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A Generalization of Metabolic Control Analysis to Conditions of No Proportionality Between Activity and Concentration of Enzymes

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The assumption currently considered in the Metabolic Control Theory that velocity of every isolated step is proportional to enzyme concentration, is analysed by considering that in some metabolic systems that condition could not be accomplished. Analysis of the main core of this theory is carried out removing this hypothesis, expressed as "$\varepsilon_{e_i}^{v_i}$ is not necessarily equal to one". The results obtained supply a more general formulation of the main theorems of the Control Theory, extending its possible application to more complex metabolic systems.

Introduction

Control Theory was formulated by Kacser & Burns (1973) and Heinrich & Rapoport (1974) for studying metabolic control *in vivo*. Further development of this theory by Fell & Sauro (1985), Heinrich & Rapoport (1975), Heinrich *et al.* (1977), Hofmeyr *et al.* (1986), Kacser (1981), Kacser & Burns (1979, 1981), Sauro *et al.* (1987), Torres *et al.* (1988*a*), and Westerhoff & Chen (1984), among others, has supplied several methods for its application to metabolic systems, and a number of data on metabolic control have been obtained by different groups of researchers (see, e.g. Dyzkhuizen *et al.*, 1987; Groen *et al.*, 1982*a*; Rapoport *et al.*, 1974, 1976; Regen & Pilkis, 1984; Salter *et al.*, 1986; Stuart *et al.*, 1986; Torres *et al.*, 1988*a*,*b*); reviews on this theory and its application can be seen in Heinrich *et al.*, 1977; Groen *et al.*, 1982*b*; Westerhoff *et al.*, 1984; Meléndez-Hevia *et al.*, 1987.

Control theory is based on the separate study of systemic and local effects, and the relationships among them. Systemic effects are described by control coefficients, C_p^V, whose formulation in the current nomenclature (see Burns *et al.*, 1985) is:

$$C_p^V = \frac{\partial V}{\partial p} \cdot \frac{p}{V}, \tag{1}$$

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where V stands for any systemic dependent variable (such as flux J, or the concentration of a given intermediate pool s_j) and p for any systemic independent variable, or parameter (currently enzyme concentration) whose variation causes the change in V. Thus, control coefficients give a quantitative measure of the effect of an infinitesimal change of a specified parameter on a given dependent variable.

Local effects are described by elasticity coefficients, whose formulation is done under the same considerations for any isolated step, in the same conditions in which it occurs in the whole system; its general formulation is (Burns *et al.*, 1985):

$$\varepsilon_x^v = \frac{\partial v}{\partial x} \cdot \frac{x}{v},$$

where v stands for any local dependent variable (usually the velocity) of the isolated step, and x for any local independent variable (usually substrate or effector concentration) of the local isolated system. Finally, response coefficients, R_x^V, describe systemic effects of any external substrates, products or effectors on the flux.

The main theoretical core of Control Theory can be considered as a set of theorems which relate the different coefficients among them. Summation theorems for flux (Kacser & Burns, 1973, 1979; Heinrich & Rapoport, 1974) and individual pools (Heinrich & Rapoport, 1974) impose given restrictions on control distribution. Connectivity and reponse theorems (Kacser &Burns, 1973; Westerhoff & Chen, 1984) describe properties which relate control and elasticity coefficients.

The usefulness of these theorems is that they describe structural relationships in metabolic control and, in addition, they have allowed the design of methods to assay the different coefficients (see, e.g. Groen *et al.*, 1982*b*; Torres *et al.*, 1986); applications of such methods to a number of metabolic systems *in vivo* and *in vitro* have supplied new information on control distribution in cell metabolism (see reviews mentioned above).

Explicit development of the Control Theory, which includes derivation of theorems, has been made on the assumption that elasticity of each enzyme for p parameter, used in eqn (1) is equal to unit, i.e. $\varepsilon_{p_i}^{v_i} = 1$ (Kacser & Burns, 1973; Heinrich & Rapoport, 1974). This means that p_i is a parameter of the enzyme E_i whose variation produces a change proportional in the velocity of its isolated step: $dv_i/v_i = dp_i/p_i$. On the other hand, since p_i is an independent variable, which must be experimentally accessible in order to determine control coefficients, enzyme concentration (e_i) has currently been used for it (Burns *et al.*, 1985). Thus, control coefficients experimentally used have currently been $C_{e_i}^V$, and therefore, experimental application of the theory has been made on the assumption that $\varepsilon_{e_i}^{v_i}$ (or ε_i, for short) is equal to one. This assumption of proportionality between enzyme concentration and isolated velocity could not be accomplished in certain cases. It has recently been shown by certain groups (see Giersch *et al.*, 1990; Heinrich, 1990; Kacser *et al.*, 1990) that different features of some biological systems could account for values of ε_i different to one, which produces that theorems of Control Theory cannot be applied in their current formulation. However, if derivation of the theorems is done without assumption of $\varepsilon_i = 1$, this fact does not leave Control Theory out of application for these systems.

The aim of this work is to extend the field of application of Control Theory to these cases, by proposing a formulation of Control Theory, where parameter p in eqn (1) is explicitly the enzyme concentration and its elasticity can have any value. In light of this consideration, we present a reformulation of the main theorems. We do not modify other assumptions of Control Theory: our analysis is applied to systems of any complexity at steady-state, with a constant value for any flux and all intermediate pools at stabilized concentrations. The mechanism of each enzyme is unspecified, allowing any degree of reversibility and saturation. In the same way, the mechanism which causes the no proportional relationship between activity and concentration of the enzyme is unspecified, allowing this formulation to be applied to any system with this feature.

Theory and Results

SUMMATION THEOREMS

Summation Theorems, described by Kacser & Burns (1973, 1979) and Heinrich & Rapoport (1974) impose a given relationship among control coefficients of any flux, or any intermediate pool, respectively, by establishing a given value of their summation for all enzymes of the system. Formulation of these theorems, on assumption that $\varepsilon_i = 1$ is:

(a) For any flux J

$$\sum_{i=1}^{n} C_{e_i}^{J} = 1. \tag{2}$$

(b) For every metabolite concentration S_j

$$\sum_{i=1}^{n} C_{e_i}^{S_j} = 0. \tag{3}$$

For cases where $\varepsilon_i \neq 1$ a different formulation is derived:

(a) Summation of flux control coefficients

Consider a metabolic system of any complexity at steady-state, where every enzyme has a different elasticity for its own concentration, ε_i. In this system let us make a certain small change in the concentration of all the enzymes of the system, simultaneously, in order to obtain a given fractional change of flux $\mathrm{d}J/J = \alpha$. Since every enzyme has a different elasticity for its concentration, ε_i, the change we ought to make in every one, $\mathrm{d}e_i/e_i = \alpha_i$, will be different but, in any case, the following condition must be fulfilled:

$$\frac{\mathrm{d}v_i}{v_i} = \alpha = \varepsilon_i \frac{\mathrm{d}e_i}{e_i}.$$

Therefore, the fractional change realized in every enzyme concentration, α_i, is given by;

$$\alpha_i = \frac{\mathrm{d}e_i}{e_i} = \frac{\alpha}{\varepsilon_i}. \tag{4}$$

On the other hand, according to expression of total differential of the flux;

$$dJ = \frac{\partial J}{\partial e_i} de_1 + \frac{\partial J}{\partial e_2} de_2 + \ldots + \frac{\partial J}{\partial e_n} de_n$$

$\partial J/\partial e_i$ being always different from zero, since if for any enzyme $\varepsilon_i = 0$, its corresponding term is obviously eliminated. This expression can be written as:

$$\frac{dJ}{J} = \alpha = \sum_{i=1}^{n} \frac{\partial J}{\partial e_i} \cdot \frac{e_i}{J} \cdot \frac{de_i}{e_i},$$

or, according to the definition of flux control coefficients,

$$\alpha = \sum_{i=1}^{n} C_{e_i}^{J} \frac{de_i}{e_i}. \tag{5}$$

By substituting de_i/e_i from eqn (4) into eqn (5) we obtain;

$$\alpha = \sum_{i=1}^{n} C_{e_i}^{J} \cdot \frac{\alpha}{\varepsilon_i}, \quad \text{and thus,} \quad \alpha = \alpha \cdot \sum_{i=1}^{n} C_{e_i}^{J} \frac{1}{\varepsilon_i}.$$

This leads to;

$$\sum_{i=1}^{n} C_{e_i}^{J} \cdot \frac{1}{\varepsilon_i} = 1, \tag{6}$$

which is the new presentation of the summation theorem for flux control coefficients. For all cases $\varepsilon_i > C_{e_i}^{J}$ because the systemic effect cannot be greater than local effect. Then, provided that in eqn (6) $\varepsilon_i > 0$, it follows that;

$$1 > C_{e_i}^{J} \cdot \frac{1}{\varepsilon_i}.$$

Therefore, all terms of the summation in eqn (6) are less than one. On the other hand, note that, since $\varepsilon_i > C_{e_i}^{J}$, if all ε_i are very small, then all $C_{e_i}^{J}$ are also very small. In these conditions, eqn (6) is also accomplished, but $\sum C_{e_i}^{J} < 1$. A system like this could not be totally controlled by modulation of enzyme concentration.

(*b*) *Summation of intermediate pool coefficients*

On the other hand, concentration of any intermediate pool, S_j, has not changed under conditions described above, dS_j/S_j being equal to zero; thus, by a similar procedure to obtain eqn (6), the reformulation of summation for flux, the summation theorem for individual metabolites is derived:

$$\sum_{i=1}^{n} C_{e_i}^{s_j} \cdot \frac{1}{\varepsilon_i} = 0. \tag{7}$$

CONNECTIVITY THEOREMS

Connectivity theorems relate control and elasticity coefficients, for all enzymes which interact with a common pool. Connectivity theorem for flux, formulated by

Kacser & Burns (1973, 1979) establishes the relationship among flux control coefficients of the whole set of enzymes E_r, which interact with a given intermediary metabolite S_k, and their elasticities for this metabolite. This theorem is expressed by;

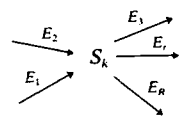
$$\sum_{i=1}^{R} C^{J}_{e_r} \cdot \varepsilon_{s_k} = 0, \tag{8}$$

ε_{s_k} being the elasticity coefficient of v_r with respect to S_k. In addition, Westerhoff & Chen (1984), have described the connectivity theorem for the concentration of any intermediary etabolite S_j. This theorem has been formulated by these authors as;

$$\sum_{r=1}^{R} C^{s_j}_{e_r} \cdot \varepsilon_{s_k} = -\delta, \tag{9}$$

where δ is the Kronecker δ which is equal to zero for any $j \neq k$ and equal to one for $j = k$. Here, considering that ε_r can have any value, connectivity theorems also have a more general expression.

Consider a metabolic system of any complexity, with a finite number n, of enzymes, in a steady-state asymptotely stable. Let $\{E_1, E_2, \ldots, E_R\}$ be the set of enzymes which interact with a given metabolite S_k; i.e. S_k is substrate, product or effector of every one of this set, not being an external or independent variable of the system (see Scheme 1).



SCHEME 1

In such a system the following properties which relate control and elasticity coefficients are accomplished:

(a) For any flux J;

$$\sum_{r=1}^{R} C^{J}_{e_r} \cdot \frac{1}{\varepsilon_r} \cdot \varepsilon_{s_k} = 0, \tag{10}$$

(b) For any intermediate pool, S_j;

$$\sum_{r=1}^{R} C^{s_j}_{e_r} \cdot \frac{1}{\varepsilon_r} \cdot \varepsilon_{s_k} = -\delta. \tag{11}$$

δ being the Kronecker δ which is equal to zero for any $j \neq k$ and equal to one for $j = k$.

Proof. In the system described above, at steady-state, let us make a small change in the concentration of S_k ($\mathrm{d}s_k \neq 0$), and in the concentration of all enzymes which interacts with S_k, some positive and others negative, in such a manner that the velocity of each individual step catalysed by $E_1, E_2, \ldots, E_R$ have been unchanged.

Thus, all fluxes and intermediate pools other than S_k will remain the same. Therefore, the change carried out in every enzyme must account for, in any case, the following relationship:

$$\frac{dv_r}{v_r} = \varepsilon_r \cdot \frac{de_r}{e_r} + \varepsilon_{s_k} \cdot \frac{ds_k}{s_k} = 0, \quad \text{being } ds_k \neq 0$$

from which is obtained;

$$\frac{de_r}{e_r} = -\frac{1}{\varepsilon_r} \cdot \varepsilon_{s_k} \cdot \frac{ds_k}{s_k}. \tag{12}$$

On the other hand, since the changes in S_k concentration, and concentration of every enzyme of the system have not changed any flux in the system, nor the concentration of any pool other than S_k, we can write:

(a) for any flux:

$$\frac{dJ}{J} = 0 = \sum_{r=1}^{R} C^J_{e_r} \cdot \frac{de_r}{e_r},$$

which, by substituting de_r/e_r from eqn (12), leads to;

$$\frac{ds_k}{s_k} \cdot \sum_{r=1}^{R} C^J_{e_r} \cdot \frac{1}{\varepsilon_r} \cdot \varepsilon_{s_k} = 0. \tag{13}$$

Regarding eqn (13), since $ds_k/s_k \neq 0$, the expression proposed in (10) is obtained.

(b) for the concentration of any intermediate pool s_j, other than S_k, since $ds_j = 0$:

$$\frac{ds_j}{s_j} = 0 = \sum_{r=1}^{R} C^{s_j}_{e_r} \cdot \frac{de_r}{e_r},$$

which, by substituting de_r/e_r from eqn (12), leads to:

$$\frac{ds_k}{s_k} \cdot \sum_{r=1}^{R} C^{s_j}_{e_r} \cdot \frac{1}{\varepsilon_r} \cdot \varepsilon_{s_k} = 0, \tag{14}$$

and by the same procedure, for the concentration of S_k we obtain

$$\frac{ds_k}{s_k} \cdot \sum_{r=1}^{R} C^{s_k}_{e_r} \cdot \frac{1}{\varepsilon_r} \cdot \varepsilon_{s_k} = -\frac{ds_k}{s_k}. \tag{15}$$

Regarding eqns (14) and (15), since $dS_k/S_k \neq 0$, it is clear that the expression proposed in (11) is obtained.

RESPONSE THEOREMS

Response theorem for flux, formulated by Kacser & Burns (1973), establishes a relationship among the flux control coefficient of any given enzyme, E_k, its elasticity for an external substrate or effector X, which specifically interacts with it, and the

effect of X on the whole system. Formulation of this theorem under the assumption that $\varepsilon_k = 1$ is:

$$R_x^J = C_{e_k}^J \cdot \varepsilon_x, \tag{16}$$

ε_x being the elasticity coefficient of v_k with respect to X.

Under consideration that ε_k can have any value, this theorem is also modified as:

$$R_x^J = C_{e_k}^J \cdot \frac{1}{\varepsilon_k} \cdot \varepsilon_x. \tag{17}$$

Proof. Since the effect of any change of X on the enzyme E_k is equivalent to a given change in the concentration of E_k, let us make a small change in X concentration, $dx \neq 0$, and simultaneously, a small change in E_k concentration, $de_k \neq 0$, in such a manner that both these are cancelled, i.e. that these have no effect on any flux nor concentration of any intermediate pool. Now, considering the result from the systemic point of view, we have

$$\frac{dJ}{J} = 0 = C_{e_k}^J \cdot \frac{de_k}{e_k} + R_x^J \cdot \frac{dx}{x}. \tag{18}$$

And considering the situation from a local point of view,

$$\frac{dv_k}{v_k} = 0 = \varepsilon_k \frac{de_k}{e_k} + \varepsilon_x \cdot \frac{dx}{x}. \tag{19}$$

Then by eliminating de_k/e_k in eqns (18) and (19), the expression proposed in (17) is obtained.

Remark. Note that by a similar procedure, an equivalent theorem of response for any intermediate pool, S_j, is also obtained:

$$R_x^{s_j} = C_{e_k}^{s_j} \cdot \frac{1}{\varepsilon_k} \cdot \varepsilon_x. \tag{20}$$

Concluding Remarks

After the formulations of the main theorems of Control Theory, presented above, it can be seen that, when $\varepsilon_i < 1$ control coefficients with respect to enzyme concentration, $C_{e_i}^V$, are only a fraction of the "absolute" control coefficients $C_{p_i}^V$:

$$C_{e_i}^V = C_{p_i}^V \cdot \varepsilon_i. \tag{21}$$

The factor ε_i which multiplies every $C_{p_i}^V$ to obtain $C_{e_i}^V$ is a normalization factor which represents the "local" response of every enzyme.

If control is analysed by modulating enzyme activity through another parameter different to enzyme concentration (e.g. by using an inhibitor which affects the affinity or the catalytic constant), the control coefficients obtained, $C_{k_i}^V$, are also related to the "absolute" coefficients $C_{p_i}^V$ by;

$$C_{k_i}^V = C_{p_i}^V \cdot \varepsilon_{k_i}. \tag{22}$$

This is the general expression which relates the "experimental" or "apparent" control coefficient to the "absolute" control coefficient. And again, where $\varepsilon_{k_i} = 1$, the value of the "absolute" coefficient will be the same as the experimentally obtained.

Discussion

A first conclusion which has arisen from the present results is the possible disagreement, in certain cases, between the assayed coefficients and the predictions of theorems. It must be observed that, for example, the summation theorem for the flux, $\sum C^J_{p_i} = 1$, is only valid if p_i is a paramter of the enzyme whose variation is proportional to the velocity of the isolated step. Note, however, that if this parameter is not proportional to the isolated velocity, then the theorem shown in eqn (6) must be applied. Therefore, in certain cases, control coefficients assayed by modulation of enzyme concentration (directly or indirectly by means of some inhibitors) could not be the "absolute" control coefficients, but only a fraction of them, which is accessible by the used procedure of modulation. There could also be cases where $\varepsilon_i > 1$. Such cases are also included in the formulation here presented.

The problem is, therefore, to accede experimentally to a proportional parameter. However, by means of the calculation described in eqn (21), enzyme concentration can be used as the modulating parameter and, after assay or estimation of ε_i, "absolute" control coefficient can be obtained. A different theme is the usefulness of the "absolute" control coefficient $C^V_{p_i}$. Consider, for example, an enzyme with a $C^V_{e_i} = 0{\cdot}12$ and $\varepsilon_i = 0{\cdot}20$; this gives, according to eqn (21) an "absolute" control coefficient, $C^V_{p_i} = 0{\cdot}6$. Then, the following question arises: What is the real capacity of control of this enzyme: 0·12 or 0·6? or, is the capacity of control of the enzyme small or large? The response to this question is given according to the meaning of control and, in particular, in respect to which parameter we refer the control. If control is refering to enzyme concentration, such an enzyme is a poor controller, since a given change in its concentration does not provoke a great change in flux. However, its total capacity of control is high (0·6) and it could be exercised by other regulation mechanism available to the cell, e.g. modulating K_m or catalytic constant. In this way, it is possible that some experimental results, where control is widely shared among many enzymes, and whose control coefficient summation is less than 1 (see, e.g. Ottaway, 1976) could be due to this fact. In such cases, calculation of ε_i and correction of $C^J_{e_i}$ by means eqn (21) can clear this situation, demonstrating that a certain fraction of control should be necessarily exercised by other mechanisms.

Enzyme activity can be modulated in several different ways (e.g. its concentration, affinity or catalytic constant), each of them being able to have a different specific elasticity. A conclusion derived from these results is that when such elasticity is less than one, then by modulating this parameter, only a certain fraction of the "absolute" value of control coefficient is accessible. This means that in certain cases a number of different regulatory mechanisms must necessarily be exercised in order to get the whole control capacity of a given enzyme. This fact leads to a further question: Has

each enzyme any chance to carry out its whole capacity of control? We think that this can occur in certain cases, specially in the so called 'regulatory enzymes", and particularly in the multimodulated enzymes. These aspects of control analysis will be explored in a further work.

REFERENCES

BURNS, J. A., CORNISH-BOWDEN, A., GROEN, A. K., HEINRICH, R., KACSER, H., PORTEOUS, J. W., RAPOPORT, S. M., RAPOPORT, T. A., STUCKI, J. W., TAGER, J. M., WANDERS, R. J. A. & WESTERHOFF, H. V. (1985). *Trends Biochem. Sci.* **10,** 16.

DYZKHUIZEN, D. E., DEAN, A. M. & HARTL, D. L. (1987). *Genetics* **115,** 25-31.

FELL, D. A. & SAURO, H. M. (1985). *Eur. J. Biochem.* **148,** 555-561.

GIERSCH, C., LÄMMEL, D. & STEFFEN, K. (1990). In: *Control of Metabolic Processes.* (Cornish-Bowden, A. & Cárdenas, M. L., eds), NATO ASI Series, Plenum Press, New York (in press).

GROEN, A. K., WANDERS, R. J. A., WESTERHOFF, H. V., VAN DER MEER, R. & TAGER, J. M. (1982*a*). In: *Metabolic Compartmentation.* (Sies, H., ed.) pp. 9-37, New York: Academic Press.

GROEN, A. K., WANDERS, R. J. A., WESTERHOFF, H. V., VAN DER MEER, R. & TAGER, J. M. (1982*b*). *J. biol. Chem.* **257,** 2754-2757.

HEINRICH, R. (1990). In: *Control of Metabolic Processes.* (Cornish-Bowden, A. & Cárdenas, M. L., eds) NATO ASI Series, Plenum Press, New York (in press).

HEINRICH, R. & RAPOPORT, T. A. (1974). *Eur. J. Biochem.* **42,** 89-95.

HEINRICH, R. & RAPOPORT, T. A. (1975). *BioSystems* **7,** 130-136.

HEINRICH, R., RAPOPORT, S. M. & RAPOPORT, T. A. (1977). *Progr. Biophys. molec. Biol.* **32,** 1-82.

HOFMEYR, J.-H. S., KACSER, H. & VAN DER MERWE, K. J. (1986). *Eur. J. Biochem.* **155,** 631-641.

KACSER, H. (1981). *Biochem. Soc. Trans.* **11,** 35-40.

KACSER, H. & BURNS, J. A. (1973). *Symp. Soc. expl. Biol.* **27,** 65-104.

KACSER, H. & BURNS, J. A. (1979). *Biochem. Soc. Trans.* **7,** 1149-1160.

KACSER, H. & BURNS, J. A. (1981). *Genetics* **97,** 639-666.

KACSER, H., SAURO, H. M. & ACERENZA, L. (1990). In: *Control of Metabolic Processes.* (Cornish-Bowden, A. & Cárdenas, M. L., eds) NATO ASI Series, Plenum Press, New York (in press).

MELÉNDEZ-HEVIA, E., RIOL-CIMAS, J. M. & TORRES, N. V. (1987). *An. Quim.* **83** C, 224-243.

OTTAWAY, J. H. (1976). *Biochem. Soc. Trans.* **4,** 371-376.

RAPOPORT, T. A., HEINRICH, R. & JACOBASCH, G. & RAPOPORT, S. (1974). *Eur. J. Biochem.* **42,** 107-120.

RAPOPORT, T. A., HEINRICH, R. & RAPOPORT, S. M. (1976). *Biochem. J.* **154,** 449-469.

REGEN, D. M. & PILKIS, S. J. (1984). *J. theor. Biol.* **111,** 635-658.

SALTER, M., KNOWLES, R. G. & POGSON, C. I. (1986). *Biochem. J.* **234,** 635-647.

SAURO, H. M., SMALL, J. R. & FELL, D. A. (1987). *Eur. J. Biochem.* **165,** 215-221.

STUART, F., PORTEOUS, D. J., FLINT, H. J. & KACSER, H. (1986). *J. gen. Microbiol.* **132,** 1159-1166.

TORRES, N. V., MATEO, F., MELÉNDEZ-HEVIA, E. & KACSER, H. (1986). *Biochem. J.* **234,** 169-174.

TORRES, N. V., MATEO, F., SICILIA, J. & MELÉNDEZ-HEVIA, E. (1988*a*). *Int. J. Biochem.* **20,** 161-165.

TORRES, N. V., MATEO, F. & MELÉNDEZ-HEVIA, E. (1988*b*). *FEBS lett.* **233,** 83-86.

WESTERHOFF, H. V. & CHEN, Y.-D. (1984). *Eur. J. Biochem.* **142,** 425-430.

WESTERHOFF, H. V., GROEN, A. K. & WANDERS, R. J. A. (1984). *Biosci. Rep.* **4,** 1-22.