An Amplified Sensitivity Arising from Covalent Modification in Biological Systems

Albert Goldbeter, and Daniel E. Koshland

PNAS 1981;78;6840-6844
doi:10.1073/pnas.78.11.6840

Proc. Natl. Acad. Sci. USA
Vol. 78, No. 11, pp. 6840–6844, November 1981
Biochemistry

An amplified sensitivity arising from covalent modification in biological systems

(protein modification/metabolic regulation/switch mechanism/enzyme cascades)

ALBERT GOLDBETER† AND DANIEL E. KOSHLAND, JR.

Department of Biochemistry, University of California, Berkeley, California 94720

Contributed by Daniel E. Koshland, Jr., August 11, 1981

ABSTRACT **The transient and steady-state behavior of a reversible covalent modification system is examined. When the modifying enzymes operate outside the region of first-order kinetics, small percentage changes in the concentration of the effector controlling either of the modifying enzymes can give much larger percentage changes in the amount of modified protein. This amplification of the response to a stimulus can provide additional sensitivity in biological control, equivalent to that of allosteric proteins with high Hill coefficients.**

Biological systems must respond to internal and external variations such as the depletion of nutrients, the variations in hormone levels, and the reception of sensory signals. The stimuli are processed to change the activities of enzymes controlling pathways in the biological system. Two basic phenomena play a large role in this processing: allosteric changes in protein conformation and covalent modification of proteins.

Since the findings of Cori and Green (1) and Krebs and Fischer (2) that glycogen phosphorylase exists in two forms, phosphorylated and dephosphorylated, the number of proteins that have been found to be controlled by covalent modification has increased steadily. Covalent modification has been identified with control in carbohydrate metabolism, fat metabolism, sensory systems, muscular contraction, protein synthesis, nitrogen metabolism, and malignant transformation (3–10).

In phenomena such as sensing, and in the regulation of metabolism, it is important that the "turning on" of one pathway and the "turning off" of another be sensitive to relatively small changes in effector concentration. One known mechanism for increasing the sensitivity of a system is through cooperative interactions. Another is the effect of a ligand that enters at more than one step in a pathway—e.g., to activate one enzyme and inhibit another, as happens in the glycogen cascade (4).

Stadtman and Chock (11–13) have made an extensive and elegant analysis of the mathematical relationships of cascades. Using simplifying assumptions such as first-order kinetics and negligible concentrations of the Michaelis complexes, they were able to quantitate the amplification caused by multiple inputs, the interrelationship of output rates to parameter changes in the modifying enzymes, and the acceleration of rate processes. To show that their conclusions were not dependent on the simplifying assumptions, they also utilized a more general quartic equation involving 200 terms, which was solved by computer approximation methods (11). This more complex equation supported the conclusions obtained from the simpler equations, but specific cases revealed kinetic curves for certain parameter values that were apparently more sensitive to changes than the initial curves. It occurred to us that further insight into the nature of covalent regulation was possible, if the differential equations could be solved analytically outside the first-order region.

This analysis has been achieved, and the results reveal that there is an added sensitivity inherent in covalent modification schemes when one or more of the converter enzymes operate in the "zero-order" region—i.e., region of saturation with respect to protein substrate. Thus there is a property of covalent systems that, in the absence of allosteric cooperativity and multiple inputs, can generate sensitivity equivalent to cooperative enzymes with high Hill coefficients. The derivations leading to and the implications of this finding are discussed below. For convenience, we shall use the term "ultrasensitivity" to describe an output response that is more sensitive to change in stimulus than the hyperbolic (Michaelis–Menten) equation.

Steady-state behavior of modification system

We shall consider a covalent modification system in which a protein can exist in the unmodified form W and the modified form W* as shown in Eqs. 1 and 2. The interconversion of the forms is catalyzed by two converter enzymes, E_1 and E_2, according to Eqs. 1 and 2.

$$W + E_1 \underset{d_1}{\overset{a_1}{\rightleftarrows}} WE_1 \overset{k_1}{\rightarrow} W^* + E_1 \qquad [1]$$

$$W^* + E_2 \underset{d_2}{\overset{a_2}{\rightleftarrows}} W^*E_2 \overset{k_2}{\rightarrow} W + E_2. \qquad [2]$$

It is assumed that the other substrates and products for modification and demodification, corresponding in specific cases to ATP, *S*-adenosylmethionine, H_2O, etc., are present at constant levels and can therefore be included in the kinetic constants without loss of generality. The kinetic equations governing the time evolution of such a system are:

$$\begin{aligned}
\frac{d[W]}{dt} &= -a_1[W][E_1] + d_1[WE_1] + k_2[W^*E_2] \\
\frac{d[WE_1]}{dt} &= a_1[W][E_1] - (d_1 + k_1)[WE_1] \\
\frac{d[W^*]}{dt} &= -a_2[W^*][E_2] + d_2[W^*E_2] + k_1[WE_1] \\
\frac{d[W^*E_2]}{dt} &= a_2[W^*][E_2] - (d_2 + k_2)[W^*E_2].
\end{aligned} \qquad [3]$$

These equations are complemented by conservation equations 4, 5, and 6.

$$W_T = [W] + [W^*] + [WE_1] + [W^*E_2] \qquad [4]$$

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

† On leave from the Faculté des Sciences, Université Libre de Bruxelles, B-1050 Brussels, Belgium.

$$E_{1T} = [E_1] + [WE_1] \quad [5]$$

$$E_{2T} = [E_2] + [W^*E_2]. \quad [6]$$

In the initial derivation we shall consider the concentration W_T to be in such large excess over E_1 and E_2 that the Michaelis complexes, WE_1 and W^*E_2 can be neglected in Eq. 4. Later we shall analyze the effect on the system if these terms are not negligible as well as the effect of nonproductive binding terms W^*E_1 and WE_2.

In the following mathematical relationships we shall use W and W* to indicate mole fractions—i.e., $[W]/W_T$ and $[W^*]/W_T$. At steady state $k_1[WE_1] = k_2[W^*E_2]$, which leads to Eq. 7 for the fraction of modified protein.

$$W^* = \frac{\left(\frac{V_1}{V_2} - 1\right) - K_2\left(\frac{K_1}{K_2} + \frac{V_1}{V_2}\right) + \left(\left[\frac{V_1}{V_2} - 1 - K_2\left(\frac{K_1}{K_2} + \frac{V_1}{V_2}\right)\right]^2 + 4K_2\left(\frac{V_2}{V_2} - 1\right)\left(\frac{V_1}{V_2}\right)\right)^{1/2}}{2\left(\frac{V_1}{V_2} - 1\right)} \quad [7]$$

In this expression

$$V_1 = k_1E_{1T},\ V_2 = k_2E_{2T},\ K_1 = \frac{d_1 + k_1}{a_1W_T} = K_{m1}/W_T,$$

$$\text{and } K_2 = \frac{d_2 + k_2}{a_2W_T} = K_{m2}/W_t.$$

Note that K_1 and K_2 are the respective Michaelis constants K_{m1} and K_{m2} divided by W_T. The conservation relationship gives $W = 1 - W^*$. Eq. 7 can be rearranged to give the relationship between V_1/V_2 and a given value of W* at steady state.

$$\frac{V_1}{V_2} = \frac{W^*[1 - W^* + K_1]}{(1 - W^*)(W^* + K_2)}. \quad [8]$$

For the special case when $V_1 = V_2$, Eq. 7 has to be replaced by Eq. 9 to obviate indeterminate forms.

$$W^* = = \frac{K_2}{K_1 + K_2}. \quad [9]$$

A plot of the mole fractions W and W* at steady state as a function of the ratio V_1/V_2 is shown in Fig. 1 for various values of K_1 in a system in which $K_1 = K_2$. At low values of K_2 and K_1—i.e., at enzyme saturation—there is an abrupt change from largely unmodified protein to largely modified protein over a very small change in the V_1/V_2 ratio. For large values of K_2 and K_1, the curve is quite shallow. This leads to the first significant conclusion of the study, that the W-to-W* transition occurs far more abruptly when the converting enzymes E_1 and E_2 are saturated than when they operate in the first-order region.

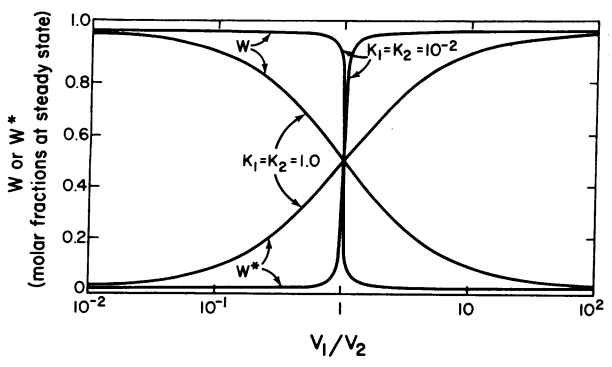


FIG. 1. Fractions of modified (W*) and unmodified (W) protein at steady state as a function of the ratio of the modification rates. The curves are established according to Eq. 7 for the values of the constants K_1 and K_2 indicated.

Response coefficient R_v

The steep transition curves of Fig. 1 illustrate an additional type of increased sensitivity. It originates from the kinetic interplay between two converter enzymes operating in opposite directions, outside the domain of first-order kinetics, and on a target protein whose total quantity is conserved. We shall therefore refer to the latter phenomenon as "zero order ultrasensitivity," meaning zero-order in the protein substrate, which saturates the enzyme surface, and ultrasensitive because it is more sensitive than a Michaelis–Menten response to stimulus.

To evaluate the steepness of the transition in covalent modification we shall use a response coefficient R_v defined in analogy to the allosteric response coefficient R_s. This coefficient is defined as $(S_{0.9}/S_{0.1})$, the ratio of the substrate concentration required to give 90% saturation relative to the concentration required to give 10% saturation (14). The analogous quantity R_v is defined as the ratio of V_1/V_2 when 90% of the protein is in the modified form to V_1/V_2 when 10% is in the modified form.

The ratio R_v is a function of K_1 and K_2 as given in Eq. 10.

$$R_v = \frac{81(K_1 + 0.1)(K_2 + 0.1)}{(K_1 + 0.9)(K_2 + 0.9)}. \quad [10]$$

In the limiting case when K_1 and K_2 are much greater than 1, the value of R_v is 81, exactly the same as the ratio of the ligand concentrations needed to go from 10% to 90% saturation of a Michaelis–Menten protein. As K_1 and K_2 decrease, the value of R_v decreases, approaching unity for the limit at which K_1 and K_2 are both much smaller than 0.1. Clearly this limit can never be reached because it would indicate an impossibly abrupt transition. However, R_v would approach a value of 1 (infinite ultrasensitivity) as the modifying enzymes both become saturated with the substrates W and W*.

Using these equations, it is now possible to compare the steepness of the covalent transition with the steepness of allosteric proteins with various Hill coefficients (14). The value of R_s derived from the Hill equation is given by the expression

$$S_{0.9}/S_{0.1} = R_s = (81)^{1/n_H}. \quad [11]$$

in which n_H is the Hill coefficient. This value is plotted in Fig. 2 as a function of n_H along with values of R_v as a function of K_1 and K_2.

A value of $R_s = 4.5$ corresponds to a Hill coefficient of 2.9, the observed coefficient for hemoglobin (15), a protein whose cooperativity is essential to performance of its function. The same degree of steepness is obtained by a reversible covalent modification system in which $K_1 = K_2 = 10^{-1}$. A covalent modification scheme having the constants $K_1 = K_2 = 10^{-2}$ would correspond in sensitivity to the saturation curve of a cooperative protein with a Hill coefficient greater than 13.

Effect of nonproductive binding and appreciable concentration of enzyme–substrate complexes

As previously mentioned, the effect of higher concentrations of the nonproductive forms (E_1W^* and E_2W) and the presence

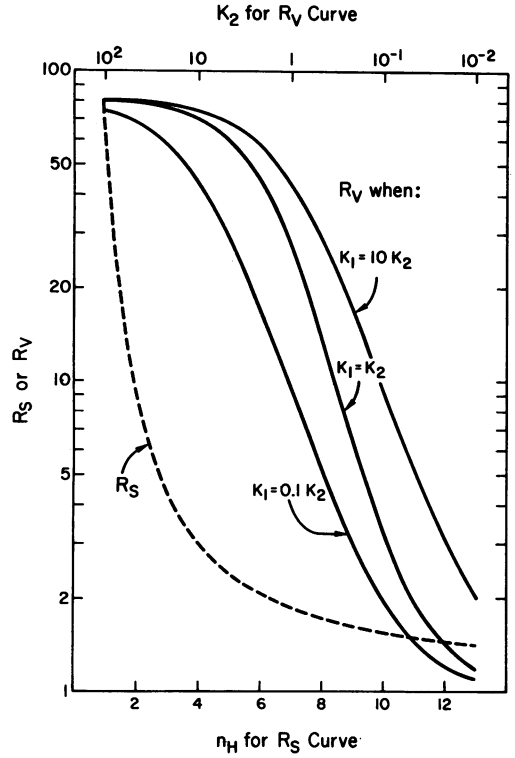


FIG. 2. Comparison of response coefficients for sensitivity in covalent modification (R_c) and allosteric (R_s) systems. R_c represents the ratio of V_1/V_2 values required to give 90% and 10% modified protein, respectively. R_s represents the ratio of ligand concentrations needed to give 90% and 10% saturation or maximal catalytic rate. R_s is varied as a function of n_H (Eq. 11) and R_c as a function of K_1 and K_2 (Eq. 12). Thus reading the curve shows that $R_s = 3$ when $n_H = 4$. To compare the respective sensitivities, one may imagine a horizontal line intersecting the curves at the same R_s and R_c values. The W* vs. V_1/V_2 curve corresponding to that value of R_c will have the same steepness as the saturation curve of the R_s protein. If the mechanism that controls V_1/V_2 leads to a situation in which V_1/V_2 is proportional to S, the W* vs. S curve can be superimposed on the saturation curve of the allosteric protein.

of appreciable concentrations of the Michaelis intermediates need to be considered. The effect of the latter on the concentrations of free W and W* are shown in Fig. 3. It can be seen that values of 0.1 or less for E_{1T}/W_T and E_{2T}/W_T do not change the sensitivity significantly, but large values reduce the steepness dramatically.

If we consider the nonproductive complexes E_1W^* and E_2W whose equilibrium dissociation constants are denoted by K_{i1} and K_{i2}, the quantity R_c previously defined is given by Eq. 12.

$$R_c = \frac{81[K_1 + 0.1(1 + 9\rho_1)][K_2 + 0.1(1 + 9\rho_2)]}{[K_1 + 0.1(9 + \rho_1)][K_2 + 0.1(9 + \rho_2)]}. \quad [12]$$

Here $\rho_1 = K_{m1}/K_{i1}$ and $\rho_2 = K_{m2}/K_{i2}$.

Eq. 12 shows that for $\rho_1 = \rho_2 = 1$, the ratio R_c is equal to 81 regardless of the value of $K_1 = K_2$. Whenever ρ_1 and ρ_2 are greater than unity, the coefficient R_c will exceed 81, analogous to negative cooperativity in allosteric enzymes. For values of $K_1 = K_2$ smaller than 0.1, an R_c value equivalent to an $n_H = 0.8$ obtains when $\rho_1 = \rho_2$ and they are close to 2—i.e., when the product inhibition constants are half the Michaelis constants of the modifying enzymes. When nonproductive binding and Michaelis complexes are considered together, the influence to diminish sensitivity is less than additive. That is to be expected

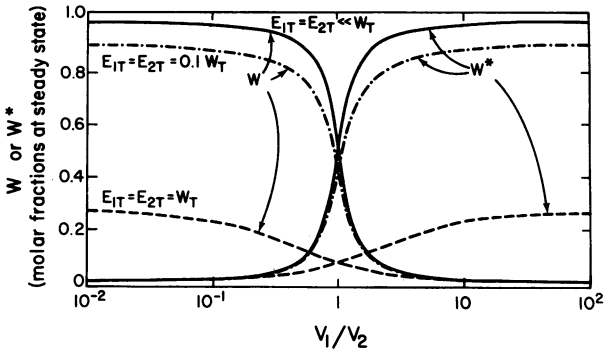


FIG. 3. Fractions of the modified and unmodified protein when concentrations of modifying enzymes E_1 and E_2 are not negligible with respect to W_T. The curves are established for $K_1 = K_2 = 10^{-1}$ by numerical resolution of a third-degree equation for W that is obtained when taking into account the complexes E_1W and E_2W^* in the conservation relationship for the target protein. Curves for W and W* are given for concentrations of the converter enzymes that are much less than, 10% of, and equal to W_T. The third-degree equation reads as follows:

$$W^3(1 - \alpha) + W^2\{(K_1 + K_2\alpha) + (1 - \alpha)[K_1 + \varepsilon_1 + \varepsilon_2\alpha - 1]\}$$
$$+ K_1W\{(K_1 + \alpha K_2) + (\alpha - 2) + (\varepsilon_1 + \varepsilon_2\alpha)\} - K_1^2 = 0$$

with $\varepsilon_1 = E_{1T}/W_T$, $\varepsilon_2 = E_{2T}/W_T$, and $\alpha = V_1/V_2$.

The other fractions are given by

$$W^* = 1 - W\left[1 + \frac{(\varepsilon_1 + \varepsilon_2\alpha)}{K_1 + W}\right],$$

$$E_1W = \varepsilon_1\left(\frac{W}{K_1 + W}\right), E_2W^* = \varepsilon_2\left(\frac{W^*}{K_2 + W^*}\right).$$

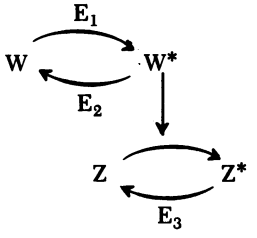
(W, W*, E_1W, and E_2W^* are the molar fractions of the corresponding species.)

because the protein complexes will be competing with each other.

How much these effects actually diminish the sensitivity of a covalent modification system to the environmental changes depends on the activity of the E_1W, E_2W^*, E_1W^*, and E_2W complexes. If, for example, W is an enzyme such as phosphorylase and its active site is free in the protein complexes, E_1W^* may be just as active as W*. In that case the plots of Fig. 1 will more closely represent the real change in the response than will the more dampened responses of Fig. 3.

Zero-order ultrasensitivity in a bicyclic cascade

The question arises as to whether the zero-order sensitivity obtained in a single cycle can be further enhanced in a multicyclic cascade. To answer this question, we consider the bicyclic system shown in Eq. 13, in which protein W*, modified in the first cycle, catalyzes the modification of a second target protein Z into Z*.



[13]

Fig. 4 illustrates the variation of the fractions of modified proteins, W* and Z*, as a function of the effector S that controls V_1/V_2. In the simplest mechanism S activates enzyme E_1 in a

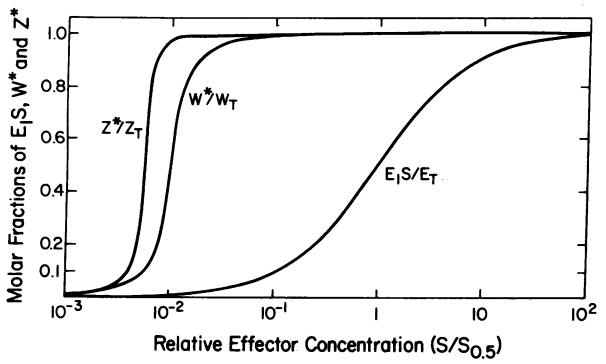


FIG. 4. Behavior of a bicyclic cascade. The molar fractions W* (product of the first cycle) and Z* (product of the second cycle) are shown as a function of the effector, S, which activates the converter enzyme E_1 (see Eq. 13). The fraction of active enzyme, E_1S, is equal to the saturation function, $S/(S_{0.5} + S)$. The values of R_s for the W* and Z* curves are 3.4 and 1.8, respectively. These R_s values correspond in sensitivity to Hill coefficients of 3.6 and 7.5 (see Fig. 2). The same values are obtained for R_c when W* and Z* are plotted with respect to (V_1/V_2). The curves for W* and Z* are obtained from Eq. 7 and from a similar equation for Z*, when taking the Michaelis constants of E_1 and E_2 equal to 0.1 W_T, and the Michaelis constants of W* and E_3 equal to 0.1 Z_T. The ratio V_1/V_2 is linked to S by the relationship $V_1/V_2 = 100S/(S_{0.5} + S)$; the ratio of modification rates for the second cycle, V_{W^*}/V_3, taken as 10W*. For these values, $W^* = Z^* = 0.01$ when $V_1/V_2 = 0.1$. The curves for W* and Z* are shifted to the right and reach lower asymptotic values for $S \gg S_{0.5}$ when $V_1/V_2 = S/(S + S_{0.5})$.

Michaelian fashion. Two significant features can be noticed. First, although the saturation of E_1 by S is Michaelian, the curves W* and Z* vs. S exhibit the form of positive cooperativity. Second, the curve for Z* is steeper than that for W*, although the same values for the normalized Michaelis constants were taken in the two cycles of the cascade. The Hill coefficients of enzymes that give curves of steepness equivalent to these would be 3.6 for the W* curve and 7.5 for Z* (see Fig. 2). Therefore additional cycles in a cascade provide the potentiality for increasing the sensitivity of an individual cycle as shown in other cases by Stadtman and Chock (5, 13).

Behavior of modification system in the first-order region

Mathematical equations can be derived to show that zero-order ultrasensitivity cannot occur when the converter enzymes operate entirely in the domain of first-order kinetics. When K_1 and $K_2 \gg 1$, the equation yielding W* at steady state in a monocyclic system reduces to

$$W^* = \frac{V_1/V_2}{(K_1/K_2) + (V_1/V_2)}. \qquad [14]$$

Because this expression is hyperbolic in form, R_c is 81, which corroborates the results for large values of K_1 and K_2 in Fig. 2. Whenever V_1/V_2 is proportional to S, the curve yielding W* as a function of S will also have an R_s of 81. Extending this reasoning, it can readily be shown that the curve for Z* in a bicyclic cascade will also be hyperbolic when all converter enzymes operate in the first-order region. Thus the condition for zero-order ultrasensitivity is that one or more of the converter enzymes operate outside the first-order region.

Time required for change

The possibility of an abrupt change from largely unmodified to largely modified enzymes, or vice versa, offers the opportunity for a highly sensitive regulatory control, but whether such a mechanism is of physiological importance depends on whether it can reach steady state within a reasonable interval of time.

The time evolution of the fraction of modified protein is governed by the differential equation 15

$$\frac{dW^*}{dt} = \frac{V_2}{W_T}\left[\frac{(V_1/V_2)(1 - W^*)}{K_1 + 1 - W^*} - \frac{W^*}{K_2 + W^*}\right] \qquad [15]$$

when a quasi-steady-state assumption is made for the various enzyme–substrate complexes in Eq. 3. Accordingly, calculations were made for some of the most sensitive systems (very low R_c) and some of the least sensitive (very large R_c) to determine the time required to change from one steady-state situation to the other. The results show that the change from the initial steady state to the final state is quite rapid and relatively independent of the steepness of the transition. When kinetic values measured for actual enzymes in the glycogen cascade are used, the shift is calculated to occur in seconds in some cases and in minutes in others. Thus the switch from one activity level to the other occurs within physiologically significant time intervals—e.g., matching those observed for the phosphorylation of glycogen phosphorylase *b* (2, 17, 18), the dephosphorylation of glycogen synthetase (19, 20), and the adenylylation of glutamine synthetase (21, 22).

Although the change in V_1/V_2 can occur rapidly, this may not always be the case. The control of E_1 and E_2 may involve several reactions, some of which may be slow steps. The effect of a slow variation in V_1/V_2 is shown in Fig. 5. In the curves marked I, V_1/V_2 rises from 0.5 to 1.2 with a half-time of 70 sec. The consequent change in W* shows an abrupt transition but only after a time lag. If the change in V_1/V_2 is not large enough to exceed the threshold of stimulation (illustrated in Fig. 5 by the curves marked II for a change in V_1/V_2 from 0.5 to 0.8) the resulting W* curve never shows a dramatic increase. In fact, the threshold effect and time lag shown here have a striking resemblance to those observed for the activation of liver glycogen synthetase by glucose (19, 20).

Discussion

The mathematical analysis of covalent modification schemes has revealed an intriguing and surprising result. It is that an amplification in response can arise from the kinetics of covalent modification analogous to the cooperativity present in allosteric enzymes with Hill coefficients greater than 1. The ingredients that lead to this sensitivity are threefold: (*i*) the kinetics operate

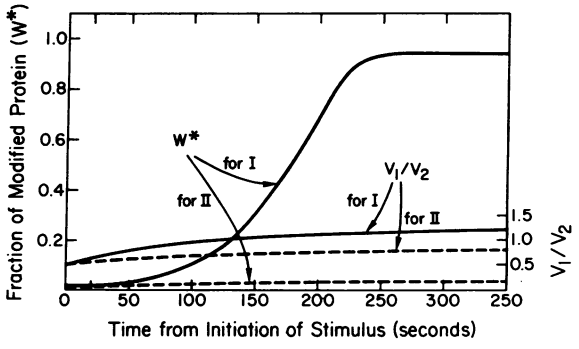


FIG. 5. Threshold effect and time lag in the time evolution of the fraction of modified protein. The curves for W* are obtained by integration of Eq. 15 with the indicated slow variation in V_1/V_2 from 0.5 to 1.2 (case I) and from 0.5 to 0.8 (case II). Other parameter values are $W_T = 100\ \mu M$, $K_1 = K_2 = 10^{-2}$, and $V_2 = 10\ \mu M\ sec^{-1}$. Similar time lags can be obtained for the bicyclic cascade (see Eq. 13 and Fig. 4) when the rise in W* from a low initial value precedes the rise in Z*.

at least in part in the zero-order region, (*ii*) the conservation relationships require W to decrease as W* increases and vice versa, and (*iii*) a steady state rather than an equilibrium is ultimately reached. This zero-order ultrasensitivity occurs even when a single effector acts in a noncooperative manner on one of the converter enzymes.

A simple explanation for the kinetic results is as follows. When both converter enzymes are saturated, the rate of formation of W* is $V_1 - V_2$—i.e., independent of W and W*. Such a rate will continue until either W or W* falls below the saturation level—i.e., W^*/K_2 or W/K_1 is 1. The rate will then drop and eventually reach the steady-state value. The smaller K_1 or K_2 or both, the more abrupt the transition. In the region of first-order kinetics, in contrast, any increase in the rate of E_1 will induce a rise in W* that will be counterbalanced immediately by the subsequent increase in the rate of E_2. Such a situation results in a less steep transition between W and W* as V_1/V_2 is varied.

In a normal Michaelian hyperbolic enzyme there must be an 81-fold change in ligand (substrate, inhibitor, or activator) to achieve an activity change from 10% maximal enzyme activity to 90% maximal enzyme activity. A cooperative enzyme with a Hill coefficient of 4 can give the same enzyme activity change with only a 3-fold variation in ligand concentration. The same sensitivity is apparently obtained in a properly designed covalent system even when the regulatory ligand binds hyperbolically to only one converter enzyme in the cascade. Moreover, if the covalent modification scheme has the optimal constants, it can achieve a sensitivity to ligand changes which exceeds that of enzymes that have Hill coefficients of 4. Such high Hill coefficients are a very great rarity among cooperative proteins, so this aspect of covalent modification offers great advantages for tight control of a biological system.

This advantage is not present if both of the modifying proteins are operating in the first-order region relative to the proteins modified. The mathematical analysis shows that in that region they will give the same kind of responsiveness as a Michaelis–Menten enzyme. In many cases, such a response may be adequate. However, in certain futile cycles or in an adaptive sensory system in which one pathway must be turned on and another pathway turned off, higher sensitivity may be needed and zero-order ultrasensitivity could provide a mechanism. Because phosphorylation has been identified with the *src* gene (23–25), it is intriguing to ask whether a change in sensitivity may be important in the loss of control identified with cancer cells.

Analysis of the kinetics shows that the time to obtain a new steady state after a stimulus can be slower than allosteric changes. This is understandable, and it is probably true of most covalent modifications. With the constants available for the cyclic AMP-dependent protein kinase–phosphorylase kinase–phosphorylase system, the new steady state can be achieved in the order of seconds. With higher turnover numbers for the converter enzymes, lower concentrations of the target proteins, or both, a millisecond time course could be achieved. The nonequilibrium steady state that is reached will be stable, because sustained temporal oscillations develop only when the converter enzymes are subjected to regulation by a target enzyme or one of its products (26).

The present findings mean that a multienzyme cascade with reversible converter enzymes such as kinases and phosphatases has three potential devices for enhancing its sensitivity beyond that expected from Michaelis–Menten kinetics: (*i*) the conventional "cooperative ultrasensitivity," which could occur for any enzyme with a Hill coefficient greater than 1; (*ii*) the opportunity for a given ligand (or its messenger) to act in more than one step, "multistep ultrasensitivity"; (*iii*) "zero-order ultrasensitivity," in which converter enzymes operating under saturating conditions amplify the response to a signal. A given pathway or cascade can use any one of these mechanisms or all three to enhance its sensitivity.

Simple extension of the mathematics shows that the sensitivity can be propagated and enhanced in a multicycle network. Such amplification is not automatic. It is important that the kinetic and binding constants for the second and third cycles be in the appropriate range or the initial sensitivity will be damped. However, the appropriate relationships cover a wide range of values and presumably are selected by evolution for those systems in which enhanced sensitivity is appropriate.

It should be emphasized that the data are not yet available to say with certainty that this device for added sensitivity is actually utilized in biological systems, although preliminary observations on phosphorylation systems in our laboratory are encouraging. The predicted relationships are consistent with the range of enzymatic values that have been observed, and knowledge of this possibility allows a search to determine whether such mechanisms exist in nature. If so, it provides a significant added mechanism for regulatory pathways to be sensitive to small changes in environmental stimuli.

The authors acknowledge valuable suggestions received from Earl Stadtman, Boon Chock, John Spudich, and William Ray. This work was supported by National Institutes of Health Grant AM 9765 and National Science Foundation Grant PCM75-16410. A.G. received support from a North Atlantic Treaty Organization Research Fellowship and from the Belgian Government: Actions de Recherche Concertées 76/81 II 3.

1. Cori, G. T. & Green, A. A. (1943) *J. Biol. Chem.* **151**, 31.
2. Krebs, E. G. & Fischer, E. H. (1956) *Biochim. Biophys. Acta* **20**, 150–157.
3. Krebs, E. G. & Beavo, J. A. (1979) *Annu. Rev. Biochem.* **48**, 923–959.
4. Cohen, P. (1979) *Curr. Top. Cell. Regul.* **14**, 118–196.
5. Chock, P. B., Rhee, S. G. & Stadtman, E. R. (1980) *Annu. Rev. Biochem.* **49**, 813–843.
6. Greengard, P. (1978) *Science* **199**, 146–152.
7. Uy, R. & Wold, F. (1977) *Science* **198**, 890–896.
8. Springer, M. S., Goy, M. F. & Adler, J. (1979) *Nature (London)* **280**, 279–284.
9. Koshland, D. E., Jr. (1979) *Physiol. Rev.* **59**, 811–862.
10. Sutherland, E. W. (1972) *Science* **177**, 401–408.
11. Stadtman, E. R. & Chock, P. B. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2761–2765.
12. Chock, P. B. & Stadtman, E. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2766–2770.
13. Stadtman, E. R. & Chock, P. B. (1978) *Curr. Top. Cell. Regul.* **13**, 53–93.
14. Koshland, D. E., Jr., Nemethy, G. & Filmer, D. (1966) *Biochemistry* **5**, 365–385.
15. Perutz, M. R. (1979) *Annu. Rev. Biochem.* **48**, 327–386.
16. Lee, E. Y. C., Silberman, S. R., Ganapathi, M. K., Petrovic, S. & Paris, H. (1980) *Adv. Cyclic Nucleotide Res.* **13**, 95–131.
17. Fischer, E. H., Heilmeyer, L. M. & Haschke, R. H. (1971) *Curr. Top. Cell Regul.* **4**, 211–251.
18. Danforth, W. H., Helmreich, E. & Cori, C. F. (1962) *Proc. Natl. Acad. Sci. USA* **48**, 1191–1199.
19. Stalmans, W., De Wulf, H., Hue, L. & Hers, H. G. (1974) *Eur. J. Biochem.* **41**, 127–134.
20. Hers, H. G. (1976) *Annu. Rev. Biochem.* **45**, 167–189.
21. Schutt, H. & Holzer, H. (1972) *Eur. J. Biochem.* **26**, 68–72.
22. Segal, A., Brown, M. S. & Stadtman, E. R. (1974) *Arch. Biochem. Biophys.* **161**, 319–327.
23. Collett, M. S. & Erikson, R. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2021–2024.
24. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1311–1315.
25. Levinson, A. D., Opperman, H., Levintow, L., Varmus, H. E. & Bishop, J. M. (1978) *Cell* **15**, 561–572.
26. Martiel, J. L. & Goldbeter, A. (1981) *Biochimie* **63**, 119–124.