Paradoxical control properties of enzymes within pathways: can activation cause an enzyme to have increased control?

Boris N. KHOLODENKO*†‡ and Guy C. BROWN†

*A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119899 Moscow, Russia, and †Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

It is widely assumed that within a metabolic pathway inhibition of an enzyme causes the control exerted by that enzyme over the flux through its own reaction to increase, whereas activation causes its control to decrease. This assumption forms the basis of a number of experimental methods. For a pathway conceptually divided into two enzyme groups connected via a single metabolite we have derived a general condition under which this assumption is false, and thus the pathway shows paradoxical control behaviour, i.e. increased control with activation and decreased control with inhibition of an enzyme or group of enzymes.

Paradoxical control behaviour occurs widely when enzyme activity is altered by changing K_m (if an enzyme is already close to saturation by its substrate), but may also occur with changes in V_{max} when the elasticity to the linking metabolite increases with its concentration (as in some cases of sigmoidal and exponential kinetics or for reactions catalysed by isoenzymes). These findings suggest that enzymes with sigmoidal kinetics may have low control in the absence of activation, but may gain control with activation, and thus have beneficial regulatory properties.

INTRODUCTION

The control exerted by an enzyme over the steady-state flux of a metabolic pathway can be quantified as a control coefficient with metabolic control analysis (MCA). The control coefficient is the fractional change in pathway flux divided by the fractional change in the enzyme's activity, extrapolated to infinitesimally small change. This quantifies the ability of an enzyme to change the pathway rate and enables the relative importance of different enzymes to control the flux to be simply assessed, since the sum of the control coefficients of all the enzymes is equal to 1 ([1,2]; see ref. [3] for a review).

Thus the control coefficients have been used to assess which enzymes in a pathway might be important in regulating its rate. However, because the control coefficients are defined for infinitesimally small change, these coefficients make no predictions about what will happen during finite (i.e. real) changes (see, however, refs. [4–6]). Thus the fact that the control coefficient of an enzyme is high or low does not itself tell us how the flux will change with a large change in enzyme activity. It is commonly assumed that activation of an enzyme either (a) will not change the control coefficients much or (b) will cause its control coefficient to fall, whereas inhibition of an enzyme will cause its control coefficient to rise. If assumption (a) were correct, then control coefficients would be accurately predictive of finite changes; however, there is much experimental evidence that the assumption is not correct in real systems (see, e.g., ref. [7]). If assumption (b) were correct, we could at least predict that, if the control coefficient of an enzyme is low, then activation of the enzyme could not cause large changes in the pathway flux, and thus the enzyme cannot be responsible for up-regulating the pathway. Similarly if assumption (b) were correct, then the finding that the control coefficient of an enzyme increased during inhibition of the pathway could be used as evidence that the enzyme had been inhibited, or correspondingly the finding that

the control coefficient of an enzyme decreased during activation of a pathway could be used as evidence that the enzyme had been activated. This type of reasoning has been used, for example, to determine which mitochondrial enzymes are activated by Ca^{2+} [8]. For all these reasons it is important to know how the control coefficient of an enzyme will change when the activity of the enzyme is changed.

There have been a number of attempts to extend MCA to allow prediction of the effects of large finite changes in enzyme activity [4–6]. Small and Kacser [4,5] derived simple relationships between the control coefficients and changes expected for large finite changes in enzyme concentrations. However, their derivation was based on the assumption of linear kinetics (that is linear relationships between enzyme rate and substrate or product concentrations), and thus would seem unlikely to apply to enzymes approaching saturation or enzymes with sigmoidal kinetics. On the other hand, they showed that their simple relations made very accurate predictions when applied to experimental data from a number of real systems, suggesting that these enzymes did obey linear kinetics [4,5]. Höfer and Heinrich [6] showed how MCA could be extended to include the second-order dependencies of variables on parameter changes to give better predictions of variable changes with finite changes in parameters (such as enzyme concentration). However, the relations derived required a knowledge of all the second-order partial derivatives of any enzyme rate with respect to concentrations and parameters, i.e. coefficients thought to be very difficult to measure experimentally.

In this paper we have investigated whether and under what conditions the control coefficient of an enzyme increases on its activation and thus the enzyme exhibits paradoxical control behaviour. We find that enzymes with sigmoidal kinetics are particularly likely to exhibit paradoxical control behaviour, and this has a number of important implications for allosteric enzymes.

Abbreviations used: MCA, metabolic control analysis; MWC model, Monod–Wyman–Changeux model; AKNF model, Adair–Koshland–Nemethy–Filmer model.

‡ To whom correspondence should be addressed at Cambridge.

RESULTS

Theoretical background

We shall consider two sequential enzymes and, more generally, the two parts of a metabolic pathway linked by a single metabolite (X) as depicted in Scheme 1, where E_1 and E_2 stand for either individual enzymes or whole groups of enzymes. The concentrations of the initial substrate (S) and the final product (P) are assumed to be constant, whereas the concentration (X) of the linking metabolite varies depending on the kinetic parameters of the enzymes.

Scheme 1

We shall designate by $J_1(X)$ and $J_2(X)$ the dependencies of steady-state fluxes through either part of the pathway on the concentration X . Each flux also depends on the kinetic parameters of the enzyme constituting the corresponding part of the pathway; however, we shall not express these dependencies explicitly in order to avoid complex designations. In the case of only two enzymes, the steady-state fluxes, J_1 and J_2 , are simply the steady-state rates of the corresponding reactions, $J_1 = v_1(X)$ and $J_2 = v_2(X)$. For a pathway that includes several enzymes, transporters and diffusion steps, the dependencies $J_1(X)$ and $J_2(X)$ are called the regulatory characteristics [9,10] or phenomenological kinetics [11,12]. These characteristics are isolated responses of either part of the pathway to changes in X . Usually the flux $J_1(X)$ decreases and the flux $J_2(X)$ increases when X is increased provided that all the kinetic parameters are kept constant. This well-known phenomenon reflects the back-pressure that the link metabolite (X) exerts on the first part of the pathway as its product and the stimulation of the second part of the pathway by X as the substrate.

We shall define the (overall) elasticity coefficients of both parts of the pathway with respect to X as the following [1,13–17]:

$$\epsilon_X^1 = \frac{\partial J_1}{\partial X} \times \frac{X}{J_1}, \quad \epsilon_X^2 = \frac{\partial J_2}{\partial X} \times \frac{X}{J_2} \quad (1)$$

In terms of these elasticities, the above condition on the dependencies J_1 and J_2 on the concentration of the link metabolite implies that:

$$\epsilon_X^1 < 0, \quad \epsilon_X^2 > 0 \quad (2)$$

At any steady state, the fluxes J_1 and J_2 through either part of the pathway are equal to each other and to the pathway flux (J):

$$J_1(X) = J_2(X) = J \quad (3)$$

In a pathway of two enzymes, there are two flux control coefficients defined in terms of modulation of the concentration (maximal activity) of either enzyme [1,2]:

$$C_i^J = \frac{dJ}{de_i} \times \frac{e_i}{J} = \frac{d \ln |J|}{d \ln e_i}, \quad i = 1, 2 \quad (4)$$

In cases when maximal enzyme activity ($V_i^{\max}$) is not proportional to the enzyme concentration (e_i), the control coefficients that are considered here should be defined by modulation of the enzyme turnover number (which is proportional to the enzyme activity). Although for simplicity we shall use the enzyme concentrations as modulation parameters, in cases when such proportionality is

lacking, e_i should be substituted by $V_i^{\max}$. Different modes by which an enzyme controls the flux, i.e. different types of control coefficients, are reviewed elsewhere [18,19].

Using eqn. (4), the (overall) control coefficient for either part of the pathway in Scheme 1 can be defined, provided that the concentrations of all the individual enzymes constituting the part i are changed in proportion, i.e.

$$de_{i1}/e_{i1} = de_{i2}/e_{i2} = de_{i3}/e_{i3} = \dots = de_i/e_i, \quad i = 1, 2 \quad (5)$$

How does the control change on inhibition or activation of an enzyme?

An enzyme can be activated and inhibited in a number of different ways depending on the particular mechanism of interactions between the enzyme and its specific effector. For instance, activation of an enzyme can increase its turnover number ($V^{\max}$) or decrease its Michaelis constant (K_m) with respect to the substrate. Allosteric enzymes can also be activated by decreasing the Hill constant or the allosteric constant in the case of kinetics described by the Hill equation [20] or by the Monod–Wyman–Changeux (MWC) model [21] respectively or through effector-induced change of the subunit conformation and subunit interactions in Adair–Koshland–Nemethy–Filmer (AKNF) models [21].

We shall designate by p_i any parameter of the phenomenological kinetic law (J_i) affected by a specific effector or the concentration of that effector itself. The elasticity coefficient with respect to this parameter is designated by ϵ_p^i :

$$\epsilon_p^i = \frac{\partial J_i}{\partial p_i} \times \frac{1}{J_i}, \quad i = 1, 2 \quad (6)$$

Note that the semilogarithmic derivative $\partial \ln |J_i| / \partial p_i$ is used in eqn. (6) in order to avoid indefiniteness connected with the log derivative when p_i is zero. The latter is the case when p_i stands for effector concentration that is equal to 0 in the original steady state [22].

If the elasticity (ϵ_p^i) is greater than 0, an increase in the parameter (p_i) corresponds to activation of the enzyme or of the corresponding part of the pathway, and if this elasticity is less than 0, an increase in p_i corresponds to inhibition. The derivative (dC_i^J/dp_i) shows whether the control coefficient will increase or decrease with the parameter (p_i). Therefore the ratio (B_p^i) of the derivative (dC_i^J/dp_i) and the local elasticity (ϵ_p^i) shows whether the control coefficient and the local rate change in the same direction or in opposite directions on a change in the parameter p_i :

$$B_p^i = (dC_i^J/dp_i) / \epsilon_p^i, \quad i = 1, 2 \quad (7)$$

We shall call the ratio B_p^i the ‘control response ratio’. If this ratio is positive, the control coefficient of the enzyme (or group of enzymes) i increases on activation of that enzyme (or group of enzymes) and decreases on its inhibition, and therefore we shall refer to this behaviour as ‘paradoxical’. When this ratio is negative, activation causes a decrease in control. When the ratio is zero, both activation and inhibition cause no change in the control coefficient.

Calculation of the control response ratio

Now we can calculate the response ratio using the summation and connectivity properties. These properties (theorems) allow one to express the (overall) control coefficients in terms of the (overall) elasticity coefficients of each part of the pathway [1,14–17]:

$$C_1^J = \epsilon_X^2 / (\epsilon_X^2 - \epsilon_X^1), \quad C_2^J = -\epsilon_X^1 / (\epsilon_X^2 - \epsilon_X^1) \quad (8)$$

Let some part (i) of the pathway of Scheme 1 be affected as the result of a change in some parameter (p_i) affecting the flux $J_i(X)$. Then the pathway will evolve to a new steady state with increased or decreased concentration of the intermediate X . From eqn. (8) it follows that changes in the control coefficients occur as the result of changes in the elasticities in the new steady state, caused by changes in both X and p_i . Therefore how the control changes with activation or inhibition of an enzyme depends on the second derivatives of the regulatory characteristics, $J_i(X)$, with respect to X and, also, to X and p_i . Using eqn. (8) one can express the derivative (dC_i^j/dp_i) that describes changes in control, in terms of the derivatives of the elasticities. Taking into consideration the fact that only the elasticity ϵ_X^i can depend on p_i explicitly, one arrives at the following equation for the control response ratio (B_p^i):

$$B_p^i = (dC_i^j/dp_i)/\epsilon_p^i = a(f + \frac{1}{p_i}) \quad (9)$$

where

$$a = (-X\epsilon_X^1\epsilon_X^2)/(\epsilon_X^2 - \epsilon_X^1)^3,$$

$$f = (1/\epsilon_X^2)(d\epsilon_X^2/dX) - (1/\epsilon_X^1)(d\epsilon_X^1/dX) =$$

$$d \ln |\epsilon_X^2|/dX - d \ln |\epsilon_X^1|/dX,$$

$$\frac{1}{p_i} = -(\epsilon_X^2 - \epsilon_X^1)(\partial \epsilon_X^i / \partial p_i) / (\epsilon_p^i \epsilon_X^i X)$$

($i = 1$ or 2 depending on whether the first or second part of the pathway in Scheme 1 is affected).

The relationships obtained exhibit a number of interesting features. One can see the similarities and differences in the control response ratios (B_p^1 and B_p^2), i.e. in the description of whether paradoxical control is displayed on activation of the first or second part of the pathway. In both cases the functions, a and f , are the same; they do not depend on the local responses to the affected parameter (p_i). The functions $\frac{1}{p_i}$ and $\frac{2}{p_i}$ differ and depend on both the local response to the parameter p_i and the second derivative of the corresponding flux with respect to the affected parameter and the linking metabolite. Because of eqn. (2), function a is positive, hence the sign of B_p^i , i.e. the type of control behaviour, is determined by the sign of the function ($f + \frac{1}{p_i}$).

When only maximal catalytic activity or the concentration of enzyme i is changed in the two-enzyme pathway, or the concentrations of all the individual enzymes constituting part i of the multienzyme pathway are changed in proportion [eqn. (5)], then only $V_i^{\max}$ in the phenomenological rate law is changed. Since the elasticity ϵ_X^i does not depend explicitly on $V_i^{\max}$; the partial derivative $\partial \epsilon_X^i / \partial V_i^{\max}$ equals 0. It follows from eqn. (9) that the control response ratio for changes in $V_i^{\max}$ of either the first or second part of the pathway is given by:

$$B_{e_1}^1 = B_{V_1^{\max}}^1 = B_{e_2}^2 = B_{V_2^{\max}}^2 = af \quad (10)$$

Therefore no matter whether the $V_1^{\max}$ or $V_2^{\max}$ is changed, the same control behaviour (i.e. paradoxical or not) will occur in both cases. We will use eqns. (9) and (10) to test whether enzymes with various kinetics exhibit paradoxical or non-paradoxical control.

Modulation of the catalytic activity: paradoxical control properties of allosteric enzymes

We begin with two Michaelis–Menten enzymes the rates of which (v_1 and v_2) in the general case are described by the following well-known equations (see, e.g., ref. [20]):

$$v_1 = \frac{V_1^f S / K_1^S - V_1^r X / K_1^X}{1 + S / K_1^S + X / K_1^X}, \quad v_2 = \frac{V_2^f X / K_2^X - V_2^r P / K_2^P}{1 + X / K_2^X + P / K_2^P} \quad (11)$$

Here V_i^f and V_i^r are the maximal rates in the forward and reverse

Figure 1 Non-paradoxical control behaviour of a pair of Michaelis–Menten enzymes

The control coefficient of the downstream enzyme (—) decreases and the pathway flux (---) increases with an increase in $V_2^{\max}$ of this enzyme. The parameter values were chosen as follows (dimensionless units): $V_1^f = 1$, $S = 1$, $K_1^S = 1$, $K_1^X = 0.2$, $K_1^{\text{eq}} = 1000$, $K_2^X = 0.1$, $P = 0.1$, $K_2^S = 1$, $K_2^{\text{eq}} = 100$. Calculations used SCAMP [23] and were checked analytically.

directions for either enzyme ($i = 1, 2$), K_1^S and K_1^X are the Michaelis constants for the substrate (S) and for the product (X) of the first enzyme, K_2^X and K_2^P are the corresponding Michaelis constants of the second enzyme.

Using eqns. (10) and (11) one can show readily that for two enzymes following Michaelis–Menten kinetics the control response ratio ($B_{e_i}^i$) is always negative. Indeed, both the (positive) elasticity of the downstream enzyme (ϵ_X^2) with respect to its substrate (X) and the (negative) elasticity of the upstream enzyme (ϵ_X^1) with respect to its product (X) decreases with increase in X . Therefore $d \ln |\epsilon_X^2|/dX < 0$, and $-d \ln |\epsilon_X^1|/dX < 0$. As a consequence, f is negative, hence $B_{e_i}^i$ is negative [see eqns. (10) and (11)]. We conclude that a pathway of any two Michaelis–Menten enzymes always exhibits non-paradoxical control behaviour when the maximal catalytic activity or the concentration of an enzyme is changed. Numerical simulation of this behaviour is shown in Figure 1 where an increase in $V_2^{\max}$ of the downstream enzyme causes a decrease in its control coefficient (solid line) and an increase in the pathway flux (dashed line). Note that an increase in $V_2^{\max}$ in eqn. (11) corresponds to a simultaneous proportional increase in V^f and V^r which leaves the equilibrium constant (K^{eq}) unchanged.

Allosteric enzymes usually display a sigmoidal dependence of the initial rate (v) on the substrate or product concentration. In some cases their kinetics can be described by the Hill equation [20]. Considering this description for the enzymes of the pathway in Scheme 1 and assuming that both enzymes are far from equilibrium, the corresponding rate equations read:

$$v_1 = \frac{V_1^f S / K_1^S}{1 + S / K_1^S + (X / K_1^X)^{h_1}}, \quad v_2 = \frac{V_2^f X^{h_2}}{K_{0.5}^{h_2} + X^{h_2}} \quad (12)$$

The (positive) elasticity of the second enzyme to its substrate, X , decreases from the maximal value equal to the Hill coefficient, h_2 , (at very low substrate concentrations, $X \ll K_{0.5}$) to 0 (at high concentrations $X \gg K_{0.5}$). The (negative) elasticity of the first enzyme to its product, X , decreases from 0 to $-h_1$. Therefore $d \ln |\epsilon_X^2|/dX < 0$, $-d \ln |\epsilon_X^1|/dX < 0$ and, in accordance with eqn. (10), the conclusion drawn above for the two Michaelis–Menten

Figure 2 Paradoxical control behaviour of an allosteric enzyme in the pathway of Scheme 1

The kinetics of the allosteric (second) enzyme is described by an MWC model [eqn. (13)], and the first enzyme is described by the Michaelis–Menten equation [eqn. (11)]. The dependencies of the control coefficient (C_2^J) of the allosteric enzyme (—) and the (common) logarithm of the flux (J) through the pathway (---) on the logarithm of $V_2^{\max}$ are shown. The parameter values were: $V_1^1 = 200$, $S = 1$, $K_1^S = 1$, $K_1^X = 0.005$, $V_1^r = 0$, $L = 10\,000$, $K_R = 1$.

enzymes remains valid for sigmoidal kinetics provided that the latter is described by the Hill equation [eqn. (12)]. Notice that both Hill coefficients, h_1 and h_2 , can assume any positive values, for instance any of them can be 1 as for the Michaelis–Menten enzyme.

However, when allosteric kinetics are more complex (and their description requires MWC or AKNF models) the corresponding elasticity coefficients can vary non-monotonically with variation in the concentration (X) of the linking metabolite. Therefore, contrary to the previous cases considered above, the derivative, $d \ln |e_X^2|/dX$ (or $-d \ln |e_X^1|/dX$), and thus the function f in eqn. (10), can be positive in some region of X . As a useful illustration, we consider a simple version of the MWC model for a four-subunit enzyme in which ligands can bind only to the active state (R) [21]:

$$v = \frac{V^r X / K_R}{[1 + (X/K_R)] \{L/[1 + (X/K_R)]^4 + 1\}} \quad (13)$$

Here L is the allosteric constant, i.e. the equilibrium constant for two different conformational states (R and T) in the absence of ligands, K_R is the dissociation constant for X binding to the R state, V^r stands for the maximal velocity which is expressed as the product of the molar concentration of sites and the turnover number.

Let the kinetics of the second enzyme in Scheme 1 be described by eqn. (13). Using eqns. (1) and (13) to calculate the elasticity coefficient (e_X^2) for large values of L , one can see readily that a range of X exists in which the (positive) value of e_X^2 increases as X increases. Therefore, in this range $d \ln |e_X^2|/dX$ is greater than 0, and, since there is no constraint relating the elasticities e_X^2 and e_X^1 , the ratio $B_{e_2}^2$ can be positive. Hence this enzyme can show paradoxical control properties on a change in its maximal catalytic activity or the concentration.

For a two-enzyme pathway, in which the first enzyme follows Michaelis–Menten kinetics and the second enzyme is allosteric displaying sigmoidal rate-dependence as described by eqn. (13), Figure 2 shows how the flux control coefficient (solid line) and

the pathway flux (dashed line) change with an increase in maximal catalytic activity ($V_2^{\max}$) of the downstream enzyme. One can see a large domain where the control coefficient of the allosteric enzyme increases with increasing $V_2^{\max}$. This example shows that the general assumption that the flux control coefficient of an enzyme over the flux through its own reaction always increases as its catalytic activity decreases is false.

It is important to note that sigmoidal kinetics cause allosteric enzymes to have low control coefficients when not saturated, because of their large elasticity coefficients. In Figure 2 the domain in which the elasticity (e_X^2) is large compared with that of the Michaelis–Menten enzyme, extends from $e_2 = 1$ to $e_2 = 100$. In this domain the control coefficient of the allosteric enzyme (C_2^J) is small. Moreover, it does not change much on a change in enzyme activity of more than two orders of magnitude (cf. refs. [24,25]).

To understand better how widely sigmoidal kinetics may cause paradoxical control to occur we shall consider another example, in which the second part of the pathway in Scheme 1 consists of two enzymes, E_{21} and E_{22} (Scheme 2).

Scheme 2

Whether paradoxical control will be observed with changes in $V^{\max}$ is determined by eqn. (10), in which e_X^1 and e_X^2 stand for the elasticity of E_1 and for the overall elasticity of the second part of the pathway, i.e. of the group of enzymes, E_{21} and E_{22} . As in the case above, if the enzyme (E_1) in the first part of the pathway follows Michaelis–Menten kinetics, the necessary condition for paradoxical control is the existence of a range of concentrations X , where the positive value of e_X^2 increases as X increases, i.e. $d e_X^2/dX$ is positive. The overall elasticity (e_X^2) can be expressed in terms of the elasticities (e_X^{21} and e_X^{22}) of the individual enzymes and their control coefficients considered within the second part of the pathway, i.e. at fixed concentrations of the link metabolite (X) and the end product (P). In most cases, only the rate, v_{21} , depends on X directly, hence the overall elasticity (e_X^2) and its derivative ($d e_X^2/dX$) with respect to X , can be expressed as [26,27]:

$$e_X^2 = {}^b C_{21}^J e_X^{21}, \quad d e_X^2/dX = (d {}^b C_{21}^J/dX) e_X^{21} + {}^b C_{21}^J (d e_X^{21}/dX) \quad (14)$$

Here the superscript, b , specifies the control coefficient of enzyme E_{21} , as determined within the second part of the pathway of Scheme 2 (which is considered in isolation from the first part). Correspondingly, in the derivative, $d {}^b C_{21}^J/dX$, the concentration, X , plays the role of a parameter (the concentration of the initial substrate) of the second part of the pathway. It follows from eqn. (14) that whether $d e_X^2/dX$ is positive depends on whether the derivative, $d {}^b C_{21}^J/dX$, is positive, i.e. on whether the control within the second part of the pathway is paradoxical (the elasticity, e_X^{21} , of enzyme E_{21} to its substrate is always positive).

When enzyme E_{21} has sigmoidal kinetics and exhibits paradoxical control behaviour within the second part of the pathway (i.e. its control increases when the initial substrate, X , increases), the control within the whole pathway will remain paradoxical with changes in $V^{\max}$ of either part of the pathway in a wide range of parameter values. Indeed, in this case the derivative $d e_X^{21}/dX$ is positive in some range of X (see above), and since $d {}^b C_{21}^J/dX$ is positive, $d e_X^2/dX$ will be also positive [eqn. (14)].

Table 1 Paradoxical increase in the overall control coefficient ($C_2^J = C_{21}^J + C_{22}^J$) of the second part of the pathway in Scheme 2 with a coherent increase (10-, 100- and 400-fold) in the maximal activities of enzymes E_{21} and E_{22}

The pathway of Scheme 2 with Michaelis–Menten enzymes E_1 and E_{21} and allosteric enzyme E_{22} was simulated. The parameter values in the rate equations of enzymes E_1 and E_{22} are indicated in the legend to Figure 2. The rate of enzyme E_{21} is described by eqn. (11), where Y is substituted for P , and the subscript, 21, is substituted for 2; $K_{21}^X = 3$, $K_{21}^Y = 2$, $K_{21}^{eq} = V_{21}^I K_{21}^Y / V_{21}^I K_{21}^X = 1/3$. An increase in $V_2^{\max}$ corresponds to simultaneous proportional increases in V^I and V^T leaving the equilibrium constant (K^{eq}) unchanged. The original (initial) values of maximal activities (the first row in the Table) were: $V_1^I = 180$, $V_{21}^I = 14$, $V_{22}^I = 4500$. $S = 1$, $P = 0$. The values in the Table are the indicated control coefficients.

	C_1^J	C_{21}^J	C_{22}^J	$C_2^J = C_{21}^J + C_{22}^J$
Original values of enzyme activities	0.617	0.091	0.292	0.383
10-fold increase in $V_1^{\max}$ and $V_{22}^{\max}$	0.590	0.039	0.371	0.410
100-fold increase in $V_1^{\max}$ and $V_{22}^{\max}$	0.553	0.022	0.425	0.447
400-fold increase in $V_1^{\max}$ and $V_{22}^{\max}$	0.545	0.018	0.437	0.455

It is less evident whether the control remains paradoxical or not when the first enzyme of the second part, E_{21} , follows Michaelis–Menten kinetics, whereas enzyme E_{22} has sigmoidal kinetics. Indeed, in this case de_X^{21}/dX is negative (the elasticity coefficient of the Michaelis–Menten enzyme decreases with an increase in its substrate; see above). Hence, only the first term in eqn. (14) is positive, because of assumed paradoxical control properties of the second part of the pathway. Only when this term exceeds the absolute value of the second negative term, the control within the whole pathway can remain paradoxical with respect to changes in $V_2^{\max}$ of the first or second part of the pathway. Clearly, this decreases the range of parameter values that would demonstrate paradoxical control. However, even in this case, paradoxical control can occur, for instance, when the control coefficient, C_{21}^J , of the Michaelis–Menten enzyme (E_{21}) is very small. Table 1 illustrates this numerically showing that the overall control coefficient ($C_2^J = C_{21}^J + C_{22}^J$) of the second part of the pathway increases with an increase in $V_2^{\max}$ [i.e. when maximal activities, $V_{21}^{\max}$ and $V_{22}^{\max}$, of enzymes E_{21} and E_{22} increase in proportion; see eqn. (5)]. Table 1 also illustrates another important feature of the control: even if the control exerted by a group of enzymes is paradoxical, this does not necessarily imply that the control of any enzyme of this group will show paradoxical behaviour. Indeed, one can see that the control coefficient C_{22}^J increases (paradoxically), whereas the control coefficient, C_{21}^J , decreases (usual control behaviour) with increasing $V_2^{\max}$. However, if one is interested in the control behaviour of the allosteric enzyme (E_{22}) only, rather than in the control exerted by the group of enzymes, E_{21} and E_{22} , then one should perform the analysis considering Y as the link metabolite (see Scheme 2). With a particular type of sigmoidal kinetics allowing for non-monotonical dependence of the elasticity of allosteric enzyme to the metabolite, Y , and with particular conditions on the overall elasticity of the first enzyme group to Y , the allosteric enzyme may exhibit paradoxical control no matter how many Michaelis–Menten enzymes precede this enzyme in a pathway. This conclusion was confirmed numerically by a simulation of a five-enzyme pathway, where four Michaelis–Menten enzymes preceded the same allosteric enzyme as in the simulations of Figure 2 and Table 1 (results not shown).

Figure 3 Paradoxical control in the pathway of Michaelis–Menten enzymes in which the link metabolite (X) is produced in two parallel reactions (Scheme 3)

The overall flux control coefficient of the second enzyme (—) and the logarithm of the total flux through the pathway [i.e. the rate of product (P) production] (---) versus the logarithm of the maximal catalytic activity ($V_2^{\max}$) of the second enzyme are shown. The parameter values were: $V_1^I = 110$, $S_1/K_{11}^S = 0.1$, $K_{11}^X = 0.09$, $V_{12}^I = 1$, $S_2/K_{12}^S = 199$, $K_{12}^X = 5$, $K_2^X = 1100$, $P = 0$.

Isoenzymes and allosteric enzymes showing negative co-operativity

There are also other important cases in which the control exhibits paradoxical behaviour on changes in $V_i^{\max}$. Let us suppose that the first part of the pathway of Scheme 1 consists of the two enzymes (E_{11} and E_{12}) both of which produce the link metabolite X (Scheme 3). The concentrations of the initial substrates (S_1 and S_2) and of the final product (P) are assumed constant. Note that E_{11} and E_{12} can consist of several enzymes, transporters, etc.

Scheme 3

We shall consider the case when both enzymes E_{11} and E_{12} are far from equilibrium and follow Michaelis–Menten kinetics with respect to their product X :

$$v_{11} = e_{11} \frac{k_1^f S_1 / K_{11}^S}{1 + S_1 / K_{11}^S + X / K_{11}^X}, \quad v_{12} = e_{12} \frac{k_2^f S_2 / K_{12}^S}{1 + S_2 / K_{12}^S + X / K_{12}^X},$$

$$J_1 = v_{11} + v_{12} \quad (15)$$

Here K_i^S and K_i^X are the Michaelis constants with respect to the substrate (S_i) and the product (X) for either enzyme, e_{1i} is the enzyme concentration, k_i^f is the catalytic constant (turnover number) in the forward direction, so that $e_{1i} k_i^f = V_{1i}^f$ is the maximal (forward) rate. It is assumed that in the range of the concentrations S_i and X under consideration, the backward rates

$[V_{1i}^r X/K_{1i}^X]$, see eqn. (11)] are negligible compared with the forward rates ($V_{1i}^r S_i/K_{1i}^S$), and that the Michaelis constants (K_{1i}^X) of the two enzymes differ.

Calculating using eqns. (1) and (15) the overall elasticity coefficient ($\epsilon_X^1 = \partial \ln |J_1| / \partial \ln X$) one can see readily that a range of X can exist in which the elasticity coefficient increases, remaining negative as X increases (i.e. the absolute value $|\epsilon_X^1|$ decreases). Therefore in this range the derivative $-d \ln |\epsilon_X^1| / dX$ is positive, and, if it exceeds the value of $d \ln |\epsilon_X^2| / dX$, the function f in eqn. (10) is positive. As a consequence, paradoxical control behaviour will occur in this range of concentrations of the linking metabolite.

Figure 3 elaborates this example numerically. For simplicity the kinetics of the second part of the pathway (the enzyme E_2) were also described by the Michaelis–Menten equation. One can see that there is a domain in which increasing the maximal catalytic activity or the concentration of enzyme E_2 (i.e. $V_2^{\max}$ of the second part of the pathway) increases its control coefficient. Note that, as in Figure 2, the domain of paradoxical control behaviour coexists with domains of non-paradoxical behaviour, in which increases in catalytic activity of an enzyme decrease its control.

In line with eqn. (10), on proportional activation of enzymes E_{11} and E_{12} ($de_{11}/e_{11} = de_{12}/e_{12} = dV_1^{\max}/V_1^{\max}$), the control coefficient of the first part of the pathway increases in the same region of the concentrations X , whereas increasing the maximal catalytic activity of the second part ($V_2^{\max}$) increases its control coefficient (not shown).

Similarly, if the Michaelis–Menten enzyme (E_2) of the second part of the pathway exists as two enzymes (E_{21} and E_{22}) with different Michaelis constants for the link metabolite X , this pathway can also display paradoxical control behaviour even if the first enzyme (E_1) follows Michaelis–Menten kinetics. The basis for such a phenomenon is non-monotonical dependence of the overall elasticity ϵ_X^2 of the second part of the pathway on the link metabolite.

These examples show that paradoxical control can occur when the first or second part of a pathway exists as two branches (Scheme 3). Importantly, this conclusion continues to apply to a pathway involving isoenzymes that catalyse the same step but have different K_m values. An example of such a pathway is shown in Scheme 4. Here the first part of the pathway is a step catalysed by enzyme E_1 , and the second part involves steps catalysed by enzymes E_{21} , E_{22} and E_{23} . The step after the link metabolite, X , is catalysed by two isoenzymes, E_{21} and E_{22} , which compete for the same substrate (X) and are inhibited by the same product (Y) which they both produce. For simplicity we shall assume that all four enzymes in the pathway of Scheme 4 follow Michaelis–Menten kinetics.

Scheme 4

When the Michaelis constants of the two isoenzymes for their common substrate (X) differ significantly, the overall elasticity (ϵ_X^2) of the second group can depend on X non-monotonically, i.e. a range of X can exist, where the (positive) value of ϵ_X^2 will

Table 2 Paradoxical control properties of a pathway involving isoenzymes (Scheme 4) with changes in $V^{\max}$ of the first and second parts

The control properties of the pathway in Scheme 4 were simulated. The parameter values for the rate equation of enzyme E_1 are indicated in the legend to Figure 2, and the rates of enzymes E_{21} , E_{22} and E_{23} are described by eqn. (11) with the corresponding substitution of the designations of the substrates and the products (see the legend to Table 1). The parameter values were: $K_{21}^X = K_{21}^Y = 1000$, $K_{21}^{S_1} = V_{21}^1 K_{21}^X / V_{21}^1 K_{21}^Y = K_{22}^{S_1} = V_{22}^1 K_{22}^X / V_{22}^1 K_{22}^Y = 2$, $K_{22}^X = 0.1$, $K_{22}^Y = 10$; $K_{23}^X = 0.1$, $K_{23}^Y = 10$, $K_{23}^{S_1} = V_{23}^1 K_{23}^X / V_{23}^1 K_{23}^Y = 100$, $S = 1$, $P = 0.1$. An increase in $V^{\max}$ was achieved by simultaneous proportional increase in $V_{21}^{\max}$, $V_{22}^{\max}$ and $V_{23}^{\max}$. The original values of enzyme activities (the first row in the Table) were: $V_1^1 = 1000$, $V_{21}^1 = 200$, $V_{22}^1 = 1$, $V_{23}^1 = 3$. The values in the Table are the control coefficients.

	C_1^J	$C_{21}^J + C_{22}^J + C_{23}^J$
Original values of enzyme activities	0.294	0.706
2-fold increase in $V_1^{\max}$	0.337	0.663
4-fold increase in $V_1^{\max}$	0.352	0.648
2-fold increase in $V_2^{\max}$	0.249	0.751
6-fold increase in $V_2^{\max}$	0.213	0.787

increase as X increases (notice that, if a single enzyme rather than two isoenzymes were present, ϵ_X^2 would always decrease with an increase in X). From eqn. (10) it follows that such a non-monotonical dependence of the overall elasticity, ϵ_X^2 , on the concentration, X , may well result in paradoxical control behaviour. A numerical illustration is given in Table 2. One can see that an increase in $V_1^{\max}$ causes the control, C_1^J , exerted by the first enzyme (E_1) to increase and the control, C_2^J , exerted by the second part (enzymes E_{21} , E_{22} and E_{23}) to decrease (the second and third rows in Table 2). An increase in $V_2^{\max}$ causes the C_2^J to increase and the C_1^J to decrease (the fourth and fifth rows in Table 2). Thus a pathway in which some enzyme steps are catalysed by isoenzymes with different binding constants to the ligands can display the paradoxical control property.

It is important to note that allosteric enzymes can mimic the kinetics of two or more independent enzymes (isoenzymes). This occurs when an allosteric enzyme possesses different non-interacting binding sites or when negative co-operativity between identical sites is occurring. The latter can be obtained within the AKNF model and cannot be obtained with the MWC model (for a review see, e.g., ref. [21]). Therefore paradoxical control may occur in the case of Michaelis–Menten kinetics, when the intermediate X is at a branch point or is produced or consumed by isoenzymes, whereas in the case of allosteric enzymes, a paradoxical increase in the control exerted by an enzyme on increasing its concentration can occur in the (linear) two-enzyme pathway (Figure 2).

Paradoxical control behaviour may also occur on changes in $V^{\max}$ when the rate of the downstream enzyme or group of enzymes has a very sharp exponential dependence on its substrate (X), thus causing the elasticity coefficient (ϵ_X^2) to increase with the concentration, X . Such exponential dependence of the rate on the membrane potential is a feature of some transport reactions. For instance, the rate of proton leak through the mitochondrial membrane increases exponentially with the protonmotive force, $\Delta\mu_H$ [28], and the rate of mitochondrial H^+ -ATP synthetase, displaying threshold behaviour with respect to $\Delta\mu_H$, can be

described in terms of an exponential dependence on $\Delta\mu_H$ [29]. However, when the upstream enzyme or group of enzymes is inhibited by its product (X) as a (negative) exponent of X, the control behaviour of the pathway is non-paradoxical.

Paradoxical control as a result of affecting the binding and/or Michaelis constants

Although we have presented a number of cases where increases in enzyme concentration (maximal catalytic activity, $V^{\max}$) in the first or second parts of a pathway increase their control coefficients, most often an increase in $V^{\max}$ decreases the corresponding control coefficient, i.e. control behaviour is non-paradoxical, since most enzymes follow Michaelis–Menten kinetics. The situation changes dramatically when an enzyme is activated by decreasing its Michaelis constant (K_m) for the substrate. Whereas the modulation of maximal catalytic activity ($V_i^{\max}$) does not change the elasticities directly ($\partial e_x^i / \partial V_i^{\max} = 0$; see above), a decrease in K_m necessarily decreases the elasticity coefficient with respect to the substrate. Therefore, even when the response to the changes in maximal catalytic activity is non-paradoxical, activation of an enzyme by decreasing its K_m can result in paradoxical control behaviour.

We shall consider again the pathway of two enzymes that follow Michaelis–Menten kinetics [see eqn. (11)]. However, now we shall modulate the Michaelis constant (K_2^X) of the second enzyme for its substrate (X), rather than the concentration (maximal catalytic activity) of the enzyme. Increasing K_2^X decreases the enzyme rate (v_2), and, at the same time, increases its elasticity (e_x^2) with respect to the substrate. Therefore designating by p_2 this specific parameter (K_2^X) one can write:

$$e_p^2 < 0, \quad \partial e_x^2 / \partial p_2 > 0 \quad (16)$$

Taking into consideration these inequalities and using eqn. (9), one can see that the function $\frac{\partial}{\partial p_2}$ is positive. Since the function f for two Michaelis–Menten enzymes is negative (see above), whether the control coefficient of the enzyme E_2 will increase or decrease when its Michaelis constant (K_2^X) decreases depends on maximal velocities (maximal catalytic activities) and the other kinetic parameters of both enzymes. Using eqns. (9) and (11) to calculate the control response ratio for changes in K_2^X , one arrives at:

$$B_{K_2^X}^2 / b^2 = (K_{m2}^X - K_{m1}^X) / (K_{m1}^X + X) + [X - (2P / K_2^{\text{eq.}}) - K_{m2}^X] \{1 / (SK_1^{\text{eq.}} - X) + 1 / [X - (P / K_2^{\text{eq.}})]\} \quad (17)$$

Here b^2 is a positive function; K_{m2}^X and K_{m1}^X are the apparent Michaelis constants of the enzymes, $K_{m2}^X = K_2^X(1 + P / K_2^P)$, $K_{m1}^X = K_1^X(1 + S / K_1^S)$; $K_1^{\text{eq.}}$ and $K_2^{\text{eq.}}$ are the equilibrium constants, $K_1^{\text{eq.}} = V_1^r K_1^X / V_1^f K_1^S$, $K_2^{\text{eq.}} = V_2^r K_2^P / V_2^f K_2^X$. Eqn. (17) shows that if the two following inequalities are fulfilled simultaneously:

$$K_2^X(1 + P / K_2^P) > K_1^X(1 + S / K_1^S); \quad X > K_2^X(1 + P / K_2^P) + 2P / K_2^{\text{eq.}} \quad (18)$$

then on changes in K_2^X the control response ratio is positive, hence the control is paradoxical. If both inequalities in eqn. (18) have the opposite sign, the second enzyme displays non-paradoxical behaviour when activated or inhibited through changes in its Michaelis constant (K_2^X). The conditions [eqn. (18)] mean that paradoxical control behaviour is likely to occur when the enzyme is saturated by the substrate.

Figure 4 illustrates this conclusion numerically showing a decrease in the control coefficient of the second enzyme (solid line) when its Michaelis constant (K_2^X) for the substrate (X) increases. This increase in K_2^X is accompanied by decrease in the

Figure 4 Paradoxical control property of the pathway of Scheme 1 of the two Michaelis–Menten enzymes occurring when the Michaelis constant of the second enzyme is modulated

The control coefficient of the second enzyme (—) and the pathway flux (---) decrease when the Michaelis constant (K_2^X) for the substrate (X) increases. The kinetic parameters of the enzymes are indicated in the legend to Figure 1.

pathway flux (dashed line). Note that in this example all the kinetic parameters are the same as in Figure 1, where activation of the same (second) enzyme by increasing its $V^{\max}$ decreased its control dramatically.

Similar arguments show that for allosteric enzymes there exists a wide range of parameter values in which allosteric inhibitors that increase the allosteric constant L will increase the control coefficient of the affected enzyme and allosteric activators that decrease L will decrease its control coefficient.

DISCUSSION

Whether an enzyme exhibits paradoxical control depends on (a) the structure of the system, (b) the kinetics of the pathway enzymes and (c) the mode by which the enzyme is activated or inhibited. We have not investigated all possible causes of paradoxical control, but rather sought to illustrate control properties in some simple but common cases. Although we have emphasized conditions under which paradoxical changes in control occur, control properties of enzymes within pathways are most often non-paradoxical, i.e. inhibition of an enzyme causes its control to increase, whereas activation causes its control to decrease. Indeed, we have shown that, when the enzymes follow Michaelis–Menten kinetics, only changes in K_m lead to paradoxical control behaviour, and even then only under special conditions [see Figures 1 and 4, and eqn. (17)]. For enzymes with sigmoidal kinetics, paradoxical changes in control occur under a wider range of conditions (a wider range of parameter values and for changes in K_m , L and $V^{\max}$), but non-paradoxical behaviour still occurs under a wide range of conditions. The occurrence of paradoxical control with changes in $V^{\max}$ can be tested by raising the enzyme concentration over a wide range of concentrations, either by genetic methods or by adding exogenous enzyme to tissue homogenates [30–33]. Paradoxical control (with changes in $V^{\max}$) would result in a sigmoidal dependence of pathway flux on enzyme concentration, whereas non-paradoxical control would in general result in a dependence of flux on enzyme concentration similar to a hyperbolic one. This has been investigated for some enzymes and systems, and in most of these

experiments the relation between pathway flux and enzyme concentration fits a hyperbolic curve reasonably well [4,5]. This result might be expected from our analysis, since we found that, with changes in $V^{\max}$, all Michaelis–Menten enzymes exhibit non-paradoxical behaviour, and, even with sigmoidal enzymes, paradoxical behaviour with changes in $V^{\max}$ is only seen with a particular type of sigmoidal kinetic and under particular conditions. Nevertheless the existence of such rare enzymes with strongly sigmoidal kinetics may be important, since it is just these enzymes that are often considered to be regulatory. It would therefore be interesting to test for paradoxical control (with changes in $V^{\max}$) in such regulatory enzymes. Note that allosteric enzymes with sigmoidal kinetics are most often regulated by changes in K_m rather than $V^{\max}$, and such changes will result in paradoxical control over a wider range of parameter values.

Paradoxical control may be an important beneficial feature for a regulatory enzyme. If the rate of a pathway is to be regulated by allosteric activation of a regulatory enzyme within the pathway by an effector external to the pathway, then it is important that activation of that enzyme does not cause the enzyme to lose control over the pathway, otherwise activation of the enzyme will only have a relatively small effect on the rate of the pathway. The paradoxical control exhibited by enzymes with sigmoidal kinetics enables the enzyme to retain control even after significant activation, and therefore enables the enzyme to regulate the pathway rate over a significant range of rates. Activation of the Michaelis–Menten enzyme by decreasing the K_m for substrate also allows the enzyme to retain its control, although in a narrower range of rates. However, if a pathway is to be regulated by allosteric inhibition of a regulatory enzyme within a pathway by an effector external to the pathway, then it is important that the changes in control are non-paradoxical so that the enzyme retains control after inhibition. Therefore such regulation would be most effective when occurring by changes in $V^{\max}$ of a Michaelis–Menten enzyme. When the allosteric effector is internal to the pathway (e.g. during feedback inhibition), then the same principles apply, but effective feedback inhibition requires that the elasticity of the regulated enzyme to the effector is high, and thus the control coefficient over the pathway flux is low (see ref. [1]).

Our study might help us to understand the ubiquitousness of isoenzyme families and to elucidate their regulatory properties. Comparing Table 2 and Figure 3 with Figure 1, one may speculate that isoenzyme families help to retain the regulatory pattern over a wide variation in the rates caused by either activation or inhibition of the enzymes.

To determine specific targets of hormones and of other effectors, measurements of changes in the control coefficients do not suffice, since the control behaviour can be paradoxical. Estimation of changes in the intermediate concentration is necessary, as in phenomenological kinetic analysis [11,12,34]. For example, to diagnose channelling or localized coupling between enzymes, double inhibitor titrations can be applied only when evidence is provided that enzymes within the pathway exhibit non-paradoxical control behaviour.

The occurrence of paradoxical control requires some caution in the practical application of MCA. Control coefficients refer to flux changes after infinitesimally small changes in enzyme activity, and therefore cannot be used to predict real changes, unless coupled with assumptions about how the coefficients will change.

We have shown that, without knowing the kinetics and structure of the pathway, the way in which the control coefficients will change cannot be predicted. Thus, for example, the fact that an enzyme has a low control coefficient cannot be used as evidence that activation of the enzyme will not significantly stimulate the pathway flux, and thus cannot be used to predict that the enzyme does not regulate the pathway. However, our findings do indicate that changes in enzyme concentration or $V^{\max}$ normally cause non-paradoxical changes in control, and thus knowledge of control coefficients does allow certain predictions of real changes when enzyme activity is changed in this way.

This work was supported by the Royal Society (London) and the Wellcome Trust.

REFERENCES

- Kacser, H. and Burns, J. A. (1973) in *Rate Control of Biological Processes* (Davies, D., ed.), pp. 65–104, Cambridge University Press, London
- Heinrich, R. and Rapoport, T. A. (1974) *Eur. J. Biochem.* **42**, 89–102
- Fell, D. A. (1992) *Biochem. J.* **286**, 313–330
- Small, J. R. and Kacser, H. (1993) *Eur. J. Biochem.* **213**, 613–624
- Small, J. R. and Kacser, H. (1993) *Eur. J. Biochem.* **213**, 625–640
- Höfer, T. and Heinrich, R. (1993) *J. Theor. Biol.* **164**, 85–102
- Schuster, R. and Holzhütter, H.-G. (1995) *Eur. J. Biochem.* **229**, 403–418
- Moreno-Sanchez, R., Devars, S., Lopez-Gomez, F., Urite, A. and Corona, N. (1991) *Biochim. Biophys. Acta* **1060**, 284–292
- Kholodenko, B. N. (1979) *Biofizika* **24**, 640–645 (Engl. transl. **24**, 659–665)
- Kholodenko, B. N. (1980) *Biofizika* **25**, 250–257 (Engl. transl. **25**, 256–264)
- Brown, G. C. (1994) in *Modern Trends in Biothermokinetics* (Schuster, S., Riogoulet, M., Ouhabi, R. and Mazat, J.-P., eds.), pp. 225–228, Plenum Press, New York
- Brown, G. C. (1995) in *Bioenergetics: A Practical Approach* (Brown, G. C. and Cooper, C. E., eds.), pp. 111–131, Oxford University Press, Oxford
- Kholodenko, B. N. (1980) *Biofizika* **25**, 258–264 (Engl. transl. **25**, 264–271)
- Kacser, H. (1983) *Biochem. Soc. Trans.* **11**, 35–40
- Westerhoff, H. V., Groen, A. K. and Wanders, R. J. A. (1983) *Biochem. Soc. Trans.* **11**, 90–91
- Brown, G. C., Hafner, R. P. and Brand, M. D. (1990) *Eur. J. Biochem.* **188**, 321–325
- Westerhoff, H. V. and Van Dam, K. (1987) *Thermodynamics and Control of Biological Free-energy Transduction*, Amsterdam, Elsevier
- Kholodenko, B. N. and Westerhoff, H. V. (1995) *Trends Biochem. Sci.* **20**, 52–54
- Kholodenko, B. N., Molenaar, D., Schuster, S., Heinrich, R. and Westerhoff, H. V. (1995) *Biophys. Chem.* **56**, 215–226
- Cornish-Bowden, A. (1976) *Principles of Enzyme Kinetics*, Butterworths, London
- Hammes, G. G. (1982) *Enzyme Catalysis and Regulation*, Academic Press, New York
- Kholodenko, B. N. and Westerhoff, H. V. (1995) *Biochim. Biophys. Acta* **1229**, 275–289
- Sauro, H. M. (1993) *Comput. Appl. Biosci.* **9**, 441–450
- Kholodenko, B. N., Dibrov, B. F. and Zhabotinsky, A. M. (1981) *Biofizika* **26**, 501–595 (Engl. transl. **26**, 510–516)
- Kholodenko, B. N. (1983) in *Mathematical Modeling in Immunology and Medicine* (Marchuck, G. I. and Belykn, L. N., eds.), pp. 311–318, North-Holland Publishing Company, Amsterdam
- Kholodenko, B. N. (1984) *Biofizika* **29**, 760–765 (Engl. transl. **29**, 831–836)
- Kholodenko, B. N. (1988) *FEBS Lett.* **232**, 383–386
- Brown, G. C. and Brand, M. D. (1986) *Biochem. J.* **234**, 75–81
- Clark, A. J., Cotton, N. P. J. and Jackson, J. B. (1983) *Biochim. Biophys. Acta* **723**, 440–453
- Niederberger, P., Prasad, R., Miozzari, G. and Kacser, H. (1992) *Biochem. J.* **287**, 473–479
- Walsh, K. and Koshland, D. E., Jr. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3577–3581
- Jensen, P. R., Westerhoff, H. V. and Michelsen, O. (1993) *Eur. J. Biochem.* **211**, 181–191
- Torres, N. V., Mateo, F., Melendez-Havia, E. and Kacser, H. (1986) *Biochem. J.* **234**, 169–174
- Korzeniewsky, B., Harper, M.-E. and Brand, M. (1995) *Biochim. Biophys. Acta* **1229**, 315–322