

Application of Computer Algebra Matrix Operation Techniques to the Enzymes Kinetics Systems

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ABSTRACT

Metabolic control analysis allows one to quantify the behaviour of a metabolic pathway in steady state in terms of dimensionless coefficients. From the definition of metabolite and flux control coefficients and elasticities we are able to derive symbolic forms of these parameters, in terms of conventional kinetic parameters. At the simplest level we are able to substitute values of these kinetic parameters, to yield values for the metabolic control coefficients. Since we are substituting into symbolic equations we can always guarantee the conservation relationships hold. The basic relationships are the summation and connectivity theorems. The ability to define the control coefficient equations in matrix form not only allows easy solution by numerical inversion, but also opens up the possibility of obtaining the algebraic solutions by symbolic manipulation of the matrix. However, for matrixes longer than rank 4 or 5, this latter possibility, if done by hand, becomes very tedious and is prone to error. The solution to this problem is to develop computer software to automatically carry out this procedure.

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1. INTRODUCTION

Metabolic control analysis allows one to quantify the behaviour of a metabolic pathway in steady state in terms of dimensionless coefficients. Higgings was one of the first authors to propose a quantitative analysis of the control of metabolic flux (Higgings (1965)). His analysis has been developed and elaborated by Kacser and Burns (1973) and independently by Heinrich and Rapoport (1974). The theoretical principles developed by these authors have greatly facilitated the quantitative determination of the extent to which fluxes are controlled in a metabolic network. Bayram has been applied

the Computer algebra system to the enzymes kinetics (Heinrich and Rapoport (1974)). Generally, two types of coefficients have been defined in the theory:

1. The control coefficients (of flux or of metabolite concentrations) which correlate the change observed in a given parameter (flux concentration) to the perturbation of a particular step in metabolic network at the steady-state.
2. The elasticity coefficients which simply express the variation of the rate of an isolated step as a function of a given metabolite.

The theory demonstrates that relationships exist between these coefficients, i.e. summation relationships between the control coefficients and connectivity relationships between the control coefficients and the elasticity coefficients. The summation relationship provides a linear constraint on the distribution of flux control coefficients. One consequence of this is that if there is a change in the steady state caused by a change in an external effectors, then the distribution of flux control coefficients will readjust so that the summation relationship is obeyed. If some reactions experience a fall in their flux control coefficients, then the flux control coefficients for other steps must rise. The summation relationship also indicates that if there are any steps which have flux control coefficients greater than one, then there must be other steps which compensate by having negative flux control coefficients. The summation relationship puts constraints on the distribution of control such that if some enzymes have high control, then others must have less. The summation relationship provides the notion that enzymes compete for the control of flux.

Connectivity relationship indicates how the intrinsic properties of the individual enzymes contribute to the properties of the whole system. From the definition of metabolite and flux control coefficients and elasticities we are able to derive symbolic forms of these parameters, in terms of conventional kinetic parameters. At the simplest level we are able to substitute values of these kinetic parameters, to yield values for the metabolic control coefficients. Since we are substituting into symbolic equations we can always guarantee the conservation relationships hold. We have done this in Bayram (1993) for a two enzyme system in *vitro* using an aspartate aminotransferase-malate dehydrogenase coupled system.

The basic relationships are the summation and connectivity theorems. They allow one to express the behaviour of the system variables in terms of the kinetic properties of the isolated enzymatic reactions that build up the metabolic network. A matrix method was derived (Fell and Sauro (1985); Higgings

(1965); Sauro *et al.* (1987)) that allows the determination of the flux and concentration control coefficients of enzymes from their kinetic properties represented by the elasticity coefficients (Delgado and Liao (1992); Kacser and Porteous (1987); Kacser *et al.* (1990); Letellier *et al.* (1991); Reder (1988); Small and Fell (1989)).

The ability to define the control coefficient equations in matrix form not only allows easy solution by numerical inversion but also opens up the possibility of obtaining the algebraic solutions by symbolic manipulation of the matrix. However for matrixes larger than rank 4 or 5, this latter possibility, if done by hand, becomes very tedious and is prone to error. The solution to this problem is to develop computer software to automatically carry out this procedure (Bayram (1996)). In this paper a brief description of such a program based on Reder's method (Letellier *et al.* (1991) is given.

2. MAPLE COMPUTER ALGEBRA SYSTEMS

MAPLE is a general-purpose commercial computer algebra system. It was first developed in 1980 by the Symbolic Computation Group at the University of Waterloo (Geddes *et al.* (2008)). Computers are usually used to manipulate numbers. However they can just as well work with other symbols, for example, algebraic variables. Computer algebra systems manipulate symbols not numbers. Rather than using the approximation methods of numerical analysis, they use exact algebraic techniques. Such systems tend to be interactive programs, commonly written in C, and they accept their input in a quasi-mathematical notation which is simple to use and remember. They can give general expressions as an answer, rather than only a numerical value.

We have used MAPLE computer algebra matrix operations and linear algebra facilities to implement Reder's algorithm for analysing metabolic networks.

3. SYSTEM AND METHODS

We have developed a program that is written in algebraic and symbolic MAPLE form (Geddes *et al.* (2008)). Using this program it is possible to calculate the control coefficients from elasticity coefficients whatever the metabolic network is. A metabolic network is a set of reactions

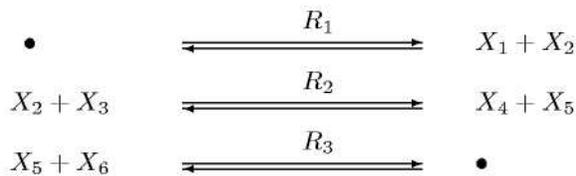
R_1, R_2, \dots, R_r between the metabolites X_1, X_2, \dots, X_m of concentrations x_1, x_2, \dots, x_m . Let us define the concentration vector x as

$$x = \begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_m \end{bmatrix} \quad (1)$$

In order to construct the model, we at first write the stoichiometric matrix \mathbf{N} of the system that describes how the metabolites X_i combine. The matrix \mathbf{N} is constructed as follows: the column j of \mathbf{N} represents the reaction j and we write in this column at row i

- $+\alpha$ if the reaction j produces α molecules of X_i ,
- $-\alpha$ if the reaction j consumes α molecules of X_i ,
- 0 if the reaction j neither produces nor consumes X_i ,

that is to say the stoichiometric coefficient of X_i in reaction j . Let us illustrate the matter using an example



The entry and exit points are represented by dots. For the example, the stoichiometric matrix is

$$\mathbf{N} = \begin{bmatrix} 1 & 0 & 0 \\ 1 & -1 & 0 \\ 0 & -1 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & -1 \\ 0 & 0 & -1 \end{bmatrix}. \quad (2)$$

We assume that the rate of change of the concentration x_i of metabolite X_i is the sum of the r reaction rates, each weighted by the corresponding stoichiometric coefficient of X_i .

The velocity of each step v_i is a function of x_i and of external parameters represented by μ :

$$v_i = v_i(x_1, x_2, \dots, x_m, \mu) \quad (3)$$

and

$$v = \begin{bmatrix} v_1 \\ v_2 \\ \vdots \\ v_r \end{bmatrix} \quad (4)$$

denotes the rate vector. Using this hypothesis, the metabolic system is expressed as

$$\frac{dx}{dt} = \mathbf{N}v \quad (5)$$

For the example, this equation can be written as follows

$$\frac{d}{dt} \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \\ x_5 \\ x_6 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 1 & -1 & 0 \\ 0 & -1 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & -1 \\ 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix}. \quad (6)$$

The concentrations x_1, x_2, \dots, x_m of the metabolites X_1, X_2, \dots, X_m at steady state are a solution of

$$\mathbf{N}v(x_1^0, \dots, x_m^0, \mu) = 0 \quad (7)$$

and the fluxes at this steady state are defined by

$$J_i = v_i(x_1^0, \dots, x_m^0, \mu). \quad (8)$$

A brief description of the Reder's method follows.

The stoichiometry matrix \mathbf{N} can be decomposed as:

$$\mathbf{N} = \mathbf{L}\mathbf{N}_R \quad (9)$$

where \mathbf{N}_R is an $m_o \times r$ matrix formed by the first m_o rows of \mathbf{N} that constitute a basis for its row space. \mathbf{L} is an $m \times m_o$ matrix that has the form

$$\mathbf{L} = \begin{bmatrix} \mathbf{I}_{m_o} \\ \mathbf{L}_o \end{bmatrix} \quad (10)$$

where \mathbf{I}_{m_o} is the $m_o \times m_o$ identity matrix and \mathbf{L}_o is $(m \times m_o) \times m_o$.

For the example, we decompose the matrix \mathbf{N} so that its first three rows are independent. Therefore,

$$\mathbf{N} = \begin{bmatrix} 1 & 0 & 0 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \\ 0 & -1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & -1 \end{bmatrix} = \underbrace{\begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ - & - & - \\ -1 & 1 & 0 \\ 1 & -1 & 0 \\ -1 & 1 & 1 \end{bmatrix}}_{\mathbf{L}} \underbrace{\begin{bmatrix} 1 & 0 & 0 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix}}_{\mathbf{N}_R} \quad (11)$$

where

$$\mathbf{I}_{m_o} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad (12)$$

and

$$\mathbf{L}_o = \begin{bmatrix} -1 & 1 & 0 \\ 1 & -1 & 0 \\ -1 & 1 & 1 \end{bmatrix}. \quad (13)$$

The elasticity matrix \mathbf{E}' is defined by

$$\mathbf{E}' = \begin{bmatrix} \epsilon'_{11} & \cdots & \epsilon'_{1m} \\ \cdots & \cdots & \cdots \\ \epsilon'_{r1} & \cdots & \epsilon'_{rm} \end{bmatrix} \quad (14)$$

where ϵ'_{ij} is

$$\epsilon'_{ij} = \frac{\delta v_i}{\delta x_j}. \quad (15)$$

The flux control coefficients are defined as

$$\mathbf{C}'_{kj} = \frac{\delta J_k / \delta \mu_j}{\delta v_j / \delta \mu_j}. \quad (16)$$

The matrix of flux control coefficients is calculated using

$$\mathbf{C}' = \mathbf{I}_r - \mathbf{E}'\mathbf{L}[\mathbf{N}_R\mathbf{E}'\mathbf{L}]^{-1}\mathbf{N}_R \quad (17)$$

where \mathbf{I}_r is identify matrix with dimension $r \times r$.

The metabolite control coefficients are defined as

$$\mathbf{S}'_{kj} = \frac{\delta x_k / \delta \mu_j}{\delta v_j / \delta \mu_j}. \quad (18)$$

The metabolite control coefficients matrix \mathbf{S}' is calculated using following relationship

$$\mathbf{S}' = -\mathbf{L}[\mathbf{N}_R\mathbf{E}'\mathbf{L}]^{-1}\mathbf{N}_R. \quad (19)$$

The summation relationships between the flux control coefficients are derived from the symbolic development of the equation

$$\mathbf{C}'\mathbf{K} = \mathbf{K} \quad (20)$$

where \mathbf{K} is a matrix which contains the vectors of a basis of the null-space of the \mathbf{N} matrix, which is also the null-space of \mathbf{N}_R .

The summation relationships between the metabolite control coefficients are

$$\mathbf{S}'\mathbf{K} = \mathbf{0}. \quad (21)$$

The connectivity relationships between the flux control coefficients and the elasticity coefficients are

$$\mathbf{C}'(\mathbf{E}'\mathbf{L}) = \mathbf{0}. \quad (22)$$

Similarly the connectivity relationships between the metabolite control coefficients and the elasticity coefficients are

$$\mathbf{S}'(\mathbf{E}'\mathbf{L}) = -\mathbf{L}. \quad (23)$$

We used here nonnormalised coefficients, however it is easy to transform them into normalised coefficients using the formulae

$$\begin{aligned} \mathbf{C}_{ij} &= \mathbf{C}'_{ij} \cdot v_j / v_i \\ \mathbf{S}_{ij} &= \mathbf{S}'_{ij} \cdot v_j / x_i \\ \epsilon_{ij} &= \epsilon'_{ij} \cdot x_j / v_i \end{aligned} \quad (24)$$

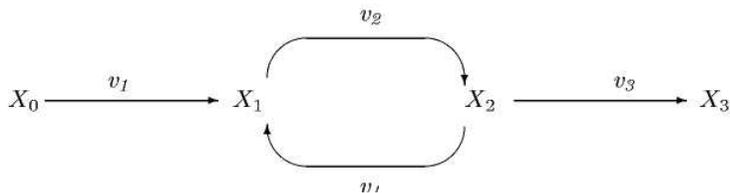


Figure 1: A substrate cycle model in a simple pathway

The matrix \mathbf{L} is determined by m_o independent columns of \mathbf{N} i.e.

$$\mathbf{L} = \mathbf{N}\mathbf{N}_{\mathbf{R}_o}^{-1}. \quad (25)$$

The matrix \mathbf{K} is an $(r \times (r - m_o))$ matrix. Its columns are built to be independent by looking for a \mathbf{K} matrix of the form

$$\mathbf{K} = \begin{bmatrix} \mathbf{A} \\ \mathbf{I}(\mathbf{r} - \mathbf{m}_o) \end{bmatrix}. \quad (26)$$

Note that $\mathbf{N}\mathbf{K} = \mathbf{N}_{\mathbf{R}}\mathbf{K} = \mathbf{0}$ by definition of \mathbf{K} (Letellier *et al.* (1991)). If \mathbf{A} exists, it then verifies

$$\mathbf{N}_{\mathbf{R}_o}\mathbf{A} + \mathbf{N}_{\mathbf{R}_1} = \mathbf{0}. \quad (27)$$

Thus,

$$\mathbf{A} = \mathbf{N}_{\mathbf{R}_o}^{-1}(-\mathbf{N}_{\mathbf{R}_1}) = \mathbf{0}. \quad (28)$$

The program uses a modified Gram-Schmidt orthogonalisation process [13] to find independent rows or columns of the matrixes such as $\mathbf{N}_{\mathbf{R}}$ and $\mathbf{N}_{\mathbf{R}_o}$, computes the nonnormalised coefficients \mathbf{C}'_{ij} , \mathbf{S}'_{ij} and ϵ'_{ij} .

An Example of Application of the Computer Program

Let us consider a substrate cycle model, Figure 1, the rate law for each enzyme is a fully reversible Michaelis-Menten mechanism. The matrix \mathbf{N} for the mechanism is

$$\mathbf{N} = \begin{bmatrix} -1 & 0 & 0 & 0 \\ 1 & -1 & 0 & 1 \\ 0 & 1 & -1 & -1 \\ 0 & 0 & 1 & 0 \end{bmatrix}.$$

The computer program calculates flux control coefficients, metabolite control coefficients, summation and connectivity relationships in terms

of elasticity coefficients from matrix \mathbf{N} for a given metabolite network. The computer result for the mechanism shown in Figure 1 is:

Flux control coefficients are

$$\mathbf{C}' = \begin{bmatrix} C'_{11} & C'_{12} & C'_{13} & C'_{14} \\ C'_{21} & C'_{22} & C'_{23} & C'_{24} \\ C'_{31} & C'_{32} & C'_{33} & C'_{34} \\ C'_{41} & C'_{42} & C'_{43} & C'_{44} \end{bmatrix}.$$

The connectivity relationships between the flux control coefficients and the elasticity coefficients, for the substrate cycle model shown in Figure 1 are

$$\mathbf{C}'(\mathbf{E}'\mathbf{L}) = \begin{bmatrix} 0 & 0 \\ 0 & 0 \\ 0 & 0 \\ 0 & 0 \end{bmatrix}.$$

The summation relationships between the flux control coefficients, for the substrate cycle model shown in Figure 1 are

$$\mathbf{C}'\mathbf{K} = \mathbf{K} = \begin{bmatrix} 0 & 1 \\ 1 & 1 \\ 1 & 0 \\ 0 & 1 \end{bmatrix}.$$

Metabolic control coefficients in the matrix form, for the substrate cycle model shown in Figure 1 are

$$\mathbf{S}' = \begin{bmatrix} S'_{11} & S'_{12} & S'_{13} & S'_{14} \\ S'_{21} & S'_{22} & S'_{23} & S'_{24} \end{bmatrix}.$$

The connectivity relationships between the metabolic control coefficients and the elasticity coefficients, for the substrate cycle model shown in Figure 1 are

$$\mathbf{S}'(\mathbf{E}'\mathbf{L}) = -\mathbf{L} = \begin{bmatrix} -1 & 0 \\ 0 & -1 \end{bmatrix}.$$

The summation relationships between the metabolic control coefficients, for the substrate cycle model shown in Figure 1 are

$$\mathbf{S}'\mathbf{K} = \begin{bmatrix} 0 & 0 \\ 0 & 0 \end{bmatrix}.$$

4. CONCLUSION

We have presented the program in this paper is more general in that it is based on the mathematical analysis of the control theory of metabolism. Using the program it is possible to calculate the control coefficients from the elasticity coefficients whatever the metabolic network is. An other advantage of the our program is that it is now possible to determine all the summation and all the connectivity relationships between direct coefficients. It is clear that the relationships between direct coefficients can be translated into the corresponding relationships between the normalised ones. This would involve using ratios of rates or ratios of concentrations over rates, and would therefore be more complex and not so easy to handle. This justifies the usage of direct coefficients which is by no means of a limitation. It is always possible to transform one type of coefficient into the other using the computer algebra system. Maple can be used to derive metabolic control coefficients for biochemical reactions even if they have unlimited steps and intermediates by eliminating the tedious process of algebraically manipulating equations.

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