Pergamon

Chemical Engineering Science, Vol. 52, No. 15, pp. 2599–2606, 1997

PII: S0009-2509(97)00076-6
0009-2509/97 $17.00 + 0.00

A general formalism for Metabolic Control Analysis

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(Accepted 29 May 1996)

Abstract—A general formalism for Metabolic Control Analysis is derived using general sensitivity analysis and structural information of the metabolic pathway inherent in the stoichiometry matrix. The equations derived provide a general procedure for calculating the control coefficients from the elasticity coefficients using matrix algebra, and is valid for any pathway stoichiometry. The procedure diminishes the risk of deriving erroneous relations and is, due to its generality, well suited for computer handling. The formalism is mathematically stringent and is a complement to the original theorems of Metabolic Control Analysis, which were derived using ad hoc reasoning. © 1997 Elsevier Science Ltd

INTRODUCTION

Metabolic Control Analysis (MCA) was developed independently in the early seventies by Kacser and Burns (1973) and by Heinrich and Rapoport (1974) [for a review see Fell (1992)] and is now a well established and important framework for quantifying how the control of steady-state fluxes and concentrations is distributed between the different reactions in a metabolic pathway.

In MCA, three types of coefficients are defined; flux control coefficients, concentration control coefficients, and elasticity coefficients. The flux control coefficients are defined as

$$C_i^{J_j} = \frac{\mathrm{d}J_j}{\mathrm{d}e_i}\cdot\frac{e_i}{J_j} = \frac{\mathrm{d}\ln J_j}{\mathrm{d}\ln e_i} \tag{1}$$

where J_j is the steady-state flux through enzyme E_j and e_i is the concentration of enzyme E_i. The value of a flux control coefficient is a measure of how a change in the concentration of enzyme E_i affects the steady-state flux through enzyme E_j, i.e. the degree of control exerted by enzyme E_i on this steady-state flux. The concentration control coefficients are similarly defined as

$$C_i^{x_j} = \frac{\mathrm{d}x_j}{\mathrm{d}e_i}\cdot\frac{e_i}{x_j} = \frac{\mathrm{d}\ln x_j}{\mathrm{d}\ln e_i} \tag{2}$$

where x_j is the steady-state concentration of metabolite X_j. The value of the concentration control coefficient is a measure of the degree of control exerted by enzyme E_i on the steady-state concentration x_j.

The third type of coefficients, elasticity coefficients, are defined as

$$\varepsilon_j^i = \frac{\partial v_i}{\partial x_j}\cdot\frac{x_j}{v_i} = \frac{\partial \ln v_i}{\partial \ln x_j} \tag{3}$$

where v_i is the reaction rate of enzyme E_i and x_j is the concentration of metabolite X_j. An elasticity coefficient is a measure of how the reaction rate v_i will respond to variations in the concentration x_j when all other concentrations are kept constant.

Both types of control coefficients are global properties in the sense that they reflect the state of the whole pathway. Making a change in a parameter of the pathway (e.g. a K_m value, an enzyme concentration, etc.) will affect all control coefficients of the pathway. An elasticity coefficient, on the other hand, is a local property (hence the partial derivative). It only reflects the state of a single enzyme and its effectors. An enzyme has as many non-zero elasticity coefficients as there are metabolites affecting it (substrate, product, inhibitor, etc.).

In the early papers of MCA (Kacser and Burns, 1973; Heinrich and Rapoport, 1974) two types of relationships were derived, called summation theorems and connectivity theorems. The flux summation theorem states that the flux control coefficients of a metabolic pathway always add up to unity and the concentration summation theorem states that the concentration control coefficients add up to zero. The flux summation theorem indicates that the term 'rate-limiting step' implies that the flux control coefficient for this step would have a value of 1, while all other flux control coefficients would be 0. This is a very

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unlikely situation, especially in a complex pathway. The term 'rate-limiting step' should therefore be replaced by quantitative measures of the controlling capability of each reaction step, given by the flux control coefficients. The connectivity theorems relate the elasticity coefficients to the control coefficients and together with the summation theorems they form a linear equation system that makes it possible to calculate the global properties, the control coefficients, from the local properties, the elasticity coefficients. If the pathway contains branches or moiety-conserved cycles, additional theorems, called branch point and substrate cycle theorems (Fell and Sauro, 1985), must be used in conjunction with the original theorems to solve the equation system.

The traditional theorems of MCA have two disadvantages. Firstly, different sets of theorems must be used for pathways with different stoichiometry. This makes it cumbersome to formulate the equation system even for moderately complex stoichiometries. Secondly, the theorems rely on the assumption that the reaction rate of an enzyme is directly proportional to the concentration of the enzyme. Reder (Reder, 1988) proposed a general formalism for the relationships between the coefficients of MCA by separating the structural relationships of a pathway from the kinetic properties. In this formalism, the unscaled equivalents of the control and elasticity coefficients are used. The method is general in the sense that it is valid for any pathway stoichiometry and it does not rely, as the original theorems of MCA do, on the assumption of direct proportionality between the enzyme concentrations and the reaction rates.

In the present paper a slightly different approach is used in deriving a general formalism for MCA. The formalism is a development and a generalisation of the results of Cascante *et al.* (1989a, b), based on general sensitivity analysis. It is, like the formalism proposed by Reder, valid for any pathway stoichiometry. Also, it does not rely on the assumption of direct proportionality between the enzyme concentrations and the reaction rates. An advantage compared with the formalism of Reder is that it uses the widely accepted scaled versions of the control and elasticity coefficients.

In the derivations to follow the term metabolite often refers, for simplicity, to a metabolite concentration and the term reaction often refers to the rate of an enzyme-catalysed reaction or to the flux in a membrane-associated transport step. In the latter case the transport can occur without chemical modification of the metabolite. The concentration of the metabolite on each side of the membrane, though, is likely to differ and both these concentrations are thus considered to be separate metabolites. A distinction is made between internal and external metabolites. The concentrations of the internal metabolites are variables of the system, while the concentrations of the external metabolites are assumed to be constant. The external metabolites are usually the substrates and products of the pathway and these concentrations must be kept constant if a steady-state or quasi-steady-state should be obtainable, which can be experimentally accomplished, for instance, in a chemostat culture.

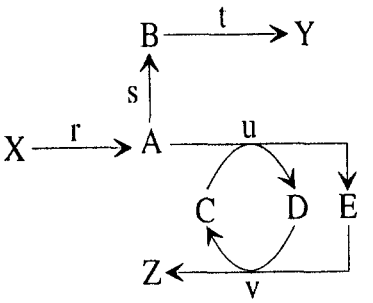

Fig. 1. Metabolic pathway used in the example.

The derivations will be presented in general terms but the use of the derived equations will be illustrated by examples. These examples apply to the pathway shown in Fig. 1. The pathway contains 5 internal metabolites, A, B, C, D, and E, with concentrations *A*, *B*, *C*, *D*, and *E*, and 5 enzymatic reactions, r, s, t, u, and v, with reaction rates *r*, *s*, *t*, *u*, *and v*. X, Y, and Z are external metabolites.

STRUCTURAL RELATIONSHIPS

Consider an arbitrary metabolic pathway containing m internal metabolites and n reactions. The stoichiometry of the pathway can be conveniently described by the $m \times n$ matrix **N**, called the stoichiometry matrix. Each row in **N** corresponds to an internal metabolite (concentration) and each column corresponds to a reaction (rate). The elements, n_{ij}, of **N** are defined as follows (Reder, 1988):

$n_{ij} = +z$ if the reaction j produces z molecules of metabolite i

$n_{ij} = -z$ if the reaction j consumes z molecules of metabolite i

$n_{ij} = 0$ if the reaction j neither produces nor consumes metabolite i.

The structural relationships of the pathway can be readily deduced from the stoichiometric matrix. Suppose that **N** has the rank m_0, where m_0 is less than or equal to the number of rows, m. This means that **N** contains m_0 independent rows and the remaining $m - m_0$ dependent rows can be expressed as linear combinations of the independent rows. Since each row in **N** corresponds to a metabolite, this also means that if m_0 is less than m, the pathway will contain dependent metabolites which can be expressed in terms of the independent metabolites. There may be several combinations of metabolites (rows in **N**) that can be chosen as independent and the choice is arbitrary. To simplify the derivation below, the metabolites are renumbered so that the independent rows are the first m_0 rows of **N**.

Since the column rank of a matrix is always equal to its row rank, the matrix **N** also contains m_0 independent columns. Again, several combinations of independent columns may exist and again the choice is arbitrary. The columns are renumbered so that the m_0 independent columns are the last columns of **N**. The number of dependent columns, equal to $n - m_0$, is denoted n_0. The dependent columns are thus the n_0 first columns of **N**.

Three new matrices are constructed from **N** for use in the derivations below. The first consists of the m_0 independent (first) rows of **N** and is denoted $\mathbf{N}_R$. The second consists of the m_0 independent (last) columns of **N** and is denoted $\mathbf{N}_C$. The third consists of the m_0 independent rows and the m_0 independent columns of **N** and is denoted $\mathbf{N}_{RC}$. The matrices are illustrated in eq. (4).

$$\mathbf{N} = \begin{pmatrix} n_{11} & \cdots & n_{1n} \\ \cdots & \cdots & \cdots \\ n_{m1} & \cdots & n_{mn} \end{pmatrix}, \quad \mathbf{N}_R = \begin{pmatrix} n_{11} & \cdots & n_{1n} \\ \cdots & \cdots & \cdots \\ n_{m_0 1} & \cdots & n_{m_0 n} \end{pmatrix}$$

$$\mathbf{N}_C = \begin{pmatrix} n_{1(n_0+1)} & \cdots & n_{1n} \\ \cdots & \cdots & \cdots \\ n_{m(n_0+1)} & \cdots & n_{mn} \end{pmatrix},$$

$$\mathbf{N}_{RC} = \begin{pmatrix} n_{1(n_0+1)} & \cdots & n_{1n} \\ \cdots & \cdots & \cdots \\ n_{m_0(n_0+1)} & \cdots & n_{m_0 n} \end{pmatrix}.$$

Note that the matrix $\mathbf{N}_{RC}$ is square and always invertible, since all rows (and columns) are independent.

Example

The pathway in Fig. 1 contains 5 metabolites and 5 reactions, i.e. $m = 5$ and $n = 5$. The rank of the stoichiometry matrix **N**, which can be found by Gauss elimination, is 3. This means that the pathway contains 3 independent rows and columns, i.e. $m_0 = 3$ and $n_0 = n - m_0 = 2$. If the three first rows and the three last columns are chosen to be independent, the metabolites and the reactions do not have to be renumbered and the matrices defined in eq. (4) can be constructed. These matrices are shown in eq. (5). In the stoichiometry matrix, **N**, the metabolites and reactions corresponding to each row and column are indicated.

$$\mathbf{N} = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \end{array} \begin{array}{c} \begin{array}{ccccc} r & s & t & u & v \end{array} \\ \begin{pmatrix} 1 & -1 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 1 & -1 \end{pmatrix} \end{array}, \quad (4)$$

$$\mathbf{N}_R = \begin{pmatrix} 1 & -1 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & -1 & 1 \end{pmatrix}$$

$$\mathbf{N}_C = \begin{pmatrix} 0 & -1 & 0 \\ -1 & 0 & 0 \\ 0 & -1 & 1 \\ 0 & 1 & -1 \\ 0 & 1 & -1 \end{pmatrix},$$

$$\mathbf{N}_{RC} = \begin{pmatrix} 0 & -1 & 0 \\ -1 & 0 & 0 \\ 0 & -1 & 1 \end{pmatrix} \quad (5)$$

Relationships between metabolites

The m metabolites of the arbitrary pathway are collected in an $m \times 1$ vector denoted **x** and the m_0 independent metabolites are collected in an $m_0 \times 1$ vector, denoted $\mathbf{x}_R$. The aim is now to express all m metabolites of the pathway in terms of the m_0 independent metabolites.

The stoichiometry matrix **N** can be decomposed as (Reder, 1988)

$$\mathbf{N} = \mathbf{L}^X \cdot \mathbf{N}_R \quad (6)$$

The $m \times m_0$ matrix $\mathbf{L}^X$ is called the concentration link matrix. If the dependent columns of **N** and $\mathbf{N}_R$ are deleted in eq. (6), $\mathbf{L}^X$ can be calculated as

$$\mathbf{L}^X = \mathbf{N}_C \cdot \mathbf{N}_{RC}^{-1} \quad (7)$$

Note that if **N** has full rank ($m_0 = m$), $\mathbf{N}_R$ will be equal to **N** and $\mathbf{L}^X$ will be the $m \times m$ identity matrix.

It can be shown that (Reder, 1988)

$$\frac{d\mathbf{x}}{dt} = \mathbf{L}^X \cdot \frac{d\mathbf{x}_R}{dt} \quad (8)$$

which can be integrated to give

$$\mathbf{x}(t) = \mathbf{L}^X \cdot \mathbf{x}_R(t) + \mathbf{A} \quad (9)$$

where **A** is an $m \times 1$ vector where the first m_0 elements are zero. The equation specifies the conservation relationships of the metabolites, i.e. how the dependent metabolites can be expressed in terms of the independent ones. One row of the matrix equation (9) may, for instance, have the appearance:

$$[\mathrm{NADH}](t) = -1 \cdot [\mathrm{NAD^+}](t) + K_{\mathrm{NAD}} \quad (10)$$

showing that the sum of the concentrations of NADH and NAD^+ is constant.

Example

Using the matrices in eq. (5), constructed for the pathway in Fig. 1, the matrix $\mathbf{L}^X$ can be constructed

from eq. (7) as

$$\mathbf{L}^X = \mathbf{N}_C \cdot \mathbf{N}_{RC}^{-1}$$

$$= \begin{pmatrix} 0 & -1 & 0 \\ -1 & 0 & 0 \\ 0 & -1 & 1 \\ 0 & 1 & -1 \\ 0 & 1 & -1 \end{pmatrix} \cdot \begin{pmatrix} 0 & -1 & 0 \\ -1 & 0 & 0 \\ -1 & 0 & 1 \end{pmatrix}$$

$$= \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & -1 \\ 0 & 0 & -1 \end{pmatrix}. \tag{11}$$

Equation (8) now gives the structural relationships between the metabolite concentrations in the pathway:

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} A \\ B \\ C \\ D \\ E \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & -1 \\ 0 & 0 & -1 \end{pmatrix} \cdot \frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} A \\ B \\ C \end{pmatrix} = \frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} A \\ B \\ C \\ -C \\ -C \end{pmatrix} \tag{12}$$

which can be integrated to give

$$\begin{pmatrix} A(t) \\ B(t) \\ C(t) \\ D(t) \\ E(t) \end{pmatrix} = \begin{pmatrix} A(t) + 0 \\ B(t) + 0 \\ C(t) + 0 \\ -C(t) + C(0) + D(0) \\ -C(t) + C(0) + E(0) \end{pmatrix}. \tag{13}$$

The first three of these relationships are trivial, while the last two are the actual structural constraints. The fourth relation, for instance, states that the sum of concentration C and concentration D is constant at all times, which can also be readily seen from Fig. 1.

Relationships between steady-state fluxes

The steady-state fluxes of the reactions in a metabolic pathway are related by structural constraints, i.e. not all steady-state fluxes are independent. For instance, in a linear pathway the steady-state fluxes through each reaction are equal and only one can be chosen as independent.

A general relation between the independent and the dependent steady-state fluxes of a metabolic pathway can be derived using the matrices defined in eq. (4) and a new matrix, $\mathbf{N}_0$, containing the m_0 independent (first) rows and the n_0 dependent (first) columns of $\mathbf{N}$:

$$\mathbf{N}_0 = \begin{pmatrix} n_{11} & \cdots & n_{1n_0} \\ \vdots & & \vdots \\ n_{m_0 1} & \cdots & n_{m_0 n_0} \end{pmatrix}. \tag{14}$$

The n_0 dependent columns will correspond to the independent fluxes of the pathway. If the steady-state flux through reaction k is denoted J_k, and all steady-state fluxes are collected in the $n \times 1$ vector $\mathbf{J}$, the following structural relationship is valid for any pathway:

$$\mathbf{N}_R \cdot \mathbf{J} = 0. \tag{15}$$

This equation can be rearranged in order to obtain the m_0 dependent fluxes, collected in the $m_0 \times 1$ vector $\mathbf{J}_0$, as functions of the n_0 independent fluxes, collected in the $n_0 \times 1$ vector $\mathbf{J}_R$:

$$\mathbf{N}_0 \cdot \mathbf{J}_R + \mathbf{N}_{RC} \cdot \mathbf{J}_0 = 0 \tag{16}$$

or

$$\mathbf{J}_0 = -\mathbf{N}_{RC}^{-1} \cdot \mathbf{N}_0 \cdot \mathbf{J}_R. \tag{17}$$

The product $-\mathbf{N}_{RC}^{-1} \cdot \mathbf{N}_0$ will have the dimensions $m_0 \times n_0$. A new matrix, $\mathbf{L}^J$, is constructed from this product and an n_0 identity matrix as:

$$\mathbf{L}^J = \begin{pmatrix} \mathbf{I}_{n_0} \\ -\mathbf{N}_{RC}^{-1} \cdot \mathbf{N}_0 \end{pmatrix}. \tag{18}$$

The matrix, $\mathbf{L}^J$, which has the dimensions $n \times n_0$, is called the flux link matrix and is equal to the matrix $\mathbf{K}$, defined by Reder (1988). All n steady-state fluxes, $\mathbf{J}$, can now be expressed as functions of the n_0 independent steady-state fluxes, $\mathbf{J}_R$, by

$$\mathbf{J} = \mathbf{L}^J \cdot \mathbf{J}_R. \tag{19}$$

This equation is actually the basis for metabolic flux analysis [for a review see Nielsen and Villadsen (1994)], which provides a method of determining all steady-state fluxes in a metabolic network by measuring some external (independent) key fluxes.

Example

The matrix $\mathbf{N}_0$ is constructed for the pathway in Fig 1.

$$\mathbf{N}_0 = \begin{pmatrix} 1 & -1 \\ 0 & 1 \\ 0 & 0 \end{pmatrix}. \tag{20}$$

Using the matrix $\mathbf{N}_{RC}$, defined in eq. (5), the relations between the steady-state fluxes of the pathway are obtained using eq. (19). If the steady-state flux through reaction r in Fig. 1 is denoted J_r,

the result is

$$\begin{pmatrix} J_r \\ J_s \\ J_t \\ J_u \\ J_v \end{pmatrix} = \mathbf{L}^J \cdot \begin{pmatrix} J_r \\ J_s \end{pmatrix} = \begin{pmatrix} \mathbf{I}_2 \\ -\mathbf{N}_{RC}^{-1} \cdot \mathbf{N}_0 \end{pmatrix} \cdot \begin{pmatrix} J_r \\ J_s \end{pmatrix}$$

$$= \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ 0 & 1 \\ 1 & -1 \\ 1 & -1 \end{pmatrix} \cdot \begin{pmatrix} J_r \\ J_s \end{pmatrix} = \begin{pmatrix} J_r \\ J_s \\ J_s \\ J_r - J_s \\ J_r - J_s \end{pmatrix}. \quad (21)$$

KINETIC RELATIONSHIPS

The concentrations of the m internal metabolites in an arbitrary pathway are denoted $x_1, x_2, \ldots, x_m$ and the n rates of the reactions are denoted $v_1, v_2, \ldots, v_n$. The concentrations and the reaction rates are collected in the $m \times 1$ vector $\mathbf{x}$ and in the $n \times 1$ vector $\mathbf{v}$, respectively.

$$\mathbf{x} = (x_1 \quad x_2 \quad \ldots \quad x_m)^{\mathrm{T}} \quad (22)$$

$$\mathbf{v} = (v_1 \quad v_2 \quad \ldots \quad v_n)^{\mathrm{T}}. \quad (23)$$

The pathway also contains r parameters, with values denoted $p_1, p_2, \ldots, p_r$. These parameters, that can be enzyme concentrations, kinetic parameters, external metabolites, etc., are collected in the $r \times 1$ vector $\mathbf{p}$:

$$\mathbf{p} = (p_1 \quad p_2 \ldots \quad p_r)^{\mathrm{T}}. \quad (24)$$

Each reaction rate can be generally expressed as a function of the m concentrations and of the r parameters, i.e.

$$v_k = f_k(\mathbf{p}, \mathbf{x}), \quad k = 1, \ldots, n. \quad (25)$$

If one of these arbitrary functions is differentiated with respect to one enzyme concentration, e_j, the result is

$$\frac{\mathrm{d}v_k}{\mathrm{d}e_j} = \frac{\partial v_k}{\partial p_1} \cdot \frac{\mathrm{d}p_1}{\mathrm{d}e_j} + \cdots + \frac{\partial v_k}{\partial p_r} \cdot \frac{\mathrm{d}p_r}{\mathrm{d}e_j} + \frac{\partial v_k}{\partial x_1} \cdot \frac{\mathrm{d}x_1}{\mathrm{d}e_j} + \cdots + \frac{\partial v_k}{\partial x_m} \cdot \frac{\mathrm{d}x_m}{\mathrm{d}e_j}. \quad (26)$$

The enzyme concentration, e_j, is present in the parameter vector $\mathbf{p}$ and if the parameters are not in any way coupled, i.e. if a change in one parameter does not affect the others, it is noted that

$$\frac{\mathrm{d}p_i}{\mathrm{d}e_j} = \begin{cases} 0, & p_i \neq e_j \\ 1, & p_i = e_j. \end{cases} \quad (27)$$

If the zero terms of eq. (26) are eliminated and the equation is multiplied by e_j/v_k, it can be rearranged to give:

$$\frac{\mathrm{d}v_k}{\mathrm{d}e_j} \cdot \frac{e_j}{v_k} = \frac{\partial v_k}{\partial e_j} \cdot \frac{e_j}{v_k} + \sum_{i=1}^{m} \left(\frac{\partial v_k}{\partial x_i} \cdot \frac{x_i}{v_k} \cdot \frac{\mathrm{d}x_i}{\mathrm{d}e_j} \cdot \frac{e_j}{x_i} \right). \quad (28)$$

If v_k is chosen to be the steady-state flux through reaction k, i.e. $v_k = (v_k)_{ss} = J_k$, the elasticity coefficients, ε_i^k, the parameter elasticity coefficients, π_j^k, the flux control coefficients, $C_j^{J_k}$, and the concentration control coefficients, $C_j^{X_i}$, can be recognised in eq. (28) as

$$\varepsilon_i^k = \frac{\partial v_k}{\partial x_i} \cdot \frac{x_i}{v_k}, \qquad \pi_j^k = \frac{\partial v_k}{\partial e_j} \cdot \frac{e_j}{v_k},$$

$$C_j^{J_k} = \frac{\mathrm{d}J_k}{\mathrm{d}e_j} \cdot \frac{e_j}{J_k}, \qquad C_j^{X_i} = \frac{\mathrm{d}x_i}{\mathrm{d}e_j} \cdot \frac{e_j}{x_i} \quad (29)$$

respectively. The parameter elasticity coefficient, π_j^k, was defined by Kacser *et al.* (1990) and is a measure of the response in the enzymatic reaction rate to variations in an enzyme concentration. In traditional MCA these coefficients are assumed to be 0 when $k \neq j$ and 1 when $k = j$. Making the substitutions given by eq. (29), eq. (28) can be written as

$$C_j^{J_k} = \pi_j^k + \sum_{i=1}^{m} \varepsilon_i^k \cdot C_j^{X_i}. \quad (30)$$

In vector form this is equal to

$$C_j^{J_k} = \pi_j^k + (\varepsilon_1^k \quad \varepsilon_2^k \quad \ldots \quad \varepsilon_m^k) \cdot \begin{pmatrix} C_j^{X_1} \\ C_j^{X_2} \\ \vdots \\ C_j^{X_m} \end{pmatrix}. \quad (31)$$

Extension to all enzymes in the pathway yields:

$$(C_1^{J_k} \quad \ldots \quad C_n^{J_k}) = (\pi_1^k \quad \ldots \quad \pi_n^k) + (\varepsilon_1^k \quad \ldots \quad \varepsilon_m^k) \cdot \begin{pmatrix} C_1^{X_1} & \cdots & C_n^{X_1} \\ \vdots & & \vdots \\ C_1^{X_m} & \cdots & C_n^{X_m} \end{pmatrix}. \quad (32)$$

Further extension to all steady-state enzyme fluxes yields:

$$\begin{pmatrix} C_1^{J_1} & \cdots & C_n^{J_1} \\ \vdots & & \vdots \\ C_1^{J_n} & \cdots & C_n^{J_n} \end{pmatrix} = \begin{pmatrix} \pi_1^1 & \cdots & \pi_n^1 \\ \vdots & & \vdots \\ \pi_1^n & \cdots & \pi_n^n \end{pmatrix} + \begin{pmatrix} \varepsilon_1^1 & \cdots & \varepsilon_m^1 \\ \vdots & & \vdots \\ \varepsilon_1^n & \cdots & \varepsilon_m^n \end{pmatrix} \cdot \begin{pmatrix} C_1^{X_1} & \cdots & C_n^{X_1} \\ \vdots & & \vdots \\ C_1^{X_m} & \cdots & C_n^{X_m} \end{pmatrix}. \quad (33)$$

If the matrices in eq. (33) are named it can be written as

$$\mathbf{C}^J = \pi + \varepsilon \cdot \mathbf{C}^X. \quad (34)$$

If it is assumed that each reaction rate is directly proportional to the concentration of the corresponding enzyme and that the concentration only affects its own reaction rate, as is assumed in traditional MCA, the matrix π becomes an $n \times n$ identity matrix.

RESULTS

Since the aim is to solve for the control coefficients in terms of the elasticity coefficients, eq. (34) is not

sufficient. It is a system of equations with $n \cdot n + m \cdot n$ unknowns (the control coefficients) and only $n \cdot n$ equations. More relations must thus be utilised in order to solve it. These relations are provided by the structural relationships derived from the stoichiometry matrix.

The relations between the independent and the dependent metabolites were shown to be described by eq. (9). Row k of this matrix equation can be written as

$$x_k = \sum_{i=1}^{m_0} l_{ki} \cdot x_i + a_k \tag{35}$$

where l_{ki} is an element of the concentration link matrix $\mathbf{L}^X$ and a_k is an element of the vector $\mathbf{A}$. If x_k and x_i denote steady-state concentrations this equation can be differentiated with respect to an enzyme concentration, e_j. Multiplying the result of this differentiation by e_j/x_k yields

$$\frac{\mathrm{d}x_k}{\mathrm{d}e_j} \cdot \frac{e_j}{x_k} = \frac{e_j}{x_k} \cdot \sum_{i=1}^{m_0} l_{ki} \cdot \frac{\mathrm{d}x_i}{\mathrm{d}e_j}. \tag{36}$$

Rearrangement and recognition of terms yields

$$C_j^{X_k} = \sum_{i=1}^{m_0} l_{ki} \cdot \frac{x_i}{x_k} \cdot C_j^{X_i}. \tag{37}$$

If a new matrix, denoted $\mathbf{L}_F^X$, is constructed from $\mathbf{L}^X$ by multiplying each element, l_{ki}, of $\mathbf{L}^X$ by x_i/x_k, and if eq. (37) is extended to all metabolites and enzymes of the pathway, the resulting equation in matrix notation reads:

$$\mathbf{C}^X = \mathbf{L}_F^X \cdot \mathbf{C}_R^X. \tag{38}$$

The $m \times n$ matrix $\mathbf{C}^X$ contains all concentration control coefficients of the pathway and the $m_0 \times n$ matrix $\mathbf{C}_R^X$ contains the concentration control coefficients for the independent metabolites. If the concentration control coefficients for the independent metabolites have been calculated, the concentration control coefficients for the remaining metabolites can be calculated using eq. (38).

The relations between the independent and the dependent steady-state fluxes were shown to be described by eq. (19). This equation can be differentiated as

$$\mathrm{d}\mathbf{J} = \mathbf{L}^J \, \mathrm{d}\mathbf{J}_R. \tag{39}$$

Exactly the same derivation can then be made as when eq. (38) was derived from eq. (35). The result is

$$\mathbf{C}^J = \mathbf{L}_F^J \cdot \mathbf{C}_R^J. \tag{40}$$

$\mathbf{C}^J$ contains all the flux control coefficients and $\mathbf{C}_R^J$ contains the flux control coefficients for the independent steady-state fluxes. $\mathbf{L}_F^J$ is the result when each element, l_{ki}, of $\mathbf{L}^J$ is multiplied by J_i/J_k.

Equations (38) and (40) can now be inserted into eq. (34) and the result is

$$\mathbf{L}_F^J \cdot \mathbf{C}_R^J - \varepsilon \cdot \mathbf{L}_F^X \cdot \mathbf{C}_R^X = \pi. \tag{41}$$

The number of unknowns has now been reduced to $n_0 \cdot n + m_0 \cdot n = n \cdot n$ and it is possible to solve the equation system.

Equation (41) can be rearranged as

$$\begin{pmatrix} \mathbf{L}_F^J \\ -\varepsilon \cdot \mathbf{L}_F^X \end{pmatrix}^{\mathrm{T}} \cdot \begin{pmatrix} \mathbf{C}_R^J \\ \mathbf{C}_R^X \end{pmatrix} = \pi \tag{42}$$

and

$$\begin{pmatrix} \mathbf{C}_R^J \\ \mathbf{C}_R^X \end{pmatrix} = (\mathbf{L}_F^J \mid -\varepsilon \cdot \mathbf{L}_F^X)^{-1} \cdot \pi. \tag{43}$$

The control coefficients for the independent fluxes and metabolites can thus be calculated using eq. (43). If required, all control coefficients can then be calculated using eqs. (38) and (40). Implicit in eq. (43) are not only the summation and connectivity theorems, but also the structural relationships of the pathway. An identical calculation procedure can be used for the calculation of the control coefficients in terms of the elasticity coefficients (and if appropriate also the parameter elasticity coefficients) for any pathway stoichiometry.

Example

Consider again the pathway depicted in Fig. 1. Using the matrices $\mathbf{L}^X$ and $\mathbf{L}^J$, derived in eqs (11) and (21), the matrices $\mathbf{L}_R^X$ and $\mathbf{L}_R^J$ can be written as

$$\mathbf{L}_R^X = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & -C/D \\ 0 & 0 & -C/E \end{pmatrix}, \qquad \mathbf{L}_R^J = \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ 0 & J_s/J_t \\ J_r/J_u & -J_s/J_u \\ J_r/J_v & -J_s/J_v \end{pmatrix}. \tag{44}$$

These matrices inserted into eq. (43) yield the control coefficients for the independent fluxes and metabolites:

$$\begin{pmatrix} C_r^{J_r} & \cdots & C_v^{J_r} \\ C_r^{J_s} & \cdots & C_v^{J_s} \\ C_r^{A} & \cdots & C_v^{A} \\ C_r^{B} & \cdots & C_v^{B} \\ C_r^{C} & \cdots & C_v^{C} \end{pmatrix} = \begin{pmatrix} 1 & 0 & \varepsilon_A^r & \varepsilon_B^r & \varepsilon_C^r - \varepsilon_D^r \cdot C/D - \varepsilon_E^r \cdot C/E \\ 0 & 1 & \varepsilon_A^s & \varepsilon_B^s & \varepsilon_C^s - \varepsilon_D^s \cdot C/D - \varepsilon_E^s \cdot C/E \\ 0 & J_s/J_t & \varepsilon_A^t & \varepsilon_B^t & \varepsilon_C^t - \varepsilon_D^t \cdot C/D - \varepsilon_E^t \cdot C/E \\ J_r/J_u & -J_s/J_u & \varepsilon_A^u & \varepsilon_B^u & \varepsilon_C^u - \varepsilon_D^u \cdot C/D - \varepsilon_E^u \cdot C/E \\ -J_r/J_v & -J_s/J_v & \varepsilon_A^v & \varepsilon_B^v & \varepsilon_C^v - \varepsilon_D^v \cdot C/D - \varepsilon_E^v \cdot C/E \end{pmatrix}^{-1} \cdot \pi \tag{45}$$

where the matrix π is usually an $n \times n$ (5×5) identity matrix.

DISCUSSION

The original theorems of MCA were derived using *ad hoc* reasoning and are only sufficient for linear pathways. Additional theorems were later derived to cover more complex pathway stoichiometries. These additional theorems make the originally simple framework of MCA a rather complicated system of equations. A more general approach to MCA is thus desirable.

The matrix equations derived in the present paper, using general sensitivity analysis and structural information of the metabolic pathway inherent in the stoichiometry matrix, establish a stringent mathematical foundation for MCA. They are valid for any pathway stoichiometry and the calculations illustrated in the examples, tedious as they might seem to be, are nevertheless straightforward and similar, regardless of the stoichiometry. This diminishes the risk of deriving erroneous relations. Furthermore, in the general approach the assumption of direct proportionality between enzyme concentration and reaction rate is relaxed.

The equations are, due to their generality, well suited for computer handling. They have been implemented in the Metabolic Interactive Simulation Tool, MIST (Ehlde and Zacchi, 1995), which is a program for dynamic simulation and calculation of control coefficients of arbitrary user-defined metabolic pathways. The program has been successfully used in evaluating experimental methods of MCA (Ehlde, 1995; Ehlde and Zacchi, 1996).

A disadvantage with the equations derived is that the illustrative summation and connectivity theorems of the original MCA are implicit. These theorems are still valuable for understanding the nature of and relations between the different coefficients of MCA.

NOTATION

$\mathbf{A}$	vector specifying moiety conservations ($m \times 1$)
$C_i^{J_j}$	flux control coefficient
$\mathbf{C}^J$	matrix containing all flux control coefficients ($n \times n$)
$\mathbf{C}_R^J$	matrix containing independent flux control coefficients ($n_0 \times n$)
$C_i^{X_j}$	concentration control coefficient
$\mathbf{C}^X$	matrix containing all concentration control coefficients ($m \times n$)
$\mathbf{C}_R^X$	matrix containing independent concentration control coefficients ($m_0 \times n$)
e_i	concentration of the ith enzyme
J_i	steady-state flux through the ith enzyme
$\mathbf{J}$	vector containing steady-state fluxes ($n \times 1$)
$\mathbf{J}_R$	vector containing independent steady-state fluxes ($n_0 \times 1$)
$\mathbf{J}_0$	vector containing dependent steady-state fluxes ($m_0 \times 1$)
$\mathbf{L}^J$	flux link matrix ($n \times n_0$)
$\mathbf{L}_F^J$	modified flux link matrix ($n \times n_0$)
$\mathbf{L}^X$	concentration link matrix ($m \times m_0$)
$\mathbf{L}_F^X$	modified concentration link matrix ($m \times m_0$)
m	number of pathway metabolites
m_0	number of independent pathway metabolites
n	number of pathway enzymes
n_0	number of independent pathway steady-state fluxes
$\mathbf{N}$	stoichiometry matrix ($m \times n$)
$\mathbf{N}_C$	the independent columns of $\mathbf{N}$ ($m \times m_0$)
$\mathbf{N}_R$	the independent rows of $\mathbf{N}$ ($m_0 \times n$)
$\mathbf{N}_{RC}$	the independent rows and independent columns of $\mathbf{N}$ ($m_0 \times m_0$)
$\mathbf{N}_0$	the independent rows and dependent columns of $\mathbf{N}$ ($m_0 \times n_0$)
p_i	value of the ith parameter
$\mathbf{p}$	vector containing parameters ($r \times 1$)
r	number of pathway parameters
v_i	reaction rate of the ith reaction
$\mathbf{v}$	vector containing reaction rates ($n \times 1$)
x_i	concentration of the ith metabolite
$\mathbf{x}$	vector containing metabolite concentrations ($m \times 1$)

Greek letters

ε_j^i	elasticity coefficient
ε	matrix containing elasticity coefficients ($n \times m$)
π_j^i	parameter elasticity coefficient
π	matrix containing parameter elasticity coefficients ($n \times n$)

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