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Modular analysis of the control of complex metabolic pathways

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Abstract

The understanding of the functioning of the intact cell would be simplified appreciably if it were possible first to analyze particular modules of cell physiology separately, and then to integrate the information so as to yield understanding of the control structure in terms of the mutual regulation of the modules. Here we develop a quantitative method based on Metabolic Control Analysis that makes this possible: The relevant properties of the modules are contained in "overall" elasticity coefficients, which reflect the changes in fluxes in the module upon a small variation of the environment of the module, allowing the latter to attain steady state. We show how overall control coefficients, which reflect the control exerted by the processes catalyzed by each module, can be expressed into the overall elasticity coefficients. We derive corresponding summation and connectivity theorems. A number of possible divisions of physiological systems into modules is discussed. This work is a generalization of previous analyses of overall control properties in that it allows for multiple fluxes to connect the modules, and reaction stoichiometries of any complexity.

Keywords: Control analysis; Metabolic pathways; Modular approach; Steady-state modeling; Oxidative phosphorylation; Protein synthesis

1. Introduction

Understanding the control and regulation of cell physiology constitutes an immense scientific challenge. Key problems are the difficulty to assess enzyme kinetic properties in the intact cell, the nonlinearity of most enzyme catalyzed reactions and the vast number of enzymes and metabolites involved. One of the consequences of the non-linearity and the complexity is that the implications of certain kinetic properties of the enzymes cannot be understood immediately, or even after considerable thought; regulation tends to involve a number of regulatory links and the result is often a complex superposition of their effects.

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Approaches such as Metabolic Control Analysis (MCA; on which we shall focus here [1–3], for review see [4]), Biochemical Systems Analysis [5,6], and Mosaic Non-Equilibrium Thermodynamics [7] have devised ways to evaluate control and regulation in complex systems. For systems at steady state, MCA defines control coefficients as the dependence of flux, concentration, or potential on the activity of one of the enzymes (or processes) in the system. The relevant enzyme properties are the elasticity coefficients, defined as the dependence of the reaction rates on the concentrations of one of the effectors of that rate (which could be the concentration of the substrate of the reaction). MCA has derived how one can calculate the control coefficients from the elasticity coefficients, provided one knows the metabolic map [8–13]. If one measures both the control coefficients and the elasticity coefficients and the values of the former as calculated from the latter correspond to the experimental values, one may claim to understand control in terms of enzyme properties.

A limitation of the existing MCA was that, even though the biochemist and cell biologist discern functional units in cell metabolism, such as glycolysis and oxidative phosphorylation, or cytosol and mitochondrion, MCA would discuss control only in terms of kinetic properties of the individual enzymes, and not in terms of control properties of these functional metabolic subunits. For instance, MCA would not explain the control of intracellular ATP as being the result of the elasticities of glycolysis and mitochondria versus ATP, but would discuss such control in terms of all the individual contributions of all enzymes in the cell. This inelegance was recognized in control systems that involve both intermediary metabolism and gene expression [14,15] and in systems with cascades of enzymes modifying each other's activity [16]. In the former, there are the pathways around mRNA synthesis, the pathways leading to protein synthesis, as well as the metabolic pathway that is catalyzed by the synthesized proteins. One would wish to discuss control in terms of how much of it resides at the particular levels (transcription, translation, intermediary metabolism itself). It was recognized that these systems essentially consist of modules which are only related by 'allosteric' influences, rather than by net flows of metabolites. A method was developed to analyze control in such systems of unconnected modules in terms of the control properties of the individual modules and the allosteric effects between the modules [16].

The latter approach is limited by the demand that there be no net flux between the modules (which can also occur in some connected systems [17,18]). Where there is no flux between modules, it is obvious how to dissect a system. However, it has been argued that in metabolic systems containing practically irreversible steps or near-equilibrium reactions [2,3,8], or saturated enzymes [1], there may be reactions that exert practically no control on some fluxes although there is a net flux between them. This suggests that other possibilities for modular decomposition of metabolic networks may exist.

An example of a modular decomposition of a connected network was provided for the case of mitochondrial oxidative phosphorylation. Here relevant elasticity coefficients would include the dependence of the rate of the adenine nucleotide translocator on the extramitochrondrial ATP/ADP ratio at constant membrane potential and constant intramitochondrial ATP/ADP ratio. Experiments to measure such dependences are difficult because of the problem to maintain the membrane potential (which usually changes quite rapidly) constant during the time of the experiment (although indirect methods have led to some results [19]). For this particular problem a solution was devised which groups all the reactions involved in mitochondrial oxidative phosphorylation into three parts: those connected with respiration and producing $\Delta\tilde{\mu}_H$, those connected with synthesis of extramitochondrial ATP and consuming $\Delta\tilde{\mu}_H$ and those that leak away $\Delta\tilde{\mu}_H$. The control of mitochondrial respiration was subsequently described as divided over these three groups [20]. The experimental values for the control coefficient of the ATP synthesizing group with respect to respiration corresponded to that calculated from measured overall elasticity coefficients, defined as the response of each group to a change in $\Delta\tilde{\mu}_H$, allowing the group to attain its

own steady state [7,20]. In this manner control of oxidative phosphorylation could be understood in terms of regulatory interactions between three black boxes (respiration, phosphorylation and leak) even though the regulations within the boxes were not completely known.

The idea to define control coefficients of enzyme sequences has also been put forward by Heinrich and Rapoport [2,3]. Kacser [21] developed it for branched metabolic pathways. Aggregation of processes has also been practised in Biochemical Systems Theory [5,6]. More recently, the approach has been developed in more detail and renamed 'top–down approach' [22–24]. As developed until now however, this approach is limited to cases where the subunits into which one divides metabolism, are bridged by a single metabolic variable.

In the present paper we generalize overall control analysis to cases where modules have several throughput fluxes and/or are connected by more than a single metabolite. This allows us to treat such modules as 'superenzymes' catalyzing multiple reactions.

2. Modular decomposition of a metabolic network

For the present analysis, it is appropriate to consider two types of module, i.e.: type 1—subsystems of which we only wish to observe the reactions that link those subsystems with their surroundings but not internal reactions and metabolites, and type 2—subsystems subject to explicit observation.

The modular decomposition we describe below, may or may not correspond to a spatial decomposition into compartments.

Although it is in some cases convenient to distinguish several modules of type 1 (cf. Section 7), we start with a decomposition into one module of type 1 and one module of type 2 (cf. Fig. 1). The reactions can be classified into three groups: reactions internal to module 1 (index 1); reactions bridging the two modules (index b), and reactions internal to subsystem 2 or linking it with the surroundings (index 2). Reactions linking module 1 with the surroundings of the whole system can formally be included in the bridging reactions (cf. Fig. 1). Let the vector $\boldsymbol{v}$ represent all the reaction rates and the vector $\boldsymbol{X}$ the molarities of all substances except for source and sink metabolites, the molarities of which are assumed to be constant and are included in the parameter vector, $\boldsymbol{p}$. Throughout the paper, vectors and matrices are denoted by bold-face italics and bold-face roman symbols, respectively.

The change in time of the molarities is related to the reaction rates by the stoichiometry matrix $\mathbf{N}$ [25]:

$$\frac{\mathrm{d}\boldsymbol{X}}{\mathrm{d}t} = \mathbf{N} \cdot \boldsymbol{v}. \tag{1}$$

For systems consisting of several compartments, one should identify $\boldsymbol{x}$ with the vector of mole numbers rather than of molarities.

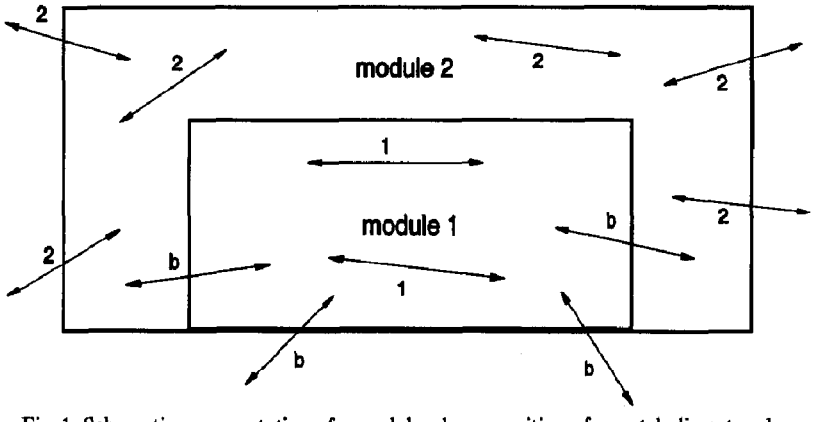

Fig. 1. Schematic representation of a modular decomposition of a metabolic network.

Following Reder [11], we choose a submatrix $\bar{\mathbf{N}}$ of $\mathbf{N}$ by using a maximum number of linearly independent rows. Then, we can write

$$\mathbf{N} = \mathbf{L} \cdot \bar{\mathbf{N}}, \tag{2}$$

where $\mathbf{L}$ is called the link matrix. X can now be reduced to a vector of independent variables $\bar{X}$, with $\mathrm{d}X = \mathbf{L}\mathrm{d}\bar{X}$. In line with the decomposition of the system into two modules, the stoichiometry matrix, molarity vector and rate vector can be partitioned as follows, respectively,

$$\mathbf{N} = \begin{bmatrix} \mathbf{N}_{11} & \mathbf{N}_{1b} & \mathbf{0} \\ \mathbf{0} & \mathbf{N}_{2b} & \mathbf{N}_{22} \end{bmatrix}, \tag{3a}$$

$$X = \begin{bmatrix} X_1 \\ X_2 \end{bmatrix}, \tag{3b}$$

$$v = \begin{bmatrix} v_1 \\ v_b \\ v_2 \end{bmatrix}. \tag{3c}$$

That $\mathbf{N}_{12}$ and $\mathbf{N}_{21}$ are zero reflects that metabolites in subsystem 1 are not metabolized by processes in subsystem 2 and *vice versa*. Consequently, the elasticities $\mathbf{D}_{21}$ are made up by the non-stoichiometric (e.g. allosteric) influences of X_1 on v_2. Since the term 'non-stoichiometric' might be misleading, we will use, in the following, the notion 'direct influences' because they are not mediated by the bridging reactions.

We take, throughout this paper, the modular decomposition to be arranged in such a way that direct effects of concentrations in module 1 on reactions in module 2 are absent:

$$\mathbf{D}_{21} = \mathbf{0}. \tag{4}$$

The matrix of non-normalized elasticities [11] can be decomposed as:

$$\mathbf{D} \underset{\text{def}}{=} \frac{\partial v}{\partial X} = \begin{bmatrix} \mathbf{D}_{11} & \mathbf{D}_{12} \\ \mathbf{D}_{b1} & \mathbf{D}_{b2} \\ \mathbf{0} & \mathbf{D}_{22} \end{bmatrix}. \tag{5}$$

The present paper will be limited to cases where conservation relations linking X_1 and X_2 are absent, that is

$$\mathbf{L} = \begin{bmatrix} \mathbf{L}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{L}_2 \end{bmatrix} \tag{6}$$

with $\mathbf{L}$ being the link matrix defined in eq. (2) and $\mathbf{L}_2$ denoting the link matrix of module 2.

Equation (6) is trivially fulfilled if there are no conservation relations at all ($\mathbf{L}$ equals the identity matrix), as in the examples shown in Scheme 1 and Fig. 2 and the systems dealt with in [22]. It is also satisfied if the conservation relations are confined to module 1 [7].

In an extended treatment, conditions (4) and (6) can be relaxed (Schuster et al., in preparation).

The assertion that the observation of module 1 is limited to interactions with its environment means that one has no knowledge about the elasticity matrices $\mathbf{D}_{11}$, $\mathbf{D}_{12}$, $\mathbf{D}_{b1}$, $\mathbf{D}_{21}$, and possibly not even about the stoichiometry matrices $\mathbf{N}_{11}$ and $\mathbf{N}_{1b}$. We now address the question as to under what conditions we are able to determine the control properties of modules 1 and 2 with module 1 considered as an aggregated 'super-reaction'.

Let J denote the vector of steady-state fluxes (i.e., $J = v$ whenever $\mathbf{N} \cdot v = 0$). J can be partitioned in accordance with the decomposition (3c).

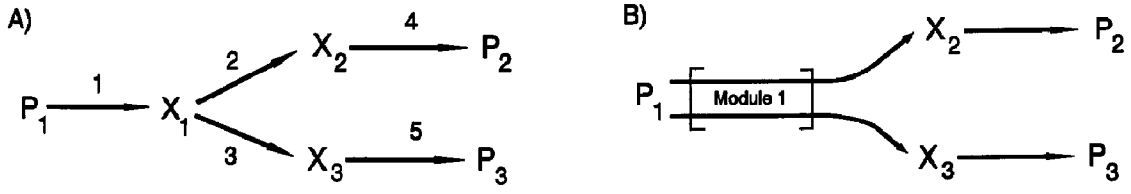

Fig. 2. System with two linearly independent bridging fluxes. A, complete scheme; B, simplified scheme with substance X_1 taken as module 1.

We distinguish between fluxes, J, attained in a steady state of the whole system and those attained when module 1 is in a steady state on its own, with the molarities of the substances belonging to module 2 kept constant experimentally. The latter fluxes are to be distinguished in symbolism by an asterisk (e.g. $^*J_b$). This distinction is only important if derivatives (with respect to parameters or concentrations) rather than the fluxes themselves are considered.

In this paper, it will be assumed that module 1 can attain a stable steady state on its own when the concentrations within module 2 are kept constant, in the neighborhood of their values attained in steady states of the whole system.

By observation of the bridging reactions (cf. above) we mean to imply that their flux response coefficients with respect to changes in X_2 (gathered in the matrix $\partial^*J_b/\partial X_2$) can be measured. The appropriate terminology for the components of $\partial^*J_b/\partial X_2$ depends on the point of view. Regarding the molarities in module 2 as external and clamped, these coefficients may be called response coefficients (cf. [1,26], see, however, [27]). On the other hand, considering the molarities X_2 as internal variables (which they are if the whole system is considered), the term 'overall elasticities' is appropriate [7,20]. Accordingly we use the notation

$$^*\mathbf{D}_{b2} = \partial^*J_b/\partial X_2. \tag{7}$$

Let us identify, by way of example, the mitochondria of a given cell as module 1 and the processes in the cytosol as module 2. The response coefficients $^*\mathbf{D}_{b2}$ can then be determined experimentally by resuspending the mitochondria in an incubation medium with a sufficiently large volume, where the substances of interest have approximately their cytosolic molarities and may thus be considered independent of the processes within module 1. Experimental alteration of these molarities and concomitant measurement of the fluxes linking the mitochondria with their surroundings (e.g. oxygen consumption) gives the abovementioned overall elasticity coefficients.

Another situation where overall elasticity coefficients $^*\mathbf{D}_{b2}$ are experimentally accessible is when module 1 is a fast subsystem, i.e., if it approaches steady state much faster than the whole system. In this situation, $^*\mathbf{D}_{b2}$ expresses the response of module 1 towards changes in X_2, with the response measured in a time scale long enough to allow module 1 to reach a new steady state but short enough so that X_2 has not yet relaxed to the original values.

3. Choice of independent bridging fluxes

Because at steady state for module 1, fluxes feeding into module 1 must balance output fluxes, the bridging fluxes are usually linearly dependent. It is therefore appropriate to reduce them to a vector of independent fluxes, J_r, such that

$$J_b = \mathbf{Q} \cdot J_r. \tag{8}$$

If the stoichiometry within module 1 is known, matrix $\mathbf{Q}$ can be constructed by analyzing the null-space matrix (kernel) [11,17] of the submatrix $(\mathbf{N}_{11} \quad \mathbf{N}_{1b})$. Balance equations for specified moieties such as the phosphate group can often be written even without knowledge of the exact reaction stoichiometries within a given module. It is worth noting that since $\mathbf{Q}$ can always be chosen to contain an identity submatrix, J_r can be chosen to encompass a subset of the component of J_b.

Consider, for example, an unbranched reaction scheme as shown in Scheme 1A.

$$P_1 \longrightarrow X_1 \longrightarrow X_2 \longrightarrow P_2$$

Scheme 1A

We treat X_1 as module 1 and the first two reactions as bridging reactions. Since at steady state, these fluxes are equal to each other, it is meaningful to lump these reactions into one overall reaction. The reaction scheme can then be depicted as shown in Scheme 1B.

$$P_1 \longrightarrow [\text{Module 1}] \longrightarrow X_2 \longrightarrow P_2$$

Scheme 1B

For this example, $\mathbf{Q}$ reads $(1 \quad 1)^T$.

In this special case, this method of aggregation is essentially identical with the 'top–down' approach [22–24,28]. In this paper, we wish to include cases with more than one independent bridging flux. Consider the exemplifying system depicted in Fig. 2A. If substance X_1 is taken as module 1, it is linked with subsystem 2 by two independent steady-state fluxes. Accordingly, it is useful to redraw the scheme as shown in Fig. 2B. Module 1 is now a 'super-reaction' involving two independent fluxes.

For the system shown in Fig. 2A, we may take J_2 and J_3 as independent fluxes, so that eq. (8) reads

$$J_b = \mathbf{Q} \cdot \mathbf{J}_r = \begin{pmatrix} 1 & 1 \\ 1 & 0 \\ 0 & 1 \end{pmatrix} \begin{pmatrix} J_2 \\ J_3 \end{pmatrix}. \tag{9}$$

The notation $^*J_r$ is used for the vector of lumped (reduced) bridging fluxes attained in a steady state of module 1 (i.e. with X_2 clamped).

It is worth mentioning that there are even 'ordinary' enzymatic reactions with several rates. Any slipping enzyme (such as F_0F_1-ATPase) exhibits at least two reaction rates, a 'useful' catalytic rate and a 'futile' slip rate [7,29–31]. Multifunctional enzymes have one or several degrees of freedom with respect to flux according to whether or not the reactions catalyzed by these enzymes lie in the same branch of the pathway [32].

Similarly as in the traditional kinetic description of biochemical reactions, the lumped rates are to be described as functions of parameters and of molarities external to the 'super-reaction' occurring in module 1:

$$^*J_r = {}^*J_r(p, X_2). \tag{10}$$

Since the concentrations X_1 have been eliminated, also $^*J_2$ has to be expressed into X_2 and p only:

$$^*J_2 = {}^*J_2(p, X_2). \tag{11}$$

Strictly speaking, even the parameter vector p should be transformed so that parameters internal to module 1 are replaced by overall parameters accessible to observation, but this distinction is of minor importance for our analysis.

Since X_1 is a function of X_2 if module 1 is at steady state, function (11) can be written as

$$^*J_2(p, X_2) = v_2[X_1(X_2), X_2, p]. \tag{12}$$

Differentiating this equation with respect to X_2, we obtain, due to condition (4),

$$\partial {}^*J_2/\partial X_2 = \mathbf{D}_{22}. \tag{13}$$

Let $^*\mathbf{D}_{r2}$ denote the matrix of overall elasticities pertaining to $^*J_r$. Through differentiation of eq. (8), this matrix is linked with $^*\mathbf{D}_{b2}$ by

$$^*\mathbf{D}_{b2} = \mathbf{Q} \cdot {}^*\mathbf{D}_{r2}. \tag{14}$$

In the following, we assume that the matrices $^*\mathbf{D}_{r2}$ and $\mathbf{D}_{22}$ are known, either from experiment or from computation. Moreover, the local properties of module 2 are assumed to be known (expressed by $\mathbf{N}_{22}$, $\mathbf{N}_{2b}$, $\mathbf{X}_2$ and $\mathbf{J}_2$).

4. Overall control coefficients

In accordance with the structural approach to control theory [11], we use the following definition for control coefficients. Be p_2 a vector of parameters for which $\partial v_2/\partial p_2$ is a non-singular square matrix. For example, p_2 may encompass parameters influencing the reaction rates specifically, so that $\partial v_2/\partial p_2$ is a diagonal matrix. In the absence of enzyme–enzyme interactions and multifunctionality of enzymes, the enzyme molarities may be chosen for these parameters [33,34]. The matrices of non-normalized control coefficients expressing the control exerted by the reactions of module 2 can be defined as

$$\mathbf{\Gamma}_{22} = (\partial X_2/\partial p_2) \cdot (\partial v_2/\partial p_2)^{-1}, \tag{15}$$

$$\mathbf{\Phi}_{x2} = (\partial J_x/\partial p_2) \cdot (\partial v_2/\partial p_2)^{-1} \tag{16}$$

with index x standing either for r or for 2. X_2 and J_x here refer to concentrations and fluxes at steady state for the entire system. Following similar notations in [11,16], we denote the matrices of non-normalized concentration control coefficients and flux control coefficients by $\mathbf{\Gamma}$ and $\mathbf{\Phi}$, respectively. In the notation advocated in [34], these matrices would be denoted by $\mathbf{C}^{rS}$ and $\mathbf{C}^{rJ}$, respectively.

In a similar way as in eqs. (15) and (16), coefficients expressing the control exerted by the lumped bridging reactions may be defined. These overall control coefficients are indicated by an asterisk,

$$^*\Gamma_{2r} = (\partial X_2/\partial p_r)\cdot(\partial^* J_r/\partial p_r)^{-1}, \quad (17)$$

$$^*\Phi_{xr} = (\partial J_x/\partial p_r)\cdot(\partial^* J_r/\partial p_r)^{-1}, \quad (18)$$

with p_r being a vector of parameters influencing the rates $^*J_r$. Here, the components of $^*J_r$ play the role of 'isolated' reaction rates, which is justified if module 1 is regarded as a 'super-reaction' embedded in a larger biochemical system. If, however, module 1 is considered as a system on its own (with the molarities X_2 clamped), $^*J_r$ plays the role of a steady-state flux vector.

Whereas in usual definitions of control coefficients [3,4,34], one considers such parameters that affect particular reaction rates specifically, it is actually sufficient that the matrices in the denominators in eqs. (15)–(18) are non-singular rather than diagonal [11]. This is of importance for the modular approach, because for a given parametrization it is not, in general, possible to find parameters influencing the components of $^*J_r$ specifically. In the system shown in Fig. 2, for example, a change of any one parameter pertaining to a particular bridging reaction usually affects both lumped bridging fluxes. This does not, however, restrict the appropriateness of definitions (17) and (18).

5. Expressing control in terms of overall elasticity and network structure

Now we will show how one can calculate the control properties of a system in terms of the overall elasticity properties of its modules. By the definition

$$(\mathbf{N}_{2b} \quad \mathbf{N}_{22}) = \mathbf{L}_2\cdot(\bar{\mathbf{N}}_{2b} \quad \bar{\mathbf{N}}_{22}), \quad (19)$$

and eq. (8), the steady-state condition for module 2 reads

$$\bar{\mathbf{N}}_{2b}\cdot\mathbf{Q}\cdot{}^*\mathbf{J}_r + \bar{\mathbf{N}}_{22}\cdot{}^*\mathbf{J}_2 = \mathbf{0}. \quad (20)$$

Differentiation of this equation with respect to any parameter vector p yields, due to eq. (13),

$$\bar{\mathbf{N}}_{2b}\cdot\mathbf{Q}\cdot\left[\frac{\partial^*\mathbf{J}_r}{\partial\mathbf{p}} + {}^*\mathbf{D}_{r2}\cdot\frac{\partial\mathbf{X}_2}{\partial\mathbf{p}}\right] + \bar{\mathbf{N}}_{22}\cdot\left[\frac{\partial^*\mathbf{J}_2}{\partial\mathbf{p}} + \mathbf{D}_{22}\cdot\frac{\partial\mathbf{X}_2}{\partial\mathbf{p}}\right] = \mathbf{0}. \quad (21)$$

Owing to condition (6) and eq. (3b) we have

$$\frac{\partial\mathbf{X}_x}{\partial\mathbf{p}} = \mathbf{L}_x\cdot\frac{\partial\bar{\mathbf{X}}_x}{\partial\mathbf{p}} \quad (22)$$

with x standing for 1 or 2. Equations (21) and (22) can be combined to obtain

$$\bar{\mathbf{N}}_{2b}\cdot\mathbf{Q}\cdot\frac{\partial^*\mathbf{J}_r}{\partial\mathbf{p}} + \bar{\mathbf{N}}_{22}\cdot\frac{\partial^*\mathbf{J}_2}{\partial\mathbf{p}} + \mathscr{J}^*\cdot\frac{\partial\bar{\mathbf{X}}_2}{\partial\mathbf{p}} = \mathbf{0} \quad (23)$$

with

$$\mathscr{J}^* = \left(\bar{\mathbf{N}}_{2b}\cdot\mathbf{Q}\cdot{}^*\mathbf{D}_{r2} + \bar{\mathbf{N}}_{22}\cdot\mathbf{D}_{22}\right)\cdot\mathbf{L}_2. \quad (24)$$

Equations (23) and (24) only contain quantities outside system 1, i.e., quantities taken to be known.

The matrix $\mathscr{J}^*$ is the Jacobian matrix of module 2 taking into account the implications of the presence of module 1 on the regulations of the metabolite molarities in module 2.

Because eq. (23) is valid both for parameters only affecting the bridging reactions and for parameters only affecting reactions in module 2, it allows the calculation of the concentration control coefficients defined in eqs. (15) and (17),

$$^*\Gamma_{2r} = -\mathbf{L}_2\cdot(\mathscr{J}^*)^{-1}\cdot\bar{\mathbf{N}}_{2b}\cdot\mathbf{Q}, \quad (25a)$$

$$\Gamma_{22} = -\mathbf{L}_2\cdot(\mathscr{J}^*)^{-1}\cdot\bar{\mathbf{N}}_{22}. \quad (25b)$$

The normalized concentration control coefficients can be calculated from:

$$^*\mathbf{C}^S_{2r} = (\mathbf{diag}\ X_2)^{-1}\cdot{}^*\Gamma_{2r}\cdot(\mathbf{diag}\ J_r), \quad (26)$$

$$C^S_{22} = (\mathbf{diag}\ X_2)^{-1}\cdot\Gamma_{22}\cdot(\mathbf{diag}\ J_2). \quad (27)$$

with **diag** standing for diagonal matrix.

To obtain the flux control coefficients, one may differentiate the equations $J_x(p) = {}^*J_x(p, X_2)$ (x = r, 2) with respect to p, to obtain

$$\frac{\partial J_x}{\partial p} = \frac{\partial {}^*J_x}{\partial p} + \frac{\partial {}^*J_x}{\partial X_2} \cdot \frac{\partial X_2}{\partial p}. \tag{28}$$

From this equation, we derive

$${}^*\boldsymbol{\Phi}_{rr} = \mathbf{I} + {}^*\mathbf{D}_{r2} \cdot {}^*\boldsymbol{\Gamma}_{2r}, \tag{29a}$$

$$\boldsymbol{\Phi}_{r2} = {}^*\mathbf{D}_{r2} \cdot \boldsymbol{\Gamma}_{22}, \tag{29b}$$

$${}^*\boldsymbol{\Phi}_{2r} = \mathbf{D}_{22} \cdot {}^*\boldsymbol{\Gamma}_{2r}, \tag{30a}$$

$$\boldsymbol{\Phi}_{22} = \mathbf{I} + \mathbf{D}_{22} \cdot \boldsymbol{\Gamma}_{22}, \tag{30b}$$

with I denoting the identity matrix.

The normalized flux control coefficients are obtained by

$${}^*\mathbf{C}^J_{xy} = (\mathbf{diag}\ J_x)^{-1} {}^*\boldsymbol{\Phi}_{xy} (\mathbf{diag}\ J_y) \tag{31}$$

with x, y standing for r and/or 2.

Now we have derived expressions for all sought control coefficients in terms of quantities assumed to be known. These results show that one is able to calculate the flux and concentration control related to module 2 even without knowing the internal details of module 1. The information of the inside of module 1 that is relevant for the entire system is apparently fully represented by the overall elasticity coefficients ${}^*\mathbf{D}_{r2}$ and matrix **Q**.

The definitions (15) and (16) pertaining to 'traditional' MCA have the favorable property that the values of control coefficients are independent of the special choice of the parameter vectors p_2, as long as they fulfill the conditions that the matrix $\partial v_2/\partial p_2$ be non-singular and that the rate functions $v_2(X_2, p)$ be the same in the entire system and for the respective reactions studied in isolation [11,34,35], unless there are enzyme-enzyme interactions and/or moiety-conserved cycles involving enzymic species and free metabolites [13]. As the expressions (25), (29) and (30) for the overall control control coefficients do not contain the parameters p_r used in definitions (17) and (18), the abovementioned invariance property applies to the overall coefficients as well.

6. Control theorems

Equations (25a, b) imply, due to eq. (20),

$$({}^*\boldsymbol{\Gamma}_{2r} \quad \boldsymbol{\Gamma}_{22}) \cdot \begin{bmatrix} J_r \\ J_2 \end{bmatrix} = \mathbf{0}, \tag{32}$$

a summation theorem for the concentration control coefficients.

For the normalized coefficients, this theorem reads

$$({}^*\mathbf{C}^S_{2r} + \mathbf{C}^S_{22}) \cdot \mathbf{1} = {}^*\mathbf{C}^S_{2r} \cdot \mathbf{1} + \mathbf{C}^S_{22} \cdot \mathbf{1} = \mathbf{0}, \tag{33}$$

with $\mathbf{1}$ denoting the unit vector, $(1\ 1\ \ldots\ 1)^T$, of the appropriate length.

By using eqs. (24) and (25), also concentration control connectivity relationships can be obtained:

$${}^*\boldsymbol{\Gamma}_{2r} \cdot {}^*\mathbf{D}_{r2} \cdot \mathbf{L}_2 + \boldsymbol{\Gamma}_{22} \cdot \mathbf{D}_{22} \cdot \mathbf{L}_2 = -\mathbf{L}_2, \tag{34}$$

$$({}^*\mathbf{C}^S_{2r} \cdot {}^*\boldsymbol{\varepsilon}_{r2} + \mathbf{C}^S_{22} \cdot \boldsymbol{\varepsilon}_{22}) \cdot (\mathbf{diag}\ X_2)^{-1} \cdot \mathbf{L}_2 = -(\mathbf{diag}\ X_2)^{-1} \cdot \mathbf{L}_2, \tag{35}$$

with $\boldsymbol{\varepsilon}$ referring to normalized elasticities.

On account of eqs. (29) and (30), the flux control coefficients fulfill the summation theorem

$$\begin{pmatrix} {}^*\boldsymbol{\Phi}_{rr} & \boldsymbol{\Phi}_{r2} \\ {}^*\boldsymbol{\Phi}_{2r} & \boldsymbol{\Phi}_{22} \end{pmatrix} \cdot \begin{pmatrix} J_r \\ J_2 \end{pmatrix} = \begin{pmatrix} J_r \\ J_2 \end{pmatrix}, \tag{36}$$

and in normalized form,

$${}^*\mathbf{C}^J_{xr} \cdot \mathbf{1} + \mathbf{C}^J_{x2} \cdot \mathbf{1} = \mathbf{1}. \tag{37}$$

The connectivity relationships can be obtained to read (combining eqs. (29), (30), and (34)),

$${}^*\boldsymbol{\Phi}_{xr} \cdot {}^*\mathbf{D}_{r2} \cdot \mathbf{L}_2 + \boldsymbol{\Phi}_{x2} \cdot \mathbf{D}_{22} \cdot \mathbf{L}_2 = \mathbf{0}, \tag{38}$$

$$({}^*\mathbf{C}^J_{xr} \cdot {}^*\boldsymbol{\varepsilon}_{r2} + \mathbf{C}^J_{x2} \cdot \boldsymbol{\varepsilon}_{22}) \cdot (\mathbf{diag}\ X_2)^{-1} \cdot \mathbf{L}_2 = \mathbf{0} \tag{39}$$

These theorems are similar to those obtained when treating the system in terms of all individual reactions. The novelty is that in the present theorems a large part of the metabolic network can be treated as a single component (with its small set of elasticity coefficients and control coefficients). In a system consisting of ten reactions, for example, grouping of eight of them into

a module of type 1 leads to summation and connectivity theorems with three terms each, while the traditional theorems would have ten terms each.

A practically important situation is when the fluxes through all lumped bridging reactions are changed by the same fractional amount, α (e.g. by changing the number of mitochondria in a suspension),

$$\delta \ln {}^*J_{r_k} = \alpha \qquad \text{for any } k. \tag{40}$$

In that case the control exerted by module 1 as a whole can, due to eqs. (26), (31) and the relation $(\mathbf{diag}\ {}^*\boldsymbol{J}_r)\boldsymbol{1} = {}^*\boldsymbol{J}_r$ be expressed by the sum of all normalized overall control coefficients pertaining to this module:

$$\delta \ln J_r = {}^*\mathbf{C}^{\mathrm{J}}_{rr}\alpha \boldsymbol{1}, \tag{41a}$$

$$\delta \ln J_2 = {}^*\mathbf{C}^{\mathrm{J}}_{2r}\alpha \boldsymbol{1}, \tag{41b}$$

$$\delta \ln X_2 = {}^*\mathbf{C}^{\mathrm{S}}_{2r}\alpha \boldsymbol{1}, \tag{41c}$$

where $\ln \boldsymbol{J}_r$ stands for the vector of logarithms of the components of $\boldsymbol{J}_r$. This result can readily be understood on the basis of the summation theorems.

7. Two black-box modules with a number of metabolites in between

We wish to consider the case where two modules of type 1 are connected by an arbitrary number of metabolites, which constitute a module of type 2, as shown in Fig. 3A. The two modules of type 1 can be regarded as the upper part and the lower part of a metabolic pathway, and are accordingly referred to by indices u and ℓ, respectively. The complete reaction scheme compares to Fig. 3B, where the upper and the lower part have each been replaced by an enzyme. The essence of the problem we address here, is to see if Fig. 3A can be treated in terms of simplified schemes such as Fig. 3B. This involves treating the upper and the lower part of the pathway as a unit each. Both of the units should then have elasticity coefficients with respect to the intermediates and should be conceived of as possibly exerting control on the fluxes through the pathway or on the intermediates, X_1, X_2 and X_3.

The simplest case is when there is only one metabolite between the upper and lower parts. For this case, the problem outlined above has been dealt with earlier [7,20–22,36,37].

In our general treatment, we now have two *vectors* of bridging fluxes, $\boldsymbol{J}_u$ and $\boldsymbol{J}_\ell$, and two *matrices* of overall elasticities,

$${}^*\mathbf{D}_{u2} = \partial {}^*\boldsymbol{J}_u / \partial \boldsymbol{X}_2, \tag{42a}$$

$${}^*\mathbf{D}_{\ell 2} = \partial {}^*\boldsymbol{J}_\ell / \partial \boldsymbol{X}_2, \tag{42b}$$

where ${}^*\boldsymbol{J}_u$ and ${}^*\boldsymbol{J}_\ell$ denote steady-state fluxes of the modules when the rest of the system is clamped. To simplify the notation, we suppose that ${}^*\boldsymbol{J}_u$ and ${}^*\boldsymbol{J}_\ell$ have already been reduced to contain independent fluxes only.

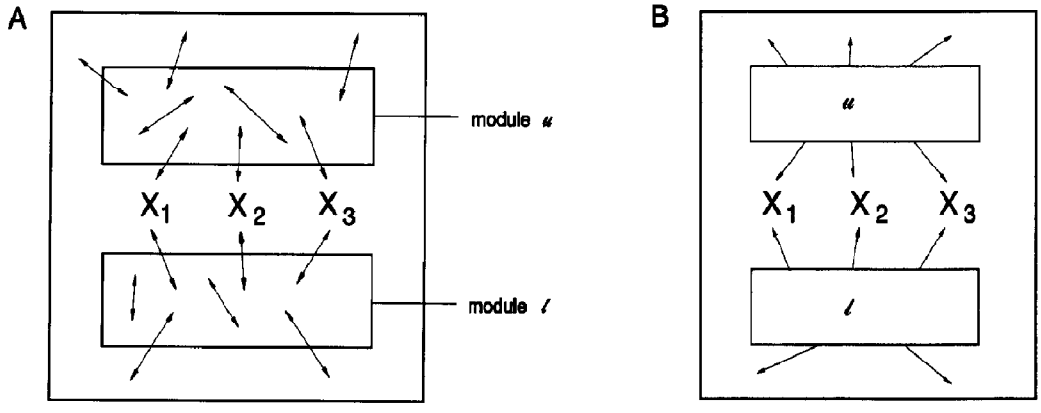

Fig. 3. Schematic picture of a system with two type 1 modules. A, scheme comprising all reactions; B, scheme showing the black-box modules as 'superenzymes'.

In definition (42), it has actually to be indicated whether the metabolites in the lower (upper) module are clamped or allowed to attain steady state when $^*D_{u2}$ ($^*D_{\ell 2}$) is determined. The absence of stoichiometric interactions between the upper and lower modules simplifies the situation; if we confine these modules so that there are no direct interactions between them either (e.g. allosteric influences), the concentrations within the upper module have no effect on the overall elasticities of the lower and *vice versa*. When interactions between the two type 1 modules do exist, one needs to define the overall elasticities such that both the ℓ and the u module regain steady state.

Since module 2 does not contain any reaction in the case considered, there is no matrix with an index 2 referring to fluxes (such as $\mathbf{D}_{2x}$, Γ_{x2}, ...). As above, we have to assume that no conservation relationship links X_2 with either the upper or the lower module. This means that matrix L can be decomposed into three diagonal blocks. From the structure of the system, it follows that there is no conservation relationship linking components of X_2; L_2 is therefore the identity matrix.

Although the upper and lower modules are not connected stoichiometrically, they can be formally combined into one module of type 1. Thus, the vectors J_u and J_ℓ can be regarded as subvectors of the vector J_r introduced in Section 3,

$$J_r = \begin{pmatrix} J_u \\ J_\ell \end{pmatrix}. \tag{43}$$

Accordingly, we have

$$^*\mathbf{D}_{r2} = \begin{pmatrix} ^*\mathbf{D}_{u2} \\ ^*\mathbf{D}_{\ell 2} \end{pmatrix}, \tag{44}$$

$$\mathscr{J}^* = \bar{\mathbf{N}}_{2u}\mathbf{Q}_u{}^*\mathbf{D}_{u2} + \bar{\bar{\mathbf{N}}}_{2\ell}\mathbf{Q}_\ell{}^*\mathbf{D}_{\ell 2}. \tag{45}$$

Thus, we obtain

$$^*\Gamma_{2r} = (^*\Gamma_{2u} \quad ^*\Gamma_{2\ell}) = -(\mathscr{J}^*)^{-1}(\bar{\mathbf{N}}_{2u}\cdot\mathbf{Q}_u \quad \bar{\bar{\mathbf{N}}}_{2\ell}\cdot\mathbf{Q}_\ell), \tag{46}$$

$$^*\Phi_{rr} = \begin{pmatrix} ^*\Phi_{uu} & ^*\Phi_{u\ell} \\ ^*\Phi_{\ell u} & ^*\Phi_{\ell\ell} \end{pmatrix} = \begin{pmatrix} \mathbf{I} + ^*\mathbf{D}_{u2}\cdot{}^*\Gamma_{2u} & ^*\mathbf{D}_{u2}\cdot{}^*\Gamma_{2\ell} \\ ^*\mathbf{D}_{\ell 2}\cdot{}^*\Gamma_{2u} & \mathbf{I} + ^*\mathbf{D}_{\ell 2}\cdot{}^*\Gamma_{2\ell} \end{pmatrix}. \tag{47}$$

$^*\Phi_{u\ell}$ and $^*\Phi_{\ell u}$ express the flux control exerted by the upper and lower modules upon each other. The normalized control coefficients are found in the same way as in eq. (31).

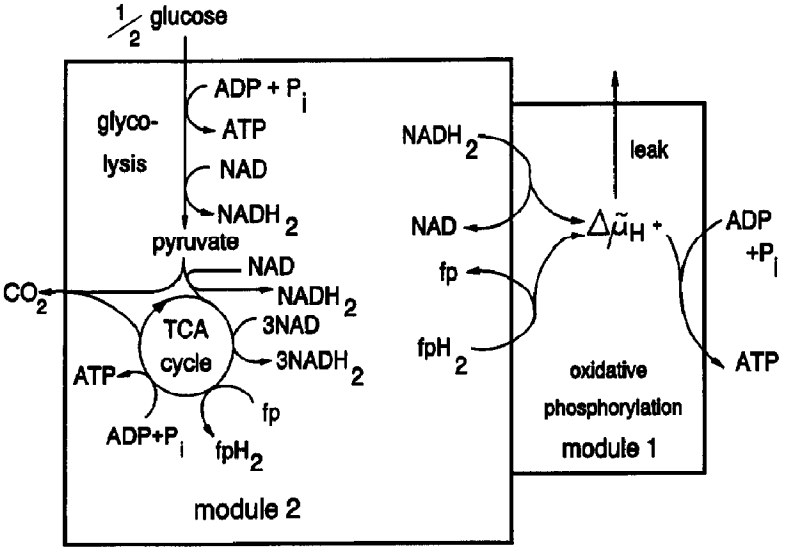

Fig. 4. Reaction scheme representing glycolysis, TCA cycle and oxidative phosphorylation as an example of a modular system. Nucleoside-diphosphokinases have been assumed to be at equilibrium.

The summation and connectivity relationships for the non-normalized control coefficients read

$$^*\Gamma_{2u} \cdot J_u + {}^*\Gamma_{2\ell} \cdot J_\ell = 0, \tag{48}$$

$$\begin{pmatrix} ^*\Phi_{uu} & ^*\Phi_{u\ell} \\ ^*\Phi_{\ell u} & ^*\Phi_{\ell\ell} \end{pmatrix} \cdot \begin{pmatrix} J_u \\ J_\ell \end{pmatrix} = \begin{pmatrix} J_u \\ J_\ell \end{pmatrix}, \tag{49}$$

$$^*\Gamma_{2u} \cdot {}^*\mathbf{D}_{u2} + {}^*\Gamma_{2\ell} \cdot {}^*\mathbf{D}_{\ell 2} = -\mathbf{I}, \tag{50}$$

$$^*\Phi_{xu} \cdot {}^*\mathbf{D}_{u2} + {}^*\Phi_{x\ell} \cdot {}^*\mathbf{D}_{\ell 2} = \mathbf{0}. \tag{51}$$

Again, these theorems contain less components than the traditional theorems containing the coefficients pertaining to all the enzymes.

Choosing a system with the black-box modules having only one degree of freedom each and with only one intermediate linking these modules, the summation and connectivity relationships given by Brown and coworkers [22] obtain as special cases from eqs. (48)–(51).

The results of this section show that the situation of several modules of type 1 can be treated by a straightforward generalization of the formalism set out in the previous sections.

8. Examples of application

Two of the major ATP generating pathways of the cell often operate in series; the product of glycolysis, pyruvate, serves as a carbon substrate for the TCA cycle, which is again hooked up to oxidative phosphorylation (Fig. 4). It is common practice to study parts of this system, i.e., glycolysis, TCA cycle and oxidative phosphorylation independently. Consequently, this paper may only serve to formalize that approach and to show how the information gained from the studies of the independent parts may be used to understand the control of the system as a whole.

The module of oxidative phosphorylation has five input fluxes ($NADH_2$, reduced flavoprotein, O_2, ADP and inorganic phosphate) and five output fluxes (NAD, oxidized flavoprotein, leaking protons, H_2O and ATP). For chemical stoichiometry reasons, these bridging fluxes are not independent of each other. A matrix **Q** relates these fluxes to independent bridging fluxes which may be chosen as the fluxes of $NADH_2$ oxidation, fpH_2 oxidation, proton leak and ATP production (J_N, J_f, J_l and J_p, respectively, with $\frac{1}{2}J_{O_2} = J_{H_2O} = J_N + J_f$ and $J_p = -J_{ADP} = -J_{P_i}$).

In terms of the treatment given above, oxidative phosphorylation may be regarded as module 1. We shall consider the rest of the system as module 2. The proton leak flux can formally be considered as a bridging flux although it does not end in module 2. An example of an elasticity coefficient of module 2 is the elasticity coefficient of the pyruvate dehydrogenase complex with respect to $NADH_2$:

$$D_{\mathrm{PdC,NADH_2}} = \frac{\partial v_{\mathrm{PdC}}}{\partial \mathrm{NADH_2}}. \tag{52}$$

This elasticity coefficient would be measured by varying the $NADH_2$ concentration at constant magnitude of all other concentrations in this system. The problem that $NADH_2 + NAD$ is constant would be taken care of by a matrix L_2, cf. above; alternatively one may immediately define this elasticity coefficient in terms of independent variables by taking the derivative at constant $NADH_2 + NAD$, giving

$$D_{\mathrm{PdC,NADH_2}} = \left(\frac{\partial v_{\mathrm{PdC}}}{\partial \mathrm{NADH_2}}\right)_{\mathrm{NAD}} - \left(\frac{\partial v_{\mathrm{PdC}}}{\partial \mathrm{NAD}}\right)_{\mathrm{NADH_2}}. \tag{53}$$

In experimental practice it would be difficult to maintain the electrochemical potential difference for protons across the inner mitochondrial membrane ($\Delta\tilde{\mu}_H$) constant. Consequently, the corresponding overall elasticity coefficient would be more accessible experimentally. This coefficient is defined as the analogous derivative, but now allowing $\Delta\tilde{\mu}_H$ to reattain steady state,

$$^*D_{\mathrm{PdC,NADH_2}} = \left(\frac{\partial v_{\mathrm{PdC}}}{\partial \mathrm{NADH_2}}\right)_{\mathrm{oxphos,ss}} = D_{\mathrm{PdC,NADH_2}} + \left(\frac{\partial v_{\mathrm{PdC}}}{\partial \Delta\tilde{\mu}_H}\right) \cdot \left(\frac{\partial \Delta\tilde{\mu}_{\mathrm{II}}}{\partial \mathrm{NADH_2}}\right)_{\mathrm{oxphos,ss}}, \tag{54}$$

where the subscript "oxphos,ss" means that module 1 (oxidative phosphorylation) is allowed to attain steady state.

Since the pyruvate dehydrogenase complex is not a transmembrane enzyme, the second term of the equation is zero; as often, the overall elasticity coefficients of the external module are equal to its standard elasticity coefficients.

The overall elasticity coefficients for the bridging reactions differ from the standard ones. They incorporate effects within module 1, such as the coupling between $\Delta\tilde{\mu}_H$ and ATP synthesis. An example is the overall elasticity coefficient of mitochondrial ATP synthesis with respect to $NADH_2$:

$$
\begin{aligned}
{}^{*}D_{p,NADH_2} &= \left(\frac{\partial v_p}{\partial NADH_2}\right)_{oxphos,ss} \\
&= \frac{\partial v_p}{\partial \Delta\tilde{\mu}_H} \cdot \left(\frac{\partial \Delta\tilde{\mu}_H}{\partial NADH_2}\right)_{oxphos,ss} \\
&= D_{p,\Delta\tilde{\mu}_H} \cdot \gamma_0^{\Delta\tilde{\mu}_H} \cdot D_{0,NADH_2}, \qquad (55)
\end{aligned}
$$

where $\gamma_0^{\Delta\tilde{\mu}_H}$ is the internal control coefficient of respiration rate with respect to proton-motive force ('internal' means under conditions of constant concentrations in module 2) [cf. 7,36,37].

The overall elasticity coefficient ${}^{*}D_{p,NADH_2}$ may be measured experimentally by controlling the $NADH_2$, ATP, etc. concentrations, but not $\Delta\tilde{\mu}_H$ and measuring the change in ATP synthesis rate when $NADH_2$ is brought to a different level. As shown by the above equation this overall elasticity coefficient can also be expressed in terms of the control properties of module 1 alone [7] (it should be noted that $\gamma_0^{\Delta\tilde{\mu}_H}$ differs from $\Gamma_0^{\Delta\tilde{\mu}_H}$, the latter comprising indirect regulation of $\Delta\tilde{\mu}_H$ by mitochondrial respiration involving changes in module 2).

Overall control coefficients of particular interest are those that quantify the notion that the process of oxidative phosphorylation controls steady-state fluxes in module 2. The overall control coefficient of (oxidative) phosphorylation upon the glycolytic flux is defined by the effect on the steady-state glycolytic flux of a small activation of the H^+-ATPase:

$$
{}^{*}\Phi_{glyc,p} = \left(\frac{\partial J_{glyc}}{\partial p_p} \Big/ \frac{\partial v_p}{\partial p_p}\right)_{oxphos,ss} \qquad (56)
$$

Similarly the control on glycolytic flux by the proton permeability of the inner mitochondrial membrane, ${}^{*}\Phi_{glyc,l}$, and that of fpH_2 and $NADH_2$ oxidation may be defined. The latter includes the Pasteur effect.

For the glycolytic flux, the summation theorem (eq. 36) for these control coefficients, reads

$$
\begin{aligned}
&\sum_{\text{module 2}} \Phi_{glyc,k} \cdot J_k + {}^{*}\Phi_{glyc,p} \cdot v_p + {}^{*}\Phi_{glyc,N} \cdot v_N \\
&+ {}^{*}\Phi_{glyc,f} \cdot v_f + {}^{*}\Phi_{glyc,l} \cdot v_1 = J_{glyc}, \qquad (57)
\end{aligned}
$$

or, in terms of normalized control coefficients,

$$
\begin{aligned}
&\sum_{\text{module 2}} C^J_{glyc,k} + {}^{*}C^J_{glyc,p} + {}^{*}C^J_{glyc,N} + {}^{*}C^J_{glyc,f} \\
&+ {}^{*}C^J_{glyc,l} = 1, \qquad (58)
\end{aligned}
$$

which may be written as

$$
\sum_{\text{module 2}} C^J_{glyc,k} + {}^{*}C^J_{glyc,op} = 1. \qquad (59)
$$

The latter control coefficient quantifies the control exerted by the process of oxidative phosphorylation as such (i.e., as a whole) on glycolysis. Operationally it quantifies the effect on steady-state glycolytic flux of a simultaneous increase of all partial processes of mitochondrial oxidative phosphorylation by the same, small percentage (cf. eq. 44b).

The connectivity relationships can now be expressed in terms of overall control and overall elasticity coefficients, further emphasizing that the process of oxidative phosphorylation, in reality consisting of more than four subprocesses, may be treated as a single process catalyzed by a 'superenzyme'. For instance, with respect to $NADH_2$ the connectivity theorem for the glycolytic flux reads:

$$
\begin{aligned}
&\sum_{\text{module 2}} C^J_{glyc,k} \cdot \varepsilon_{k,NADH_2} + {}^{*}C^J_{glyc,N} \cdot \varepsilon_{N,NADH_2} \\
&+ {}^{*}C^J_{glyc,f} \cdot \varepsilon_{f,NADH_2} + {}^{*}C^J_{glyc,p} \cdot \varepsilon_{p,NADH_2} \\
&+ {}^{*}C^J_{glyc,l} \cdot \varepsilon_{1,NADH_2} = 0. \qquad (60)
\end{aligned}
$$

This may be written as

$$
\begin{aligned}
&\sum_{\text{module 2}} C^J_{glyc,k} \cdot \varepsilon_{k,NADH_2} \\
&+ \left({}^{*}C^J_{glyc,l}\right)^T \cdot \varepsilon_{1,NADH_2} = 0, \qquad (61)
\end{aligned}
$$

where $^*\varepsilon_{1,NADH_2}$ is the vector (column matrix) $(^*\varepsilon_{N,NADH_2}, {}^*\varepsilon_{f,NADH_2}, {}^*\varepsilon_{p,NADH_2}, {}^*\varepsilon_{1,NADH_2})^T$ and $(^*C^J_{glyc,1})^T$ is the vector (row matrix) $(^*C^J_{glyc,N}, {}^*C^J_{glyc,f}, {}^*C^J_{glyc,p}, C^J_{glyc,1})$. It is noteworthy that the 'superenzyme' that catalyzes oxidative phosphorylation cannot be fully characterized by a single (scalar) overall control coefficient and a single (scalar) overall elasticity. Indeed, although $\epsilon_{p,NADH_2}$ is probably zero, the corresponding overall elasticity coefficient $^*\varepsilon_{p,NADH_2}$ is not, as can be seen from the equation expressing the corresponding absolute elasticity coefficient (eq. 55); as $NADH_2$ is changed, $\Delta\tilde{\mu}_H$ will change and affect the rate of phosphorylation.

By use of the equations given above, the overall control coefficients can be expressed into the overall elasticity coefficients. The procedure is straightforward, though somewhat tedious, which is why we do not elaborate on it here. The result can be summarized by stating that the four processes involved in oxidative phosphorylation can be grouped together and treated as if they constitute a single process, catalyzed by a single superenzyme.

An alternative to the subdivision of Fig. 4 discussed above is to group all intramitochondrial processes, i.e., TCA cycle plus oxidative phospho-

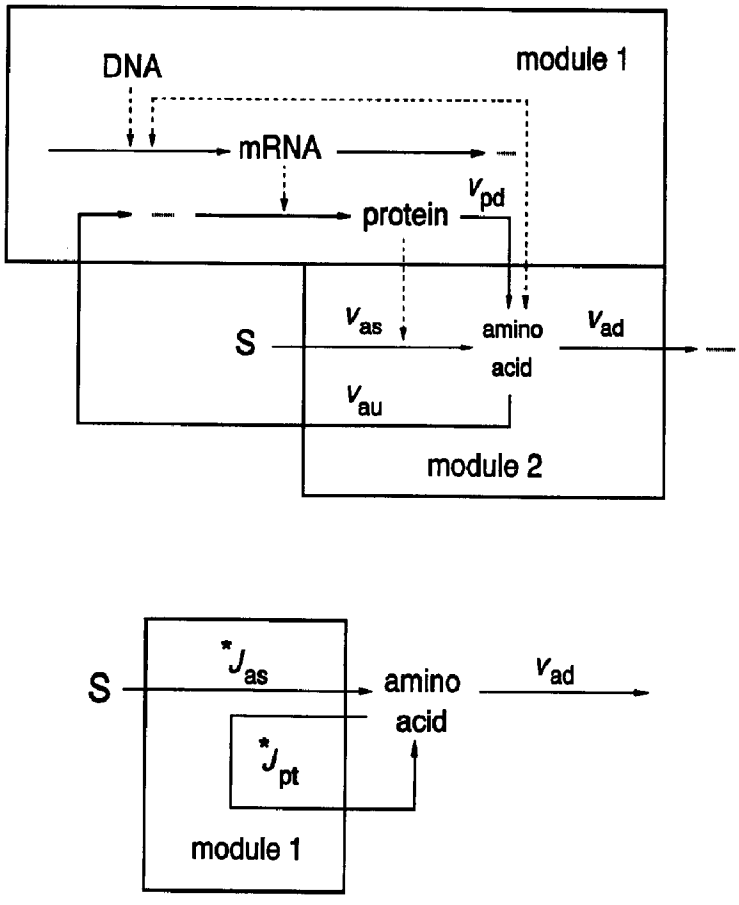

Fig. 5. Metabolic regulation involving gene expression as an example of connected modules. (A) complete scheme, (B) simplified scheme. v_{as}, rate of amino acid synthesis; v_{au}, rate of amino acid utilization for biosynthesis of enzymes involved in amino acid synthesis; v_{ad}, rate of amino acid utilization other than v_{au} (e.g. degradation of amino acid, synthesis of other proteins); v_{pd}, rate of protein degradation; $^*J_{pt}$, flux of protein turnover.

rylation together into an inner module; all extramitochondrial processes constituting the external module ('module 2'). In this case the independent bridging fluxes are the import of pyruvate, $NADH_2$ (involving a shuttle), ADP (in exchange for ATP), and the export of CO_2. Otherwise the treatment is similar to the one given above.

A completely different example of modular control analysis is regulation of a metabolic flux through gene expression (see Fig. 5). Previously such a type of network was discussed in terms of unconnected modules [14–16]. The treatment developed here, however, allows us *not* to neglect the fact that the substrates for protein synthesis are metabolites themselves and may thus affect this process. From the point of view of metabolic regulation, one may consider the processes related to mRNA and enzyme synthesis together as a black-box module, the internal characteristics of which are unknown. In this case the two modules are connected by direct effects, one corresponding to the regulation of transcription (and/or other processes in module 1, this is of no importance for the treatment) by the amino acid, the other to the effect of the enzyme on the rate of amino acid synthesis. To cope with the latter effect, one can either restrict the changes in module 1 in such a way that the concentration of the considered protein is not affected, or redraw the scheme in more detail, such that the direct (in this case, catalytic) effect is represented as a 'stoichiometric' interaction. After simplification of module 1 to a black-box, this gives rise to the scheme shown in Fig. 5B. Note that there is no conservation relation linking the two modules because the amino acid is subject to turnover.

The overall elasticity coefficients now contain the cross effects between the modules. For instance:

$$*D_{as,A} = \frac{\partial v_{as}}{\partial A} + \frac{\partial v_{as}}{\partial P} \cdot \left(\frac{dP}{dA}\right)_{ss-1} \qquad (62)$$

(A and P referring to the amino acid and protein of interest). The latter factor, $(dP/dA)_{ss-1}$, corresponds to change occurring in the concentration of protein when the concentration of amino acid is changed, at constant values of the other concentrations in module 2 (such as S) but allowing module 1 to reattain steady state.

Because module 1 would tend to a be a slower subsystem than module 2, the measurement of $*D_{as,A}$ is not straightforward. It may, however, be accomplished *in situ* by maintaining the concentrations of module 2 constant by combining various modulations, or an *in vitro* system comprising v_{as} plus module 1, may be studied. Alternatively $(dP/dA)_{ss-1}$ can be measured by studying module 1 in isolation (though under the same conditions), or it can be calculated from independently determined control properties of module 1 (cf. [7]).

The modules are also connected by the flow of amino acid A into protein P (and, in steady state, back to A). This bridging flux will have a direct as well as an overall elasticity coefficient with respect to A:

$$*D_{au,A} = D_{au,A} + D_{au,P} \cdot \left(\frac{dP}{dA}\right)_{ss-1} \qquad (63)$$

Thus, these two elasticities differ in the event that protein P affects its own synthesis. A similar equation holds for the elasticities with respect to protein degradation. By eq. (14), we obtain the equation

$$*D_{pt,A} = *D_{au,A} = *D_{pd,A}, \qquad (64)$$

which implies a relation among the direct elasticities.

In this system the control coefficients of module 1 may again be grouped together in terms of overall control coefficients. In terms of the relative control coefficients:

$$*C^J_{as,as} + C^J_{as,ad} + *C^J_{as,pt} = 1, \qquad (65)$$

and similiarly for the coefficients expressing the control of the protein turnover flux. A connectivity theorem reads

$$*C^J_{as,as} \cdot *\varepsilon_{as,A} + C^J_{as,ad} \cdot \varepsilon_{ad,A} + *C^J_{as,pt} \cdot \varepsilon^*_{pt,A} = 0. \qquad (66)$$

This again shows that the module of gene expression may just be treated as another enzyme present in the metabolic system.

Additional examples where the modular approach should be useful include the control of metabolism as it is governed by an interplay of various tissues. For instance, one module could be the liver, synthesizing glucose from lactate and glycogen. The other module could be peripheral organs converting the glucose to lactate. A third module representing glucose uptake by the intestines would subsequently be added to the analysis. The treatments are analogous to those given above. Now entire organs may be treated as enzymes in an otherwise virtually standard control analysis.

9. Discussion

Traditional methods in MCA serve to express control coefficients in terms of all the molecular elasticity coefficients. Here we acknowledge that many molecular elasticity coefficients may not be known or may not be readily measurable [18,36,37] and that it is important to express control in terms of more global properties of some specified modules and of the regulatory interactions between the modules. Moreover, even if detailed information about all reactions and metabolites were available, modularization of a system can facilitate the analysis and understanding by structuring information.

Another method for calculating control coefficients without knowledge of all elasticity coefficients is the dynamic approach of Delgado and Liao [38,39]. This method requires, however, knowledge of transient concentrations of all metabolites, which is hardly available if some parts of the system are not directly accessible to observation.

By our approach, control may be understood in terms of a few properties of the modules and the interactions between them, rather than in terms of the possibly myriad of properties within the modules. Not only does this allow for a simpler, i.e., stepwise approach to the understanding of control and regulation. It also allows one to analyze control even if one does not (or cannot) know the details of all the molecular properties of parts of the system. Such parts can remain 'black boxes' of which only the input–output characteristics need to be known.

In traditional MCA, the global properties of metabolic networks are calculated in terms of the local properties of enzymes. In the frequently occurring cases where the local properties are not completely known, it is more appropriate to derive the global features from 'semi-global' properties of certain subsystems (modules) of the network. Such kind of approach has been referred to as 'overall approach' [7,36,37] or 'top-down approach' [22,23].

The present analysis requires consideration of a number of fluxes which link the particular modules with each other ($^*J_r$ in our notation). These fluxes are obtained by a suitable choice of linearly independent bridging reactions. This reduction is necessary in order that the matrix $(\partial {}^*J_r/\partial p_r)$, the inverse of which is involved in the definition of overall control coefficients, be nonsingular, or in other words, in order for these control coefficients to be defined uniquely. Unlike in Savageau's aggregation method [5,6], the number of degrees of freedom with respect to steady-state flux is retained in the present reduction method.

The cardinal quantities of the present approach are the overall control coefficients, which differ from the traditional control coefficients both conceptually and mathematically (unless particular enzymatic reactions are treated as modules, see below). The former express the control as exerted by the lumped reactions of module 1 as a whole. Mathematically, the two types of coefficients differ in that the denominator in the definitions of overall coefficients is a matrix of *overall* π-elasticities, $\partial {}^*J_r/\partial p_r$. Note that, in modification to a basic idea of the top-down approach [7,22,23,36,37], the overall control coefficients as defined by eqs. (17) and (18) are not restricted to the situation that all enzymes belonging to one module (nor even to one lumped reaction) are changed by the same fractional amount.

As was recently done also by other authors [24,40], we restricted the analysis to situations when substances within the black-box modules do not exert any direct influences on their environment (i.e. influences not mediated by the bridging

reactions). This allows to define overall control coefficients independent of choice of the parameters perturbed. For the same reason, one has to exclude conservation relations linking modules of different type. If the parameter independence of control coefficients is not invoked, the above restrictive assumptions can be dropped (Schuster et al., in preparation).

In Section 7, we have shown that a system consisting of two modules of type 1 that are connected through a number of metabolic intermediates, may be described as a system consisting of two 'enzymes', with the extra complication that each of these enzymes carries out several reactions, which are not strictly coupled.

In situations with several modules of type 1, one has to distinguish two cases according to whether the overall elasticities of each of these modules are determined by clamping the concentrations in all other 'black-box' modules or by allowing them to attain steady state. In the former case, direct influences from within one module of type 1 leading into other modules of type 1 have to be excluded.

For the overall coefficients, we established summation and connectivity theorems. Together with the classical summation relations, these theorems can be used to establish relations between the overall control coefficients and those pertaining to the particular reactions of module 1. For example, if module 1 is an unbranched reaction chain, the overall control coefficients are simply the sum of the individual control coefficients [2,3,28]. For brevity's sake, we did not elaborate on these relations, all the more as we considered the modules of type 1 as black-boxes throughout this paper.

Since an enzymatic reaction as composed of elemental steps can often be treated as a steady-state module in the sense defined above, our analysis may be considered as a generalization of conventional MCA. In this view, conventional control coefficients are identical to the overall control coefficients pertaining to the catalytic cycle of a particular enzyme. It is therefore not surprising that equations involving overall control coefficients have a similar structure to classical MCA equations. This generalization may be particularly useful when dealing with slipping enzymes (i.e. enzymes that catalyse several fluxes), a difficult problem in conventional MCA [7,41,42].

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