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Sensitivity amplification in biochemical systems

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I. INTRODUCTION 556
Definition of terms 557

II. RESULTS 558
(A) *Sensitivity amplification in co-operative and Michaelian enzymes* 558
Link between A_S *and* R_S 562
Comparison with differential expression for co-operative enzymes 562
(B) *Amplification by enzymes subjected to covalent modification* 562
Amplification in terms of change in the ratio of modification rates 564
Amplification factor A_{II} *related to change in effector level* 569
Total amplification factor (A_S) *for covalent modification scheme* 570
Propagation of sensitivity amplification in multicyclic cascades 573
(C) *Sensitivity amplification in substrate cycles* 581
(D) *Sensitivity amplification in multiple steady-state transitions* 581
(E) *Amplification in time-dependent processes* 584

III. CONCLUSIONS 585

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IV. SUMMARY 589

V. ACKNOWLEDGEMENTS 589

VI. REFERENCES 589

I. INTRODUCTION

The sensitivity of biological systems to changes in environmental stimuli is connected with their regulatory properties. In order to achieve efficient control, these systems must respond to minute environmental variations by amplifying external stimuli to yield a significant response. To that end, biochemical systems have often evolved to a cascade organization in which the product of the nth reaction in a chain acts as a catalyst in subsequent transformations. The amplification properties of such cascades were first noticed in the process of blood clotting (MacFarlane, 1964, 1969) and visual excitation (Wald, 1965). Later on, a similar organization was noticed in hormonal control of metabolism (Bowness, 1964; Stadtman & Chock, 1977, 1978; Chock, Rhee & Stadtman, 1980).

In parallel with these studies on amplification, several authors have attempted to quantify the sensitivity of biochemical systems to changes in some control parameter. The idea of expressing sensitivity in terms of the relative variation of a response ϕ (e.g. the rate of an enzymic reaction) caused by a relative variation in stimulus S was first introduced by Higgins (1965), who took the ratio of relative changes, written in the above notations as

$$C = \mathrm{d}\ln\phi/\mathrm{d}\ln S \tag{1}$$

as a measure of 'control strength'.

Later on, Kacser & Burns (1968, 1973), Savageau (1971, 1976), and Heinrich & Rapoport (1974) used definitions similar to (1) to find the control points in a sequence of enzymic reactions. Ratio (1) or related versions of it, were termed *sensitivity coefficient* (Kacser & Burns, 1968), *response* or *controllability coefficient* (Kacser & Burns, 1973), *parameter sensitivity* (Savageau, 1971, 1976), *effector strength* (Heinrich & Rapoport, 1974), *sensitivity* and *signal amplification* (Stadtman & Chock, 1978)*.

Savageau (1971, 1976) explicitly linked the sensitivity of biochemical

* A similar measure of sensitivity has been used in other fields such as economics, where the relative change in responding variable compared to the relative change in stimulating variable is sometimes called *elasticity* (Boulding, 1970).

systems to their amplification properties by calling ratio (1) the *logarithmic gain*. Although the system originally considered by Savageau is of the cascade type, the concept of logarithmic gain is also applicable to a single reaction. As noted by many authors, the effect of a cascade organization serves to multiply the amplification factors obtained at each stage of the reaction sequence (MacFarlane, 1964; Wald, 1965; Levine, 1966; Savageau, 1971; Stadtman & Chock, 1978; Chock & Stadtman, 1977; Banks, Miech & Olson, 1980).

The purpose of this work is to quantify the amplification properties of biochemical systems which can cause a larger percentage change in output response relative to the percentage change in input stimulus. In the case of an enzyme, the output is an enzymic rate and the stimulus can be a substrate concentration. In general, however, the stimuli can be inhibitors, activators, light, sound, etc. and the output response can be an ion flow across a membrane, a voltage response in a synapse, the release of a hormone, etc. The mechanism of amplification and its limitations depend not only on the individual properties, such as co-operative subunit interactions and covalent modification, but also on the way these individual units are combined in the system. In the pages which follow, we will evaluate the amplification of finite changes in stimulus and find explicitly the relation between the size of the step, the initial and final values of the stimulus, and the resulting amplification. We will determine how a multi-step cascade can enhance or diminish the amplification observed in the discrete steps. In this way, we can obtain both the optimal value of stepwise increases and the decrease in the maximum amplification factor as one departs from optimal conditions.

Definition of terms

We will use ϕ to represent the output response and S to represent the input stimulus. The subscripts i and f will refer to the initial and final values respectively.

The sensitivity amplification factor, A_S, will be defined as shown in equation 2:

$$A_S = \frac{(\Delta\phi/\phi)}{(\Delta S/S)} = \frac{(\phi_f - \phi_i)/\phi_i}{(S_f - S_i)/S_i}. \tag{2}$$

This discrete form of the ratio of output to input reduces to the logarithmic expression of equation (1) in the limit of infinitesimal changes in stimulus. The main reason which prompted Kacser & Burns (1968, 1973), Savageau (1971, 1976) and Heinrich & Rapoport (1974) to consider the continuous expression was the search for a

constant coefficient, independent of the size of variation in stimulus. The amplification factor defined by equation (2) indeed exhibits a nonlinear dependence on S_i and ΔS. With regard to the real situation in physiological processes, however, the discrete formalism of equation (2) appears more informative, and we shall analyse the dependence of A_S on the initial stimulus and on the size of the step in various biological systems.

Equation (2) cannot apply to a change from a zero background stimulus as it would involve division by zero. The processes involved in sensitivity amplification are therefore related but distinct from the catalytic amplification of a single enzyme molecule giving rise to the synthesis of a large number of product molecules. Similarly, a positive effector which opens an ion gate present in a membrane can elicit the leakage of 10^4 molecules across a synaptic gap. In contrast with the definition in equation (2), the latter relates the absolute number of molecules produced to the number of effector molecules (Bowness, 1964). This type of enhancement will therefore be referred to as magnitude amplification (Koshland, Goldbeter & Stock, 1982). Such a definition depends on the concentration scale and yields information on the turnover properties of the enzyme or pore, rather than on its sensitivity to environmental changes. Magnitude amplification can, of course, be coupled to sensitivity amplification.

II. Results

(A) *Sensitivity amplification in co-operative and Michaelian enzymes*

An approximate expression for the reaction rate, v, of an enzyme having a Hill coefficient n_H is shown in equation (3),

$$\frac{v}{V_M} = \frac{(S/S_{0.5})^{n_H}}{1+(S/S_{0.5})^{n_H}}, \tag{3}$$

where $S_{0.5}$ equals the stimulus concentration which gives the half-maximal rate. Defining v/V_M as the response ϕ, the amplification factor for the co-operative or Michaelian enzyme is given by equation (4),

$$A_S = \frac{(S_f/S_i)^{n_H}-1}{[(S_f/S_i)-1][1+(S_f/S_{0.5})^{n_H}]}. \tag{4}$$

For a Michaelian enzyme ($n_H = 1$), this reduces to equation (5):

$$A_S = \frac{1}{1+(S_f/S_{0.5})}, \tag{5}$$

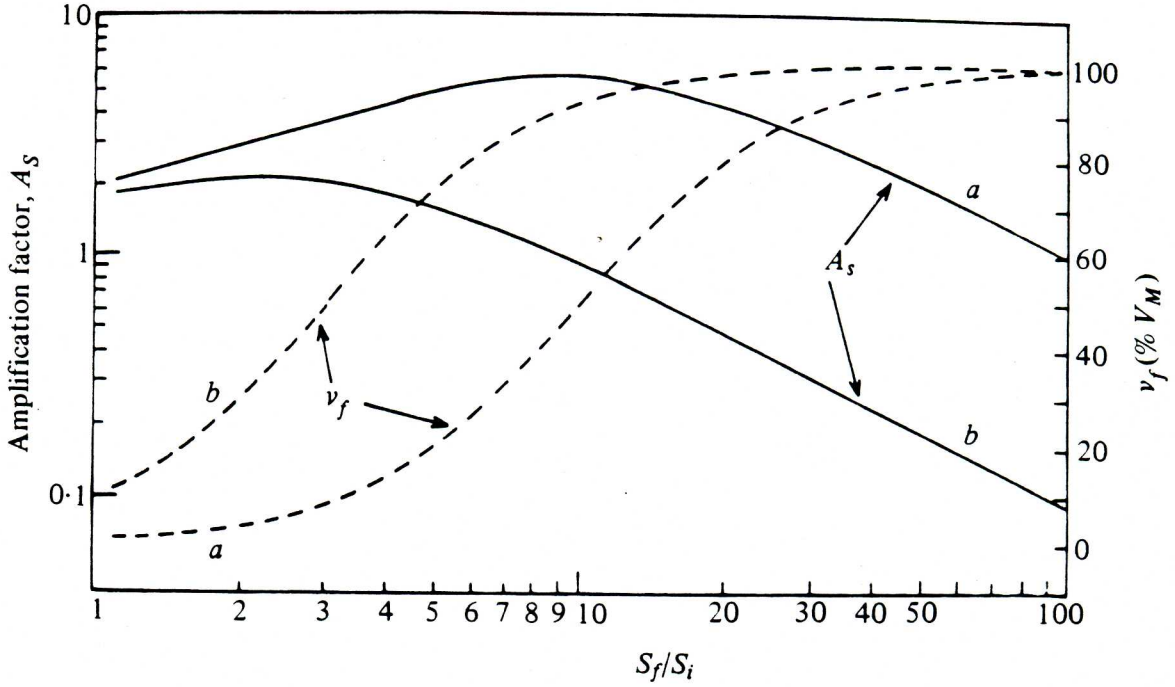

Fig. 1. Amplification by an allosteric enzyme having a Hill coefficient of 2. The amplification factor (solid line) is obtained as a function of the ratio of initial and final stimuli, S_f/S_i, according to equation (4), for (*a*) $v_i = 1\%\ V_M$. and (*b*) $v_i = 10\%\ V_M$. Dotted lines give the value of the reaction rate v_f corresponding to S_f. The stimulus can represent either the substrate or a positive effector of the enzyme.

which shows that the percentage of change in the rate of a Michaelian enzyme will never exceed the percentage of change in substrate, regardless of the initial and final values of S. In other words, the sensitivity amplification factor is always less than unity for any finite value of S_f for a Michaelian enzyme. A further feature of equation (5) is that the Michaelian amplification factor does not depend on the initial level of stimulus.

In the case of co-operative enzyme kinetics ($n_H > 1$), the dependence of A_S *on* (S_f/S_i) and S_i is shown in Fig. 1, for $v_i = 0{\cdot}01\ V_M$ and $v_i = 0{\cdot}10\ V_M$, in the case $n_H = 2$. In both instances, the amplification factor passes through a maximum as S_f/S_i increases for a given S_i. The value v_f of the reaction rate corresponding to S_f increases with S_f/S_i and rises more rapidly for $v_i = 10\%\ V_M$ than for $v_i = 1\%\ V_M$, since the value of S_i is larger in the former case.

The appearance of a maximum amplification factor is important, since it suggests that a biological system should operate over a particular range to set optimal amplification. To extend this conclusion to co-operative enzymes in general, equation (4) can be differentiated

TABLE 1. *Maximum amplification factor and properties as a function of n_H for an allosteric protein*

	n_H	$S_i/S_{0.5}$	S_f/S_i	ϕ_f/ϕ_M	A_S
Values when $\phi_i = 0.01\ \phi_M$	2	0·1	9	0·45	5·5
	4	0·32	3·74	0·66	23·9
	6	0·46	2·54	0·73	46·8
	8	0·56	2·05	0·76	71·3
	12	0·68	1·64	0·79	122·3
Values when $\phi_i = 0.1\ \phi_M$	2	0·33	2·16	0·34	2·1
	4	0·58	1·81	0·54	5·5
	6	0·69	1·55	0·61	9·2
	8	0·76	1·41	0·64	13
	12	0·83	1·27	0·67	20·8

S_i = initial stimulus concentration, S_f = final stimulus concentration, $S_{0.5}$ = stimulus at which half-maximum response is observed. ϕ_i, ϕ_f and ϕ_M are initial, final and maximum response; A_S is sensitivity amplification factor (equations (2) and (4)).

and the derivative set equal to zero. The result is equation (6), which can be used to calculate the specific values

$$\frac{S_f^{2n_H}}{S_{0.5}^{n_H} S_i^{n_H}} - \left[(n_H + 1)\left(\frac{S_i}{S_{0.5}}\right)^{n_H} + n_H - 1\right]\left(\frac{S_f}{S_i}\right)^{n_H} + n_H\left[1 + \left(\frac{S_i}{S_{0.5}}\right)^{n_H}\right]\left(\frac{S_f}{S_i}\right)^{n_H - 1} - 1 = 0 \quad (6)$$

given in Table 1.

In Table 1 are shown the values of S_f/S_i and A_S for systems with various Hill coefficients. As expected, the maximum amplification increases with the degree of enzyme co-operativity. It also becomes larger as the basal reaction rate diminishes. However, the ratio S_f/S_i corresponding to maximum amplification increases when S_i diminishes, since the basal stimulus has to be multiplied by a larger number to yield the value of S_f for which v_f is of the order of 50 % V_M.

As indicated by equation (4), for large values of (S_f/S_i), the amplification factor becomes inversely proportional to (S_f/S_i) and finally becomes less than unity. This decreasing phase, illustrated in Fig. 1, stems from the fact that further rise in S_f does not increase the response v, since it has already essentially reached V_M. Conversely, A_S rises with (S_f/S_i) when $S_f < S_{0.5}$. The dependence of the amplification factor on the size of the step in stimulus is shown in

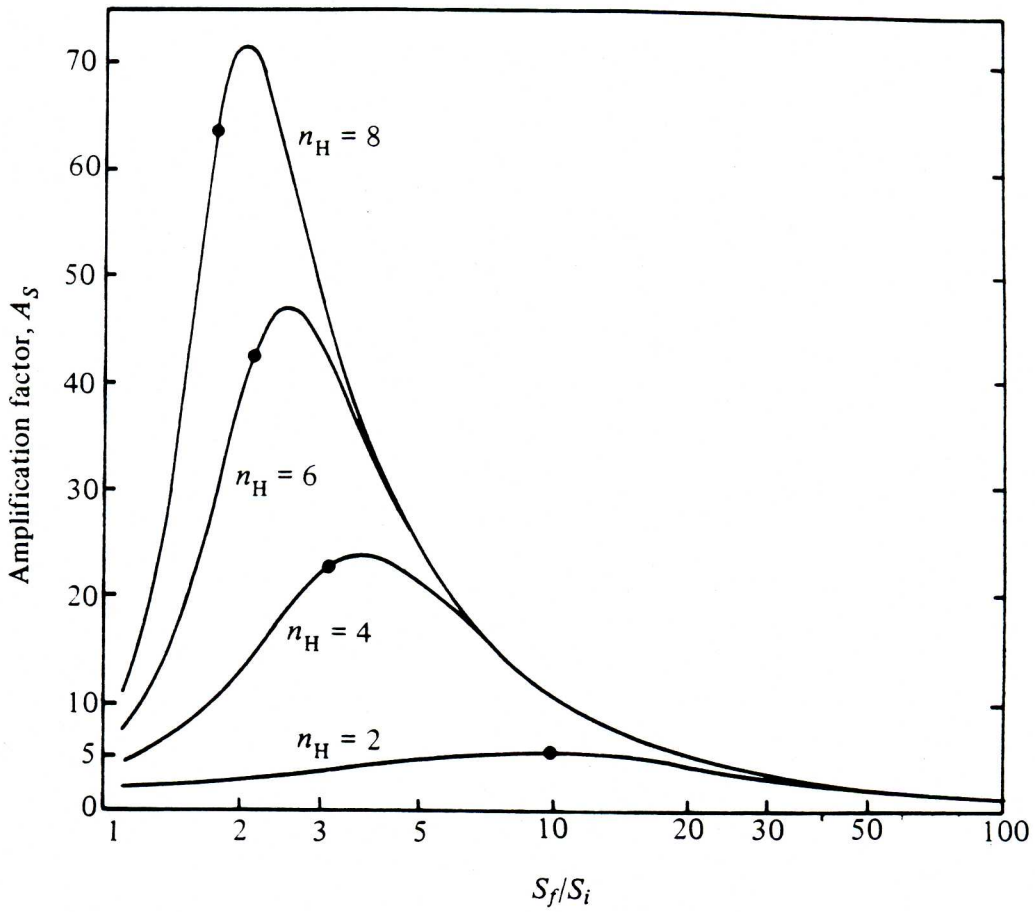

Fig. 2. Amplification factor for an allosteric enzyme obeying the Hill equation. The value of A_S is computed as a function of the step in stimulus, according to equation (4), for values of the Hill coefficient n_H ranging from 2 to 8. The value of S_i is chosen in each case so that the initial rate v_i is 1 % of the maximum rate V_M (see Table 1). The dot on each curve indicates the point where S_f yields half-maximum rate. It can be shown that A_S tends to 1 when (S_f/S_i) approaches 100 regardless of n, for the particular value $v_i = 1\ \%\ V_M$ considered.

Fig. 2 for values of the Hill coefficient ranging from 2 to 8. The dot on each curve corresponds to the point where $v = V_M/2$.

As the Hill equation represents an approximation which does not hold at low ligand concentrations, we have determined the sensitivity amplification factor, using a rate expression derived under conditions of maximum co-operativity for the concerted model for allosteric enzymes (Monod, Wyman & Changeux, 1965). For finite values of the basal stimulus, the amplification factors thus obtained are very close to those shown in Table 1. In addition, A_S tends to a value close to unity when S_i goes to zero.

In conclusion, the maximum amplification in allosteric enzymes is generally associated with a transition in stimulus in which the final response is close to or larger than 50 % of the maximum response, regardless of the initial condition. Amplification augments when the

basal response diminishes, but this tendency is limited due to the fact that the step in stimulus required to bring about the optimal amplification also becomes larger.

Link between A_S and R_S

A measure of the co-operativity of a protein can be given by the response coefficient, R_S ($= S_{0.9}/S_{0.1}$) (Koshland, Nemethy & Filmer, 1966), where $S_{0.9}$ and $S_{0.1}$ represent, respectively, the substrate (or effector) concentrations corresponding to 10% and 90% of the maximum reaction rate. Application of the Hill equation yields the well-known result that $R_S = 81^{1/n_H}$. It is of interest to compare the amplification factor for this range, which is of great physiological interest, with the otpimal amplifaction. The amplification factors for the range $S_{0.1}$ to $S_{0.9}$ are 1, 4 and 7·4 for enzymes with Hill coefficients of 2, 4 and 6. These values are significantly smaller than the maximum amplification factors listed in Table 1 for the same basal response $v_i = 0{\cdot}1\ V_M$. Thus, the symmetrical range $S_{0.1}$ to $S_{0.9}$ around the 50% maximal rate may not be optimal for sensitivity amplification.

Comparison with differential expression for co-operative enzymes

Application of the continuous definition of equation (1) to the Hill equation yields equation (7) for the amplification factor (A_{ds}) of an infinitesimal variation in stimulus at a given value of S.

$$A_{ds} = \frac{n_H}{1 + (S/S_{0.5})^{n_H}}. \tag{7}$$

This value is appreciably different from the discrete value of equation (4). It can never be greater than n_H and approaches this value only at low values of $(S/S_{0.5})$ as shown in Fig. 3.

(B) *Amplification by enzymes subjected to covalent modification*

The control of an enzyme W by a reversible covalent modification catalysed by enzymes E_1 and E_2 can be represented by the scheme in Fig. 4. W^* represents the modified form, eg. phosphorylated, of the target protein in which case E_1 and E_2 represent the active forms of a kinase and of a phosphatase, respectively. The dotted lines in Fig. 4 refer to the possible control of the modifying enzymes by some effector S which could activate or inhibit either or both steps. The kinetics of such a process has been defined with analytical equations by Stadtman & Chock (1977, 1978) for the first-order region, and by Goldbeter & Koshland (1981), for both first-order and zero-order

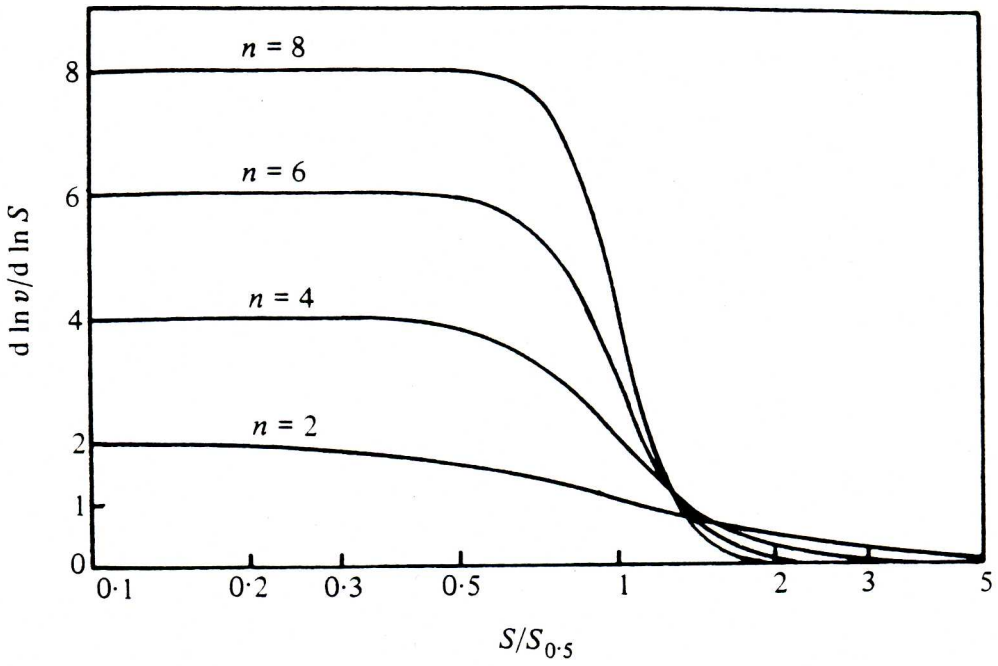

Fig. 3. The differential expression of amplification factor ($d \ln \phi / d \ln S$) as a function of stimulus S for allosteric proteins with Hill coefficients ranging from 2 to 8.

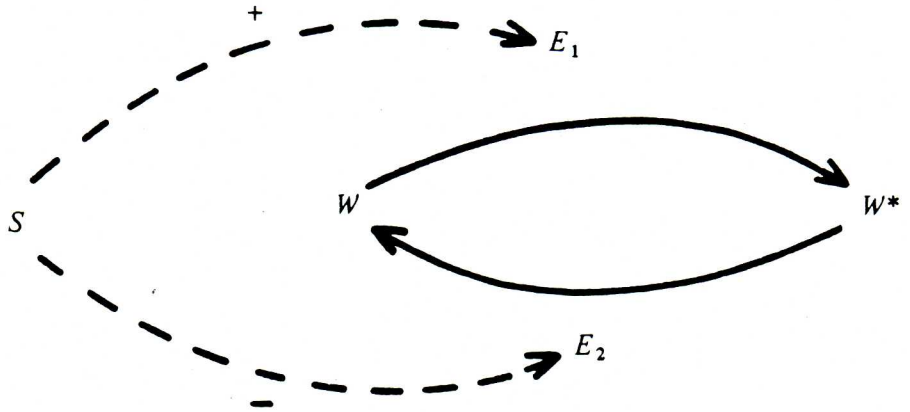

Fig. 4. Reversible covalent modification of a target protein W into W^*, catalysed by enzymes E_1 and E_2. The dashed lines represent the putative activation of E_1 and inhibition of E_2 by an effector S.

regions. It was found that sensitivity above the hyperbolic level occurred only when the zero-order region was involved when S binds in Michaelian manner to one of the two enzymes (Goldbeter & Koshland, 1981). Sensitivity greater than Michaelian can be obtained in the first-order region only when both steps are controlled by S, and then only under special conditions (Koshland, Goldbeter & Stock, 1982).

If the modified protein is active and the unmodified protein inactive, the sensitivity amplification factor can be defined as in equation (8),

$$A_S = \frac{\Delta W^*}{W_i^*} \Big/ \frac{\Delta S}{S_i}. \tag{8}$$

For mathematical reasons it is convenient to divide this amplification factor into a product of two partial amplification factors, A_{I} and A_{II} as shown in equation (9):

$$A_S = \left(\frac{\Delta W^*}{W_i^*} \Big/ \frac{\Delta \alpha}{\alpha_i}\right)\left(\frac{\Delta \alpha}{\alpha_i} \Big/ \frac{\Delta S}{S_i}\right) = A_{\mathrm{I}} A_{\mathrm{II}}, \tag{9}$$

where $\alpha = (V_1/V_2)$ is the ratio of maximum modification rates at fixed effector concentration. Equation (9) shows that the total amplification can be determined in two successive steps by linking (i) the relative variation in target protein to the relative variation in the activity of the modifying enzymes (A_{I}), and (ii) the variation in activity of modifying enzymes to the relative change in effector level (A_{II}). The calculation of each factor is carried out below.

Amplification in terms of change in the ratio of modification rates

When the kinetics of the modifying enzymes are of the Michaelian type, the molar fraction of modified protein at steady-state, (W^*), is given by

$$W^* = \frac{[(\alpha-1)-(K_1+K_2\alpha)]+\{[(\alpha-1)-(K_1+K_2\alpha)]^2+4K_2\alpha(\alpha-1)\}^{\frac{1}{2}}}{2(\alpha-1)}, \tag{10}$$

where $\alpha = V_1/V_2$ denotes the ratio of the maximum rates of enzymes E_1 and E_2, and $K_1 = K_{m1}/W_T$, $K_2 = K_{m2}/W_T$ represent the normalized Michaelis constants of these modifying enzymes. Equation (10) has been obtained (Goldbeter & Koshland, 1981) under the assumption that $W_T = W + W^*$, i.e. that the complexes EW and E_2W^* are small with respect to W and W^*. Throughout this discussion we shall use the terms W and W^* to refer to mole fractions so that they will range in values from 0 to 1. The variation in W^* as a function of α is shown in Fig. 5, for two sets of normalized Michaelis contants. The modification curves become extremely steep when the modifying enzymes are saturated by the target protein. The effect of non-negligible amounts of modifying enzymes, as well as that of non-productive binding of W to E_2 and W^* to E_1, is to reduce the sharpness of the transition which occurs between the unmodified and modified forms of the target protein as the ratio of rates V_1/V_2 increases from a low initial value. As we wish to determine here the maximum amplification possible in a covalent modification system, we shall restrict the present analysis to the case where the terms due

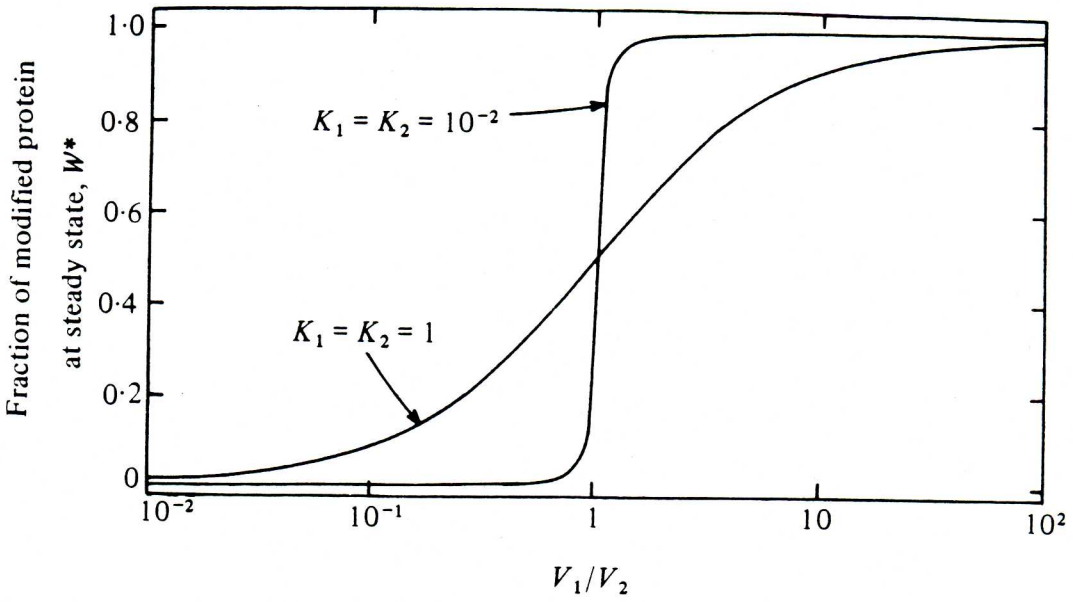

Fig. 5. Variation of the fraction of modified protein W^* as a function of the ratio of rates $\alpha = V_1/V_2$ in a monocyclic modification system, for different values of the normalized Michaelis constants of the modifying enzymes E_1 and E_2 ($K_1 = K_{m1}/W_T$, $K_2 = K_{m2}/W_T$) (redrawn from Goldbeter & Koshland, 1981).

to non-productive binding and to complexes between modifying enzymes and target protein can be neglected in the conservation relations for E_1, E_2 and W.

Equation (11), which holds in these conditions, permits us to express the ratio of modification rates corresponding to the molar fraction of modified target protein at steady state (Goldbeter & Koshland, 1981):

$$\alpha = \frac{W^*(1 - W^* + K_1)}{(1 - W^*)(W^* + K_2)}. \qquad (11)$$

The first amplification term (A_I) appearing in equation (9) can now be defined by relating the relative variation in amount of modified target protein to the relative variation in the ratio of modification rates:

$$A_I = \left(\frac{W_f^*}{W_i^*} - 1\right) \Big/ \left(\frac{\alpha_f}{\alpha_i} - 1\right), \qquad (12)$$

where α_i and α_f denote the values of the ratio V_1/V_2 giving a molar fraction W_i^* or W_f^* at steady state, respectively.

The dependence of W^* and A_I on (α_f/α_i) is shown in Fig. 6 for $K_1 = K_2 = 10^{-2}$ (curves *a*) and 10^{-1} (curves *b*). Starting with $\alpha_i = 0{\cdot}5$, i.e. $V_1 = V_2/2$, the ratio (α_f/α_i) is increased; such an increase can be achieved by activation of E_1 and/or by inhibition of E_2 (see below). The transition from $W_i^* < 0{\cdot}5$ to $W_f \approx 1$ becomes sharper as K_1 and K_2 decrease below unity (see also Fig. 5).

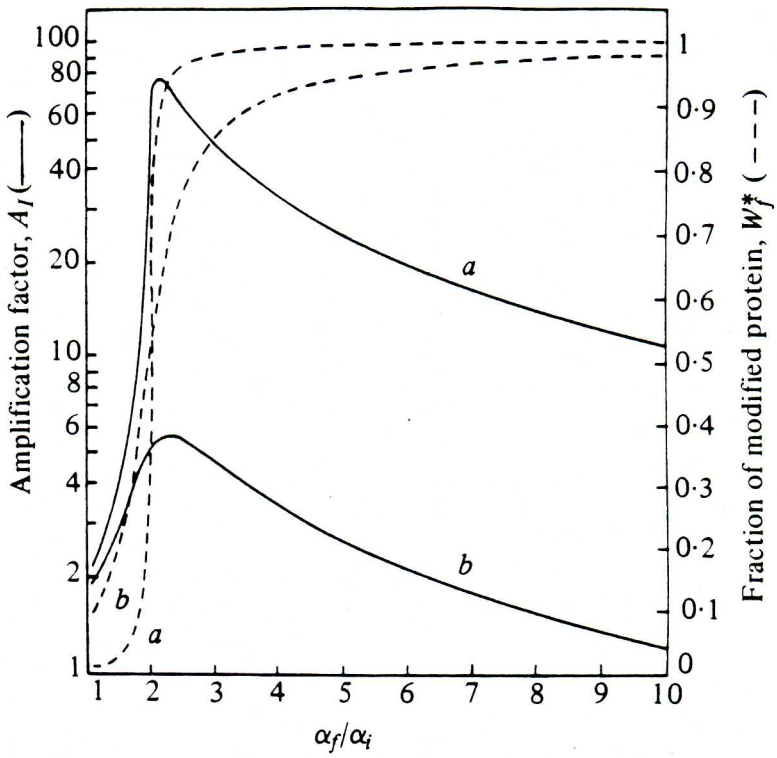

Fig. 6. Amplification by an enzyme subjected to reversible, covalent modification. The amplification factor (solid line) is determined according to equation (12) as a function of α_f for (*a*) $K_1 = K_2 = 0{\cdot}01$ and (*b*) $K_1 = K_2 = 0{\cdot}1$, with $\alpha_i = 0{\cdot}5$. The parameters α_i and α_f are the initial and final values of the ratio (V_1/V_2). The value of the fraction of modified protein corresponding to α_f is given by the dashed line for the same two cases.

As to the amplification factor, it can be seen that A_I passes through a maximum and then decreases, because a further rise in (α_f/α_i) does not result in significant increase in W^*.

From (11) and (12) we obtain the following expression for the amplification factor A_I:

$$A_I = \frac{(1 - W_f^*)(W_f^* + K_2)(1 - W_i^* + K_1)}{K_2[K_1 + (1 - W_i^*)(1 - W_f^*) + (K_1/K_2)W_i^* W_f^*]}. \qquad (13)$$

To obtain the value of W_f^* yielding the maximum amplification for a given W_i^*, we can solve the equation $(dA_I/dW_f^*) = 0$, which gives the second-degree equation:

$$W_f^{*2}\left[1 - \left(1 + \frac{K_1}{K_2}\right)W_i^*\right] - 2W_f^*[K_1 + (1 - W_i^*)] + [K_1(1 - K_2 - W_i^*) + (1 - W_i^*)] = 0. \qquad (14)$$

This equation admits only one physically acceptable solution, given that $0 < W_f^* < 1$. The values of α corresponding to W_i^* and to the

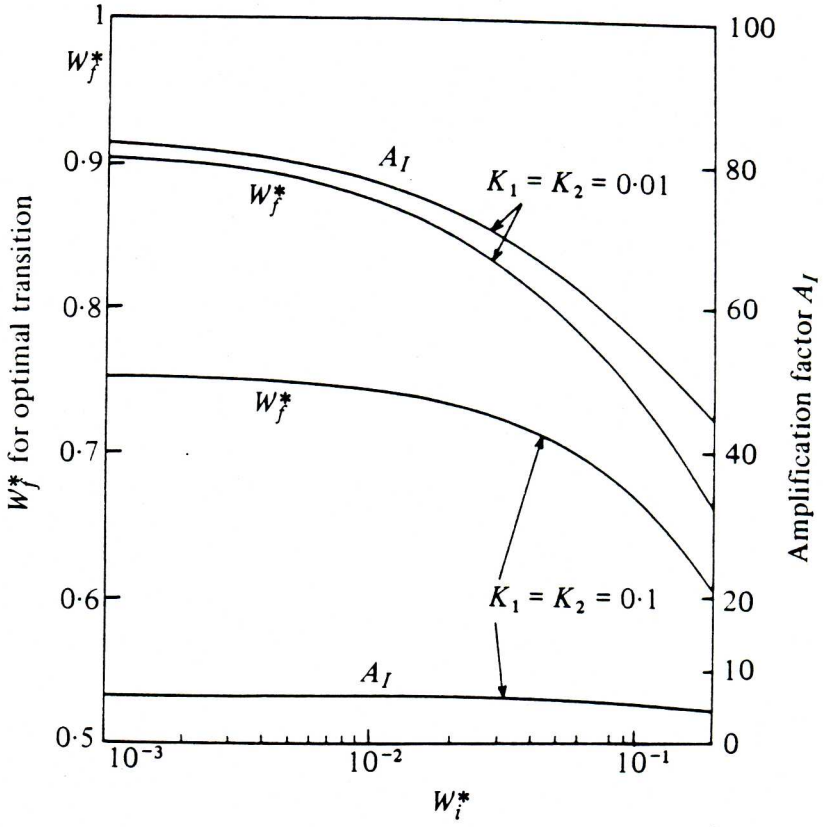

Fig. 7. Maximum amplification factor in a monocyclic modification cascade, when the amplification factor A_I is defined by equations 12 and 13. The value of W_f^* yielding maximum amplification is obtained by solving the second-degree equation (14).

optimal W_f^* can be obtained from equations (11) and (14). The maximum value of A_I and the corresponding value of W_f^* are shown in Fig. 7 as a function of W_i^*.

In Table 2, the maximum amplification factor is listed as a function of $K_1 = K_2$ with the corresponding values of W_f^* and (α_f/α_i), for $W_i^* = 0{\cdot}01$ and $0{\cdot}1$. These values of the initial fraction W_i^* are chosen so as to facilitate comparison with the data shown in Table 1 for allosteric enzymes. The molar fraction of modified protein yields indeed a measure of the response, as the rate of reaction does for an enzyme. Whereas the response for an allosteric enzyme is governed by the variation in the level of substrate (or effector), it is governed here through the variation in the ratio of modification rates V_1/V_2.

The data of Table 2 show that the amplification factor rises as the constants K_1 and K_2 decrease below unity. This results from the fact that the increase in modified protein becomes sharper as the normalized Michaelis constants of the modifying enzymes diminish (Goldbeter & Koshland, 1981) (see Fig. 5). Equation (13) indicates that A_I becomes inversely proportional to K_2 when K_1 and K_2 are much smaller than

TABLE 2. *Maximum amplification factor A_I in a monocyclic covalent modification system*

	$K_1 = K_2$	$\alpha_i = (V_1/V_2)_i$	α_f/α_i	W_f^*/W_T	A_I
Values when $W_i^* = 0{\cdot}1\ W_T$	10^{-2}	0·92	1·1	0·74	55·8
	10^{-1}	0·55	2·0	0·67	5·5
	1	0·19	2·1	0·22	1·05
Values when $W_i^* = 0{\cdot}01\ W_T$	10^{-2}	0·5	2·1	0·87	77·6
	10^{-1}	0·1	12·2	0·74	6·5
	1	0·02	24·7	0·26	1·1

Nomenclature: $\alpha = V_1/V_2$ where V_1 and V_2 are maximum velocities under given conditions. K_1 and K_2 are normalized Michaelis constants, K_{m1}/W_T and K_{m2}/W_T for enzymes E_1 *and* E_2, respectively, in Fig. 4. A_I is defined in equation (12) as partial amplification factor for covalent cascade.

unity. In contrast to the data of Table 1 where A_I drops as the initial response v_i increases from 1 to 10% V_M, the value of the maximum amplification factor in the covalent modification system does not change much when the initial fraction of modified target protein passes from 1 to 10%. The calculations of Fig. 7 and Table 2 show that the maximum amplification factor is more sensitive to such a change in W_i^* when $K_1 = K_2 = 0{\cdot}01$. It should be noted that the maximum value of A_I drops below unity when K_1 and K_2 surpass 1. This shows that no amplification can obtain when the modifying enzymes E_1 and E_2 function in the domain of first-order kinetics where K_{m1} and K_{m2} are much greater than W_T.

As in the case of allosteric enzymes, the optimal transition covers a range of physiological interest. The results show indeed that the maximum amplification corresponds to a transition in which the final value of the fraction of modified protein exceeds 50%. As to the step in stimulus, we note that the ratio (α_f/α_i) needed to bring about this transition diminishes when the initial value of W^* increases. Then the value α_i rises, and the ratio (α_f/α_i) approaches more rapidly the point of mid-transition where the target protein exists in equal amounts in the modified and unmodified states. Consequently, an approximate expression for the ratio (α_f/α_i) yielding maximum amplification, when $K_1 = K_2$, is

$$(\alpha_f/\alpha_i) \approx \frac{1}{\alpha_i}. \qquad (15)$$

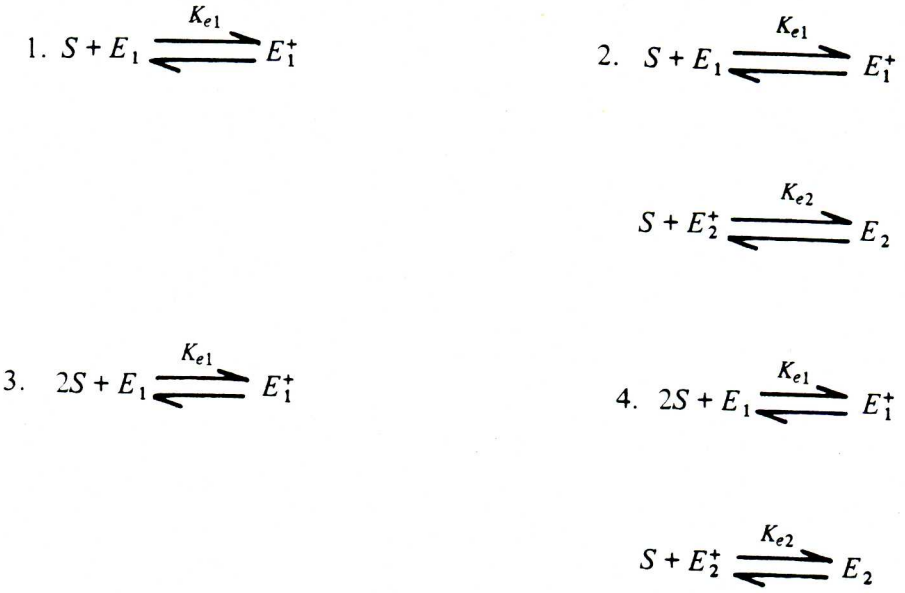

Fig. 8. Four possible mechanisms for the control of the modifying enzymes E_1 and E_2 by effector S in the modification cycle of Fig. 4. The presence or absence of a superscript + denotes the active and inactive forms of the enzymes, respectively.

Amplification factor A_{II} related to change in effector level

To obtain the overall amplification factor for covalent modification it is necessary to relate the level of V_1/V_2 to the level of S, the stimulus. As described above, this means relating $(V_1/V_2) = \alpha$ to S for obtaining the amplification factor A_{II}.

To this end, four mechanisms will be considered (see Fig. 8): Michaelian activation of E_1 by S; co-operative activation of E_1 by S; Michaelian activation of E_1 by S and inhibition of E_2 by S; and co-operative activation of E_1 by S and Michaelian inhibition of E_2 by S. Constants K_{e1} and K_{e2} denote the dissociation constants of the complexes formed by S with enzymes E_1 and E_2, respectively. The effector concentration yielding 50 % activation of E_1 is denoted $S_{0.5}$. The total enzyme concentrations E_{1T} and E_{2T} must be replaced by the amounts of active enzymes E_1 and E_2 available at a given effector concentration S. In mechanisms 1–4 of Fig. 8, the ratio $\alpha = V_1/V_2$ is given as a function of S by the expressions listed in Table 3. Also shown in this table are the expressions for A_{II}, i.e. the amplification factor relating the change in α to the variation in S.

The control of E_1 according to mechanism 1 never leads to amplification *per se*, since $A_{II} < 1$. In contrast, A_{II} is larger than unity at low values of S_i and smaller than unity at values of $S_i > S_{0.5}$ in mechanisms 2–4.

TABLE 3. *Dependence of ratio of rates* $\alpha = V_1/V_2$ *and the amplification factor* A_{II} *on the effector concentration, in the four mechanisms depicted in Fig.* 8 *for the control of* E_1 *and* E_2 *by* S. *The ratio* (V_{m1}/V_{m2}) *is the ratio of rates* $(k_1 E_{1T}/k_2 E_{2T})$ *when* E_1 *and* E_2 *are maximally active*

Mechanism for control of E_1 and E_2 by S	Ratio $\alpha = V_1/V_2$	Amplification factor $A_{II} = \dfrac{\Delta\alpha}{\alpha_i}\Big/\dfrac{\Delta S}{S_i}$
1	$\dfrac{V_{m1}}{V_{m2}}\dfrac{S}{K_{e1}+S}$	$\dfrac{K_{e1}}{K_{e1}+S_f}$
2	$\dfrac{V_{m1}}{V_{m2}}\dfrac{S(K_{e2}+S)}{K_{e2}(K_{e1}+S)}$	$\dfrac{K_{e1}K_{e2}+S_iK_{e1}+S_f(K_{e1}+S_i)}{(K_{e1}+S_f)(K_{e2}+S_i)}$
3	$\dfrac{V_{m1}}{V_{m2}}\dfrac{S^2}{K_{e1}+S^2}$	$\dfrac{1+(S_f/S_i)}{1+(S_f^2/K_{e1})}$
4	$\dfrac{V_{m1}}{V_{m2}}\dfrac{S^2(K_{e2}+S)}{K_{e2}(K_{e1}+S^2)}$	$\dfrac{S_iK_{e2}(S_i+S_f)+S_i[(S_f-S_i)^2+S_iS_f+(S_iS_f)^2/K_{e1}]}{(K_{e1}+S_i^2)(K_{e1}+S_f^2)}$

When compared to the factor A_I previously computed for a given ratio (α_f/α_i), the total amplification factor A_S, equal to the product $A_I\ A_{II}$ (see equation 9) is thus smaller when E_1 is controlled according to mechanism 1, and larger, equal or smaller, depending on the value of S_i, S_f and K_{e2}/K_{e1} in mechanisms 2–4. A case of particular interest obtains in mechanism 2 when $K_{e1} = K_{e2}$. Then $A_{II} = 1$, regardless of S_i and S_f: the variation in α is always equal to the variation in the effector concentration, and the total amplification factor is equal to A_I. A similar result is obtained in mechanism 1 only when S_f is much less than $S_{0.5}$. Mechanism 2 therefore provides a buffer capacity such that the system maintains its amplification properties (measured by A_I) over a wide range of S_i and S_f values.

Total amplification factor (A_S) *for covalent modification scheme*

In order to compare the effect of mechanisms 1–4 on the total amplification factor A_S, we may pick a particular transition, for example $W^* = 0.066 \rightarrow 0.934$. For $K_1 = K_2 = 10^{-2}$, this transition yields the amplification factor $A_I = 44.13$ and is associated with a variation in $\alpha = V_1/V_2$ such that $(\alpha_f/\alpha_i) = 1.297$. By means of optimization procedures similar to those utilized above, it can be shown

that the $W_i^* = 0{\cdot}066$ to $W_f^* = 0{\cdot}934$ transition is the W_i^* to $(1 - W_i^*)$ transition which yields the maximum amplification factor when $K_1 = K_2 = 10^{-2}$; as shown in Table 2, this factor is generally smaller than the maximum possible factor which is associated with a value $W_f^* \neq (1 - W_i^*)$.

The value of A_S here is determined according to the equation

$$A_S = \left(\frac{2W_f^* - 1}{1 - W_f^*}\right) \Big/ \left(\frac{S_f}{S_i} - 1\right), \tag{16}$$

where $W_f^* = 0{\cdot}934$. The ratio (S_f/S_i) is determined in mechanisms 1–4 by the equations of Table 3. To each value of S_i there corresponds a value of W_i^* which depends on the choice of $(V_{m1}/V_{m2}) = (k_1 E_{1T}/k_2 E_{2T})$. For a given value of this ratio, the values of S_i and S_f will produce a pair of values α_i, α_f which, in turn, will correspond to a certain transition W_i^* to W_f^*. Here, let us choose (V_{m1}/V_{m2}) such that $W_i^* = 0{\cdot}066$. Then, S_f is determined so that $(\alpha_f/\alpha_i) \approx 1{\cdot}3$; this variation in the ratio of rates V_1/V_2 is indeed required for the transition $W^* = 0{\cdot}066 \rightarrow 0{\cdot}934$.

For mechanism 1, this condition yields the expression

$$\frac{S_f(S_{0{\cdot}5} + S_i)}{S_i(S_{0{\cdot}5} + S_f)} = 1{\cdot}3. \tag{17}$$

Hence, in mechanism 1, the variation in effector required for the transition $W^* = 0{\cdot}066 \rightarrow 0{\cdot}934$ is given by

$$\left(\frac{S_f}{S_i}\right) = \frac{1{\cdot}3}{1 - 0{\cdot}3(S_i/S_{0{\cdot}5})}. \tag{18}$$

For mechanisms 2–4, the value of the ratio (S_f/S_i) corresponding to the value of (α_f/α_i) required for the transition can be computed in a similar way, by means of the relations linking α to S in Table 3.

The total amplification factor thus determined from equation (16) is shown in Fig. 9 as a function of the initial effector concentration S_i. For values of S_i less than or close to $0{\cdot}1\ S_{0{\cdot}5}$, the value of A_S remains practically constant; it is twice as high in mechanisms 3 and 4 than it is in mechanisms 1 and 2. This additional amplification results from the co-operative activation of enzyme E_1 by the effector. Whereas A_S remains buffered in mechanisms 2 and 4 as S_i increases, it sharply drops in mechanisms 1 and 3 which lack the inhibition of enzyme E_2 by the effector. This drop is due to the fact that the value of S_f needed to produce the step $(\alpha_f/\alpha_i) = 1{\cdot}3$ becomes very large as S_i rises. When S_i becomes too large, i.e. $(S_i/S_{0{\cdot}5}) = 3{\cdot}37$ in mechanism

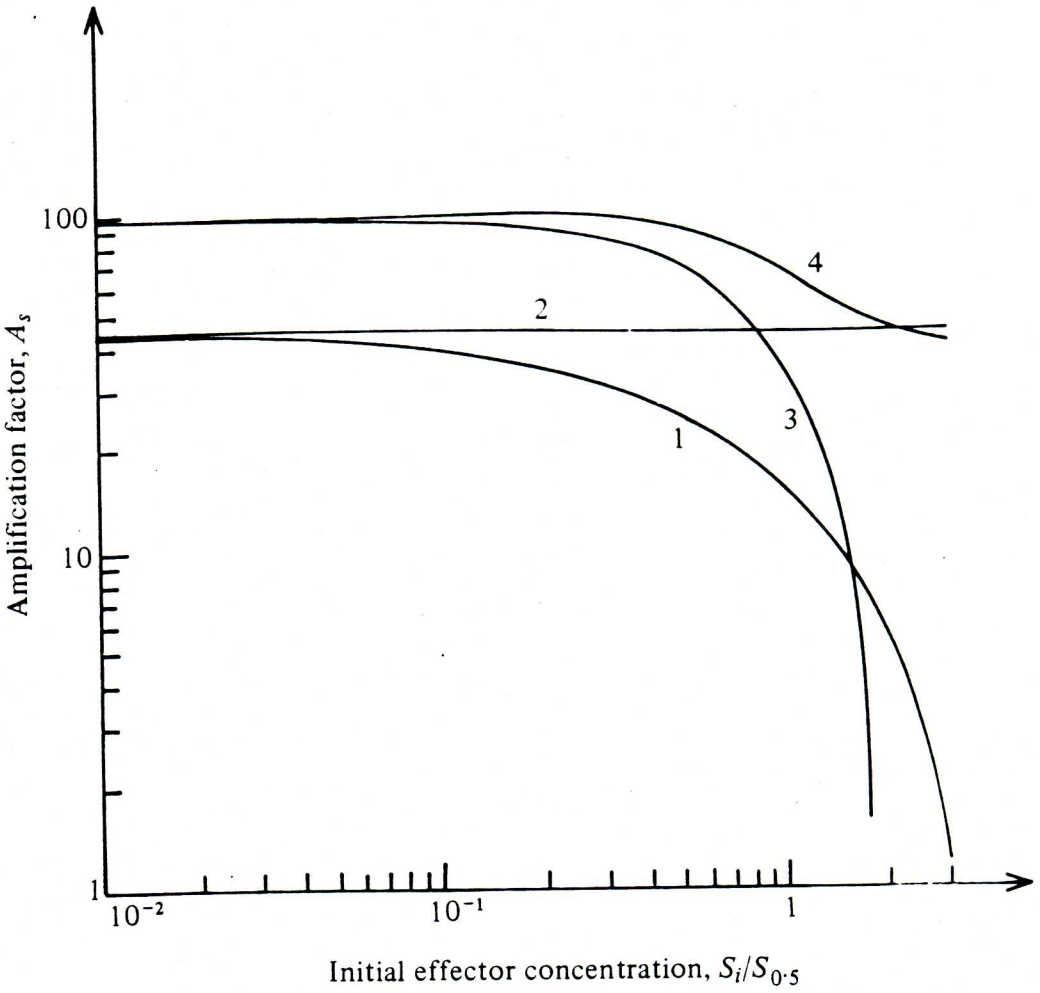

Fig. 9. Amplification of a change in stimulus leading to the transition in the fraction of modified protein W^* from 6·6% to 93·4% of W_T. The curves are numbered according to the four different mechanisms for the control of E_1 and E_2 by an effector S (see Fig. 8). The amplification factor ($A_S = A_I A_{II}$) is determined according to equation (16) as a function of the initial effector concentration S_i; the value of S_f is computed from the expressions of Table 3 so as to yield the change $\alpha_f/\alpha_i = 1{\cdot}3$ required for the transition in W^* when $K_1 = K_2 = 0{\cdot}01$. The curves for mechanisms 2 and 4 are established for $K_{e1} = K_{e2}$.

1 and $(S_i/S_{0.5}) = 1{\cdot}84$ in mechanism 4, no value of S_f can be found which yields the required variation in (α_f/α_i). The optimal range of basal effector levels clearly corresponds to concentrations of effector smaller than K_{e1}, i.e. S_i is much less than $S_{0.5}$. The simultaneous activation of E_1 and inhibition of E_2 by S provides a means of preserving the amplification capability over a wide range of basal effector concentrations.

It should be noted that amplification factors smaller than those shown in Fig. 9 are found for larger values of the normalized Michaelis constants K_1 and K_2. The dependence of A_I on these

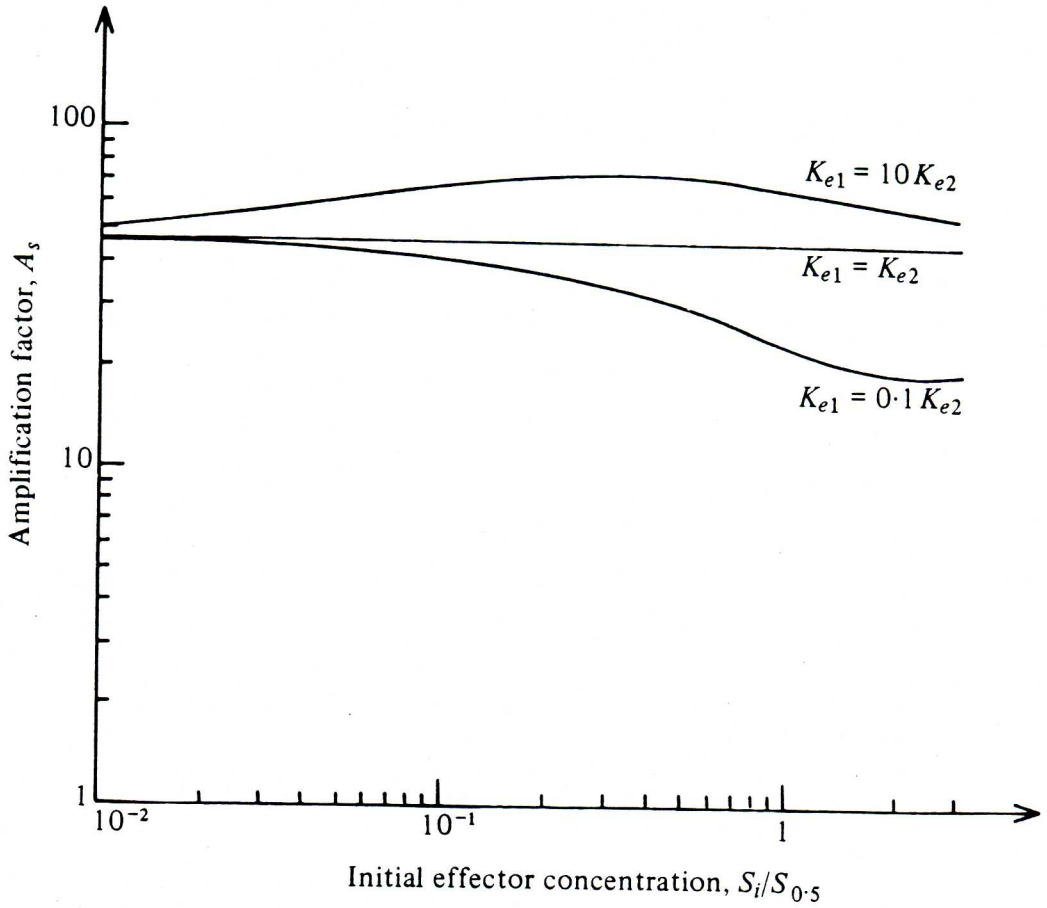

Fig. 10. Amplification factor in terms of change in effector level in a monocyclic modification system, when the converter enzymes are controlled by effector S according to mechanism 2 of Fig. 8. The curves are established for three different values of K_{e2}/K_{e1}, which is the ratio of the dissociation constants for the complexes formed by S with E_2 and E_1, respectively. The amplification factor is computed for the parameter values and the transition in W^* considered in Fig. 9.

constants is illustrated in Table 2 (A_S coincides with A_I in mechanism 2 when $K_{e1} = K_{e2}$). Finally, when compared to the data obtained for $K_{e1} = K_{e2}$, A_S is larger when $K_{e1} > K_{e2}$ and smaller when $K_{e1} < K_{e2}$. In the case of Fig. 10, the variation in A_S remains less than threefold when K_{e1} varies from 0·1 K_{e2} to 10 K_{e2}.

Propagation of sensitivity amplification in multicyclic cascades

Biochemical systems controlled by covalent modification are often organized as multicyclic cascades (Stadtman & Chock, 1977, 1978). The question arises as to whether sensitivity amplification can be propagated and enhanced along such cascades. We shall show how this is possible in the simple case of a bicyclic cascade (this analysis can readily be extended to cascades comprising more than two cycles).

Let us consider that W^*, product of the first modification cycle,

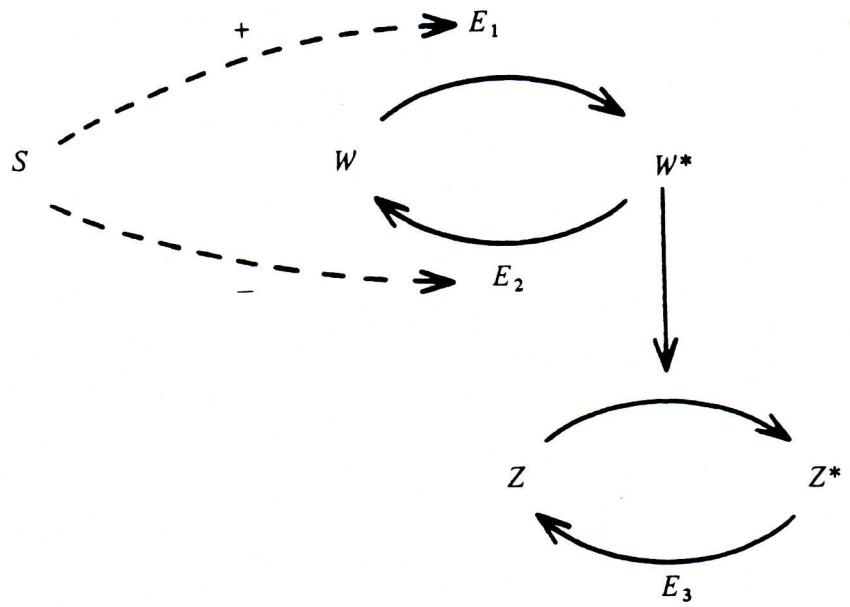

Fig. 11. Bicyclic modification cascade. The product of the first modification cycle, W^*, catalyses the modification of a second target protein, Z, into Z^*. This modification is reverted by enzyme E_3. It is assumed that effector S controls the modifying enzymes of the first cycle according to mechanism 2 of Fig. 8.

acts as a modifying enzyme for the conversion of a protein Z into Z^*, while enzyme E_3 catalyses the reverse reaction (Fig. 11).

For the control of this system by an effector S, we consider the simple situation (defined above as mechanism 2) in which S activates E_1 and inhibits E_2 in Michaelian manner, with equal activation and inhibition constants $K_{e1} = K_{e2}$.

In the bicyclic cascade α and α' denote, respectively, the two ratios of maximum modification rates, at a given effector concentration. For the first and second cycles we use the definitions of equation (19),

$$\alpha = V_1/V_2, \quad \alpha' = V_{W^*}/V_3. \tag{19}$$

We wish to determine the relative change in Z^* resulting from a variation in S from S_i to S_f. Hence, the total amplification factor A_{1+2} is here the product of four factors:

$$\begin{aligned} A_{1+2} &= \left(\frac{\Delta Z^*}{Z_i^*}\Big/\frac{\Delta\alpha'}{\alpha_i'}\right)\left(\frac{\Delta\alpha'}{\alpha_i'}\Big/\frac{\Delta W^*}{W_i^*}\right)\left(\frac{\Delta W^*}{W_i^*}\Big/\frac{\Delta\alpha}{\alpha_i}\right)\left(\frac{\Delta\alpha}{\alpha_i}\Big/\frac{\Delta S}{S_i}\right) \\ &= \left(\frac{\Delta Z^*}{Z_i^*}\Big/\frac{\Delta W^*}{W_i^*}\right)\left(\frac{\Delta W^*}{W_i^*}\Big/\frac{\Delta S}{S_i}\right) = \left(\frac{\Delta Z^*}{Z_i^*}\Big/\frac{\Delta W^*}{W_i^*}\right) A_1 \\ &= \left(\frac{\Delta Z^*}{Z_i^*}\Big/\frac{\Delta S}{S_i}\right), \end{aligned} \tag{20}$$

where A_1 denotes the amplification for the first cycle.

For definiteness, let us consider the case of a change $S_i \rightarrow S_f$ yielding the variation $W_i^* = 0{\cdot}1 \rightarrow W_f^* = 0{\cdot}9$. This transition corresponds to certain values of α_i (S_i) and α_f (S_f) which are functions of K_1 and K_2 (see the preceding section). The relation between S and α when E_1 and E_2 are controlled by the effector according to mechanism 2 reduces to $\alpha = S$ when $K_{e1} = K_{e2}$ and $k_1 E_{1T} = k_2 E_{2T}$ (Table 3). We shall consider this simple case for the numerical evaluation of amplification in the bicyclic cascade.

To evaluate the total amplification factor defined in equation (21),

$$A_{1+2} = \left(\frac{Z_f^*}{Z_i^*} - 1\right) \Big/ \left(\frac{S_f}{S_i} - 1\right), \tag{21}$$

we first obtain the ratio (S_f/S_i). Since E_1 and E_2 are controlled by S according to mechanism 2 in a way such that $\alpha = S$, for the particular transition chosen $(W_i^* = 0{\cdot}1 \rightarrow W_f^* = 0{\cdot}9)$, the ratio S_f/S_i is obtained from equation (11) and given as a function of K_1 and K_2 by the relation of equation (22):

$$\frac{S_f}{S_i} = \frac{\alpha_f}{\alpha_i} = \frac{81\,(0{\cdot}1 + K_1)\,(0{\cdot}1 + K_2)}{(0{\cdot}9 + K_1)\,(0{\cdot}9 + K_2)}. \tag{22}$$

For $K_1 = K_2 = 10^{-2}$ and 10^{-1}, (S_f/S_i) equals 1·18 and 3·2 respectively.

Now, our purpose is to determine the dependence of A_{1+2} on Z_i^*. The initial value of the fraction Z^* is set by the value of α_i' which is obtained from equation (23):

$$\alpha_i' = \frac{Z_i^*(1 - Z_i^* + K_{W^*})}{(1 - Z_i^*)\,(Z_i^* + K_3)}. \tag{23}$$

Here, K_{W^*} and K_3 denote the Michaelis constants of enzymes W^* and E_3, divided by the total concentration Z_T. Since the transition considered occurs from $W_i^* = 0{\cdot}1$ to $W_f^* = 0{\cdot}9$, there is a ninefold change in the activity of enzyme W^* in the second modification cycle; hence $\alpha_f' = g\alpha_i'$. The value of Z_f^* is therefore given by equation (24), which is similar in form to equation (10) established for a monocyclic modification system:

$$Z_f^* = \frac{[(\alpha_f' - 1) - (K_{W^*} + K_3\alpha_f')] + \{[(\alpha_f' - 1) - (K_{W^*} + K_3\alpha_f')]^2 + 4K_3\alpha_f'(\alpha_f' - 1)\}^{\frac{1}{2}}}{2(\alpha_f' - 1)}. \tag{24}$$

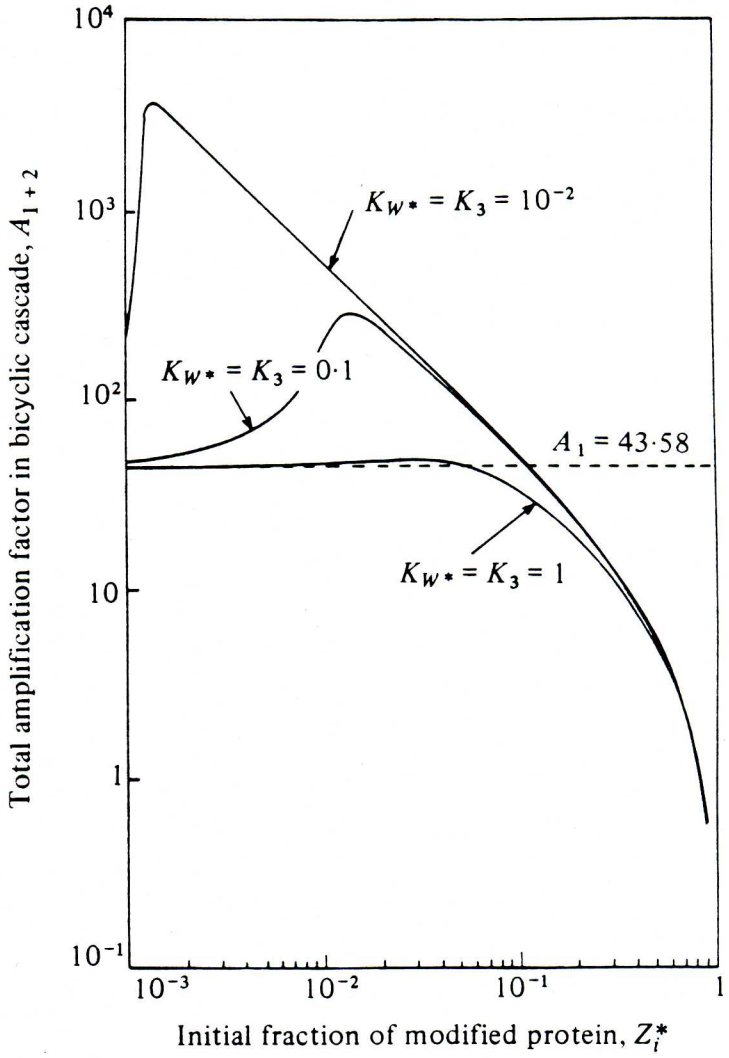

Fig. 12. Total amplification factor (A_{1+2}) in a bicyclic cascade, as a function of the initial level, Z_i^* of modified, second target protein. Amplification is computed from equations (21) and (24) for a change $(S_f/S_i) = 1{\cdot}18$ which produces a variation $W_i^* = 0{\cdot}1 \rightarrow W_f^* = 0{\cdot}9$ in the first modification cycle. The amplification factor A_1 corresponding to this transition in the first cycle is 43·58 (dashed line). The normalized Michaelis constants of E_1 and E_2 are $K_1 = K_2 = 0{\cdot}01$; for W^* and E_3, the values of these constants range from 0·01 to 1 as indicated on the curves.

The value of the total amplification factor in the bicyclic system is plotted as a function of Z_i^* in Fig. 12, for $K_{W^*} = K_3 = 10^{-2}$, 10^{-1} and 1. This figure is established for $K_1 = K_2 = 10^{-2}$, in which case the amplification factor A_1 for the transition $W_i^* = 0{\cdot}1$ to $W_f^* = 0{\cdot}9$ is 43·6.

Several conclusions can be drawn from the data of Fig. 12. First, enhancement of sensitivity amplification by as much as two orders of magnitude is possible when the normalized Michaelis constants of the second cycle, K_{W^*} and K_3, as well as the initial value Z_i^*, are sufficiently low. Second, there exists an optimal value of Z_i^* yielding

maximum amplification; this value is a function of K_{W*} and K_3, and increases with these parameters.

Thirdly, the curves yielding A_{1+2} as a function of Z_i^* drop below the value of A_1 and converge to a common curve when Z_i^* approaches 0·1. Therefore, a bicyclic cascade offers the possibility of an enhancement of sensitivity but can also give rise to a damping of the amplification obtained in the first modification cycle. The main factor responsible for the enhancement or damping effect is the value of Z_i^*. Indeed, the condition for enhancing sensitivity, i.e. $A_{1+2} > A_1$, can be written as

$$\frac{Z_f^*}{Z_i^*} > \frac{W_f^*}{W_i^*}. \tag{25}$$

Here, this condition reduces to $Z_i^* < Z_f^*/9$. Since Z_f^* is bounded by and generally close to 1, A_{1+2} will exceed A_1 when Z_i^* is less than 11 % of Z_T, whereas A_{1+2} will drop below A_1 when Z_i^* becomes larger than this value.

The above results can readily be extended to a cascade containing more than two cycles. If Z^* catalyses the modification of a third target protein Y into Y^*, enhancement of amplification by the latter cycle will be favoured when Y_i^* is less than W_i^*. As shown by Fig. 12, the amplification gained in the first cycle diminishes and is eventually lost (i.e. $A_{1+2} < 1$) as the initial value of the modified, final target protein approaches unity.

In the case depicted in Fig. 12, parameters $K_1 = K_2 = 10^{-2}$ of the first modification cycle are such that sensitivity amplification is high at the beginning of the cascade. Thus a change of $(S_f/S_i) = 1{\cdot}18$ is sufficient to bring about a variation from $W_1^* = 0{\cdot}1$ to $W_f^* = 0{\cdot}9$. If $K_1 = K_2 = 10^{-1}$, a ratio $(S_f/S_i) = 3{\cdot}24$ is needed to elicit the same change in W^*. Calculations similar to those performed in Fig. 12 show that the amplification factor of the first cycle ($A_1 = 3{\cdot}57$) can be enhanced by up to one order of magnitude in the second cycle for sufficiently low values of Z_i^*. Since the ratio (S_f/S_i) governs the amplification properties of the whole cascade, it might seem that the optimal structure of a modification cascade – i.e. that yielding maximum total amplification – is to have the first modification cycle as most sensitive. This would imply that either the K_m of enzymes E_1 and E_2 are low, or the total amount of protein W^* is large, so that K_1 and K_2 are much smaller than unity.

To complete our discussion of the bicyclic cascade we must consider the effect of another parameter, namely $\alpha'_M = (k_{W^*} W_T / k_3 E_{3T})$, i.e. the ratio of maximum rates of W^* and

E_3. This parameter links α' to the product of the first modification cycle:

$$\alpha' = \alpha'_M W^*. \tag{26}$$

Thus α'_M is the maximum value that the ratio of modification rates in the second cycle can reach when the target protein of the first cycle is completely modified (whether W^* goes to unity as α increases depends on whether α is much larger than unity when S is much greater than $S_{0.5}$; this, in turn, depends on the value of $V_{m1}/V_{m2} = k_1 E_{1T}/k_2 E_{2T}$, as shown in Table 3).

Numerical simulations show that the effect of an increase in K^*_W and K_3 on the steepness of the Z^* curves – and hence on the amplification properties of the system – strongly depends on the value of α'_M. When $\alpha'_M < 1$, the modification curve for Z^* *vs* S becomes steeper as K_{W^*} and K_3 increase, regardless of the value of K_1 and K_2 (see the curves for $\alpha'_M = 0{\cdot}8$ in Fig. 13). Note that the asymptotic value reached by Z^* when S is much greater than $S_{0.5}$ remains less than 0·5 since the maximum value of α' is less than unity. In contrast, the steepness of the Z^* curve increases as K_{W^*} and K_3 diminish when $\alpha'_M > 1$ (see the data in Fig. 13 for $\alpha'_M = 10$).

The reason for the puzzling behaviour of the modification cascade at values of α'_M less than 1 can be found in the curves presented in Fig. 5 for a monocyclic modification system. In a bicyclic system, similar results hold for Z^* when replacing $\alpha = V_1/V_2$ by $\alpha' = V_{W^*}/V_3$ and $K_1 = K_2$ by $K_{W^*} = K_3$. It can be seen that the fraction of modified protein for $K_{W^*} = K_3 = 1$ will be larger than for $K_{W^*} = K_3 = 0{\cdot}01$ as long as $\alpha' < 1$, and that the reverse becomes true as soon as $\alpha' > 1$. Therefore, if α'_M is less than unity, the curves for Z^* will tend to larger values upon increasing K_{W^*} and K_3; this will result in steeper modification curves as shown in Fig. 13 for $\alpha'_M = 0{\cdot}8$. Whenever $\alpha'_M > 1$, steeper modification curves will obtain as K_{W^*} and K_3 diminish below unity.†

Several additional features of the curves of Fig. 13 are worthy of mention. First, for $\alpha'_M > 1$, the curves for Z^* begin to rise at lower values of S when K_1 and K_2 pass from 0·01 to 1. This is due once again to the fact that the rise in W^* occurs at lower values of S (or α) when K_1 and K_2 are large (see Fig. 5). Secondly, the curves for $\alpha'_M = 10$ and $K_1 = K_2 = 1$ illustrate well how a small increase in W^* causes a dramatic increase in Z^* when K_{W^*} and K_3 are smaller than

† The results on the steepness of the modification curves as a function of the asymptotic value of the ratio of modification rates also apply to a monocyclic modification system.

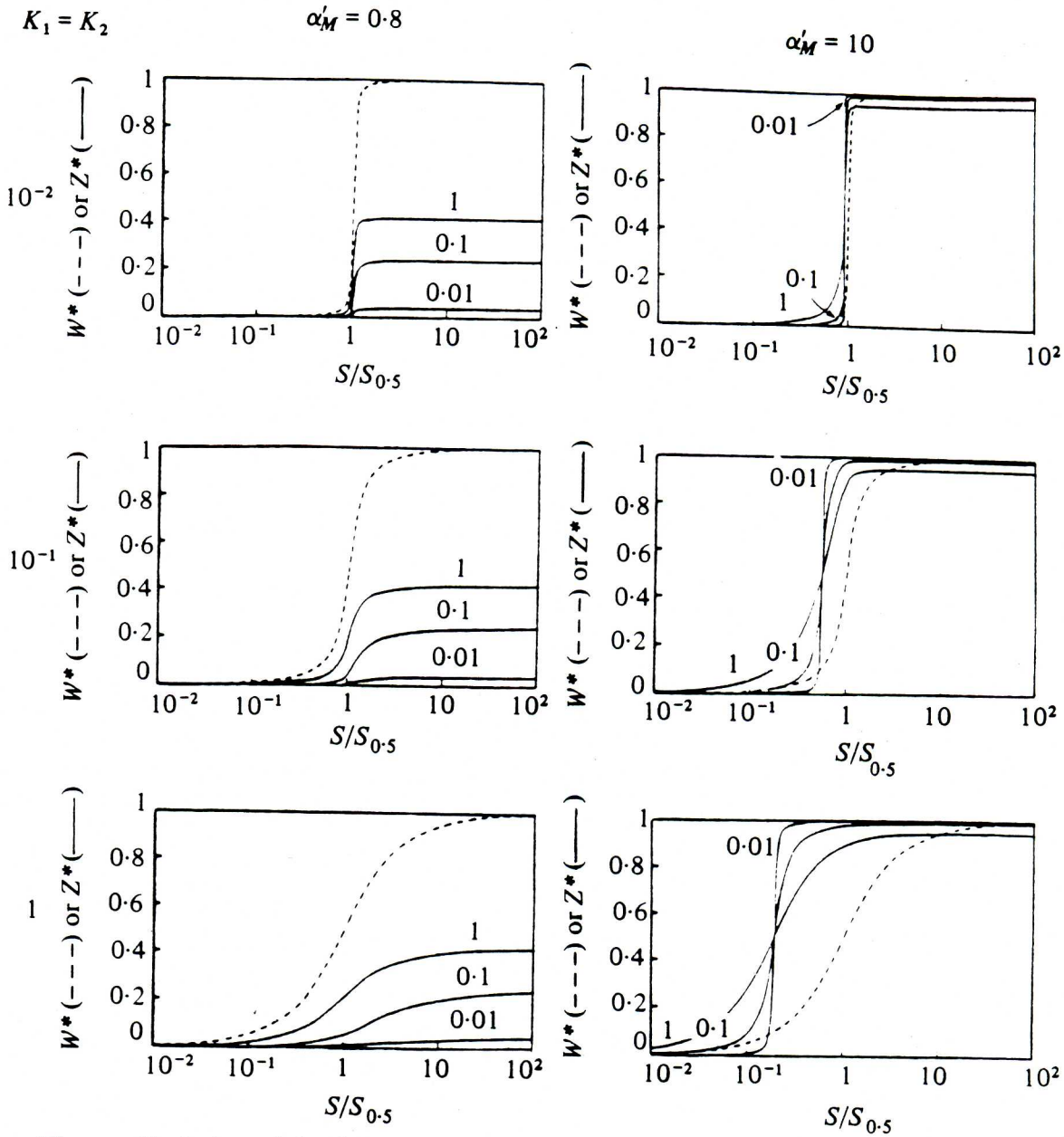

Fig. 13. Variation of the fractions of modified proteins W^* (dashed line) and Z^* (solid lines) in the bicyclic cascade of Fig. 11 as a function of effector S. It is assumed that the effector controls the converting enzymes of the first modification cycle according to mechanism 2 of Fig. 8, with $K_{e1} = K_{e2}$. The curves were established for three different values of constants $K_1 = K_2$ and $K_{W^*} = K_3$ (the values of the latter are indicated on each Z^* curve), and for two values of α'_M.

1. This allows for sensitivity amplification despite the absence of steepness in the modification curve for the product of the first cycle.

Finally, it should be noted that the curves of Fig. 12 are obtained when varying the initial fraction of modified protein Z_i^* at a fixed value of W_i^* (this value equals 0·1 in Fig. 12). Therefore, since Z_i^* is governed by $\alpha'_i = \alpha'_M W_i^*$, a variation in Z_i^* implies a variation in α'_M. In contrast, the curves of Fig. 13 are established for fixed values of α'_M.

TABLE 4. *Sensitivity amplification in a bicyclic cascade as a function of the characteristics of the first and second cycles*

(The amplification factor A_{1+2} is given for the variation in S which produces a transition in Z^* from 10% to 90% of the asymptotic value Z^*_∞ obtained for $S \gg S_{0.5}$. Data are obtained assuming that S controls E_1 and E_2 according to mechanism 2 of Fig. 8, with $K_{e1} = K_{e2}$, and taking $W^* \simeq 1$ for $S \gg S_{0.5}$.)

$K_1 = K_2$	$K_{W^*} = K_3$	$\alpha'_M = 0.8$	$\alpha'_M = 1.2$	$\alpha'_M = 10$
		Amplification factor in bicyclic cascade		
10^{-2}	10^{-2}	17.9	188.4	513.1
	10^{-1}	26.5	55.5	67.9
	1	47.2	50.0	15.6
10^{-1}	10^{-2}	1.8	25.1	89.4
	10^{-1}	2.5	5.7	10.0
	1	3.9	4.3	1.8
1	10^{-2}	0.2	8.3	45.4
	10^{-1}	0.3	0.9	4.0
	1	0.4	0.4	0.4
		Value of Z^* when $S \gg S_{0.5}$ (Z^*_∞)		
	10^{-2}	0.04	0.95	1
	10^{-1}	0.24	0.73	0.99
	1	0.42	0.57	0.95

The question still arises as to how sensitivity amplification factors for $\alpha'_M < 1$ compare to those obtained for $\alpha'_M > 1$. We have determined the amplification factor in the bicyclic cascade, according to equation (21), for the transition bringing Z^* from 10% to 90% of the asymptotic value Z^*_∞ obtained for $S \gg S_{0.5}$. The data, shown in Table 4, were computed for $\alpha'_M = 0.8$, 1.2 and 10 (the corresponding curves for Z^* are shown in Fig. 13 for $\alpha'_M = 0.8$ and 10), as a function of $K_1 = K_2$ and $K_{W^*} = K_3$ which were varied from 0.01 to 1.

In agreement with the preceding discussion of the steepness of the Z^* curves as a function of K_{W^*} and K_3 for α'_M values smaller or larger than 1, we note that sensitivity amplification augments with K_{W^*} and K_3 when $\alpha'_M < 1$, and decreases as these Michaelis constants rise when $\alpha'_M > 1$. The changes in amplification factors are, however, more significant at large values of α'_M. Also, the largest value of sensitivity amplification obtains when α'_M is largest and when K_1, K_2, K_{W^*} and K_3 are much smaller than unity.

For optimal amplification the configuration of a bicyclic cascade should therefore be such that the modifying enzymes of each cycle

should operate under zero-order conditions, with catalytic parameters V_{m1}/V_{m2} and α'_M allowing each target protein to be completely modified as the level of effector controlling the modification system rises. If the catalytic parameters are such that less than 50 % of target protein can be modified upon increasing the effector level, then higher amplification could obtain when the modifying enzymes operate in the first-order domain.

(C) *Sensitivity amplification in substrate cycles*

Newsholme and coworkers (1973, 1976) have pointed out that amplification can occur in futile cycles under certain special conditions. If the substances A and B are intermediates in a pathway as shown in equation (27),

$$\rightarrow A \underset{k_2, E_2}{\overset{k_1, E_1}{\rightleftharpoons}} B \overset{k_3, E_3}{\rightarrow} \tag{27}$$

then the flux through that step is $v_1 - v_2$, where v_1 is the rate for the step catalysed by the enzyme E_1 and v_2 is the rate for the step catalysed by enzyme E_2. Newsholme defines amplification as the ratio of the final flux over the initial flux, assuming that a stimulus S activates E_1 and inhibits E_2. Quite large numbers can be obtained when $v_1 - v_2$ is close to zero and v_1 and v_2 are large, but a careful analysis shows that additional assumptions are required. If Newsholme's assumption that the level of B stays constant is retained, then k_3 must increase proportional to the amplification in $v_1 - v_2$ or k_3 will become the rate-determining step. Thus the stimulus S must directly or indirectly activate E_3 allosterically while it is also activating E_1 and inhibiting E_2. Alternatively the concentration of B can be allowed to vary while assuming that E_1 and E_2 are operating in the zero-order region. The level of B would then rise and would increase the flux through E_3, provided that E_3 were operating in the first-order region.

(D) *Sensitivity amplification in multiple steady-state transitions*

In the mono- and multicyclic systems discussed in the preceding section, regulatory processes were considered explicitly only in connection with the control of the modifying enzymes E_1 and E_2. Biochemical pathways nevertheless represent integrated systems in which multiple feedback processes ensure the mode of operation best suited to environmental conditions.

Owing to the nonlinear kinetics of regulated systems, self-organization phenomena can occur in biochemical reactions operating far from thermodynamic equilibrium. These phenomena, referred to

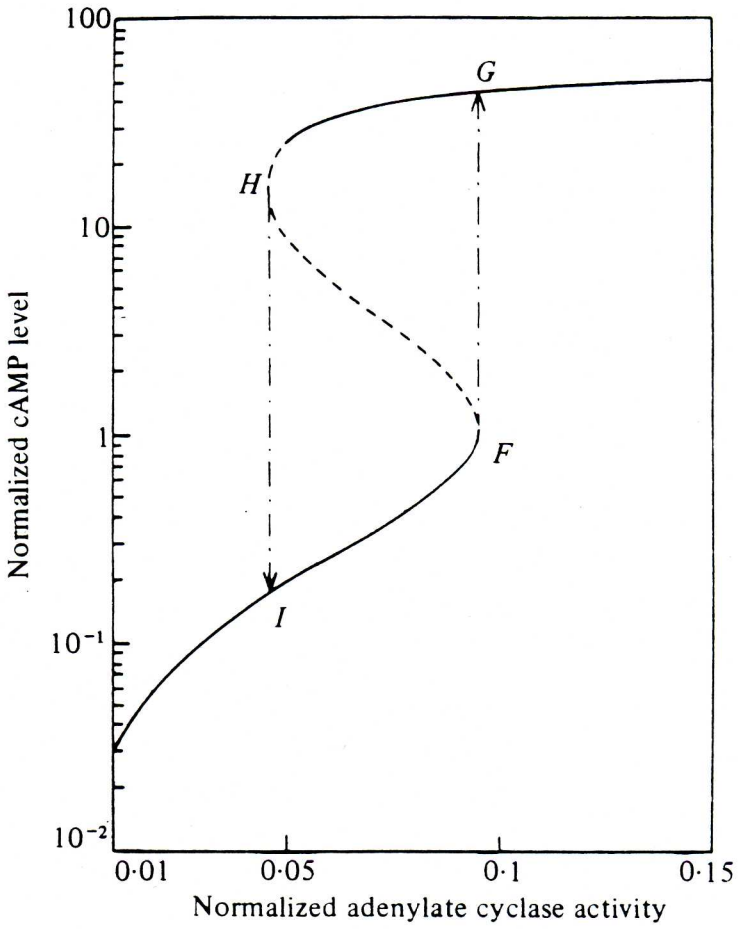

Fig. 14. Multiple steady states in a model for the cyclic AMP signalling system of *Dictyostelium discoideum* (redrawn from Goldbeter, 1980). The normalized level of cyclic AMP is given as a function of maximum adenylate cyclase activity. The curve shows the existence of a region where three steady states coexist; these states are either stable (solid line) or unstable (dashed line). Amplification of an increase in enzyme activity is largest around the transition $F \rightarrow G$.

as dissipative structures (Nicolis & Prigogine, 1977), occur beyond a critical point of instability and correspond to a temporal (sustained oscillations) or spatiotemporal (chemical waves) organization. Several oscillatory enzyme reactions are known, the best example being that of glycolytic oscillations, which are due to the positive feedback exerted on phosphofructokinase by a reaction product (Hess & Boiteux, 1971; Goldbeter & Caplan, 1976).

A third type of self-organization is of direct relation to amplification. For a given set of conditions, i.e. substrate or effector levels, and enzyme activities, some chemical systems may evolve to more than one stable steady state. The most common situation is that of bi-stability, in which two stable steady states are separated by one unstable state. For definiteness, such a situation is illustrated on a specific biochemical example in Fig. 14, where extracellular cAMP level at steady state is computed in a model for the cAMP signalling

system in the slime mould *Dictyostelium discoideum*. This model, based on the activation of adenylate cyclase which follows the binding of extracellular cAMP to a cell surface receptor, accounts for the oscillations and relay of cAMP which control slime mould aggregation after starvation (Goldbeter & Segel, 1977). The relay capability can be associated with the excitability of the adenylate cyclase reaction in *D. discoideum* (see next section below).

In certain conditions, there exists a range of adenylate cyclase concentration in which the model for the signalling system admits three steady-state values for intracellular and extracellular cAMP, for a given concentration of the cyclase. Then, if the adenylate cyclase activity increases from a low initial level, the cAMP steady-state concentration rises along the lower branch of the S-shaped curve in Fig. 14. Upon further increasing adenylate cyclase until the system reaches the point F where two steady states coalesce, one observes a discontinuous jump from F to G on the upper branch of stable steady states.

An infinitesimal variation in enzyme activity around the value corresponding to F elicits a jump of finite magnitude in cAMP level. The amplification factor associated with such variation thus goes to infinity in the immediate vicinity of F. Since it is unlikely that the system is located precisely in this point before a putative increase in adenylate cyclase, it is of interest to determine the order of magnitude of the amplification factor for a finite variation in adenylate cyclase activity on both sides of the value corresponding to F. Thus, for a normalized enzyme activity varying from 0·09 to 0·1 in Fig. 14, the normalized cAMP level goes from 0·667 to 43·14. The amplification factor associated with this transition is 573, which is much larger than the factors obtained for allosteric or covalently modified enzymes in the absence of feedback processes.

All-or-none transitions due to multiplicity of steady-state solutions therefore represent the most sensitive device by which biochemical systems can respond to changes in their environment. Besides the cAMP signalling system such phenomena have been invoked in a variety of biological processes (see Nicolis & Prigogine, 1977, for a review). They have been observed in several biochemical reactions such as the peroxidase reaction (Degn, 1968), a pH-controlled, immobilized enzyme system (Naparstek *et al.* 1974) and a partially reconstituted glycolytic system (Eschrich, Schellenberger & Hofmann, 1980).

(E) *Amplification in time-dependent processes*

In many instances the variation in stimulus from S_i to S_f elicits a time-dependent response $\phi_i \rightarrow \phi_M \rightarrow \phi_f$ where ϕ_M represents the maximum in the response, and ϕ_f the final steady-state value to which the system settles. The stimulus can either remain at the level S_f – as for many hormones, or for chemotactic stimuli – or decrease in time, owing to hydrolysis as in the case of cAMP signals in *D. discoideum*. Even when the stimulus remains constant, the ensuing response can decrease in time. This *adaptation* phenomenon, known in most sensory and hormonal systems, often results from the modification or desensitization of the receptor upon constant stimulation (Koshland, 1981).

Since the physiological significance of the response is generally linked to the initial variation from ϕ_i to ϕ_M, amplification can be defined in such a time-dependent process as

$$A = \left(\frac{\phi_M - \phi_i}{\phi_i}\right) \Big/ \left(\frac{S_f - S_i}{S_i}\right). \tag{28}$$

As an example, we may consider the response of the cAMP signalling system in *D. discoideum* to an increase in stimulus, i.e. extracellular cAMP. Roos *et al.* (1975) have quantified the amplification capability of this system by relating the number of cAMP molecules synthesized intracellularly to the number of molecules of cAMP added into the extracellular medium. Such a definition embodies both the magnitude and sensitivity amplifications discussed above and elsewhere (Koshland *et al.* 1982). A measure of sensitive amplification can be gained by application of equation (28). However, since the basal extracellular cAMP level prior to stimulation is not precisely known, this question can best be investigated in the model for the cAMP signalling system discussed in the preceding section with respect to multiple steady-state transitions.

In certain well-defined conditions the adenylate cyclase reaction in *D. discoideum* behaves as an excitable system. Then the dose-response curve linking the level of intracellular cAMP to the extracellular cAMP stimulus exhibits a sharp threshold (Goldbeter & Segel, 1977). A variation in normalized cAMP stimulus from 1 to 1·848 results in the synthesis of a pulse of intracellular cAMP, the latter going from a normalized value of 10 to a maximum value close to 200. The cAMP response is transient as both intra- and extracellular cAMP return to their pre-stimulation levels. The amplification achieved by the

excitable system yields a value of $A = 22{\cdot}4$. This order of magnitude compares with the highest amplifications seen in allosteric or covalently modified enzymes.

The reason for the particular amplification properties of the system lies in the fact that cAMP in *D. discoideum* acts both as primary and second messenger. The resulting autocatalytic regulation of adenylate cyclase is responsible for excitability. Amplification factors closer to (or smaller than) 1 are to be expected for the temporal response of non-excitable biochemical systems.

III. Conclusions

The amplification of a stimulus is a necessity in biological systems. Whether the stimulus be a substrate, an effector, a sensory signal, a hormone or a neurotransmitter, it will be necessary in many circumstances to amplify the initial signal in order to get an appropriate biological response. There are roughly two categories into which amplification can be divided which we will call magnitude amplification and sensitivity amplification.

Magnitude amplification will refer to any process in which the number of the output molecules which carry the response to the next component of the system is much greater than the input stimulus. Processes such as the activation of an enzyme, which converts many molecules of a compound such as ATP to cyclic AMP, or an ion gate, which allows many molecules of sodium to enter a cell under the stimulus of a neurotransmitter, would be examples. It should be emphasized that in magnitude amplification the inhibition of a reaction can be as effective as the acceleration of one; in fact in a visual receptor it is the turning off of the sodium ions which is effective in propagating the initial stimulus. An analysis of these systems indicates that the cell has little trouble in achieving very large numbers for magnitude amplification. It in fact almost invariably designs automatic adaptive mechanisms to shut down the amplification before it becomes excessive.

Magnitude amplification can be achieved either by a single enzyme or by a cascade process, and indeed it appears to be carried out by both processes in a cell or in an organism; blood clotting and complement fixations are examples that are known of such cascade processes leading to magnitude amplification. It is not apparent whether this is the main purpose of such cascades, since in many cases the same process might well be achieved by a single enzymic reaction.

Sensitivity amplification is defined as the percentage change in a response relative to the percentage change in the stimulus, and is more difficult for the cellular system to achieve. In the foregoing analysis we have described five basic mechanisms for such sensitivity amplification. The first of these is the classical allosteric co-operativity. In this mechanism, a single enzyme by subunit interactions can give a higher percentage change in the output, for example the molecules of product generated by an allosteric enzyme, than the percentage change in the allosteric effector. Although very high numbers could be obtained from highly co-operative enzymes, Hill coefficients above 4 are rarely observed as most regulatory proteins contain four or less subunits, putting a practical limit on the theoretical factor for a Hill coefficient. As shown in Table 1, the maximum amplification factor for a Hill coefficient of 4 is 24. These values were calculated for an initial velocity which was 1 % of the maximum velocity, and therefore cover a reasonable physiological range. If a much lower starting point were chosen, this number could be increased slightly. Since this figure is for an enzyme with a very large Hill coefficient operating over an optimal range, in practical terms allosteric co-operativity will probably give numbers appreciably lower than this most of the time.

The second type of sensitivity amplification can be achieved by multistep sensitivity. In the cascade process, a given effector or its messenger can influence many steps in the pathway. The simplest example would be if a single effector activates an enzyme in the forward pathway and inhibits an enzyme in the reverse pathway. Another example would be a single effector activating a number of different enzymes in the same pathway. Thus, cyclic AMP acts on the cyclic AMP-dependent protein kinase to activate the phosphorylation and inhibit the action of glycogen synthetase. It also enters at five points in these two pathways, activating not only phosphorylase kinase but also causing phosphorylation of inhibitor I, which inhibits the phosphatase in three separate steps in the pathway. Multi-step sensitivity could give a sensitivity proportional to the number of steps in which the effector acts, as indicated by the expressions listed in Table 3 for the case of two effector inputs. Moreover, each of the steps would have to be in the optimal range for the effects to be multiplicative. One can calculate the maximum amplification available by this mechanism by consulting Table 1 again, in which now the value n for the number of multiple entries is substituted for the Hill coefficient. Such a calculation would depend of course on the detailed kinetics, but it would give a fairly good indication of the optimal numbers.

The third potential device for sensitivity amplification is zero-order ultrasensitivity (Goldbeter & Koshland, 1981). This type of co-operativity depends on the kinetic characteristics of the modifying enzymes and the fact that the quantity of the target protein is conserved. It is found that outside the first-order region large amplification factors could be observed in a monocyclic cascade. A value of 78 is obtained in a system in which the target protein exists in quantities which are 100-fold greater than the K_m of the converter enzymes. If this factor is obtained, this number would be achieved if a single effector activated (or inhibited) in a Michaelian manner a single converter enzyme in a monocyclic modification system. A somewhat larger number could be obtained if the effector molecule inhibited one converter enzyme and activated another one, for example inhibiting a phosphatase and activating a kinase. The amplification factor would decrease, however, as the converter enzymes shift towards the first-order region, or other suboptimal conditions such as non-productive binding occur. Thus zero-order ultrasensitivity, like the two other alternatives, requires a system which operates over an appropriate range with the proper kinetic constants. It has, however, a major advantage in that it can be enhanced in a cascade process so that the amplification factor in one cycle can be multiplied by those achieved in the next cycles to produce very large numbers.

The mathematical analysis indicates that any such features of multiplicative cascades require rather stringent conditions for the various enzyme molecules. As was pointed out in the calculations, the initial quantities of the target proteins must initially be in the 1 % or less than 1 % range to obtain the optimal amplification. Moreover, the target proteins in the second and third cascade must be in sufficient quantities to saturate the converter enzymes of the previous cascades. Hence, conditions of enzyme concentration and enzyme kinetics will be required to obtain multi-cascade effects. This may not be a serious limitation, however, since an overall amplification factor of greater than 100 would seem to have little value in a physiological process. The fourth method involves substrate cycles, with the qualifications of zero-order rates or allosteric effects as described above. The fifth method is multi-steady-state transitions, which can give amplification factors greater than those mentioned above but which require stringent conditions on the kinetics and the parameters of the physiological system.

These results show that sensitivity amplification can be achieved up to factors of 10–100 for a single step, but they contrast with the

amplification factors available in magnitude amplification which can occur in the 10^3–10^5 range. Thus sensitivity amplification is in a different scale from magnitude amplification generally. However, there are similarities in the application of each of these factors. Most of the time, it is probably unnecessary to have sequential, large-magnitude amplification factors (Koshland *et al.* 1982). In most cases, a large amplification will be followed by a large diminution, for example a large amount of epinephrine is produced by the adrenal medulla and it is then diluted extensively as it travels through the bloodstream. In the same way, sensitivity amplification probably does not need sequential amplification factors, but is probably designed to provide a sensitivity amplification followed by a sluggish system which needs large factors to activate it. Thus, alternating amplification and diminution is probably the most prevalent physiological organization. Finally, the question arises as to when the physiological system needs magnitude amplification and sensitivity amplification. It is clear that magnitude amplification will be needed whenever a small amount of signal must be translated into a significant output response. The amount of hormone travelling through the blood is obviously too small in quantity to cause major shifts in the enzymes directly involved in metabolic processes. The signal therefore must be amplified, e.g. through the adenylate cyclase system, to produce a significant response.

Two circumstances requiring percentage changes over background level can immediately be envisaged. In one case, an adaptive system operates at a dampened response level because the receptor system has been desensitized to prevent excessive stimulation. The second example would be a futile cycle system in which both pathways are operating at a subdued level. It will frequently be important for these systems to be sensitive to small fluctuations in the levels of effectors in the environment. In that case, sensitivity amplification would provide an excellent device to distinguish a true signal over the background noise.

The problem which arises in each of these cases is that the cyclic process required to maintain the system in readiness for a new signal is frequently expensive in terms of energy. Calculations of the level of chemoeffectors in cells indicate that futile cycles are not entirely shut off, and that the textbook assumption that the activation of one pathway inhibits the other pathway to zero velocity is not correct. That calculation, however, is based on classical Michaelis–Menten-type kinetics. In the kinetic analysis shown above there is the

opportunity, however, to have a pathway essentially shut down in the absence of a signal. As shown in Fig. 13, the levels of chemoeffector operating in the Michaelis–Menten region can change the level of the protein Z from 99 % inactive to 99 % active over a very modest range of effector concentration. Thus, one of the important uses of sensitivity amplification may well be to preserve the economy of the system in the absence of stimuli and yet have a system which can be turned on to optimal functioning in the presence of small changes in the environmental stimuli.

IV. Summary

The potentialities and limitations of signal amplification are examined. Sensitivity amplification, defined as the ratio of percentage change in response to percentage change in stimulus, is extremely important in propagating responses to stimuli which exist at background levels in the environment, in the communication system between cells, and in the metabolic control within cells. The phenomena which can provide sensitivity amplification are allosteric proteins, covalent modification cascades, multistep inputs, substrate cycles, and multi-steady-state transitions. The amplification factors identified with each type of process are evaluated. In general, it is concluded that quite large factors can exist but that the conditions necessary to obtain the large factors are more stringent than previously expected.

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