Eur. J. Biochem. *210*, 147 – 153 (1992)

Control of the metabolic flux in a system with high enzyme concentrations and moiety-conserved cycles

The sum of the flux control coefficients can drop significantly below unity

Boris N. KHOLODENKO[1], Arkadii E. LYUBAREV[2] and Boris I. KURGANOV[3]

[1] A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Russia
[2] Vitamin Research Institute, Moscow, Russia
[3] A. N. Bakh Institute of Biochemistry, Russian Academy of Science, Moscow, Russia

(Received April 2/July 2, 1992) – EJB 92 0470

In a number of metabolic pathways enzyme concentrations are comparable to those of substrates. Recently it has been shown that many statements of the 'classical' metabolic control theory are violated if such a system contains a moiety-conserved cycle. For arbitrary pathways we have found: (a) the equation connecting coefficients $C^J_{E_i}$ (obtained by varying the E_i concentration) and $C^J_{v_i}$ (obtained by varying the k_i^{cat}), and (b) modified summation equations. The sum of the enzyme control coefficients (equal to unity under the 'classical' theory) appears always to be below unity in the systems considered. The relationships revealed were illustrated by a numerical example where the sum of coefficients $C^J_{E_i}$ reached negative values. A method for experimental measurements of the above coefficients is proposed.

A quantitative approach to the study of cellular metabolism control is now widely accepted (for review see, for example, [1 – 3]). One of its important branches, the metabolic control theory, was first formulated in [4 – 7]. In the framework of this theory the contribution of the enzyme (E_i) to the control of the flux (J) can be characterized by the fractional change $\partial J/J$ in the metabolic flux induced by the fractional change $\partial E_i/E_i$ in the enzyme concentration:

$$C^J_{E_i} = \frac{\partial J/J}{\partial E_i/E_i} = \frac{\partial \ln |J|}{\partial \ln E_i}, \qquad (1)$$

where E_i is the concentration of the ith enzyme. The dimensionless coefficient, $C^J_{E_i}$, is named the flux control coefficient of the concentration of enzyme i. It corresponds to the logic of the experiment, in which enzyme concentrations are varied in turn and the changes in the steady-state fluxes obtained are compared in order to estimate the contributions of different enzymes to the flux control. Similarly metabolite concentration control coefficients are defined [5 – 7].

An important assumption of the 'classical' metabolic control theory is that each reaction rate is proportional to the corresponding enzyme concentration [4 – 7]. Furthermore, in the 'classical' theory, as well as in common enzyme kinetics, free substrate (metabolite) concentrations are identified by their total concentrations. In other words, it is supposed that, due to low enzyme concentrations, the concentrations of enzyme-bound metabolites can be neglected as compared to their free concentrations in solution.

According to these suppositions any steady-state flux is a homogeneous first-order function of the enzyme concentrations, i.e. if these concentrations change by factor α ($E_i \rightarrow \alpha\ E_i$), all fluxes in the system will change by factor α too ($J \rightarrow \alpha\ J$). In view of Euler's theorem this results in the remarkable property for the sum of the control coefficients:

$$\sum_{i=1}^{n} C^J_{E_i} . \qquad (2)$$

This equation was named 'the summation theorem' [4].

For metabolic pathways with branches and cycles additional (linear relative to $C^J_{E_i}$) summation relations exist (one for every branching point or cycle). In these additional relations the coefficients $C^J_{E_i}$ are multiplied by 'weighting' factors. The 'weighting' factors are expressed through the branching point fluxes (see [8 – 10]).

Cellular metabolic systems exist in which enzyme concentrations are comparable or even significantly exceed substrate concentrations. Glycolysis may serve as an example [11 – 13]. The present paper deals with exactly such systems. Some researchers have already noticed that, in such systems, certain definitions and theorems of the metabolic control analysis may be not valid [14, 15]. Reder has shown [16] that this is the case only for a system with a moiety-conserved cycle. In particular, the sum (2) of the enzyme control coefficients may be not equal to unity. This effect was reported by Ottaway [17, 18] during numerical modeling of the tricarboxylic acid cycle. Fell and Sauro [19] studied how the binding of the moiety-conserved cycle metabolites with the enzyme affected the control coefficients. However, their elegant equation, connecting true values of control coefficients and those determined without considering the substrate binding effect, is valid only for a pathway with a single enzyme present at a high concentration. Moreover, this enzyme has to catalyze the irreversible Michaelis-Menten reaction. It is also suggested that other moiety-conserved cycle metabolites (except for substrate) cannot bind to this enzyme, i.e. their effector action on this reaction is ruled out. In the present work we abandon these restrictions and consider general metabolic pathways

Correspondence to B. N. Kholodenko, A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119899 Moscow, Russia

with an arbitrary geometry of connections and any number of conserved sums of intermediates. We also do not impose any limitations on the kinetics or number of enzymes present at high concentrations. For these systems, we derive modified summation theorems for flux and concentration control coefficients.

Theoretical background

We consider an arbitrary metabolic system including m metabolites ($X_1, \ldots, X_m$), whose concentrations ($X_1, \ldots, X_m$) are completely determined by n enzyme reactions of the system. The chemical equation of the jth reaction is written as:

$$\sum_{i=1}^{m} \alpha_{ij} X_i \rightleftarrows \sum_{i=1}^{m} \beta_{ij} X_i, \quad j = 1, \ldots, n$$

where X_i is the chemical symbol of the ith metabolite, α_{ij} and β_{ij} are the numbers of X_i molecules in the left and right-hand sides of the chemical equation, respectively. It is convenient to associate with the reaction scheme the stoichiometric matrix Γ of m rows and n columns constructed as follows:

$$\gamma_{ij} = \beta_{ij} - \alpha_{ij},$$

where γ_{ij} is the stoichiometric coefficient of X_i in the jth reaction, $\gamma_{ij} < 0$, if X_i is the substrate, and $\gamma_{ij} > 0$, if X_i is the product of the reaction.

We will denote concentrations of metabolite X_i, free or bound to enzyme, as X_i and X_i^b, respectively. Total concentrations X_i^t are equal to $(X_i + X_i^b)$. Only the values X_i are variables in the 'classical' control theory, disregarding the X_i^b values. We assume that any reaction rate (v_j) can be written as:

$$v_j = k_j^{cat} \cdot E_j \cdot \omega_j(X), \tag{3}$$

where ω_j is the function of free metabolite concentrations: $X = X_1, \ldots, X_m$. Such a representation of the rate equation implies the absence of direct enzyme–enzyme interactions in the system [20, 21]. Also, the parameter k_j^{cat} is assumed to vary independently of the concentration E_j and parameters in ω_j. It is noteworthy that the parameter, k_j^{cat}, so defined, may correspond to none of the elementary steps, i.e. it may have a formal (not physical) character. Variation in the parameter k_j^{cat} is equivalent to the same relative change in all the constants of the elementary steps of the jth reaction.

As known from the enzyme kinetics, functions ω_j in the rate equations are determined using the steady-state assumption [22], i.e. in the approximation of steady state over all enzyme-containing compounds. The concentrations ($[X_jE_k]$) of enzyme-bound forms of metabolite X_j determined according to this assumption can be represented as:

$$X_j^b = \sum_{k=1}^{n} [X_jE_k] = \sum_{k=1}^{n} E_k \kappa_{kj}(X), \tag{4}$$

where κ_{kj} is the saturation function of the enzyme, E_k, by metabolite X_j. We emphasize especially the major property of Eqn (4): the steady-state concentrations of bound intermediates X_j^b are linear homogeneous functions of concentrations E_k and do not depend on parameters k_j^{cat}.

If the rank (r) of the stoichiometric matrix Γ is less than the number of metabolites m, the concentrations, X_k and X_k^b, are connected by the m-r linear conservation relationships:

$$\sum_{k=1}^{m} \mu_{ik} \cdot (X_k + X_k^b) = T_i, \quad i = 1, \ldots, m\text{-}r \tag{5}$$

where T_i is the conserved sum of different forms of the ith chemical moiety, μ_{ik} is the stoichiometric coefficient of metabolite X_k in the ith moiety conserved sum (T_i). T_i, as well as E_i and k_i^{cat}, are independent parameters of the system. The T_i values can be represented as: $T_i = T_i^f + T_i^b$, where T_i^f and T_i^b are the sums of concentrations of free and enzyme-bound metabolites, correspondingly (note that T_i^f and T_i^b are variables rather than parameters of the system).

The steady-state system is determined by the equations:

$$\sum_{j=1}^{n} \gamma_{ij} \cdot v_j(X) = 0, \quad i = 1, \ldots, m, \tag{6}$$

which should be considered together with Eqns (4) and (5).

It is evident that in the steady state of the system Eqns (3) and (4) become exact rather than approximate. Any metabolic flux J in the steady state can be represented as:

$$J = \sum_{i=1}^{n} \beta_i \cdot v_i(X)|_{s.s.} \tag{7}$$

where β_i are constant coefficients, at least one of them is not equal to zero. It is clear from Eqns (4–6) that the steady-state flux is the function of system parameters E_i, k_i^{cat}, T_j.

In the 'classical' theory, control coefficients $C_{E_i}^J$ can be found not only through a change in concentration E_i, but by varying any parameter (for example, k_i^{cat}) which affects rate v_i as well. However, in our case, the coefficients thus obtained do not coincide with $C_{E_i}^J$, since at varying k_i^{cat} or E_i the concentrations of metabolites bound to E_i change differently. It is noteworthy that an analogous situation also arises in the case of violation of the postulate of the 'classical' theory on linear dependence of rate v_i on E_i [3, 20, 21]. The coefficients obtained by changing k_i^{cat} are denoted as $C_{v_i}^J$ [20]:

$$C_{v_i}^J = \frac{\partial \ln |J|}{\partial \ln k_i^{cat}}. \tag{8}$$

Further we will refer to C_{v_i} as the flux control coefficients with respect to the rate (or specific enzyme activity) and C_{E_i} as those with respect to the enzyme concentration [19, 20]. The 'classical' summation theorems prove to be valid only for coefficients $C_{v_i}^J$ (see [9] and Results), but not for $C_{E_i}^J$.

RESULTS

Summation theorems for flux and concentration control coefficients

Let us assume that as a result of a perturbation in parameters the system comes to a new steady state with the reaction rates v_i shifted by Δv_i and free metabolite concentrations X unchanged. Obviously, Δv_i have to satisfy Eqn (6). For any such Δv_i perturbations ΔE_i and ΔT_i determining the new steady state are defined by the following equations (see Eqns 3–5):

$$\begin{aligned} v_i + \Delta v_i &= k_i^{cat} \cdot (E_i + \Delta E_i)\, \omega_i(X) \\ \Delta T_i &= \sum_{j=1}^{m} \left[\mu_{ij} \cdot \sum_{k=1}^{n} \Delta E_k \cdot \kappa_{kj}(X) \right] \end{aligned} \tag{9}$$

where X_i and v_i are the concentrations and rates in the initial steady state. We present changes in the rates in the form:

$$\Delta v_i = \xi \cdot l_i, \tag{10}$$

where $l = l_1, \ldots, l_n$ is the vector defining the direction of the shift of the steady state in the space of the reaction rates. According to Eqns (6) and (10) the vector l components should satisfy the following equations:

$$\sum_{j=1}^{n} \gamma_{ij} \cdot l_j = 0, \quad i = 1, \ldots, m. \tag{11}$$

The perturbed steady state flux J appears to be the function of ξ and, according to Eqns (7) and (10), one can write:

$$\frac{d \ln |J|}{d\xi} = \frac{1}{J} \sum_{i=1}^{n} \beta_i \cdot l_i. \tag{12}$$

On the other hand, the derivative $d \ln |J|/d\xi$ can be expressed in terms of the system control coefficients:

$$\frac{d \ln |J|}{d\xi} = \sum_{i=1}^{n} C_{E_i}^{J} \cdot \frac{d \ln E_i}{d\xi} + \sum_{i=1}^{m-r} R_{T_i}^{J} \cdot \frac{d \ln T_i}{d\xi} \tag{13}$$

where $R_{T_i}^{J}$ are the response coefficients of the flux J toward a change in the conserved sums T_i:

$$R_{T_i}^{J} = \frac{\partial \ln |J|}{\partial \ln T_i}, \quad i = 1, \ldots, m\text{-}r.$$

The derivatives of the perturbed parameter values with respect to ξ can be found from Eqns (9) and (10):

$$\frac{d \ln E_i}{d\xi} = \frac{d \ln \upsilon_i}{d\xi} = \frac{l_i}{\upsilon_i}$$

$$\frac{d \ln T_i}{d\xi} = \frac{1}{T_i} \sum_{j=1}^{m} \mu_{ij} \cdot \sum_{k=1}^{n} [X_j E_k] \cdot \frac{l_k}{\upsilon_k} = \sum_{k=1}^{n} \frac{[T_i E_k]}{T_i} \cdot \frac{l_k}{\upsilon_k}$$

where $[T_i E_k] = \sum_{j=1}^{m} \mu_{ij} \cdot [X_j E_k]$ denotes the E_k-bound part of the sum T_i. By substituting the latter relations in Eqn (13) and equation the right-hand sides of Eqns (12) and (13), we finally obtain the n-r summation relations for the flux control coefficients $C_{E_i}^{J}$:

$$\sum_{i=1}^{n} C_{E_i}^{J} \cdot \frac{l_i^{(\nu)}}{\upsilon_i} + \sum_{i=1}^{m-r} R_{T_i}^{J} \cdot \sum_{k=1}^{n} \frac{[T_i E_k]}{T_i} \cdot \frac{l_k^{(\nu)}}{\upsilon_k} = \sum_{i=1}^{n} \beta_i \cdot \frac{l_i^{(\nu)}}{J} \tag{14}$$

where vectors $l^{(\nu)} = (l_1^{(\nu)}, \ldots, l_n^{(\nu)})$, $\nu = 1, \ldots, n\text{-}r$ are the linearly independent solutions of Eqns (11).

Eqns (14) differ from the summation theorems of the 'classical' theory in additional items, including response coefficients of flux J, toward a change in the value of T_i. As is obvious from the above proof, they appear because, which variations occur in E_i, the steady-state values of X remain unchanged only with simultaneous variations in T_k (due to variations in the concentrations of metabolites bound to E_i, i.e. T_k^{b}). However, if the new steady state with perturbed values of $\upsilon_i + \Delta\upsilon_i$ and invariable values of X is realized due to variation of $k_i^{cat} + \Delta k_i^{cat}$ (but not E_i), the X^{b} values and, consequently, the T_i values also remain unchanged as is clear from Eqn (4). Therefore, the 'classical' summation equations will be valid for the coefficients $C_{\upsilon_i}^{J}$ [9, 10]:

$$\sum_{i=1}^{n} C_{\upsilon_i}^{J} \cdot \frac{l_i^{(\nu)}}{\upsilon_i} = \sum_{i=1}^{n} \beta_i \cdot \frac{l_i^{(\nu)}}{J}, \quad \nu = 1, \ldots, n\text{-}r. \tag{15}$$

A concrete form of Eqns (14) and (15) depends on the choice of n-r linearly independent vectors $l^{(\nu)}$. For example, the vector of steady-state reaction rates $l_i = \upsilon_i$ can always be chosen as one of the vectors $l_i^{(\nu)}$. By substituting it into Eqn (15) we obtain the 'classical' summation theorem for $C_{\upsilon_i}^{J}$:

$$\sum_{i=1}^{n} C_{\upsilon_i}^{J} = 1, \tag{16}$$

and by substituting $l_i = \upsilon_i$ into Eqn (14) and taking into account the obvious relation:

$$T_i^{b} = \sum_{k=1}^{n} [T_i E_k],$$

we obtain for the sum of $C_{E_i}^{J}$ (compare with Eqn 2):

$$\sum_{i=1}^{n} C_{E_i}^{J} = 1 - \sum_{i=1}^{m-r} R_{T_i}^{J} \cdot \frac{T_i^{b}}{T_i}. \tag{17}$$

For metabolic pathways with branching points or cycles, the vectors, $l^{(\nu)}$, can be found using simple considerations based on the graphic representation of the metabolic pathway. For each branching point of the metabolic chain we will set $l_i = 0$ for the reactions preceding the branching point, $l_i = +1$ for all the reactions belonging to branch 1, and $l_i = -1$ for all the reactions belonging to branch 2. The relationship corresponding to this choice of l_i in Eqns (14) takes the form:

$$\frac{1}{J_1}\left(\sum_{i \in br1} C_{E_i}^{J} + \sum_{i=1}^{m-r} R_{T_i}^{J} \cdot \frac{T_i^{b,br1}}{T_i}\right) = \frac{1}{J_2}\left(\sum_{i \in br2} C_{E_i}^{J} + \sum_{i=1}^{m-r} R_{T_i}^{J} \cdot \frac{T_i^{b,br2}}{T_i}\right)$$

where J_1 and J_2 are the steady-state fluxes through branches 1 and 2, i.e. $J = J_1 + J_2$, $T_i^{b,br1}$ and $T_i^{b,br2}$ are the parts of conserved sum T_i bound to enzymes of branches 1 and 2, respectively. At the same time the relation for $C_{\upsilon_i}^{J}$ corresponding to this choice of l_i coincides (see Eqn 15) with the common branching point equation obtained in [8].

Using Eqns (9) and (10) one can also obtain the summation relations for concentration control coefficients. Taking into account that concentrations of free metabolites (X_S) do not vary in a new steady state, i.e. $d \ln X_S/d\xi = 0$, and representing this derivative by control and response coefficients (by analogy with Eqn 13) one can obtain n-r summation relations for the coefficients $C_{E_i}^{X_S}$:

$$\sum_{i=1}^{n} C_{E_i}^{X_S} \cdot \frac{l_i^{(\nu)}}{\upsilon_i} + \sum_{i=1}^{m-r} R_{T_i}^{X_S} \cdot \sum_{k=1}^{n} \frac{[T_i E_k]}{T_i} \cdot \frac{l_k^{(\nu)}}{\upsilon_k} = 0, \quad \nu = 1, \ldots, n\text{-}r. \tag{18}$$

By choosing the vector of steady-state rates υ_i as one of the vectors, $l_i^{(\nu)}$, we obtain for the sum of concentration control coefficients:

$$\sum_{i=1}^{n} C_{E_i}^{X_S} = -\sum_{i=1}^{m-r} R_{T_i}^{X_S} \cdot \frac{T_i^{b}}{T_i}. \tag{19}$$

Similarly it is not difficult to show (by changing k_i^{cat} but not E_i) that $C_{\upsilon_i}^{X_S}$ satisfy usual summation relations:

$$\sum_{i=1}^{n} C_{\upsilon_i}^{X_S} \cdot \frac{l_i^{(\nu)}}{\upsilon_i} = 0, \quad \nu = 1, \ldots, n\text{-}r. \tag{20}$$

Taking into account that bound metabolite concentrations (X_i^{b}) vary by perturbation (9) of the steady state, one can see that summation relations for total concentration (X_s^{t}) control coefficients ($C_{E_i}^{x_t}$) differ from Eqns (18). In the right-hand side of these relations, the term $\left(\frac{1}{X_s^{t}} \cdot \sum_{k=1}^{n} [X_s E_k] \cdot \frac{l_k^{(\nu)}}{\upsilon_k}\right)$ occurs instead of zero.

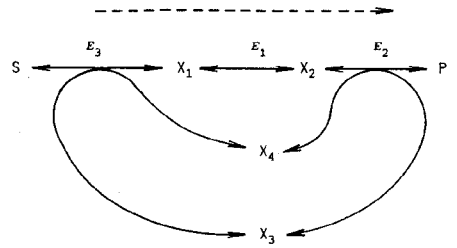

Fig. 1. Model of metabolic pathway with two moiety-conserved cycles used for numerical illustration. The dashed line with arrow indicates the direction chosen as positive.

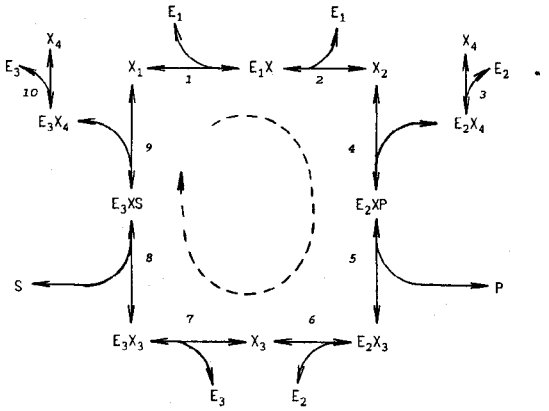

Fig. 2. Elementary steps of model metabolic pathway. E_1X, E_2XP, etc. are enzyme-substrate complexes. Numbers of steps correspond to those in Table 1. The dashed line with arrow indicates the direction chosen as positive.

How to compare the coefficients C_{E_i} and C_{v_i}?

Of special interest is the question of how the control coefficients with respect to enzyme concentration (C_{E_i}) and specific activity (C_{v_i}) are interrelated. In order to answer it, we alter the concentration of any enzyme E_i by ΔE_i and adjust k_i^{cat} so that at the same X values the rate of the ith reaction (v_i) does not change. In the new steady state all the rates and free concentrations remain unchanged if the perturbed parameter values satisfy the following Eqns (21) (compare with Eqns 9):

$$v_i = (k_i^{cat} + \Delta k_i^{cat}) \cdot (E_i + \Delta E_i)\, \omega_i(X) \qquad (21)$$

$$\Delta T_k = \sum_{j=1}^{m} (\mu_{kj} \cdot \Delta E_i \cdot \kappa_{ij}(X), \quad k = 1, \ldots, m\text{-}r.$$

Taking into consideration that after such a perturbation the flux J does not vary, we can write:

$$C_{E_i}^J + C_{v_i}^J \cdot \frac{d \ln k_i^{cat}}{d \ln E_i} + \sum_{k=1}^{m-r} R_{T_k}^J \cdot \frac{d \ln T_k}{d \ln E_i} = 0\,. \qquad (22)$$

Expressing the derivatives in Eqn (22) by virtue of Eqn (21) we finally obtain:

$$C_{E_i}^J = C_{v_i}^J - \sum_{k=1}^{m-r} R_{T_k}^J \cdot \frac{[T_k E_i]}{T_k}, \quad i = 1, \ldots, n. \qquad (23)$$

Since free metabolite concentrations did not change either (see Eqn 21), similar equations can be written for free concentration control coefficients $C_{E_i}^{X_k}$. Taking into consideration that bound metabolite concentrations (X_i^b) vary at perturbation in E_i one is readily convinced that in the right-hand side of the corresponding equations for total concentration X_S^t control coefficients an additional term, $[X_S E_i]/X_S^t$, is present.

It follows from Eqn (23) that the control coefficients C_{E_i} coincide with coefficients C_{v_i} for all the enzymes with low concentrations as compared to metabolite concentrations. So, for an arbitrary metabolic pathway the control coefficients with respect to the enzyme concentration and enzyme activity will differ only for the enzymes present at high concentrations comparable with sums of intermediate concentrations.

NUMERICAL EXAMPLE

The above results are illustrated here by example of systems with one and two moiety-conserved cycles. The metabolic pathway from Fig. 1 involves three enzymes (E_1, E_2 and E_3), a substrate (S), a product (P), two intermediates (X_1 and X_2), and two coenzymes (X_3 and X_4). This system includes two moiety-conserved cycles: $X_1 \rightarrow X_2 \rightarrow X_3 \rightarrow X_1$ and $X_3 \rightarrow X_4 \rightarrow X_3$. The former ($T_1$) involves free and bound forms of intermediates X_1 and X_2 and coenzyme X_3 ($T_1 = X_1^t + X_2^t + X_3^t$), the latter ($T_2$) involves free and bound forms of coenzymes X_3 and X_4 ($T_2 = X_3^t + X_4^t$).

Assume that the concentrations of all the three enzymes are comparable with the metabolite concentrations. Since functions κ_{ij} and v_j have a rather complicated form (for two-substrate reactions), our model system has been described at the level of elementary steps of enzyme reactions to simplify the calculation (see Fig. 2). For two-substrate reactions, catalyzed by enzymes E_2 and E_3, the ordered mechanism is accepted (for the definition, the coenzyme is assumed to bind first).

The structure of this model system resembles the simplified structure of the lower part of the glycolytic pathway, where coenzymes NAD and NADH react in turn with glyceraldehyde-phosphate dehydrogenase and lactate dehydrogenase. Therefore, the data on glycolytic enzyme and metabolite concentrations from [12] were used to choose initial values of parameters. The kinetic constants of elementary stages, substrate, product, and enzyme concentrations as well as conserved sum values are given in Table 1 (part A).

The system of equations describing the steady state of all the stages together with material balance conditions was solved by the numerical method [23]. In order to calculate the control ($C_{E_i}^J$ and $C_{v_i}^J$) and response ($R_{T_i}^J$) coefficients this system of equations was differentiated in the vicinity of the calculated steady state relative to parameters E_i, k_i^{cat} and T_i, respectively. The obtained systems of linear (with respect to the desired coefficients) equations differed only in vectors of free terms. These systems were solved by the matrix method.

The values of the control coefficients are shown in Table 2. The sum of coefficients $C_{v_i}^J$ is equal to unity, as expected, while the sum of coefficients $C_{E_i}^J$ is about 0.5. As is obvious from Table 2, the latter sum, calculated by the direct method, coincides with that calculated by Eqn (17). As mentioned above, a significant deviation of this sum from unity is explained, first, by a large fraction of bound metabolite forms and, secondly, by high values of the coefficients of flux response towards a change in conserved sum values (see Table 2). An increase in the enzyme concentrations results in a further decrease in the sum of control coefficients $C_{E_i}^J$. Moreover, under certain conditions some enzyme control coefficients acquire negative values. Fig. 3 shows that $C_{E_2}^J$ becomes nega-

Table 1. Parameter values used in numerical examples. k_{+i} and k_{-i} have the units s^{-1} or $M^{-1}\,s^{-1}$.

Step	A Example with two conserved sums		B Example with one conserved sum	
	k_{+i}	k_{-i}	k_{+i}	k_{-i}
1	0.5	23	100	200
2	5.8	0.01	2	0.001; 10
3	0.375	10	1000000	550000
4	0.01	6.6	111.111	200
5	57	0.001	74000	0.01
6	26	0.1	500	5555.56
7	0.01	22	200	1600
8	0.1	12	3000	2000
9	9	0.1	2000	3000
10	28	0.05	550000	1000000

Concentration	Value	
	μM	
E_1	200	1
E_2	300	0.01
E_3	1400	0.01
T_1	990	3
T_2	600	–
S	80	80
P	3700	3700

Table 2. Results of calculations for the model with two conserved sums.

Coefficient	Value	Coefficient	Value
$R^J_{T_1}$	0.2929	T^b_1/T_1	0.3636
$R^J_{T_2}$	0.5712	T^b_2/T_2	0.6333
$C^J_{v_1}$	0.1492	$C^J_{E_1}$	0.1196
$C^J_{v_2}$	0.3891	$C^J_{E_2}$	0.1851
$C^J_{v_3}$	0.4618	$C^J_{E_3}$	0.2270
$\sum_{i=1}^{3} C^J_{v_i}$	1.0000	$\sum_{i=1}^{3} C^J_{E_i}$	0.5137
$1 - R^J_{T_1} \cdot T^b_1/T_1 - R^J_{T_2} \cdot T^b_2/T_2$			0.5137

tive with the increase in the concentration of E_2. Similarly, $C^J_{E_1}$ and $C^J_{E_3}$ become below zero when concentrations E_1 and E_3 increase by a factor of 2 or 2.5, respectively, as compared to their initial values (data not shown).

The results proved to be more interesting when the total concentrations of all the enzymes increase in the same proportion: $E_i \rightarrow \alpha E_i$, $i = 1, 2, 3$. It is clear that in this case the 'classical' flux and concentration control coefficients [C_{E_i} (app) according to the terminology of [19], bound metabolites being neglected upon their calculation] do not change. The behavior of the true control coefficients differs in principal. Fig. 4 shows that when enzyme concentrations increase by a factor of 4, not only do the coefficients $C^J_{E_1}$ and $C^J_{E_2}$ become negative but so does the sum of $C^J_{E_i}$. In other words, a further increase in the concentrations of all the enzymes (by the same factor) leads to a drop but not a rise of the flux. Similar results were obtained for this model with other sets of kinetic constants (data not shown), so the observed phenomenon is not a consequence of a poor choice of system parameters.

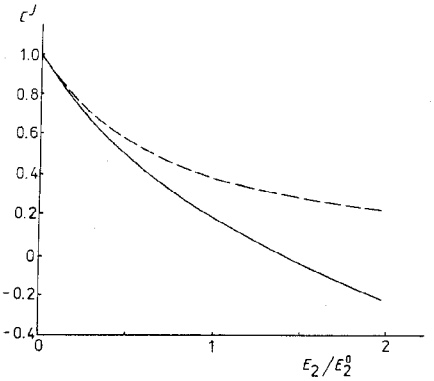

Fig. 3. Variation of the flux control coefficients with respect to enzyme 2 at varying concentrations of the enzyme. (----) Flux control coefficients with respect to the specific enzyme activity; (——) flux control coefficients with respect to the enzyme concentration. E_2^0 is the original enzyme concentration (see Table 1, part A).

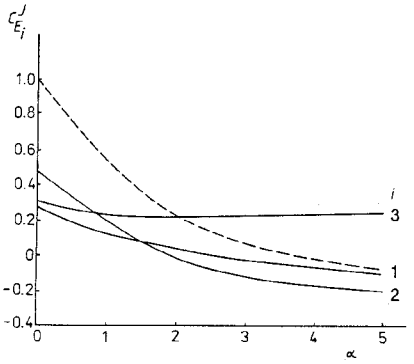

Fig. 4. Variation of the flux control coefficients with respect to enzyme concentration (——) and their sum (----) at varying enzyme concentrations. All enzymes increase or decrease by the same factor α.

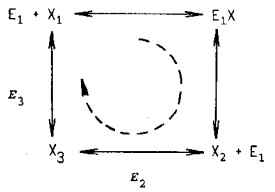

Fig. 5. Model of metabolic pathway with one moiety-conserved cycle used for numerical illustration. E_1X is the enzyme-substrate complex whose concentration is comparable with those of enzyme E_1 and metabolites X_i. Concentrations of enzymes E_2 and E_3 are much lower than those of metabolites. The dashed line with arrow indicates the direction chosen as positive.

Fell and Sauro [19] analyzed the model with one moiety-conserved cycle and one enzyme having a concentration comparable with metabolite concentrations. To check whether the effect of changing the $C^J_{E_i}$ sign can proceed in the case of such a simple system, we reduced the above model. For this purpose we gave up the conservation of sum T_2, and the concentration of coenzyme X_4 was accepted to be constant. Constant concentrations X_4, S and P were included in the apparent kinetic constants of reactions 2 and 3, so that our system was transformed to a simple three-step cycle with three variable metab-

Table 3. Results of calculations for the model with one conserved sum. For true control coefficients, the left value was obtained by numerical procedures (different in [19] and in this work), the right value was obtained by formulae proposed in [19]. Coefficients $C^J_{E_i}$ (app) were calculated as in [19] taking into account that elasticity ε^1_2 was not equal to 0 in our case.

Parameter	Data from [19]	Data from this work with reaction 1			
		practically irreversible		reversible	
X_1	0.3689	0.3670		1.9982	
X_2	2.039	2.032		0.1920	
X_3	0.4379	0.4376		0.2979	
E_1X	0.1542	0.1538		0.4997	
ε^1_1	0.846	0.852		24.899	
ε^1_2	0	−0.006		−24.399	
ε^2_2	0.940	0.940		1.010	
ε^2_3	−0.667	−0.667		−0.636	
ε^3_1	−0.305	−0.303		−2.606	
ε^3_3	1.075	1.074		3.003	
$C^J_{E_1}$ (app)	0.2142	0.2120		0.0314	
$C^J_{E_2}$ (app)	0.4346	0.4356		0.7868	
$C^J_{E_3}$ (app)	0.3513	0.3524		0.1818	
$\sum_{i=1}^{3} C^J_{E_i}$ (app)	1.0001	1.0000		1.0000	
$C^J_{E_1}$	0.2081	0.2078	0.2058	0.2056	−0.0381	0.0291
$C^J_{E_2}$	0.4218	0.4215	0.4208	0.4226	0.7872	0.7307
$C^J_{E_3}$	0.3410	0.3408	0.3416	0.3419	0.1796	0.1689
$\sum_{i=1}^{3} C^J_{E_i}$	0.9709	0.9701	0.9682	0.9700	0.9287	0.9287
$C^J_{E_1}$	0.2381	0.2378	0.2356	0.2356	0.0324	0.1004
$C^J_{E_2}$	0.4218	0.4215	0.4222	0.4226	0.7889	0.7307
$C^J_{E_3}$	0.3410	0.3408	0.3422	0.3419	0.1796	0.1689
$\sum_{i=1}^{3} C^J_{E_i}$	0.9999	1.0001	1.0000	1.0000	1.0000	1.0000

olite concentrations. Thus it became completely analogous to the model system considered in [19] (Fig. 5). The only difference was that in our case reaction 1 could be reversible.

The total enzyme concentration E_1 and the conservation total T_i were taken as in [19]. Then we chose the kinetic constants and other parameters and, in order to obtain the system identical to that in [19], we made k_{-2} small enough to keep reaction 1 practically irreversible (Table 1, part B, the first value for k_{-2}). As expected, our calculations completely confirmed the results of [19] (see Table 3, left-hand and middle columns).

However, increasing k_{-2} (Table 1, part B, the second value) and thus making reaction 1 reversible, we ascertained that the relations obtained in [19] were not true for this case (Table 3, right-hand column). In particular, control coefficient $C^J_{E_1}$ was found to be negative in the reduced system, while it remained positive if calculated by the formula proposed in [19]. Although the difference was only slight, it could become significant at other values of the parameter. For example, if E_1 increased by a factor of 10 and all kinetic constants of reaction 1 decreased by the same factor (to hold V_{max} and K_m constant, see [19]), the true $C^J_{E_1}$ value was −0.5654, while it was equal to 0.0123 if calculated by the method of [19].

DISCUSSION

In the present work we have considered metabolic systems in which enzyme concentrations are comparable with those of their substrates and products. When analysing such systems we gave up one of the assumptions of the 'classic' metabolic control theory according to which one can ignore bound metabolite concentrations as compared to their free concentrations. Simultaneously we preserved another assumption about the linear dependence of the reaction rates on the concentrations of the corresponding enzymes.

In case such systems lack moiety-conserved cycles all the concepts of the 'classical' metabolic control analysis remain valid. In this case coefficients $C^J_{E_i}$ determined by Eqn (1) coincide with $C^J_{v_i}$ determined by Eqn (8) and with the control coefficients calculated without taking into account the mentioned difference between free and total metabolite concentrations.

In the case where the systems under consideration contain the moiety-conserved cycles, an increase in enzyme concentrations results in transition of some metabolite molecules from the free state to the enzyme-bound one, and hence the available pool of free metabolites decreases. Therefore the control coefficients with respect to enzyme concentration $C^J_{E_i}$ do not coincide with the control coefficients with respect to enzyme activity $C^J_{v_i}$ (except for the enzymes whose concentrations are low as compared to metabolite concentrations, see Eqn 23).

Although the 'classical' summation relations (15) hold good for coefficients $C^J_{v_i}$, the connectivity relations (see [4, 9]) have to be modified taking into account the corrections due to the binding of the moiety-conserved cycle metabolites to enzymes. As a result, coefficients $C^J_{v_i}$ depend not only on the elasticity coefficients as in the 'classical' case, but also on the derivatives of functions $\kappa_{kj}(X)$ with respect to free metabolite concentrations. So coefficients $C^J_{E_i}$ and $C^J_{v_i}$ differ not only from each other but from control coefficients $C^J_{E_i}$ (app) and $C^J_{v_i}$ (app) calculated in the framework of the 'classical' theory. In the general case of an arbitrary metabolic system one cannot obtain equations for coefficients $C^J_{E_i}$ and $C^J_{v_i}$ via $C^J_{E_i}$ (app) and $C^J_{v_i}$ (app) in a simple and visible form by analogy with equations in [19]. However, for an arbitrary system we can compare coefficients $C^J_{E_i}$ and $C^J_{v_i}$ (see Eqn 23). Usually fluxes increase with the increase in conserved sum T_k, i.e. $R^J_{T_k} \geq 0$, so according to Eqn (23) the control coefficient with respect to enzyme concentration $C^J_{E_i}$ is less than the control coefficient with respect to enzyme activity $C^J_{v_i}$.

We obtained the modified summation relations for the flux ($C^J_{E_i}$) and concentration ($C^{X_s}_{E_i}$) control coefficients. As obvious from Eqn (17), the difference between the sum of the flux control coefficients and unity becomes significant, if at least one of the conserved sums (T) limits the flux (i.e. R^J_T is about 1) and the sums of concentrations of bound (T^b) and free (T^f) metabolites are comparable in magnitude. Such a case was demonstrated in the numerical example.

The above example shows that the flux control coefficients and even their sum can take negative values in case their 'classical' analogs are positive. This phenomenon does not contradict the above theoretical results. Indeed, it is evident from Eqn (17) that the sum of control coefficients is equal to unity minus an additional term (absent in the 'classical' the-

ory). This term is positive if coefficients $R^J_{T_k}$ are positive and, generally speaking, it can exceed unity. On the contrary, the equations obtained in [19] permit no negative values for the sum as well as for individual control coefficients, if their 'classical' analogs [$C^J_{E_i}$ (app)] and coefficients $R^J_{T_k}$ (app) are positive.

It should be noted that the control coefficient can change its sign for the simpler system with a single moiety-conserved cycle considered in [19], if the enzyme in a high concentration catalyzes a reversible reaction (see Table 3). A negative value of the control coefficient appears to be due to the enzyme binding not only with its substrate but also with the product. This product is in turn the substrate for the next moiety-conserved cycle reaction. In the case where this reaction significantly contributes to the control, the lowering of its free substrate concentration results in a flux decrease. Thus, an excess of the enzyme serves as a metabolite-sequestering agent. The possibility that the enzyme could play such a role was discussed by Sols and Marco [11]. By modeling the tricarboxylic acid cycle at high enzyme concentrations the negative flux control coefficients were found and the sum of the control coefficients appeared to be below unity. This phenomenon was named as the 'Sols-Marco effect' [17].

Fell and Sauro [19] concluded that the theorems of metabolic control analysis are less affected by the existence of enzyme concentrations comparable to metabolite concentrations than some authors have implied. However, their calculations refer to the particular case and give no grounds to draw such a strong conclusion. Indeed, the above examples suggest that a significant violation of the 'classical' summation theorem can occur in the systems modeling physiological situations.

The enzyme control coefficients can be determined experimentally. Methods of titration by specific inhibitors, for example, may be used for this purpose [24]. Affecting the system by a purely non-competative inhibitor [22] of enzyme E_i, which changes k_i^{cat} only (i.e. V_i^{max}), we can determine $C^J_{v_i}$. Using an irreversible inhibitor with a very high affinity to the enzyme, which replaces substrates (products) in the active site, we find $C^J_{E_i}$ (an example of such inhibitors is carboxyatractyloside, a specific irreversible inhibitor of adenine nucleotide translocase of the inner mitochondrial membrane [25]). In the reconstituted system coefficients $C^J_{E_i}$ can be determined by directly varying the concentration of E_i. The response coefficients $R^J_{T_i}$ can also be measured by adding extra T_i or by trapping the metabolites of the moiety-conserved cycle. If any of the control coefficients ($C^J_{E_i}$ or $C^J_{v_i}$) cannot be determined experimentally, but the enzyme-bound parts of T_i are known, the control coefficient can be calculated using Eqn (23). On the other hand, when the control and response coefficients can be measured in a system with a single moiety-conserved cycle, Eqns (17) and (23) allow one to estimate enzyme-bound fraction of T. It seems attractive to demonstrate directly the effects of high enzyme concentrations in experimental studies.

REFERENCES

1. Westerhoff, H. V. & Van Dam, K. (1987) *Thermodynamics and control of biological free-energy transduction*, Elsevier, Amsterdam.
2. Savageau, M. A. (1976) *Biochemical systems analysis: a study of function and design in molecular biology*, Addison-Wesley Publ. Co., London.
3. Kholodenko, B. N. (1991) *Modern metabolic control theory*, VINITI, Moscow (in Russian).
4. Higgins, J. (1965) in *Control of energy metabolism* (Chance, B., Estabrook, R. W. & Williamson, J. R., eds) pp. 13–46, Academic Press, New York.
5. Kacser, H. & Burns, J. A. (1973) in *Rate control of biological processes* (Davies, D. D., ed.) pp. 65–104, Cambridge University Press, London.
6. Kacser, H. & Burns, J. A. (1979) *Biochem. Soc. Trans. 7*, 1149–1160.
7. Heinrich, R. & Rapoport, T. A. (1974) *Eur. J. Biochem. 42*, 89–95.
8. Fell, D. A. & Sauro, H. M. (1985) *Eur. J. Biochem. 148*, 555–561.
9. Kholodenko, B. N. (1988) *Mol. Biol. (Mosc.) 22*, 1238–1256.
10. Kholodenko, B. N. (1990) *J. Nonlinear Biol. 1*, 107–126.
11. Sols, A. & Marco, R. (1970) *Curr. Top. Cell. Regul. 2*, 227–273.
12. Srivastava, D. K. & Bernhard, S. A. (1986) *Curr. Top. Cell. Regul. 28*, 1–68.
13. Albe, K. R., Butler, M. H. & Wright, B. E. (1990) *J. Theor. Biol. 143*, 163–195.
14. Heinrich, R., Rapoport, S. M. & Rapoport, T. A. (1977) *Prog. Biophys. Mol. Biol. 32*, 1–82.
15. Giersch, C. (1988) *Eur. J. Biochem. 174*, 509–513.
16. Reder, C. (1988) *J. Theor. Biol. 135*, 175–201.
17. Ottaway, J. H. (1976) *Biochem. Soc. Trans. 4*, 371–376.
18. Ottaway, J. H. (1979) *Biochem. Soc. Trans. 7*, 1161–1167.
19. Fell, D. A. & Sauro, H. M. (1990) *Eur. J. Biochem. 192*, 183–187.
20. Kacser, H., Sauro, H. M. & Acerenza, L. (1990) *Eur. J. Biochem. 187*, 481–491.
21. Sauro, H. M. & Kacser, H. (1990) *Eur. J. Biochem. 187*, 493–500.
22. Cornish-Bowden, A. (1976) *Principles of enzyme kinetics*, Butterworths, London.
23. Powell, M. J. D. (1968) *A Fortran subroutine for solving systems of nonlinear algebraic equations*, Atomic Energy Research Establishment, Harwell.
24. Groen, A. K., Wanders, R. J. A., Westerhoff, H. V., Van der Meer, R. & Tager, J. M. (1982) *J. Biol. Chem. 257*, 2754–2757.
25. Vignais, P. V., Block, M. R., Boulay, F., Brandolin, G. & Lauquin, G. J.-M. (1985) in *Structure and properties of cell membranes* (Bengha, G., ed.) pp. 139–179, CRC Press, New York.