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Response coefficients of interconvertible enzyme cascades towards effectors that act on one or both modifier enzymes

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Explicit expressions have been derived for the response coefficients for the effect of activator and inhibitor concentrations on the fraction in the active state of the target enzyme of a monocyclic interconvertible enzyme cascade. These allow one to assess the adequacy of such a cascade for producing a highly sensitive response to an effector. Numerical studies indicate that this type of system can readily generate response coefficients of about seven, even without requiring both modification reactions to be modulated simultaneously, and without requiring all of the parameters that characterize the system to have their optimum values. Thus, a monocyclic cascade can constitute a highly effective on/off switching device in a linear pathway.

Interconvertible enzyme systems, in which an inactive form of an enzyme can be converted by a covalent modification to an active form, and back to the inactive form by a different reaction, have been known for more than a quarter of a century (Krebs and Beavo, 1979). For example, glutamine synthetase from *Escherichia coli* can be converted to an inactive form by adenylylation and reactivated by deadenylylation (Chock et al., 1980). Numerous examples are known of cycles in which phosphorylation catalysed by a kinase can be reversed by hydrolysis catalysed by a phosphatase (Cohen, 1985). As both reactions in such a cycle are effectively irreversible, they can be independently regulated to vary the steady-state degree of modification over a wide range. Such cycles of reactions have been termed cascades by Stadtman and coworkers (Stadtman, 1970; Stadtman and Chock, 1977; Chock and Stadtman, 1977), or monocyclic cascades for the simplest type involving only one cycle; their terminology is now well established. Mathematical analysis by these workers of the possible behaviour of cascades has shown that they constitute a major mechanism for enzyme regulation.

Goldbeter and Koshland (1981, 1984) have shown that cascades can generate much higher sensitivity of response (e.g. enzyme activity) to signal (e.g. metabolite concentration) than is possible with single-enzyme mechanisms such as cooperativity and allostery. More recently, we have tried to define as precisely as possible the conditions that the enzymes catalysing the interconversion reactions must satisfy if the system as a whole is to display very high sensitivity (Cárdenas and Cornish-Bowden, 1989). When they are fulfilled, even the simplest kind of cascade can display a degree of sensitivity far outside the range of behaviour possible for a single cooperative enzyme responding to an allosteric effector.

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Although any understanding of the contribution of an interconvertible enzyme system to metabolic control must begin with a study of how such a system compares with the behaviour of an individual enzyme, that is by no means sufficient. Nearly all enzymes operate as components of metabolic pathways, and, to analyse their contribution to control, it is necessary to consider them not as systems in their own right but as components of larger systems: a highly sensitive response of one enzyme to an effector will have no practical consequences if the enzyme in question contributes to a negligible extent to the total control of the system. We shall consider this question in relation to the form of metabolic control analysis derived from the work of Kacser and Burns (1973), as this is probably the most accessible to most readers and certainly the most widely used; nonetheless, the interested reader should have no difficulty in converting our results to any alternative theoretical framework, such as those of Savageau (1969a, b), Heinrich and Rapoport (1974) and Crabtree and Newsholme (1987). As recently shown by Groen and Westerhoff (1990), the differences between these various frameworks are more apparent than real: to a considerable degree, they are simply different ways of saying the same thing.

In our previous work, as also in that of Goldbeter and Koshland (1981), sensitivity of response was examined in relation to the standard measures of protein cooperativity, the Hill coefficient and the cooperativity index of Taketa and Pogell (1965). These are certainly necessary for relating the behaviour of cascades to that of cooperative and allosteric enzymes, but for integrating this behaviour into metabolic control analysis we need an expression for the response coefficient of the target enzyme to the effector, i.e. an expression for the derivative of the catalytic activity of the target enzyme with respect to the effector. Once this expression is available, the details of the cascade become irrelevant to the control

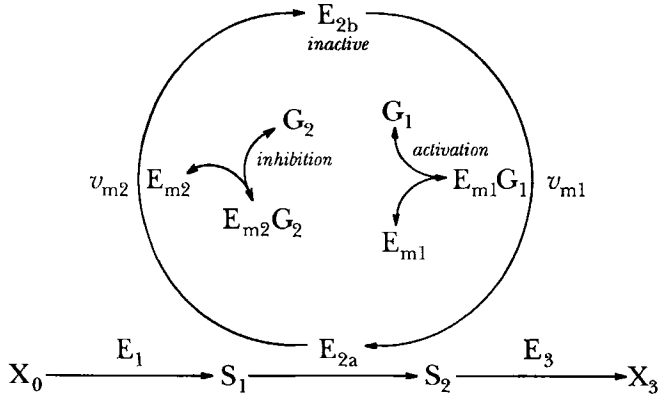

Fig. 1. Model of a monocyclic interconvertible enzyme cascade. E_2 is an enzyme that in its active form, E_{2a}, catalyses the conversion of S_1 to S_2, a step in a metabolic pathway connecting two reservoirs X_0 and X_3. It exists in both active and inactive states, E_{2a} and E_{2b}, respectively, which are interconverted in two irreversible reactions catalysed by two modifier enzymes E_{m1} and E_{m2}, of which E_{m1} is activated by an effector G_1 and E_{m2} is inhibited by a second effector G_2. The symbols used here agree with those in Cornish-Bowden and Szedlacsek (1990), but they differ from those in Cárdenas and Cornish-Bowden (1989, 1990), where the target enzyme was left unnumbered (as its existence as one of the enzymes of a pathway was not explicit), and the symbol E_2 referred not to it but to the modifier enzyme shown here as E_{m2}.

structure of the pathway, as one can consider the effect of the effector on the target enzyme exactly as if it acted directly rather than indirectly by modulating the interconversion reactions. In this paper, therefore, we show how to derive expressions for the response coefficients, and we examine how their numerical values vary under different conditions. A preliminary account of this work was presented at a symposium of the *Gesellschaft für physikalische und mathematische Biologie der DDR* in 1989 (Cornish-Bowden and Szedlacsek, 1990).

DEFINITIONS

The monocyclic cascade illustrated in Fig. 1 can generate a very high degree of apparent cooperativity, even if none of the enzymes composing the system individually shows any cooperativity (Goldbeter and Koshland, 1981, 1984). However, this is by no means guaranteed by the mere existence of a cascade, and the conditions for high sensitivity (or even for exceeding the degree of sensitivity shown by a single non-cooperative enzyme) are quite restrictive, as discussed elsewhere (Cárdenas and Cornish-Bowden, 1989, 1990). We may summarize these as follows: (a) both effectors should act primarily on the apparent catalytic constants of the modifier enzymes, i.e. they should display catalytic rather than specific effects (in the terminology of inhibition, this implies uncompetitive rather than competitive effects); (b) the activation of E_{m1} should not be appreciable until inhibition of E_{m2} is virtually complete; (c) both modifier enzymes should operate under conditions approaching saturation by their substrate ['zero-order ultrasensitivity' in the terminology of Goldbeter and Koshland (1981)]. Provided that there is a significant catalytic component in the inhibition or activation, a highly sensitive response is possible even if the effector acts on only one modifier enzyme.

In previous work (Cárdenas and Cornish-Bowden, 1989, 1990), we treated G_1 and G_2 as a single effector G acting in opposite senses on both modifier enzymes, but for fuller analysis it is convenient to be able to 'decouple' the different effects, especially as it appears common for an effector to modulate only one of the two modifier enzymes (Krebs and Beavo, 1979; Cohen, 1985); one can, of course, regenerate the simpler system at any moment by assuming that the concentrations of G_1 and G_2 are in constant ratio. In addition, we now consider the target enzyme as embedded in a metabolic pathway, and it is accordingly denoted as E_2, a symbol previously used for the modifier enzyme denoted here as E_{m2}.

The response coefficient for the fraction of E_2 existing in the active state, E_{2a}, as a function of either effector concentration, which provides a measure of sensitivity that is directly related (by multiplication by the flux control coefficient of E_{2a}) to the sensitivity of the target pathway to this concentration, may be defined as follows:

$$R^f_{G_{1(2)}} = \frac{d\ln f}{d\ln[G_{1(2)}]}, \text{ where } f = \frac{[E_{2a}]}{[E_{2a}]+[E_{2b}]}. \quad (1)$$

Goldbeter and Koshland (1982) have defined slightly different response coefficients that consider finite rather than infinitesimal changes: these may well be preferable for some applications, as a full understanding of metabolic control will require the capacity to assess finite responses. However, for incorporating the behaviour of cascades into metabolic control analysis, which is at present confined to infinitesimal effects, we need the derivatives defined here.

THEORY

If both modification reactions obey Michaelis-Menten kinetics and respond non-cooperatively to the effectors, their rates can be expressed as follows:

$$v_{m1} = \frac{(V'_1 + V_1 K'_{G_1}/[G_1])[E_{2b}]}{K'_{m1}(1 + K_{G_1}/[G_1]) + [E_{2b}](1 + K'_{G_1}/[G_1])}, \quad (2)$$

$$v_{m2} = \frac{(V_2 + V'_2[G_2]/K'_{G_2})[E_{2a}]}{K_{m2}(1 + [G_2]/K_{G_2}) + [E_{2a}](1 + [G_2]/K'_{G_2})}. \quad (3)$$

Unprimed symbols V and K_m refer to the limiting rates and Michaelis constants, respectively, of E_{m1} or E_{m2} (according to the numerical subscript) in the absence of effector G, whereas the corresponding primed symbols refer to the enzyme with effector bound to it. The effector constants K_{G_1} and K'_{G_1} refer to dissociation of G_1 from $E_{m1}G_1$ and $E_{m1}E_{2b}G_1$, respectively; K_{G_2} and K'_{G_2} refer to dissociation of G_2 from $E_{m2}G_2$ and $E_{m2}E_{2a}G_2$, respectively. In each equation, the second numerator term reflects the possibility that the enzyme in question has some activity when not activated (E_{m1}) or when maximally inhibited ($E_{m2}G_2$). In the latter case, this is perhaps an unnecessary complication, as many enzymes are known for which the activity is indistinguishable from zero when the inhibitor is bound, but it is certainly necessary for the sake of realism in the case of E_{m1}, because enzymes capable of activation normally show some activity when not activated; for example, Hunter (1987) noted that protein kinases rarely show changes greater than 20-fold between basal and active states.

The rate laws expressed in Eqns (2) and (3) are the most general that one can have without introducing cooperativity. Thus, they encompass a wide range of possible kinds of be-

haviour, but one could readily envisage more complex equations, allowing for cooperativity with respect to either substrates or effectors. One would certainly expect effector cooperativity to occur in some real systems; but it is not strictly relevant to a comparison between interaction via a cascade and direct interaction between effector and target enzyme, because any effector cooperativity that can occur in one kind of system can equally well occur in the other, as discussed in more detail elsewhere (Cárdenas and Cornish-Bowden, 1989). Substrate cooperativity in a cascade is another matter, because no corresponding interaction exists in the simpler system. However, such behaviour has not to our knowledge been reported in real systems and we shall not consider it further in the present discussion.

The steady state of the cascade is defined as a state in which the two rates v_{m1} and v_{m2} are equal to one another and to the cycling flux J_m. One can calculate this steady state, therefore, by solving for $[E_{2a}]$ after setting equal the expressions in Eqns (2) and (3), replacing $[E_{2b}]$ by $([E_2]_{total}-[E_{2a}])$, where $[E_2]_{total}$ is the total concentration of the active and inactive forms and is taken as a constant parameter of the system.

The resulting expressions are greatly simplified if one begins by defining apparent Michaelis-Menten parameters as follows:

$$V_1^{app} = \frac{V'_1 + V_1 K'_{G_1}/[G_1]}{1 + K'_{G_1}/[G_1]}, \quad (4)$$

$$K_{m1}^{app} = \frac{K'_{m1}(1 + K_{G_1}/[G_1])}{1 + K'_{G_1}/[G_1]}, \quad (5)$$

$$V_2^{app} = \frac{V_2 + V'_2[G_2]/K'_{G_2}}{1 + [G_2]/K'_{G_2}}, \quad (6)$$

$$K_{m2}^{app} = \frac{K_{m2}(1 + [G_2]/K_{G_2})}{1 + [G_2]/K'_{G_2}}. \quad (7)$$

Replacing $[E_{2b}]$ by $([E_2]_{total}-[E_{2a}])$, Eqns (2) and (3) can now be equated for the steady state as follows:

$$\frac{V_1^{app}([E_2]_{total}-[E_{2a}])}{K_{m1}^{app} + [E_2]_{total}-[E_{2a}]} = \frac{V_2^{app}[E_{2a}]}{K_{m2}^{app} + [E_{2a}]}, \quad (8)$$

which may be rearranged to give the following quadratic equation in $[E_{2a}]$:

$$\Omega([E_{2a}], [G_1], [G_2]) = a[E_{2a}]^2 + b[E_{2a}] + c = 0, \quad (9)$$

where

$$a = V_2^{app} - V_1^{app}, \quad (10)$$

$$b = V_1^{app}([E_2]_{total} - K_{m2}^{app}) - V_2^{app}([E_2]_{total} + K_{m1}^{app}), \quad (11)$$

$$c = V_1^{app} K_{m2}^{app}[E_2]_{total}. \quad (12)$$

After solving this for $[E_{2a}]$, one may use the theory of implicit functions to obtain the response coefficients:

$$R^f_{G_1} = R^{E_{2a}}_{G_1} = \frac{\partial \ln[E_{2a}]}{\partial \ln[G_1]} = -\frac{[G_1]}{[E_{2a}]}\left(\frac{\partial \Omega}{\partial [G_1]} \Big/ \frac{\partial \Omega}{\partial [E_{2a}]}\right), \quad (13)$$

$$R^f_{G_2} = R^{E_{2a}}_{G_2} = \frac{\partial \ln[E_{2a}]}{\partial \ln[G_2]} = -\frac{[G_2]}{[E_{2a}]}\left(\frac{\partial \Omega}{\partial [G_2]} \Big/ \frac{\partial \Omega}{\partial [E_{2a}]}\right). \quad (14)$$

Evaluation of these expressions is then straightforward. They are numerically quite manageable and can be used to determine how the active fraction responds to variations in one or other effector concentration, or to simultaneous variations in both.

Improper analogy with the 'chain rule' for writing down the derivative of a function of a function could lead one to think that the minus signs on the right-hand sites of Eqns (13) and (14) are incorrect. However, this rule is not appropriate here, as $[E_{2a}]$ is not written as an explicit function of $[G_1]$ and $[G_2]$, though a relationship is implicit in the fact that Ω, a function of all three variables, has a value of zero (Eqn 9). The procedure for dealing with this kind of relationship is often encountered in thermodynamics, and is discussed in textbooks such as that of Margenau and Murphy (1956).

RESULTS

Dependence of response coefficients on effector concentrations

Fig. 2 shows the response coefficient $R^f_{G_2}$ for dependence of the fraction of E_2 in the active state on the concentration of G_2 at different concentrations of the two effectors. The dimensionless ratios that characterize the system (V_2/V'_1, K_{G_1}/K'_{G_1}, K_{G_2}/K'_{G_2}, $[E_2]_{total}/K'_{m1}$, $[E_2]_{total}/K_{m2}$) are listed in the legend. They were optimized to produce the highest possible sensitivity [according to the criteria determined previously; Cárdenas and Cornish-Bowden, 1989], within the tight constraint that each must be within the range 0.1 – 10; in addition, each modifier enzyme is assumed to have 5% of full activity in its 'inactive' state, as appears to be the case for protein kinases (Hunter, 1987). An additional ratio $\overline{K}_{G_1}/\overline{K}_{G_2}$ was defined in our earlier study (Cárdenas and Cornish-Bowden, 1989) but does not need to be specified here because G_1 and G_2 are considered to be separate effectors with concentrations that can be varied independently.

In the interests of realism, we did not attempt to define the greatest possible degree of responsiveness of the system, but used values for the dimensionless parameters that are well within the range of behaviour found with real enzymes. Both modifier enzymes have appreciable activity in their 'inactive' states, and the catalytic (uncompetitive) components of the inhibition and activation are not greatly emphasized. There is no doubt that much higher response coefficients would be possible in a less constrained system. Despite these constraints, and the fact that only one modifier enzyme is modulated by the effector, in this case by inhibition of the inactivation reaction, a remarkably high response coefficient can be obtained: the high point of the ridge has $R^f_{G_2} = 8.8$.

The actual surface is a very sharp ridge; its apparent spikiness in Fig. 2 (which shows two views of the same data to give a more complete impression of the surface) is an artefact of the sampling of each of the curves that compose it at discrete points rather than continuously; even with many more sampling points than the 1800 plotted, most individual curves would fail to be sampled near to their extremely sharp maxima. Although the spikiness is an artefact, it has the serendipitous side effect of allowing one to see that the ridge is very steep on the far as well as on the near face, and that the profile is not symmetrical. The reason for the asymmetry is discussed below in the context of Fig. 5.

The location of the highest point along the ridge at a high value of $[G_2]$ (around 10) but a low value of $[G_1]$ (around 0.1) reflects the fact that high sensitivity occurs if E_{m2} is strongly inhibited when E_{m1} is barely activated.

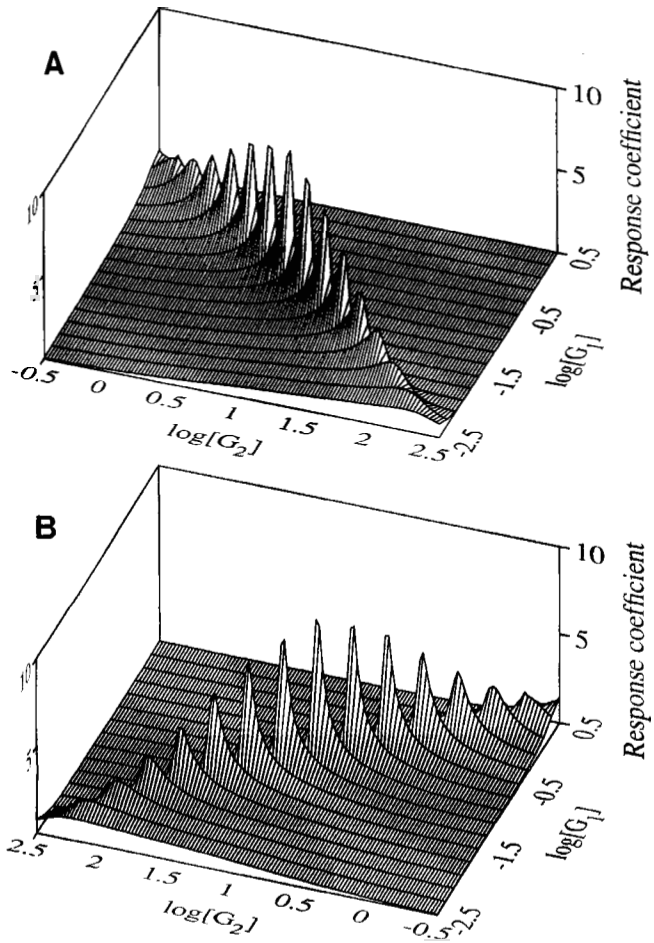

Fig. 2. Effect of the inhibitor G_2 on the response coefficient for the target activity. The response coefficient for the fraction of the target enzyme E_2 in the active state is shown as a function of the concentrations of G_1 and G_2. The following values were assumed for the dimensionless ratios that characterize the system: $V_1/V'_1 = V'_2/V_2 = 0.05$ (both modifier enzymes have 5% activity in their inactive states); $V_2/V'_1 = 1$ (the two modifier enzymes have the same maximum activity); $K_{G_1}/K'_{G_1} = 0.1$ and $K_{G_2}/K'_{G_2} = 10$ [activation of E_{m1} and inhibition of E_{m2} are predominantly catalytic (uncompetitive)]; $[E_2]_{total}/K'_{m1} = [E_2]_{total}/K_{m2} = 10$ (both modifier enzymes are approaching saturation). Effector concentrations are expressed in relation to the arithmetic mean activation constant, $\overline{K}_{G_1} = (K_{G_1} + K'_{G_1})/2$, and the harmonic mean inhibition constant, $\overline{K}_{G_2} = 2/[(1/K_{G_2}) + (1/K'_{G_2})]$. (A) and (B) show the same results, but with opposite directions in the $\log[G_2]$ axis to provide different views. The reasons for the artefactual spikiness of the ridge are discussed in the text; suffice it to note here that the true locus of the ridge is a smooth curve with a single maximum in the centre of the plot.

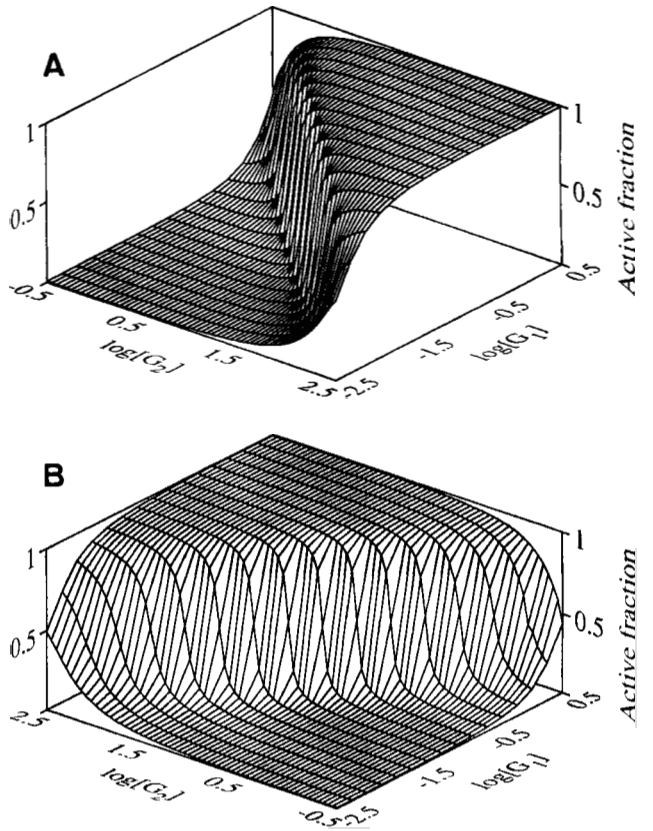

Fig. 3. Fraction of the target enzyme E_2 in the active state as a function of the concentrations of the effectors G_1 and G_2. The parameters were the same as in Fig. 2, and (A) and (B) have the same relation to one another.

Range of effector concentrations in which the sensitivity is very high

The sharpness of the surface in Fig. 2 implies that a very sensitive response is only possible in narrow ranges of effector concentrations. The ranges of very high sensitivity are nonetheless wide enough to span a wide range of activity in the target enzyme, as may be seen by comparing Fig. 2 with Fig. 3, which shows the fraction of target enzyme in the active state over the same ranges of effector concentrations. Comparing any slice through Fig. 2 with the corresponding slice through Fig. 3, one may see that the range of inhibitor concentrations in which the response coefficient is more than 50% of its peak value is sufficient to increase the target enzyme activity more than fivefold. Thus, the sharpness of the ridge in Fig. 2 reflects the fact that the transformation of target enzyme to the opposite state requires only a small change in effector concentration, not that the cascade becomes ineffective before the transformation is complete.

Magnitude of the cycling flux

Fig. 4 shows corresponding results for the cycling flux, a measure of the energetic cost of the responses shown in Figs 2 and 3. (This flux through the modification cycle should not be confused with the productive flux through the target enzyme; there is no direct relationship between their magnitudes and they may be very different.) The smooth and almost featureless surface makes a striking contrast to that shown in Fig. 2, especially if one compares the regions corresponding to the highest values of $R^L_{G_2}$. This contrast underlines the essential point that the highest response coefficients do not require the greatest cycling fluxes. On the contrary, the highest sensitivity is obtained by creating conditions where E_{m1} is barely activated but E_{m2} is strongly inhibited: in this 'poised' state, switching between the active and inactive states of the target enzyme is associated with only a slight change in the cycling flux.

Simultaneous action of one effector on both modifier enzymes

Although for generality we have allowed the two effectors to act independently, a single effector may act in opposite

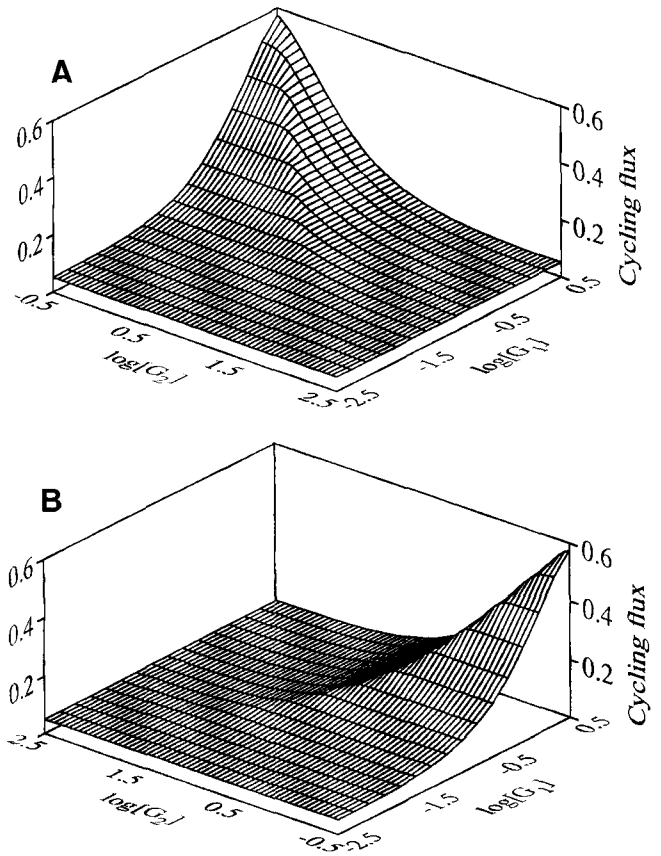

Fig. 4. Magnitude of the cycling flux as a function of the concentration of the inhibitor G_2. The parameters were the same as in Fig. 2, and (A) and (B) have the same relation to one another. The two limiting rates were equal ($V_2/V_1 = 1$), and the ordinate values represent fractions of the maximum possible flux. Note, by comparing this figure with Fig. 2, that the highest sensitivity occurs at a very low cycling flux.

directions on the two opposing modifier reactions (Chock et al., 1990). This is illustrated in Fig. 5 by taking a diagonal slice from each of Figs 2–4, from the lowest to the highest effector concentrations considered.

The definition of the response coefficient R_G^f in Fig. 5 can still be taken as given by Eqn (1), provided it is realized that whereas before it was assumed that $[G_1]$ or $[G_2]$ was varied with the other held constant, now it is assumed that both are varied simultaneously in constant ratio, with $[G] = [G_2] = 100[G_1]$ in Fig. 5. With simultaneous activation of E_{m1} and inhibition of E_{m2}, this response coefficient now attains much larger values. Less obvious (and not easily seen in Fig. 2) is that the profile for the response coefficient is highly unsymmetrical, even though all of the other curves shown in Fig. 5 are symmetrical: the curve for the cycling flux has a mirror plane through the maximum; each of the others is superimposable on itself if rotated through 180° about the point where $f = 0.5$. This disparity arises because each of the other curves represents a linear property of the system (concentration or flux) as a logarithmic function of concentration, but a response coefficient is not a linear function of the response considered: a change in f (or any other response) from 0.05 to 0.1 is equal in linear terms to a change from 0.9 to 0.95, but is about 10 times more important as a fractional change.

Relationship to the analysis of Small and Fell (1990)

Small and Fell (1990) used metabolic control analysis of the model discussed in our earlier paper (Cárdenas and Cornish-Bowden, 1989) to analyse the conditions for high sensitivity that we had reported. They showed that our conditions all 'acted to minimise the elasticities of the converting enzymes and maximised the values of the effector elasticities'. Although we find this form of wording somewhat confusing (because 'converting enzymes' could be taken to refer to the enzymes that catalyse the conversion reactions rather than to the substrates of these enzymes), we believe that the message they convey is very important: it means that the requirement is to minimize the elasticities of the modification reactions with respect to the target enzyme, and to maximize the elasticities of these reactions with respect to the effector, i.e. operate in conditions as close to zero order as possible with respect to both forms of target enzyme and as high an order as possible with respect to the effector.

Small and Fell (1990) also made the important point that our second condition could be partitioned into separate requirements for the effector concentration during the transition to be large compared with the catalytic (uncompetitive) inhibition constant of the inactivating reaction, and small compared with the catalytic activation constant of the activating reaction. This partition is especially important when two different effectors act on the two reactions, the case we have principally examined in the present paper.

DISCUSSION

With the expressions for response coefficients given here, it becomes a straightforward matter to incorporate the properties of a cascade into metabolic control analysis. Although, as we have shown, cascades allow numerical values of response coefficients that are enormous by comparison with the values that are typical of direct effects on target enzymes, the algebraic treatment of a pathway in metabolic control analysis is independent of whether responses of systems to effectors arise by direct interaction or by more complex mechanisms involving interactions between several enzymes. In a direct non-cooperative interaction the response coefficient cannot exceed one, and this limit is approached only when the target enzyme is almost completely inhibited or barely activated. Cooperativity can increase the limit, but this is irrelevant to a comparison between direct interactions and interaction via a cascade, because cooperativity can equally well occur in the modifier reactions of a cascade with a similar increase in sensitivity. Thus the results given here mean that even in a tightly constrained cascade with the effector acting on only one enzyme the sensitivity is at least sevenfold greater than direct interaction can provide.

Despite the artefactual character of the apparent spikiness of the surface in Fig. 2 (see above), one can readily imagine that small fluctuations in effector concentration could generate similar spikes in nature and that a cascade of the kind we have discussed might be too unstable to be usable. All this means, however, is that it would be difficult or impossible to maintain the activity of the target enzyme at an intermediate value: it must be either on or off. With domestic electrical switches this is not seen as a disadvantage, however, but as a necessity for efficient functioning: such switches contain mechanical springs whose function is to prevent them from being stable in an intermediate state, and in high-voltage industrial applications engineers go to some trouble to avoid

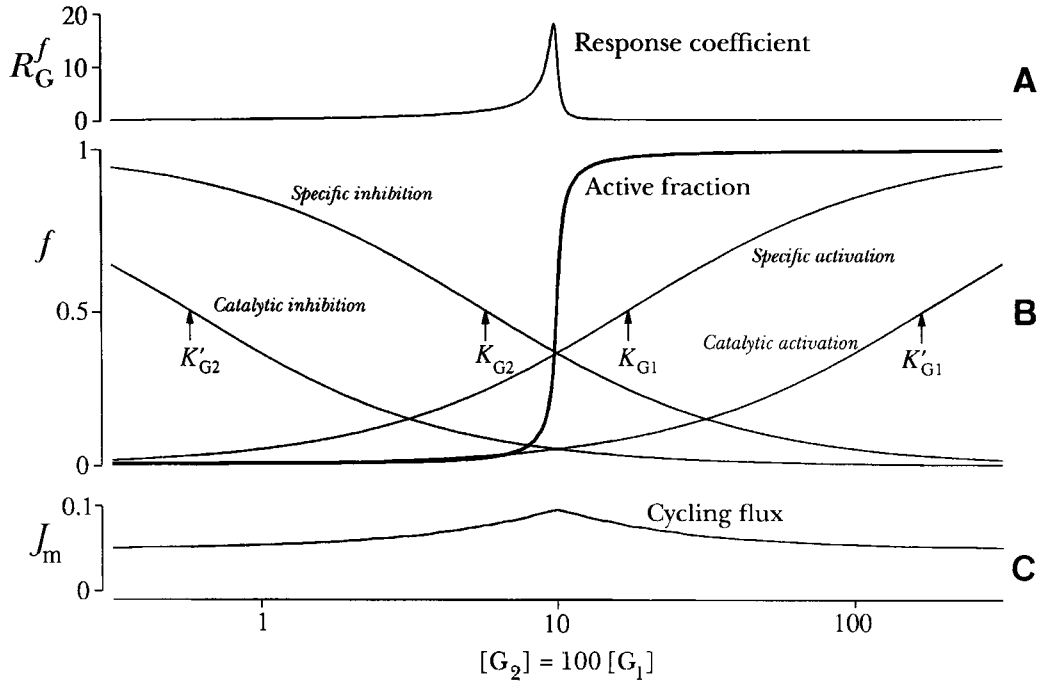

Fig. 5. Simultaneous variation of both effector concentrations $[G_1]$ and $[G_2]$. (A, C) Diagonal cuts through the surfaces shown in Figs 2 and 4, respectively, from the lowest to the highest concentrations of the effectors. (B) The heavy curve shows the same for Fig. 3, with the addition of four light curves showing the variation in activity of each modifier enzyme E_{m1} or E_{m2} that each effect would have if it acted alone: the curve labelled catalytic inhibition, for example, is calculated as $f = 1/(1 + [G_2]/K'_{G_2})$; thus, although in the full model K'_{G_2} refers to a case of catalytic inhibition, the curve is just a generic inhibition curve that takes no account of the inhibition type. The actual curve of the activity of E_{m2} as the two effector concentrations increase simultaneously (not shown) approximates to the specific inhibition curve at low concentrations, but switches abruptly to a low value when its substrate E_{2a} abruptly achieves a nearly saturating concentration when the active fraction rises; the actual activation curve for E_{m1} is the mirror image of this. The fact that the abrupt rise in active fraction occurs close to the intersection of the two specific curves is not significant: one could move the specific inhibition curve arbitrarily to the right, or the specific activation curve arbitrarily to the left, with negligible effect on the rest of the figure.

the arcing that occurs in not-quite-closed circuits. Similar considerations must apply to metabolic systems, so the difficulty of maintaining the activity of the target enzyme of a cascade at an intermediate value should likewise be seen as a design feature and not as a fault.

In contrast, Stadtman and co-workers have emphasized that covalent interconversion of enzymes allows their activities to be varied progressively over a wide range (e.g. Chock et al., 1990). Although they recognize that in some circumstances it can provide a mechanism for an on/off switch (e.g. concluding remarks in Chock et al., 1990), their main view is based on the observation that the adenylylation of glutamine synthetase and the phosphorylation of the mammalian pyruvate dehydrogenase complex are not all-or-none processes. It deserves some comment, as it could appear to contradict the conclusions of this work, as well as the views of Hunter (1987), Goldbeter and Koshland (1990) and others that interconvertible enzyme systems offer effective on/off switching devices. However, the cyclic cascade model was developed by Stadtman's group through detailed studies on the regulation of *E. coli* glutamine synthetase, a rather special enzyme in the sense that it catalyses the biosynthesis of the common precursor, glutamine, of numerous products of bacterial nitrogen metabolism. Far from catalysing the first committed step of a pathway, glutamine synthetase catalyses the last common step of several different biosynthetic pathways. The regulation of a linear biosynthetic pathway presents no serious conceptual problems, but the regulation of a highly branched biosynthetic pathway, involving a variety of different end products, is far more complicated. The modulation of the activity of glutamine synthetase must satisfy the demands of the organism for the different end products; none of them should individually inhibit the enzyme completely, exactly as observed: feedback inhibition is cumulative, i.e. excess of each end product causes only partial inhibition, but in combination the inhibitory effects are cumulative (Stadtman, 1970). This cumulative feedback inhibition of glutamine synthetase is modulated by the interconversion of adenylylated and unadenylylated forms of the enzyme.

Thus although in linear pathways cyclic cascades provide a sensitive mechanism for switching the pathway on and off, regulation of the last common step of a highly branched pathway requires a smooth transition over a wide range of activities in response to numerous different signals.

The pioneering studies of Umbarger (1956) and Yates and Pardee (1956) led to recognition of the basic mechanisms of regulation: feedback inhibition at the first committed step, allosteric and cooperative interactions, etc. These classic mechanisms are often given little emphasis in papers in the domain of metabolic control analysis, with, sometimes, the implication that they are unimportant for analysing the control structures of pathways. However, in a recent study (Hofmeyr and Cornish-Bowden, 1991), computer simulation was used to examine the effect of cooperativity in feedback regulation of a linear pathway. Although cooperative and non-cooperative inhibitions were about equally effective for

regulating the flux in response to demand over a 25-fold range, they led to very different degrees of regulation of the end-product concentration: with a Hill coefficient of four, this concentration varied less than threefold for a 25-fold range of fluxes, but with a Hill coefficient of one, it was essentially unregulated, varying 50-fold for a flux range of only about threefold. Thus, the classical limit of cooperativity, with a Hill coefficient of four, provides an enormous improvement over non-cooperative feedback, but it still falls short of strict regulation of concentrations: this requires higher response coefficients at individual steps than the classical mechanisms can provide, and so the switching devices made possible by interconvertible enzyme systems are likely to satisfy an indispensable need in metabolism.

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