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Characteristics necessary for an interconvertible enzyme cascade to generate a highly sensitive response to an effector*

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A monocyclic interconvertible enzyme cascade, in which active and inactive states of an enzyme are interconverted by two opposing enzyme-catalysed reactions, does not necessarily produce a greater degree of sensitivity to an effector than one could expect from direct interaction between effector and target reaction. On the contrary, a cascade in which an effector acts on one of the enzymes catalysing the interconversion reactions by altering the apparent value of its specificity constant will always generate a less sensitive response than direct interaction would give. Nonetheless, even if both interconversion reactions obey Michaelis–Menten kinetics with the ordinary types of inhibition and activation, one can easily generate an enormous sensitivity in which a 0.5% change in concentration can increase the proportion of target enzyme in the active state from 10% to 90%: this corresponds approximately to a Hill coefficient of 800. To maximize the sensitivity, the following conditions must be satisfied: (1) both modifier enzymes must act under conditions of near saturation; (2) the effector must act on both of them in opposite directions; (3) it must alter the apparent values of their catalytic constants; (4) the enzyme subject to inhibition by the effector must respond at much lower effector concentrations than the enzyme subject to activation. As the last of these conditions appears to be counter-intuitive, it suggests that feeble activation of modifier enzymes in real systems may have passed unnoticed, or been dismissed as physiologically insignificant, although in reality crucial to the effective response of the system.

INTRODUCTION

Many examples are now known of pairs of enzymes with different degrees of catalytic activity that can be interconverted by a pair of irreversible reactions (Cohen, 1982), often phosphorylation by ATP in one direction and hydrolysis in the other. In an important series of papers Stadtman and co-workers (Stadtman & Chock, 1977, 1978; Chock & Stadtman, 1977; Chock *et al.*, 1980) have argued that such systems, known as 'interconvertible enzyme cascades', permit a far more sensitive response to a stimulus such as a hormone than is possible for the response of a single enzyme to an effector. However, there has been only limited investigation of the properties that the enzymes catalysing the conversion reactions must have if the cascade is to generate a more sensitive response than is possible for a single enzyme (Goldbeter & Koshland, 1981, 1984; Fell & Small, 1986). The problem is not trivial, because cascades with the sort of properties often implicit in discussions generate a less sensitive response to effectors than a single enzyme would have, as we demonstrate below. We have therefore made a systematic examination of the response to an effector of a catalytic system consisting of two interconvertible components with different catalytic activities, the interconversion reactions being catalysed by two modifier enzymes, one or both activated or inhibited by an effector. This is the simplest type of interconvertible enzyme cascade that one can consider and is termed a 'monocyclic cascade'. We have determined the kinetic parameters needed for such a system to generate a highly sensitive response.

TERMINOLOGY

The monocyclic interconvertible enzyme cascade that we shall consider is shown in Fig. 1. An allosteric effector G acts on one or both of two modifier enzymes E_1 and E_2, which catalyse the formation of a target enzyme E_a from an inactive form E_b and (respectively) the reverse conversion of E_a into E_b. Both modification reactions are assumed to obey Michaelis–Menten kinetics, and G acts only by its effects on E_1 and E_2; it has no direct effect on the target enzyme. Effects on the specificity constants ($k_{cat.}/K_m$) of E_1 and E_2 are termed specific activation or inhibition, whereas effects on the catalytic constants ($k_{cat.}$) are termed catalytic activation or inhibition (Nomenclature Committee of IUB, 1983). In the case of inhibition, specific and catalytic effects are more familiarly known as competitive and uncompetitive effects respectively, but these terms will not be used here as they have no meaning in relation to activation. The additional cofactors (e.g. ATP and water) needed to make the modification reactions irreversible in both directions do not have to be considered explicitly when discussing the sensitivity properties of the cascade, though they become crucial for assessing the energy cost of the system (Shacter *et al.*, 1984; Goldbeter & Koshland, 1987), an aspect that we shall not deal with here.

For discussing control theory we shall use the terminology and symbolism agreed by several groups (Burns *et al.*, 1985; Kacser & Porteous, 1987). In particular, we shall use the term 'response coefficient', with the symbol R_g^f for the derivative $\partial \ln f / \partial \ln g$ that

* Dedicated to Professor Hermann Niemeyer on the occasion of his 70th birthday, 26 October 1988.

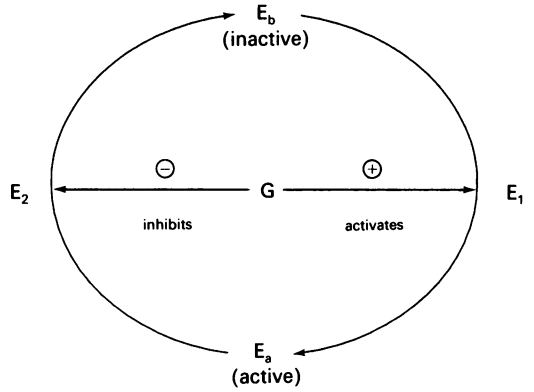

Fig. 1. Monocyclic interconvertible enzyme cascade

A target enzyme exists in a catalytically active state E_a and an inactive state E_b. These can be interconverted by the action of two modifier enzymes, E_1 and E_2. An effector G has no direct effect on the target enzyme, but controls its activity indirectly by activating E_1 or inhibiting E_2, or both.

expresses the fractional response of an output variable f of the system to a change in an external parameter g. For simplicity we shall treat the activity of the target enzyme as the output variable, effectively regarding the target reaction as a one-step pathway. In reality, of course, the target enzyme is likely to have a control coefficient less than unity for the flux through the reaction that it catalyses (Fell & Small, 1986; Kacser & Porteous, 1987), and the true response coefficient will be decreased accordingly.

Our usage of the term 'response coefficient' and the symbol R_g^f should be distinguished from the use by Goldbeter & Koshland (1981, 1984, 1987) of the same name and a similar symbol for the co-operativity index of Taketa & Pogell (1965), which defines the ratio of g values needed to span the middle 10% to 90% of the range of possible f values. We shall also have occasion to refer to this index, but we shall use the symbol r_G and the name sensitivity index to avoid confusion with the response coefficient. We shall also restrict the term 'co-operativity' to the properties of single enzymes, using 'sensitivity' in a more general way to refer to corresponding responses of systems of two or more enzymes.

THEORY

Modifier enzymes subject to specificity effects only

We begin by examining the properties of a simple cascade in which the modifier enzymes are susceptible only to effects on the apparent values of their specificity constants. We shall also assume that both modifier enzymes have the same limiting rate V, that E_1 has no activity when G is not bound to it and that E_2 has no activity when G is bound to it. As we shall see later, the restriction to specificity effects is a major limitation, whereas the other assumptions are less important: they allow great simplification of the algebra with little change to the range of behaviour possible. For these assumptions, the rates v_1 ($E_b \rightarrow E_a$) and v_2 ($E_a \rightarrow E_b$) of the forward and reverse modification reactions in Fig. 1 may be expressed as follows:

$$v_1 = V \cdot e_b/[K'_{m1}(1 + K_a/g) + e_b] \quad (1)$$

$$v_2 = V \cdot e_a/[K_{m2}(1 + g/K_i) + e_a] \quad (2)$$

where K'_{m1} and K_{m2} are the Michaelis constants for fully activated E_1 and uninhibited E_2 respectively. The first of these symbols is primed because with more general assumptions it will be convenient to use unprimed symbols to refer to enzyme without bound effector. In addition, g is the concentration of G, and e_a and e_b are the total concentrations of E_a and E_b respectively. The specific activation constant of E_1 is K_a and the specific (competitive) inhibition constant of E_2 is K_i.

The steady state of the system may be determined by setting $v_1 = v_2$ and solving the resulting equation for the fraction f of target enzyme in the active state:

$$f = e_a/(e_a + e_b) = 1/\{1 + [K'_{m1}(1 + K_a/g)]/[K_{m2}(1 + g/K_i)]\} \quad (3)$$

We now need to compare the effect that G has on this fraction via the cascade with the effect that it might have if it acted directly on the target enzyme. This may be assessed by examining the response coefficient R_g^f, defined as the partial derivative of $\ln f$ with respect to $\ln g$ (Burns *et al.*, 1985; Kacser & Porteous, 1987):

$$R_g^f = \frac{\partial \ln f}{\partial \ln g} = \frac{\dfrac{g}{K_i + g} + \dfrac{K_a}{K_a + g}}{1 + \dfrac{K_{m2}(1 + g/K_i)}{K'_{m1}(1 + K_a/g)}} \quad (4)$$

If G acted directly as a specific activator of a target enzyme E_o, without the intermediacy of a cascade, the rate v of the reaction catalysed by E_o at a concentration s of its substrate would be given by an equation of the following form:

$$v = V'_0 s/[K'_{m0}(1 + K_{a0}/g) + s] \quad (5)$$

in which V'_0, K'_{m0} and K_{a0} are constants. Partial differentiation with respect to g shows that the response coefficient corresponding to eqn. (4) has a value that approaches 1 at low values of g:

$$R_g^v = \frac{\partial \ln v}{\partial \ln g} = 1/[1 + (g/K_{a0})(1 + s/K'_{m0})] \quad (6)$$

The question that must be asked in relation to eqn. (4), therefore, is whether it permits values of the response coefficient greater than 1, and under what conditions.

As each of the two terms in the numerator of the expression on the right of eqn. (4) must be less than 1, whereas the denominator must be greater than 1, it is evident that the response coefficient cannot exceed 2 for a monocyclic cascade with the specified properties, and can only exceed 1 if G affects both of the modifier enzymes: if E_1 is fully activated at all concentrations of G, or if E_2 is not detectably inhibited at any concentration of G, the corresponding numerator term is effectively zero, and the response coefficient cannot then exceed 1.

It follows, therefore, that a cascade in which the effector acts only on the specificity constants of the modifier enzymes, and acts on only one of these enzymes, will produce a less sensitive response than one would have with direct interaction. If it acts on both modifier enzymes the response of the cascade can be somewhat more sensitive than one would obtain with direct interaction, but only if additional conditions are satisfied: K_a must be large compared with K_i, i.e. E_2 must be capable of being strongly inhibited by G at concentrations where activation of E_1 is still slight, so that both

numerator terms in eqn. (4) can approach their limiting values simultaneously; to prevent a large numerator from being effectively nullified by a large denominator at the same values of g, it is also necessary for K'_{m1} to be large compared with K_{m2}.

The conclusions would not be qualitatively different if all of the effects of G discussed (i.e. both with the modifier enzymes and in the direct interaction considered for comparison) were co-operative. This can be seen by replacing g by g^h throughout, where h is the Hill coefficient and is taken as greater than 1. Analysis of exactly the same type as we have considered shows that the response coefficient approaches h for the direct interaction, and cannot exceed h if the interaction occurs via a cascade in which the effector acts on one enzyme only. If it acts on both enzymes with the same Hill coefficient, the limit is $2h$. It follows, therefore, that the introduction of co-operative interactions does not alter the general conclusion that interaction through a cascade provides no improvement on direct interaction unless both enzymes are affected, and may not do so even then.

Catalytic effects

Goldbeter & Koshland (1984) reported that non-competitive inhibition of E_2 by the effector gave a higher sensitivity than competitive inhibition, an observation that suggests that one ought to consider catalytic as well as specificity effects. We now examine, therefore, a more general model in which the enzymes in the cascade may have different limiting rates, the effector may act on their catalytic constants, and they may have some activity in the absence of activator or when saturated with inhibitor. However, all of these complications bring the model out of the range of simple algebraic analysis, and we therefore study them by numerical simulation.

For the more general models eqn. (1) and (2) for the rates of the two modification reactions must be replaced by the following equations:

$$v_1 = (V'_1 + V_1 K'_{G1}/g)\, e_b / [K'_{m1}(1 + K_{G1}/g) + e_b(1 + K'_{G1}/g)] \quad (7)$$

$$v_2 = (V_2 + V'_2 g/K'_{G2})\, e_a / [K_{m2}(1 + g/K_{G2}) + e_a(1 + g/K'_{G2})] \quad (8)$$

Unprimed symbols V and K_m refer to the limiting rates and Michaelis constants respectively of E_1 or E_2 (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_{G1}, K_{G2}, K'_{G1} and K'_{G2} refer to dissociation of G from E_1G, E_2G, E_1E_bG and E_2E_aG respectively.

As eqns. (7) and (8) contain three concentrations and ten parameters, determining the possible range of behaviour might appear a hopeless task, requiring exploration in 13 dimensions. The dimensionality can, however, be decreased to nine by considering appropriate ratios of parameters, as defined in Table 1, and by considering not the whole range of f values but the two values 0.1 and 0.9.

RESULTS

Conditions necessary for very high sensitivity

The dimensionless parameters that give the smallest possible value of the sensitivity index r_G were found by calculating r_G for many sets of parameters with values assigned at random within the specified constraints (i.e. with each of the dimensionless ratios defined in the previous section in the range 0.01–100), and using those giving small values of r_G as starting points for varying the parameters systematically to make r_G as small as possible. Whatever the starting point, the final set of values was always the same, as listed in Table 1, leaving little doubt that it is, in fact, the optimum.

The smallest r_G value obtained in this way was 1.0053, implying that a 0.5% increase in effector concentration is sufficient to bring the proportion of the target enzyme in the active state from 10% to 90%. The enormous sensitivity implied by this value is evident from the fact that it corresponds to a Hill coefficient of around 800 (calculated as $\log 81/\log 1.0053$), whereas even the most highly co-operative of individual enzymes do not show Hill coefficients greater than 4.

To examine the importance of maintaining each ratio at or near its best value, each was varied in the range 0.01–100 with the other seven ratios held at the optimum. The results are shown in Fig. 2, from which it may be seen that a highly sensitive response, taken as one with a value of r_G less than 1.55 (i.e. $81^{0.1}$, corresponding to a Hill coefficient of around 10), requires the following characteristics, which are listed in decreasing order of importance (though all are important if very high sensitivity is to be possible).

(1) Modifier enzymes near saturation. $K'_{m1}/(e_a + e_b)$ and $K_{m2}/(e_a + e_b)$ must both be as small as possible, and in any event less than 1.75; i.e. both modifier enzymes should operate at more than 36% saturation [$0.36 = 1/(1 + 1.75)$]. This agrees with the idea of 'zero-order ultrasensitivity' emphasized by Goldbeter & Koshland (1981, 1982, 1984), though the term zero-order normally implies a state rather closer to saturation than 36%.

(2) Catalytic rather than specific effects. K'_{G1}/K_{G1} must be as large as possible, at least 0.055, and likewise K'_{G2}/K_{G2} must be as small as possible, no greater than 17.5. Thus, although the model can tolerate substantial degrees of specific activation of E_1 or inhibition of E_2, it is better for the catalytic components to predominate. Even if the catalytic components appear trivial or pass unnoticed in studies of the isolated modifier enzymes they may be crucial for generating an adequate response.

(3) Activation weaker than inhibition. $\frac{1}{4}(K_{G1} + K'_{G1}) \cdot [(1/K_{G2}) + (1/K'_{G2})]$ must be as large as possible, no less than 0.01; thus ideally E_1 should not be appreciably activated at the effector concentrations at which E_2 begins to be appreciably inhibited, though a large departure from this ideal can be tolerated. It follows, therefore, that weak activation needs to be considered seriously as a regulatory mechanism, even, or perhaps especially, when it appears to be insignificant by comparison with inhibition of the enzyme that catalyses the opposing reaction.

The values of the other ratios, which define the limiting rates of the two enzymes in relation to one another and to their less active states, are less important, and may vary over wide ranges (provided that the other parameters are close to their optimal values).

All of the tolerances noted above are somewhat unrealistic in that they assume that all parameters except one are at their optimal values. Consequently one should pay more attention to the verbal descriptions than to the numerical values given. The right-hand column of Table

Table 1. Ratios of parameters that produce very high sensitivity

Parameter ratio*	Description	Optimum†	Adequate‡
V_2/V'_1	Ratio of limiting rates for fully activated E_1 and uninhibited E_2	1.0	3.16
$K'_{m1}/(e_a+e_b)$	Scaled Michaelis constant for fully activated E_1	0.01	0.316
$K_{m2}/(e_a+e_b)$	Scaled Michaelis constant for uninhibited E_2	0.01	0.0316
K'_{G1}/K_{G1}	Ratio of catalytic and specific activation constants for E_1	100	31.6
K'_{G2}/K_{G2}	Ratio of catalytic (uncompetitive) and specific (competitive) inhibition constants for E_2	0.01	0.1
$\frac{1}{4}(K_{G1}+K'_{G1})[(1/K_{G2})+(1/K'_{G2})]$	Ratio of the arithmetic mean§ activation constant of E_1 and the harmonic mean§ inhibition constant of E_2	100	10
$100V_1/V'_1$	Limiting rate of unactivated E_1 as a percentage‖ of the limiting rate of activated E_1	0.01	0.01
$100V'_2/V_2$	Limiting rate of maximally inhibited E_2 as a percentage‖ of the limiting rate of uninhibited E_2	0.01	0.1

* In exploring the range of behaviour possible each of the ratios listed was constrained to the range 0.01–100.

† Set of ratios giving $r_G = 1.005$, the smallest value of the sensitivity index possible within the specific constraints.

‡ Set of ratios giving $r_G = 1.55$ (corresponding approximately to a Hill coefficient of 10). This set is not unique; it is shown to illustrate the degree of departure from the optimum that can be tolerated while still giving a small r_G value.

§ For inhibition the harmonic mean gives a more realistic measure of 'average' behaviour than the arithmetic mean, whereas the reverse is true for activation.

‖ The factors of 100 in these definitions ensure that G acts only as an activator of E_1 and only as an inhibitor of E_2.

1 shows an example of a set of ratios that give $r_G = 1.55$, and illustrates the rapidity with which very high sensitivity is lost when all the ratios are simultaneously allowed to vary from their optimum values.

Non-linear inhibition or activation

The results in Fig. 2 imply that it is of little importance whether the activation of E_1 and inhibition of E_2 are 'linear', i.e. that the unactivated and maximally inhibited enzymes have essentially no activity. Although this is correct provided that the other ratios are optimized, it is somewhat misleading as a general statement, because non-linearities in the activation and inhibition greatly decrease the tolerance for the ratio V_2/V'_1.

If unactivated E_1 and maximally inhibited E_2 have negligible activity this ratio has little effect on the degree of sensitivity that can be obtained. However, if $V'_1/V_1 = 0.01$, i.e. if unactivated E_1 has 1% activity, which would need considerable care to detect in studies of the isolated enzyme, this has the effect of erecting a 'wall', so that decreasing V_2/V'_1 below about 0.03 causes a steep decrease in sensitivity (Fig. 3); if $V'_2/V_2 = 0.01$, i.e. if maximally inhibited E_2 has 1% activity, there is a similar wall for V_2/V'_1 above about 30. If the unactivated and maximally inhibited enzymes have 10% activity the walls are brought much closer together, so that V_2/V'_1 needs to be within a range of about 0.3–3 to produce a high degree of sensitivity. Although non-linearities of this magnitude ought to be easily detectable in studies of the isolated enzymes, it is common practice to assume, at least in the case of inhibition, that only linear effects are present, and non-linearities may pass unnoticed. Thus the tolerable range of V_2/V'_1 may be narrower in practice than Fig. 2 might suggest.

Effector acting on one modifier enzyme only

When the modifier enzymes are subject to specific effects only, no additional sensitivity beyond that of a single enzyme is possible unless the effector acts on both enzymes, as is evident from eqn. (4). We now examine whether the same is true if the enzymes follow the more complex kinetics, including catalytic effects, defined by eqns. (7) and (8). To answer this, searches similar to that described above were carried out to identify the sets of parameter ratios that give the minimum values of the co-operativity index r_G when G inhibits E_2 but has no effect on E_1, and when it activates E_1 but has no effect on E_2. In contrast with the results with the simpler assumptions, a very high degree of sensitivity, with $r_G = 1.091$, or a Hill coefficient of about 50 ($= \log 81/\log 1.091$), is now possible even if G interacts with only one enzyme of the cascade. The conditions that give this result are the same as for the more general model described above, except that now the ratio of limiting rates V_2/V'_1 can no longer take almost any value: instead, the enzyme not acted on by G must have as small a limiting rate as possible within the constraints.

The same minimum value of 1.091 for r_G is obtained whichever of the two enzymes responds to G, but one should not be misled thereby into supposing that the two cases are of equal biological value. The two curves showing the dependence on g of e_a, the concentration of the active state of the target enzyme, are highly unsymmetrical about their half-conversion points (Fig. 4). Either curve may be transformed into the other by translation and rotation through 180° about the half-conversion point, but, whereas the no-activation curve is very steep at low values of e_a and much less steep at high values, the opposite is true of the no-inhibition curve. To increase e_a 10-fold from 5% to 50% requires an increase in g of only 1.3% for the no-activation curve, whereas it requires an 18% increase for the no-inhibition curve. To obtain a large response coefficient, therefore, which measures the relative change in output as a function of a relative change in stimulus, the inhibition of E_2 is much more important than the activation of E_1.

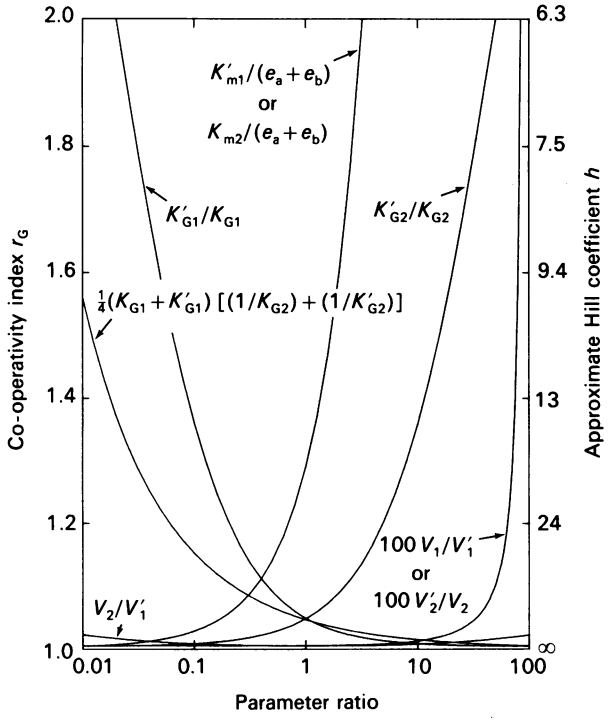

Fig 2. Dependence of the co-operativity index r_G on the individual parameter ratios

Each curve shows the value of r_G as a function of one parameter ratio, the other seven parameter ratios being held at their optimum values (within the constraint that no parameter ratio be outside the range 0.01–100), as listed in Table 1. The right-hand axis shows approximate values of the Hill coefficient, calculated as $h = \log 81/\log r_G$.

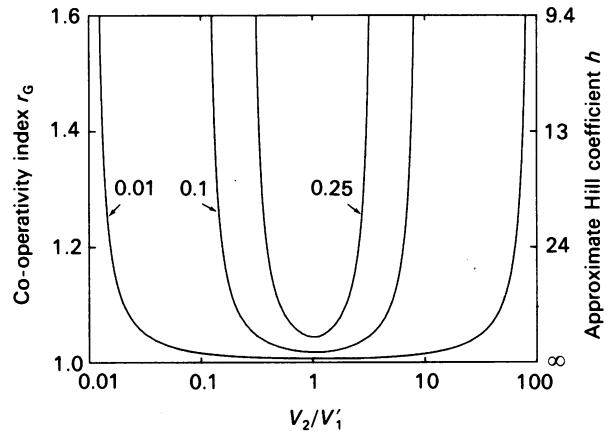

Fig 3. Effect of non-linear activation or inhibition

The wide tolerance for V_2/V'_1 evident in Fig. 2 is decreased when E_1 has non-negligible activity in the absence of activator (i.e. $V_1/V'_1 > 0$), or E_2 has non-negligible activity when saturated with inhibitor (i.e. $V'_2/V_2 > 0$). The symmetrical U-shaped curves, which are labelled with the values of V_1/V'_1 and V'_2/V_2, result from assuming that both modifier enzymes have the same degree of residual activity in their 'inactive' states, but the two 'walls' depend separately on properties of the two enzymes: if E_1 has significant activity without activation, whereas E_2 has negligible activity when inhibited [a common situation in practice: cf. Nimmo & Nimmo (1984)], the right-hand 'wall' disappears.

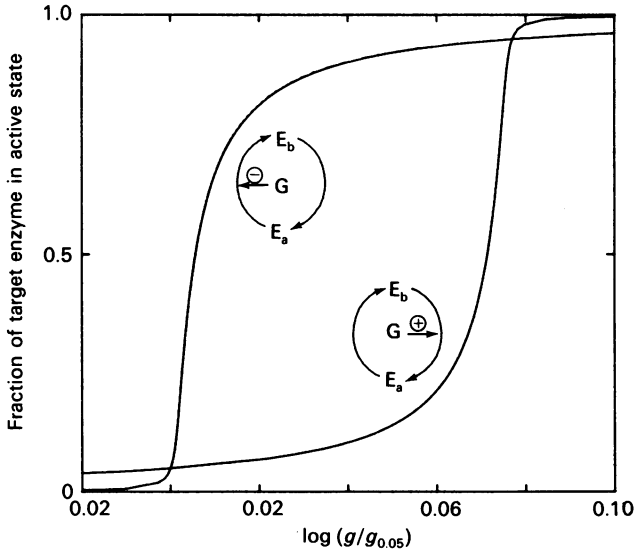

Fig 4. Effector acting on one enzyme only

The curves show the dependence of f, the fraction of the target enzyme in the active state, on $\log(g/g_{0.05})$, where $g_{0.05}$ is the value of g when $f = 0.05$, for cases where G acts only as an inhibitor of E_2, or only as an activator of E_1. For the inhibition-only curve, terms in g are omitted from eqn. (7) and $V_2/V'_1 = 100$; for the activation-only curve, terms in g are omitted from eqn. (8) and $V_2/V'_1 = 0.01$. Other ratios have the optimum values listed in Table 1. Note the gross departure of both curves from symmetry about the points at which $f = 0.5$.

Sensitivity possible with tighter constraints

Although the ranges 0.01–100 that we have allowed for each of the dimensionless ratios defined in the Theory section are reasonable in relation to the known properties of enzymes, the degree of sensitivity that they permit, corresponding to a Hill coefficient of 800, is so enormous that it invites the question of how great a degree of sensitivity would be possible with a more tightly constrained system. We have examined this question with each ratio constrained to the range 0.1–10 (instead of 0.01–100), and with the additional assumption of the unactivated form of E_1 has 10% of the activity of the activated form, i.e. $V_1/V'_1 = 0.1$.

With this more tightly constrained system the qualitative results are the same as before, i.e. the same types of conditions for maximizing the sensitivity still apply, but the smallest value of r_G that one can obtain is now 1.4 rather than 1.005. This corresponds approximately to a Hill coefficient of 13: much smaller than 800, but still far larger than the values observed for single enzymes. It is evident, therefore, that even with tightly constrained parameters a monocyclic cascade can, with proper choice of parameters, generate a very large degree of sensitivity.

DISCUSSION

The protein kinase and phosphatase that catalyse the phosphorylation and dephosphorylation of isocitrate dehydrogenase (EC 1.1.1.42) in *Escherichia coli* provide a useful context for examining the theoretical ideas that we have considered. Both modifier enzyme activities

occur on a single bifunctional protein, the product of a single gene (LaPorte & Koshland, 1982; LaPorte & Chung, 1985), and their responses to several metabolites have been studied (Nimmo & Nimmo, 1984). Several of these act in opposite directions on the two activities, and for some effectors (e.g. isocitrate and phosphoenolpyruvate, both considered important controlling metabolites *in vivo*) half-activation of the phosphatase requires much higher concentrations than are needed to half-inhibit the kinase. For other effectors (e.g. AMP) the concentration for half-activation of the phosphatase is only slightly higher than that for half-inhibition of the kinase. For each effector, saturation of the kinase results in complete loss of activity, but the phosphatase has a substantial activity in the absence of effector. These results are thus in reasonable accord with the ideas that we have put forward here, though it would be useful to have some information about the degree to which the effects are catalytic rather than specific.

Various other covalent modification systems have been studied, but in most of these the catalytic activities in the forward and reverse directions are associated with different proteins, and have been studied under different experimental conditions, making it difficult to make the proper comparisons. As the substrates of the cascade enzymes are normally also proteins, not always well characterized, the difficulties of separating the catalytic and specific components of the activation and inhibition are further compounded. We hope that more detailed and appropriate experimental data will become available that will allow a more penetrating analysis of real systems in relation to the ideas that we are putting forward than is possible at present. In particular, it will be helpful to have much more information about the actions of effectors on both directions of the cascade, including especially effects that may have been observed but dismissed as too weak to be important: this applies both to activation of one enzyme that may have appeared trivial in relation to inhibition of the other, and also to an apparently trivial uncompetitive component in an inhibition that is predominantly competitive.

The crucial importance of the catalytic components in the activation and inhibition of the modifier enzymes may appear surprising if one views it from the perspective of the kinetics of isolated enzymes. In studies of individual enzymes the uncompetitive component of inhibition may easily (indeed, often does) pass unnoticed if the competitive component is much stronger, and it is only at very high or very low concentrations of substrate that one can expect to see large differences between the effects of competitive and uncompetitive inhibitors with equal inhibition constants. At substrate concentrations near the Michaelis constant equal concentrations of such inhibitors produce nearly equal degrees of inhibition.

The important difference from physiological conditions is that experiments on isolated enzymes are normally done at substrate concentrations fixed by the experimenter, whereas in the cell these concentrations vary with the activities of the enzymes in the system. In the extreme case of constant flux, suggested by Atkinson (1977) as a useful antidote to the usual assumption of fixed concentrations, the difference between uncompetitive and competitive effects can become infinite (Cornish-Bowden, 1986), and may be extremely large even with the intermediate (and more realistic) assumption that both fluxes and concentrations can vary in response to the concentration of an external inhibitor. The herbicide Glyphosate or Roundup (*N*-phosphonomethylglycine) is an uncompetitive inhibitor of 3-phosphoshikimate 1-carboxyvinyltransferase (EC 2.5.1.19; Boocock & Coggins, 1983), and almost certainly owes its high toxicity to this type of consideration. Seen in this light the crucial importance of catalytic effects in producing very high sensitivity in the output of an interconvertible enzyme cascade appears less surprising.

For enzymes with non-protein substrates, the inhibitors that are commonly studied usually bear some structural similarity to the substrates and products of the reaction, and their behaviour is often reported to be wholly or largely competitive. This is hardly surprising, but it is unwise to assume that the same applies to enzymes with protein substrates. The absence of structural similarity between substrate and effector means that there is no reason to expect the effector to bind at the active site or to bind only when substrate is not bound, and the dramatic effects on metabolite concentrations that uncompetitive inhibition can generate (Cornish-Bowden, 1986) suggests that it may have a far greater role to play in metabolic control than has been assumed.

The Hill coefficient of around 800 that we have found to be the limit for a monocyclic cascade with reasonable constraints is far beyond any value reported for a single enzyme; it may also be far beyond the needs of the cell and may instead be a potential source of instability. (Even the value of 13 observed with tight constraints is much larger than is ever found in direct interaction.) Thus, provided that the modifier enzymes are capable of showing a catalytic response to effectors, and that they show weaker activation than inhibition and have other appropriate properties, then a simple monocyclic cascade can provide as much sensitivity as required in most circumstances. Moreover, this can be achieved without any need for co-operativity in the individual modifier enzymes. The explanation for bicyclic and more complex systems that exist in nature, such as the one that regulates glutamine synthetase in *Escherichia coli* (Garcia & Rhee, 1983), must therefore be sought in other aspects than their ability to give a highly sensitive response to individual effectors.

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