y), and then compares the result to the control coefficient of that enzyme over the flux J_b estimated within the pathway, one finds that the ratio of these control coefficients is identical for all the pathway enzymes:

$$C^Y_i / C^{J_b}_i(\text{path}) = C^Y_{\text{path}} \quad (6)$$

As a consequence, the relative distribution of the control exerted by enzymes of the pathway on *any metabolic variable* (Y) in the surroundings can be measured *within the pathway* (in isolation of the rest of the network) by evaluating the control coefficients over *its output flux* (J_b) only. Also the absolute control exerted by *any* pathway enzyme (i) on the rest of the cell can be determined from this relative control distribution by estimation of the control coefficient of just one, arbitrarily chosen enzyme (j) [10,16]:

$$C^Y_i = C^Y_j \cdot (C^{J_b}_i(\text{path}) / C^{J_b}_j(\text{path})) \quad (7)$$

Eqns. 6 and 7 demonstrate the composite control properties. Moreover, Eqn. 6 suggests that to estimate the overall control coefficient of the pathway over any variable (Y) ourside, one can modulate the activity of *any* pathway enzyme and estimate the ratio of the change in Y at clamped concentrations of the surroundings to such a change when these concentrations are allowed to relax to a new steady state.

Applying Eqn. 4 to any two variables Y and Z in the surroundings and to any two enzymes i and k in the pathway one arrives at:

$$C^Y_i / C^Z_i = C^Y_k / C^Z_k = C^Y_{\text{path}} / C^Z_{\text{path}} \quad (8)$$

Therefore, the ratio of control coefficients over the two outside variables, Y and Z, is identical for any enzyme of the pathway, and coincides with the ratio of pathway *overall* control coefficients. For such a ratio, the term 'co-response' coefficient of two variables Y and Z has been suggested [19,20]. When an enzyme i is perturbed, the ratio C^Y_i / C^Z_i is designated as ${}^iO^Y_Z$. In these notations Eqn. (8) reads:

$${}^iO^Y_Z = {}^kO^Y_Z = {}^{\text{path}}O^Y_Z \quad (9)$$

Here ${}^{\text{path}}O^Y_Z$ designates the co-response coefficient of two variables Y and Z when any enzyme (or even any parameter) belonging to the pathway is perturbed.

Although our reasoning here is absolutely rigorous, a formal analytical proof of the above statements (Eqns. 4–6) which uses the matrix machinery of control analysis can be found elsewhere [10,16] (Rohwer et al., submitted). The Appendix presents an additional proof that uses a perturbation method originated in [1,21], and provides deeper insight into the invariance phenomenon.

3. Discussion

In the present paper we delineated the composed control of an enzyme within a particular metabolic pathway on the phenomena in the rest of the cell. This composed control is determined by the relative importance of the enzyme for the control of the output pathway flux and by the control exerted by the entire pathway on its surroundings. We formulated and explained the three conditions under which the impact of the pathway on the rest of the cell can be described by its *single* overall control coeficient and by the (relative) control properties of the enzymes within that pathway 'in isolation'. In terms of the pathway stoichiometry this requires: (i) constant stoichiometric relations between the output reaction rates at steady state, i.e. a single bridging flux; and (ii) that the pathway shares no conserved moieties (e.g. coenzymes) with the surroundings. In terms of the local kinetic properties of enzymes condition (iii) requires that the rates of reactions in the surroundings must not depend directly on metabolites of the pathway.

The constraints make it possible to estimate the relative contributions of pathway enzymes to the control of *any metabolic variable* in the rest of the cell by measuring the control coefficients of those enzymes *within the pathway* over the output flux. This property is useful when pathway 'borders' differ between experimental conditions or between vitrum and vivum [22]. For example, under constant lactate/pyruvate and redox ratios the contribution of any mitochondrial enzyme to the control of gluconeogenesis will differ only by a constant factor from the control coefficient of that enzyme over mitochondial ATP production flux.

When the three conditions ensuring that a pathway affects its surroundings with a single degree of freedom are fulfilled, the co-response coefficients over any two fluxes, any flux and concentration or any two concentrations of the surroundings do not depend on a particular enzyme that has been modulated in the pathway (see Eqn. 9). As a consequence, such a pathway can be replaced by a single (super)reaction with (quasi)steady-state rate J_b. This 'pathway' rate depends on the concentrations of metabolites of the surroundings and on internal parameters of the pathway only. Importantly, if any of the conditions stated above is violated the remarkable properties discussed above are also violated [10,22].

Conceptual and methodological advances discussed in this paper may be particularly important in the realm of 'non-ideal' metabolism (see [7] for a review). All protein–protein interactions should be black-boxed within subsystems and if the three conditions ensuring a single degree of freedom in pathway links to the environment are fulfilled, one can define the overall control coefficient of a pathway as the sum of the *elemental* control coefficients [7].

Appendix

When the above-mentioned constraints on the links between the subsystem and its surroundings are fulfilled, one can consider the steady state bridging flux (J_b) as a function of the concentrations of metabolites of the surroundings (x^{surr}) and of internal parameters (e.g. the enzyme concentrations, e_i) of the pathway only:

$$J_b(x^{\text{surr}}, e_i, \xi) = \xi \cdot J_b(x^{\text{surr}}, e_i) \quad (A1)$$

Here ξ is a special parameter introduced to modulate the bridging flux J_b. Let Y be any metabolic variable of the surroundings. The control coefficient of any enzyme i over Y is defined by Eqn. 3 of the main text. Such a definition presumes that after

a small modulation (δe_i) of the activity of that enzyme the entire network relaxes to a new steady state in which a change in Y is determined. After such a perturbation (δe_i) in e_i we now change simultaneously the parameter ξ to such an extent that the bridging flux J_b returns to its initial non-perturbed value under the initial non-perturbed values of x_i^{surr}, i.e.

$$0 = C_i^{J_b}(\text{path}) \cdot (\delta e_i/e_i) + \delta\xi/\xi \qquad \textbf{(A2)}$$

The newly attained state is again steady and notwithstanding the perturbed values of e_i and ξ, any variable (Y) of the surroundings will be the same as in the initial steady state (since the pathway affects the surroundings via only a single flux J_b which does not change). Taking into consideration Eqn. A1, the absence of changes in the variable (Y) can be written as follows:

$$0 = C_i^Y \cdot (\delta e_i/e_i) + \left(\frac{\mathrm{d}\ln|Y|}{\mathrm{d}\ln|J_b|}\right) \cdot \delta\xi/\xi \qquad \textbf{(A3)}$$

Eqn. 4 of the main text follows from Eqns. A2 and A3.

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