Volume 232, number 2, 383–386 FEB 05889 May 1988

How do external parameters control fluxes and concentrations of metabolites?

An additional relationship in the theory of metabolic control

B.N. Kholodenko

Research Institute for Technology and Safety of Drugs, 23 Kirov Street, Kupavna, 142450 Moscow Region, USSR

Received 25 March 1988

The flux through a metabolic pathway can be controlled by external signals from the environment. These signals are formally described as changes in external parameters, such as concentrations of external metabolites (substrates or effectors) or physical parameters, e.g. temperature, pH, ionic strength. It was proved that the response coefficient of the flux (or of the concentration) to a change in an external parameter is the weighted average of external elasticities of pathway enzymes towards this parameter; weight factors are the control coefficients of corresponding enzymes. As compared with the previously known relationships these ones are applicable to the more common case of parameters acting on more than one enzyme. Along with other applications, the use of the obtained relationships for control analysis of moiety-conserved cycles is considered.

Metabolic control analysis; Response coefficient; External elasticity; Control coefficient; Moiety-conserved cycle

1. INTRODUCTION

Metabolic control analysis, as developed by Kacser and Burns [1,2], Higgins [3], Heinrich and Rapoport [4], has clarified a previous semi-intuitive understanding of metabolic regulation. Metabolic control analysis allows one to obtain quantitative measures of control in metabolic pathways in terms of dimensionless coefficients. Two different types of coefficients, 'global' and 'local', are distinguished. Local coefficients describe the kinetic properties of 'isolated' functional units in an enzyme system, which are the individual reaction steps. Global coefficients detail how altering the activity of any enzyme or another parameter affects system variables, such as fluxes or metabolic concentrations.

An actual problem of control analysis is to relate the systemic response to a disturbance with the local responses of individual reactions [1–6]. This paper deals with the responses of system variables to the changes of external parameters, such as concentrations of external metabolites (substrates or effectors) or various physical parameters (temperature, pH, ionic strength, etc.). An external parameter often plays the role of the control signal, which makes the system change its behaviour in accordance with the demands of the environment. Previously in the framework of control analysis the influence of an external parameter was considered for the particular case when this parameter only affects one enzyme reaction of the system [1]. In many cases, however, this assumption is unapplicable. This work generalizes the relationship for a 'single-point-action' parameter for the general situation.

Correspondence address: B.N. Kholodenko, Research Institute for Technology and Safety of Drugs, 23 Kirov Street, Kupavna, 142450 Moscow Region, USSR

2. THEORETICAL BACKGROUND

We shall consider an arbitrary metabolic system

Published by Elsevier Science Publishers B.V. (Biomedical Division)

consisting of m metabolites S_1, S_2, ..., S_m and n enzymes E_1, E_2, ..., E_n. The amount of control exerted by an enzyme E_i on any system flux J can be characterized by the fractional change in metabolic flux induced by a fractional change in the concentration or the activity of the enzyme under consideration provided all other parameters are held constant [1–4]:

$$C_i^J = \frac{E_i}{J}\left(\frac{\partial J}{\partial E_i}\right) = \frac{\partial \ln |J|}{\partial \ln E_i},\ i = 1, 2, \ldots, n$$

This coefficient C_i^J is called the flux control coefficient in accordance with the agreed new terminology. Similarly, the concentration control coefficient is defined as:

$$C_i^{Sk} = \frac{E_i}{S_k}\left(\frac{\partial S_k}{\partial E_i}\right) = \frac{\partial \ln S_k}{\partial \ln E_i},$$
$$i = 1, 2, \ldots, n;\ k = 1, 2, \ldots, m$$

These global coefficients (C_i^J, C_i^{Sk}) describe systemic properties.

Local coefficients deal with the response of any reaction rate (v_i), when the enzyme is 'isolated' from the rest of the system, towards an infinitesimal change in any metabolite. These local coefficients are called elasticity coefficients to metabolite S_k and are defined as:

$$\epsilon_k^i = \frac{S_k}{v_i}\left(\frac{\partial v_i}{\partial S_k}\right) = \frac{\partial \ln |v_i|}{\partial \ln S_k},$$
$$i = 1, 2, \ldots, n;\ k = 1, 2, \ldots, m$$

The external elasticitiy coefficient, which describes the local response to a change in an external parameter P, is defined similarly:

$$\chi\epsilon_P^i = \frac{\partial \ln |v_i|}{\partial \ln P},$$

where superscript χ indicates that ${}^{\chi}\epsilon_P^i$ is the elasticity towards an external parameter [6].

There are global coefficients, which quantify the action of external parameters on the system variables. They are the response coefficients of the flux, R_P^J, and of the concentrations, R_P^{Sk} [6]:

$$R_P^J = \frac{P}{J}\left(\frac{\partial J}{\partial P}\right) = \frac{\partial \ln |J|}{\partial \ln P},$$

$$R_P^{Sk} = \frac{P}{S_k}\left(\frac{\partial S_k}{\partial P}\right) = \frac{\partial \ln S_k}{\partial \ln P}$$

If an external parameter P affects only one of the system enzymes, say E_i, then the response coefficient equals the product of the control coefficient of the enzyme E_i and the external elasticity of this enzyme towards P [1]. Will this statement remain correct when the external parameter affects many reactions in the system? To answer this question an additional consideration is needed.

3. RESULTS AND DISCUSSION

3.1. *Theorem*

The response coefficient of the flux J to a change in an external parameter P is equal to the weighted average of the external elasticity coefficients to P for all the enzymes, where weight factors are the flux control coefficients of corresponding enzymes:

$$R_P^J = \sum_{i=1}^{n} C_i^J \cdot {}^{\chi}\epsilon_P^i \qquad (1)$$

Similar relationships are valid for the concentration response coefficients:

$$R_P^{Sk} = \sum_{i=1}^{n} C_i^{Sk} \cdot {}^{\chi}\epsilon_P^i \qquad (2)$$

To prove this theorem we use a method resembling that of Kacser and Burns [1], which consists of joint change of P and the activities E_i of all the affected enzymes, so that all the rates v_i and metabolite concentrations S_k remain unchanged:

$$v_i = E_i w_i(S_1, \ldots, S_m, P) = E_i^{(0)} w_i(S_1, \ldots, S_m, P^{(0)}),$$
$$i = 1, \ldots, n \qquad (3)$$

where w_i describes the dependence of the i-th reaction rate on metabolite concentrations and the external parameter; E_i, $E_i^{(0)}$, P, $P^{(0)}$ are new and initial values of the i-th enzyme activity and the external parameter, respectively. It should be emphasized that the decomposition of v_i in a product of two factors, only one of which depends on P, is formally possible for any concrete mechanism of external parameter action including irreversible inhibition of the enzyme or action of the change in

pH, temperature, etc. Differentiation of eqn 3 gives:

$$\frac{\mathrm{dln}E_i}{\mathrm{dln}P} = -{}^{x}\epsilon_P^i \qquad (4)$$

Since the variation of P does not change the flux J and the concentrations S_k, we can write

$$\frac{\mathrm{dln}|J|}{\mathrm{dln}P} = \sum_{i=1}^{n} C_i^J \frac{\mathrm{dln}E_i}{\mathrm{dln}P} + R_P^J = 0$$

$$\frac{\mathrm{dln}S_k}{\mathrm{dln}P} = \sum_{i=1}^{n} C_i^{Sk} \frac{\mathrm{dln}E_i}{\mathrm{dln}P} + R_P^{Sk} = 0 \qquad (5)$$

Combined with eqn 4, this yields the relationships expressed by eqns 1 and 2.

There exist various applications of the theorem proved. Suppose that an external parameter is the concentration of the coenzyme or the effector, which takes place in several reactions of the pathway and plays the role of the metabolic signal determining the steady-state flux through the pathway. Then the response coefficient of the flux to the change in the coenzyme concentration can be considered as group elasticity of the overall pathway. Eqn 1 gives its expression through the control coefficients and elasticities of individual reactions with respect to the metabolic signal.

A quite different example of using eqns 1 and 2 relates to the metabolic system with a conservation relationship:

$$\sum_{k\in T} \mu_k S_k = T, \qquad (6)$$

where μ_k are stoichiometric coefficients, subscript $k\in T$ indicates that the sum only involves the metabolites which obey the conservation constraint, and T is constant. Usually T represents the total concentration of a chemical moiety which is conserved in the reactions of the system. Systems with moiety-conserved cycles have been considered in the literature within the framework of control analysis. Hofmeyr et al. [6] investigated the response coefficients R_T^J, R_T^{Sk} of the system variables to changes in the conserved sum T. Since T can vary on larger time scales due to reactions of metabolic environment, R_T^J, R_T^{Sk} are important system characteristics along with control coefficients. As described [6] the response coefficients have been expressed in terms of cycle elasticity and control coefficients characterizing the cycle as a 'catalytic black box'. However, such an approach is self-consistent provided the cycle metabolites do not affect the reaction rates beyond the cycle. Using the theorem proved, one can obtain alternative relationships for response coefficients, which remain valid in the general case.

For our purposes T should be treated as an 'external' parameter. However, it is essentially different from that in eqns 1 and 2. The peculiarity of T is that the rates of reactions, when isolated from the system, do not depend on T. The explicit dependence on T appears if we take into account the conservation law (eqn 6) and express one of the concentrations in eqn 6, say S_k , through the remaining concentrations and T. Substituting the expression for the S_k in the rate laws and using eqn 1 one obtains:

$$R_T^J = \frac{T}{\mu_k S_k} \cdot \sum_{i=1}^{n} C_i^J \cdot \epsilon_k^i, \; k\in T \qquad (7)$$

Eqn 7 is valid for any metabolite S_k participating in the conserved sum, eqn 6. Multiplication of eqn 7 by $\mu_k S_k$ and summation over all metabolites within the T yield:

$$R_T^J = \sum_{i=1}^{n} C_i^J \cdot \left(\sum_{k\in T} \epsilon_k^i \right)$$

Similarly one can obtain the expression for the response coefficients of metabolite concentrations:

$$R_T^{Sl} = \sum_{i=1}^{n} C_i^{Sl} \left(\sum_{k\in T} \epsilon_k^i \right) + \delta_{lT}$$

where $\delta_{lT} = 1$, if S_l belongs to T, otherwise $\delta_{lT} = 0$.

In addition, it is worth mentioning that some heuristic value of the theorem proved is related to the possibility of making a preliminary conclusion about the comparative values of control coefficients. Such evaluations assume that comparison of the response of the system flux towards the external metabolite with the local responses of individual steps is made. Let us consider the regulation of oxidative phosphorylation when extramitochondrial adenine nucleotide status (ATP and ADP concentrations, or ATP/ADP ratio) is held constant [7]. The proximity of the apparent Michaelis constants for the external ADP (or

ADP/ATP ratio [8]) of the adenine nucleotide translocator and of the overall oxidative phosphorylation [9,10] suggests that the control coefficient of the translocator at intermediary respiration rates (30–70% of the State 3 respiration) is sufficiently high [11,12].

REFERENCES

[1] Kacser, H. and Burns, J.A. (1973) in: Rate Control of Biological Processes (Davies, D.D. ed.) pp.65–104, Cambridge University Press, London.
[2] Kacser, H. and Burns, J.A. (1979) Biochem. Soc. Trans. 7, 1149–1160.
[3] Higgins, J. (1965) in: Control of Energy Metabolism (Chance, B. et al. eds) pp.13–46, Academic Press, New York.
[4] Heinrich, R. and Rapoport, T.A. (1974) Eur. J. Biochem. 42, 89–104.
[5] Westerhoff, H.V. and Chen, Y. (1984) Eur. J. Biochem. 142, 425–430.
[6] Hofmeyr, J.-H.S., Kacser, H. and Van der Merve, K.J. (1986) Eur. J. Biochem. 155, 631–641.
[7] Kholodenko, B.N. (1984) J. Theor. Biol. 107, 179–188.
[8] Wanders, R.J.A., Groen, A.K., Van Roermund, C.W.T. and Tager, J.M. (1984) Eur. J. Biochem. 142, 417–424.
[9] Barbour, R.L. and Chan, S.H. (1981) J. Biol. Chem. 256, 1940–1948.
[10] Jacobus, W.E., Moreadith, R.W. and Vandegaer, K.M. (1982) J. Biol. Chem. 257, 2397–2402.
[11] Davis, E.J. and Davis-Van Thienen, I.A. (1984) Arch. Biochem. Biophys. 233, 573–581.
[12] Kholodenko, B., Zilinskiene, V., Borutaite, V., Ivanoviene, L., Toleikis, A. and Praskevicius, A. (1987) FEBS Lett. 223, 247–250.