

Metabolic Control Analysis in Enzymes Kinetics

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ABSTRACT

Mathematical modelling of metabolic pathway provides us a wide variety of information about behaviour of the system. The kinetic approach to the modelling of the systems is sometimes hampered by the fact that kinetic properties are imperfectly know that makes the structural approach more attractive. In order to make kinetic analysis of a metabolic pathway, construction of mathematical model describing its kinetics is a major part of the work. In the framework of metabolic kinetic theory, it is assumed that the rate of changes in the concentration x_i of a metabolite X_i is the sum of the r reaction rates, each weighted by corresponding stoichiometric coefficient of X_i . Using v and x to denote the rate vector and concentration vector

respectively, mathematical model for kinetics of a system can be written as $\frac{dx}{dt} = Nv$

where N is stoichiometric matrix which represents how the metabolites involved in the system combine. Derivation of conservation relationship which mainly depends on decomposition of stoichiometric matrix N plays important roles in constructing mathematical model of the system.

Keywords: Mathematical Modelling, metabolic kinetic theory, kinetic properties.

1. INTRODUCTION

Mathematical modelling and metabolic control analysis of metabolic pathway provides us a wide variety of information about behaviour of the system. The kinetic approach to the modelling of the systems is sometimes hampered by the fact that kinetic properties are imperfectly know that makes the structural approach more attractive (Bayram (1996); Yildirim (2000); Yildirim and Bayram (2000)). The structural property of a metabolic pathway is the characteristics of the system that depends only on the structure of the pathway.

Although mathematical description of dynamic of a metabolic system is a system of nonlinear differential equation including a number of parameters that is difficult to solve, the structure of the system imposes linear constraints which can be analyzed independently of the nonlinear kinetics (Fell (1997)). In a living organism, of course, very few reactants are external, but there are so many reactions to be considered that the entire system is difficult to comprehend. To make metabolism manageable for analysis, therefore, the system must be defined as just part of the whole organism and it must be taken into consideration as a metabolic pathway and then the metabolites at the interfaces with the rest of the organism must be defined as external (Bowden (1995)).

In order to analyze a metabolic pathway, Reder has emphasized on the structural properties of the pathway (Reder (1988)). The advantages of this approach lies of course in the fact that structure of the metabolic pathway depends neither on the environment nor the internal state of the system (Schuster and Hilgetag (1995)).

Basic Definition

The chemical changes that take place in a cell or an organism that produce energy and basic materials needed for important life processes. These are:

Definition 1 *Anabolism - Synthesis of complex molecules from simpler ones (e.g amino-acid synthesis) and*

Definition 2 *Catabolism - Breaking down of complex molecules to simpler, smaller molecules (e.g. glycolysis).*

Definition 3 *A chemical reaction is a process that leads to the transformation of one set of chemical substances to another.*

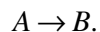


Figure 1: A chemical reaction

Definition 4 *Metabolic pathways are series of chemical reactions occurring within a cell. In each pathway, a principal chemical is modified by chemical reactions.*

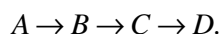


Figure 2: A metabolic pathway

Definition 5 *A metabolic network is the complete set of metabolic and physical processes that determine the physiological and biochemical properties of a cell. As such, these networks comprise the chemical reactions of metabolism as well as the regulatory interactions that guide these reactions.*

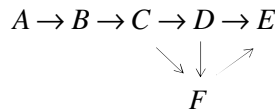


Figure 3: A metabolic network

Definition 6 *Flux - The production or consumption of mass per unit area per unit time.*

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 .

Hence, kinetics of a metabolic system can be written as a system of ordinary differential equations of the form

$$\frac{dx}{dt} = Nv(x, k, \mu)$$

where N is a stoichiometric matrix, x 's are metabolite concentrations, k 's are kinetic parameters and μ 's are some external parameters.

The method based on decomposition of a stoichiometric matrix of metabolic pathways provides us a wide variety of information for comprehending the complex topology of metabolic pathways in a systematic way (Reder (1988)). One of the main advantage of using conservation relationships is to reduce computation loads in kinetic analyzes of a metabolic pathway (Bayram (1993)).

2. METABOLIC CONTROL ANALYSIS

Metabolic control analysis is a method for analysing how the control of fluxes and intermediate concentrations in a metabolic pathway is distributed among the different enzymes that constitute the pathway. Instead of assuming the existence of a unique rate-limiting step, it assumes that there is a definite amount of flux control and that this is spread quantitatively among the component enzymes. Metabolic control analysis was formerly known as metabolic control theory and is closely related to the engineering discipline known as sensitivity analysis. Alternative approaches to studying the kinetic behaviour of multi-enzyme systems are flux-oriented theory and biochemical systems theory.

Metabolic control analysis is concerned with the effect of changes in enzyme concentration or activity on the steady state metabolite concentration and fluxes of the metabolic systems.

The theoretical principles developed by Kacser and Burns (1973) and Heinrich and Rapoport (1974) have greatly facilitated the quantitative determination of the extent to which certain enzymes control the flux through a metabolic pathway. Much work has been done on this subject (Acerenza *et al.* (1989); Bayram (1993); Cascante *et al.* (1990); Kacser (1990); Small and Fell (1990); Westerhoft and Chen (1984)).

A matrix method has been derived (Fell and Sauro (1985)) and (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. A survey of metabolic control analysis has been given by Fell (1992).

We will consider the following metabolic control coefficients;

- Flux Control Coefficients
- Concentration Control Coefficient
- Elasticity Coefficient

Flux Control Coefficients

The flux control coefficient is a measure of flux control, i.e, how an enzyme's activity has influence over the pathway flux.

The flux control coefficient is defined with respect to one specified enzyme and for one defined flux in the system.

A control coefficient is defined as:

$$C_{E_i}^v = \frac{\partial v/v}{\partial E_i/E_i} = \frac{\partial \ln v}{\partial \ln E_i}$$

where E_i *i* the step (enzyme) and v its steady-state rate of the step perturbed.

In a linear pathway, the flux control coefficient can take on any value between zero and one, larger and negative values are possible in non-linear pathways. A value of zero indicates no control and a value of one complete proportional control. For more complex pathways, e.g. branches, or cycles, the flux control coefficient is not limited by this range. It can be greater than one; or less than zero; coefficients less than zero simply indicate that a rise in enzyme activity induces a fall in flux. A flux control coefficient is smaller than zero indicate that increase in enzyme activity will lead to a decrease in speed of the system.

Concentration Control Coefficient

Concentration control coefficient describes how a change in an enzyme's activity affects the concentration of a metabolite in the system. A positive coefficient would indicate an increase in a metabolite level (for example, the product of an enzyme would rise in response to a rise in the enzyme's activity), whilst a negative coefficient would be the result of a fall in a metabolite concentration (for example, the substrate for an enzyme would fall if the enzyme's activity were increased).

- While a positive concentration control coefficients value indicates an increase in the enzyme activity level and metabolic level,
- A negative concentration control coefficients value indicates an increase in the enzyme activity together with a decrease in the metabolic level.

A concentration control coefficient is the corresponding quantity that defines effects on metabolite concentrations:

$$C_{E_i}^S = \frac{\partial S/S}{\partial E_i/E_i} = \frac{\partial \ln S}{\partial \ln E_i}$$

where S metabolite and E_i , i th step enzyme.

Elasticity Coefficient

An elasticity is a local property of an isolated enzyme that expresses how its rate varies with the concentration of any metabolite that affects it: this can be its substrate, product, or any other metabolite. The degree to which these factors change the reaction rate is described by the elasticity coefficient.

The metabolite product should have a negative elasticity coefficient.

This coefficient is defined as follows

$$C_{E_i}^{v_i} = \frac{\partial v_i/v_i}{\partial S/S} = \frac{\partial \ln v_i}{\partial \ln S}$$

where v_i i th reaction rate and S metabolite (substrate, product, or any other metabolite).

The flux and concentration control coefficients are related with overall system. Therefore, they are called the system properties. An elasticity is a local property of an isolated enzyme.

Summation Theorem

The flux control coefficient does not give any insight into metabolic control. All it states is how changes to enzyme activities of particular steps affect flux, and thereby gives information on how control is distributed amongst the different enzymes of the system. For further insight, one must turn to the theorems of control theory. One of them is the summation theorem.

This states that the sum of all the flux control coefficients of a reaction system is unity. The summation theorem 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 theorem is obeyed.

Theorem 7 *The summation theorem for the system containing n-enzyme*

$$\sum_{i=1}^n C_{E_i}^v = 1$$

where v is overall flux and E_i , i th enzyme concentration.

There are as many concentration control coefficients for any one metabolite as there are enzymes and substrates in the system.

The sum of all concentration control coefficients is always zero.

Theorem 8 *The summation theorem for Concentration Control Coefficients is*

$$\sum_{i=1}^n C_{E_i}^v = 1$$

where the system sustained by n enzymes, E_i i th enzyme concentration and S_j , j th metabolite concentration.

Connectivity Relations

Concentration control coefficients and Control coefficients are properties of the system and they depend on all the enzymes and parameters that make up the system.

The elasticity coefficient is a local property connected with an isolated enzyme in the system.

Connectivity relations relate the elasticity coefficients and control coefficients. An unbranched multi-enzyme system with n enzymes consists of n^2 unknown variables. With the summation theorems, there are n relations defined, with one being related to Control coefficients and the remaining $n-1$ related to Concentration control coefficients. So, there are a total of $n^2 - n$ connectivity relations for such a system.

The relation connecting Control coefficients and elasticity coefficients was first discovered by Kacser and Burns (1973), Kacser *et al.* (1990), Sauro *et al.* (1987) and with S_j denoting the j th metabolite, the formulization is as follows

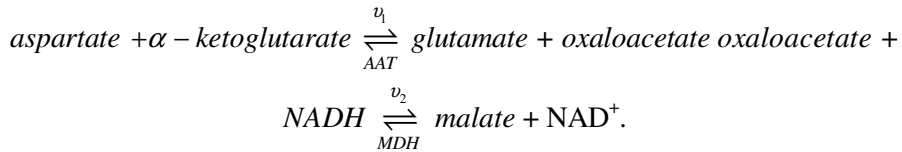
$$C_{E_1}^v \epsilon_{S_j}^{v_1} + C_{E_2}^v \epsilon_{S_j}^{v_2} + C_{E_3}^v \epsilon_{S_j}^{v_3} + \dots = 0$$

The relation between Concentration control coefficients and elasticity coefficients was first discovered by Wasterhoft and Chen (1984), Yildirim (2000) and with S_j and S_k each denoting a metabolite, the formulization is as follows

$$C_{E_1}^{S_k} \epsilon_{S_j}^{v_1} + C_{E_2}^{S_k} \epsilon_{S_j}^{v_2} + C_{E_3}^{S_k} \epsilon_{S_j}^{v_3} + \dots = \begin{cases} -1 & \text{if } k = j \\ 0 & \text{if } k \neq j \end{cases}$$

3. APPLICATIONS

Let us consider the following two enzymes system: aspartate aminotransferase (AAT) and malate dehydrogenase (MDH), that is



The system given above contain two substrates and two products and the system at steady state, that is

$$v - v_1 = 0$$

$$v - v_2 = 0$$

where v is overall flux.

Flux control coefficients for the given system

$$C_{AAT}^v = \frac{[AAT]}{v} \times \frac{\partial v}{\partial [AAT]} = 0.993544$$

and

$$C_{MDH}^v = \frac{[MDH]}{v} \times \frac{\partial v}{\partial [MDH]} = 0.006456.$$

Sum of all the flux control coefficients of a reaction system is

$$C_{AAT}^v + C_{MDH}^v = 0.993544 + 0.006456 = 1.0.$$

Metabolic control coefficients (Concentration control coefficients):

$$C_{AAT}^{oaa} = \frac{[AAT]}{oaa} \times \frac{\partial [oaa]}{\partial [AAT]} = 0.468897$$

and

$$C_{MDH}^{oaa} = \frac{[MDH]}{oaa} \times \frac{\partial [oaa]}{\partial [MDH]} = -0.468897$$

Sum of metabolic control coefficients

$$C_{AAT}^{oaa} + C_{MDH}^{oaa} = 0.468897 - 0.468897 = 0.$$

Finally, Elasticity control coefficients

$$\epsilon_{oaa}^{v_1} = \frac{[oaa]}{v_1} \times \frac{\partial [v_1]}{\partial [oaa]} = -0.0137677$$

and

$$\epsilon_{oaa}^{v_2} = \frac{[oaa]}{v_2} \times \frac{\partial [v_2]}{\partial [oaa]} = 2.11890.$$

Connectivity theorem

$$C_{AAT}^{v_1} \times \epsilon_{oaa}^{v_1} + C_{MDH}^{v_1} \times \epsilon_{oaa}^{v_2} \approx 0.000000$$

The connectivity theorem is provided.

4. CONCLUSION

In this article, we discussed the enzymes kinetic system and metabolic control coefficients. We also presented several existing strategies for their derivation. The mathematics employed in these strategies was considered (Frank (1996)). We showed that the basic relations can be obtained by simply solving the steady-state rate equations.

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