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Superiority of interconvertible enzyme cascades in metabolic regulation: Analysis of multicyclic systems

(cyclic cascade/covalent modification/allosteric effector/glutamine synthetase/glycogen phosphorylase)

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ABSTRACT *Escherichia coli* glutamine synthetase and glycogen phosphorylase are prototypes for models of "closed" and "opened" bicyclic cascade systems. Steady-state functions relating the fractional activation of interconvertible enzymes to the concentrations of allosteric effectors and to the catalytic constants of the several converter enzymes in such cascades were determined. The study shows that when the active form of an interconvertible enzyme in one cycle catalyzes the covalent modification of the interconvertible enzyme in a second cycle, the two cycles become coupled, such that the fractional activity of the second interconvertible enzyme is a multiplicative function of all parameters in both cycles, i.e., of 14 and 18 parameters for the closed and the opened bicyclic cascade, respectively. Therefore, from the standpoint of cellular regulation, bicyclic cascades are superior to the monocyclic cascades analyzed previously [E. R. Stadtman & P. B. Chock (1977) *Proc. Natl. Acad. Sci. USA* 74, 2761–2765], because: (*i*) they can respond to a greater number of allosteric effectors; (*ii*) they can achieve much greater amplification of responses to primary stimuli (e.g., with only 2-fold changes in each parameter the amplification factors of one-cycle and two-cycle cascades are 320 and 102,400, respectively); (*iii*) they can generate a sigmoidal response (Hill numbers of >2) of interconvertible enzyme activity to increasing concentrations of an allosteric effector. This is because there are more steps in a bicyclic cascade at which a given effector can interact. A similar analysis of multicyclic cascade systems shows that the capacity for amplification increases exponentially as the number of cycles in the cascade increases. In addition, regulation by cyclic cascades can achieve enormous variability of the fractional activity of the interconvertible enzyme by shifting the steady-state distribution between active and inactive forms. One equivalent of ATP is consumed in each interconversion cycle to provide the energy needed to maintain the steady-state activity of the modified enzyme at a metabolically required level. Therefore, the decomposition of ATP associated with the cyclic cascade is not a wasteful process.

In a previous communication (1), we reported the steady-state analysis of monocyclic cascade models consisting of two oppositely directed "converter" enzymes that catalyze the interconversion of a single interconvertible enzyme. The analysis showed that such simple cascade systems are endowed with extraordinary capacities to be regulated by multiple metabolites; they are remarkably flexible with respect to the response patterns elicited by increasing concentrations of individual effectors; and they can achieve huge amplification of responses to primary allosteric stimuli.

More elaborate bicyclic cascades are involved in the regulation of *Escherichia coli* glutamine synthetase (2, 3) and glycogen phosphorylase (4). These cascades consist of two linked interconvertible enzyme cycles. The present report describes a steady-state analysis of such bicyclic cascade models. In addition we report the results of a more general study of multicyclic cascade systems, i.e., systems composed of n cycles. These studies demonstrate that compared to the monocyclic cascades previously discussed (1), the bicyclic and multicyclic cascade systems are endowed with considerably greater allosteric control potential, amplification potential, and overall flexibility, and in addition are capable of generating highly cooperative types of response to increases in effector concentrations.

RESULTS

Opened bicyclic system

Fig. 1*A* shows an opened bicyclic system consisting of two interconvertible enzymes, I_1 and I_2. The first cycle involves the ATP-dependent covalent modification (not shown) of I_1, which converts it from the inactive form I_{1i} to its active form I_{1a}. This conversion is catalyzed by the active form, E_a, of a "converter" enzyme, E. The conversion of I_{1i} to I_{1a} is opposed by the action of another converter enzyme R_a that catalyzes the regeneration of I_{1i} from I_{1a}. The second cycle involves a similar interconversion of I_2 between catalytically inactive (I_{2i}) and active (I_{2a}) forms. Coupling of the two cycles derives from the fact that the active form of the first interconvertible enzyme, I_{1a}, is the "converter" enzyme that catalyzes the conversion of I_{2i} to I_{2a}.

To demonstrate the allosteric control potential of the bicyclic cascade, it is assumed that binding of the allosteric effectors e_1, e_2, and e_3 to inactive forms of the converter enzymes E_i, R_{1i}, and R_{2i} is necessary to generate the active forms E_a, R_{1a}, and R_{2a}, respectively. In variations of the model (discussed later), it is assumed that R_{1a} and R_{2a} are inhibited by e_1 and that e_1 must also bind to I_{1a} to produce a catalytically active enzyme for the conversion of I_{2i} to I_{2a}. It is evident from Fig. 1*A* that coupling of the covalent modification and demodification reactions will lead to a dynamic process in which I_1 and I_2 undergo cyclic interconversions. For a given metabolic activation, the specific activity of the target enzyme, I_2, will be determined by the steady-state distribution between I_{2i} and I_{2a}.

In deriving the steady-state expressions, the assumptions made are: (*i*) rapid equilibrium is maintained for the formation of enzyme–enzyme and enzyme–effector complexes, (*ii*) the concentrations of enzyme–enzyme complexes are negligibly low so that $[I_2] \simeq [I_{2i}] + [I_{2a}]$; $[I_1] \simeq [I_{1a}] + [I_{1i}]$; $[E] \simeq [E_i] + [E_a]$; $[R_1] \simeq [R_{1i}] + [R_{ia}]$; and $[R_2] \simeq [R_{2i}] + [R_{2a}]$, in which $[I_2]$, $[I_1]$, $[E]$, $[R_1]$, and $[R_2]$ are total concentrations of I_2, I_1, E, R_1, and R_2, respectively; and (*iii*) the concentrations of the allosteric effectors e_1, e_2, and e_3 are maintained at constant levels for a given metabolic state. Furthermore, the ATP dependency is ignored because the concentration of ATP in the cell is normally maintained at a constant level that is several orders of magnitude higher than the substrate enzyme concentrations. The results obtained from the expressions derived with these assumptions are compatible with those obtained with a much more complex expression derived from a less restricted assumption (1).

With the above assumptions, the opened bicyclic cascade

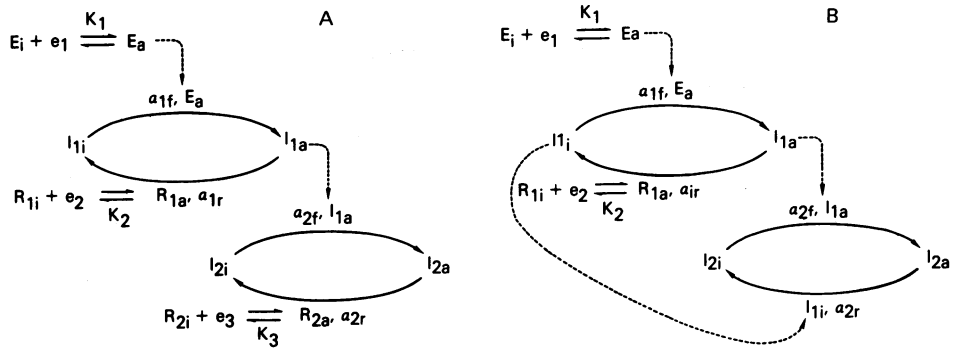


FIG. 1. Schematic representation of (*A*) an opened bicyclic cascade system and (*B*) a closed bicyclic cascade system. The parameters α_{nf} and α_{nr} are defined as: $\alpha_{nf} = (k_{nf}/K_{nf})$, $\alpha_{nr} = (k_{nr}/K_{nr})$, in which $n = 1, 2$; k_{nf} and k_{nr} are specific rate constants for the forward and reverse reaction designated. K_1, K_2, K_3, K_{nf}, and K_{nr} are dissociation constants for the designated equilibria. The indexes *i* and *a* indicate the enzyme as inactive or active, respectively.

system involves the following covalent modification reactions:

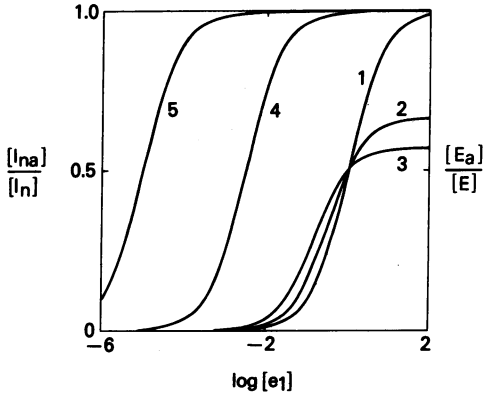
$$I_{1i} + E_a \overset{K_{1f}}{\rightleftharpoons} E_a I_{1i} \xrightarrow{k_{1f}} E_a + I_{1a}$$

$$I_{1a} + R_{1a} \overset{K_{1r}}{\rightleftharpoons} I_{1a} R_{1a} \xrightarrow{k_{1r}} R_{1a} + I_{1i}$$

$$I_{2i} + I_{1a} \overset{K_{2f}}{\rightleftharpoons} I_{2i} I_{1a} \xrightarrow{k_{2f}} I_{1a} + I_{2a}$$

$$I_{2a} + R_{2a} \overset{K_{2r}}{\rightleftharpoons} I_{2a} R_{2a} \xrightarrow{k_{2r}} I_{2i} + R_{2a}$$

in which K_{1f}, K_{2f}, K_{1r}, and K_{2r} are dissociation constants for the enzyme–enzyme complexes and k_{1f}, k_{1r}, k_{2f}, and k_{2r} are the specific rate constants for the reactions as indicated. At steady state the fractional activity of the target enzyme is given by Eq. 1,



Curve	K_1	n	$K_{(n+1)}$ E, I_1	$e_{(n+1)}$ R_n	a_{nf}	a_{nr}
1	1	0				
2	1	1	1	1	1	1
3	1	2	1	1	1	1
4	1	1	2	0.5	4	0.25
5	1	2	2	0.5	4	0.25

FIG. 2. Computer simulated curves [obtained with an interactive curve fitting and graphic program, MLAB, developed at NIH (see ref. 5)] for comparing normal saturation functions of monocyclic and opened bicyclic systems when each parameter, except K_1, is varied by a factor of 2.

$$\frac{[I_{2a}]}{[I_2]} = \left[\frac{\alpha_{1r}\alpha_{2r}(K_1 + [e_1])[R_1][R_2][e_2][e_3]}{\alpha_{1f}\alpha_{2f}(K_2 + [e_2])(K_3 + [e_3])[E][I_1][e_1]} + \frac{\alpha_{2r}[R_2][e_3]}{\alpha_{2f}[I_1](K_3 + [e_3])} + 1\right]^{-1} \quad [1]$$

in which $\alpha_{nr} = k_{nr}/K_{nr}$, $\alpha_{nf} = k_{nf}/K_{nf}$, and n is 1 or 2. This equation shows that the value of $[I_{2a}]/[I_2]$ is a function of numerous parameters that can be varied by appropriate interaction between the enzymes involved and one or more allosteric effectors. To demonstrate the amplification potential and the flexibility of the bicyclic cascade, the computer-simulated curves generated from Eq. 1 are shown in Fig. 2. Curve 1 represents the fractional activation of E when K_1 is kept constant at 1.0. Curves 2 and 3 depict the fractional activation, $[I_{1a}]/[I_1]$ and $[I_{2a}]/[I_2]$, as a function of $\log[e_1]$ for one cycle and two cycles, respectively, when all parameters in Eq. 1 are set at 1.0 (for the one-cycle system, the parameters $[R_2]$, $[e_3]$, α_{2r}, α_{2f}, $[I_1]$, and $([K_3] + [e_3])$ are deleted in the first and second terms in Eq. 1). With these conditions, the maximum level of the fractional activation for I_n decreases from 0.67 to 0.57 from a one-cycle to a two-cycle system. However, when $[I_{na}]/[I_n]$ is less than 0.5, the concentration of e_1 required to obtain the same level of fractional activation of I_n is significantly lower for a two-cycle system than for a one-cycle system. For comparative purposes, the amplification factor* is defined as the ratio

$$\frac{[e_1]_{0.5E}}{[e_1]_{0.5I_n}}$$

in which $[e_1]_{0.5E}$ and $[e_1]_{0.5I_n}$ are the concentrations of e_1 required to activate 50% of [E] and $[I_n]$, respectively. For all the calculations used here it is assumed that $K_1 = 1.0$. Therefore, the amplification factor with respect to I_n in these studies is $1/[e_1]_{0.5I_n}$. The enormous amplification factor of the bicyclic cascade is evident if K_1 is held constant at a value of 1.0 and each of the other eight variables for the one-cycle system and the 16 variables for the two-cycle system (α_{nf} and α_{nr} consist of two parameters each) are varied by a factor of 2, so as to favor the formation of I_{1a} and I_{2a}. Under these conditions, the relative concentrations of e_1 required to obtain 50% activation of E, I_1, and I_2 are 1.0, 3.12×10^{-3}, and 9.76×10^{-6}, respectively (see curves 1, 4, and 5). The amplification factor for a one-cycle system is therefore 320, and for a two-cycle system is 102,400.

* This factor refers to a time-independent signal amplification which reflects the concentration of the primary stimulus, e_1, required to obtain a 50% activation of the interconvertible enzyme.

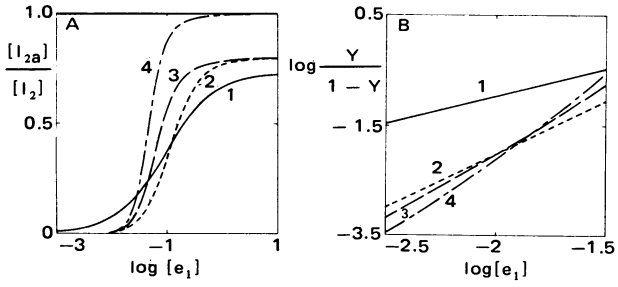


FIG. 3. (*A*) Computer-simulated curves utilizing Eqs. **2, 3, 4,** and **5** by setting K_1, α_{nf}, α_{nr}, [E], [R_1], and [R_2] equal to 1.0; [I_1] = 2.0, [e_2] = [e_3] = K_2 = K_3 = 0.01, and K_4 = 0.1. Eq. **2** gives curve 1, Eq. **3** gives curve 2, Eq. **4** gives curve 3, Eq. **5** gives curve 4. (*B*) Hill plots for the corresponding curves at *A*.

It is noteworthy that this high amplification factor is obtained with only a 2-fold variation in each parameter. Such changes are well within the range of allosteric effects. Because any one, more than one, or all parameters can be varied independently, in response to changes in the concentrations of various effectors, the flexibility of the bicyclic cascade system to metabolite control is enormous.

Variations in the patterns of opened bicyclic control

As for the monocyclic system (1), multiple patterns of regulation for the bicyclic cascades are obtained by varying the role of effectors in the activation or deactivation of the converter enzymes. For situations in which the role of e_1 is enhanced by making it an activator of both E and I_{1a} and also by allowing it to substitute for e_3 and/or e_2 as inactivators of R_2 and R_1, the activation of I_{1a} and inactivation of R_1 and R_2 are governed by the equilibria:

$$I_{1a} + e_1 \overset{K_4}{\rightleftharpoons} I_{1a} \cdot e_1$$

$$R_{1a} + e_1 \overset{K_2}{\rightleftharpoons} R_{1i}$$

$$R_{2a} + e_1 \overset{K_3}{\rightleftharpoons} R_{2i}.$$

Assuming that only the I_{1a}·e_1 complex can catalyze the conversion of I_{2i} to I_{2a} and that [I_1] $\simeq$ [I_{1i}] + [I_{1a}] + [I_{1a}·e], the steady-state equilibrium equations were derived for each of the following situations: (*i*) e_1 activates E only, while e_2 and e_3 inactivate R_{1a} and R_{2a}, respectively, (*ii*) e_1 activates both E and I_{1a}, (*iii*) e_1 activates both E and I_{1a} and inhibits R_{1a}, and (*iv*) e_1 activates E and I_{1a} and inhibits both R_{1a} and R_{2a}. Equations for each of these four situations, listed in the above order, are as follows:

$$\frac{[I_{2a}]}{[I_2]} = \left[\frac{\alpha_{1r}\alpha_{2r}(K_1 + [e_1])[R_1][R_2]K_2K_3}{\alpha_{1f}\alpha_{2f}(K_2 + [e_2])[E][I_1][e_1](K_3 + [e_3])} + \frac{\alpha_{2r}[R_2]K_3}{\alpha_{2f}[I_1](K_3 + [e_3])} + 1\right]^{-1} \quad [2]$$

$$\frac{[I_{2a}]}{[I_2]} = \left[\frac{\alpha_{1r}\alpha_{2r}(K_1 + [e_1])[R_1][R_2]K_2K_3K_4}{\alpha_{1f}\alpha_{2f}[I_1][E][e_1]^2(K_2 + [e_2])(K_3 + [e_3])} + \frac{\alpha_{2r}(K_4 + [e_1])[R_2]K_3}{\alpha_{2f}[I_1][e_1](K_3 + [e_3])} + 1\right]^{-1} \quad [3]$$

$$\frac{[I_{2a}]}{[I_2]} = \left[\frac{\alpha_{1r}\alpha_{2r}[R_1][R_2](K_1 + [e_1])K_2K_3K_4}{\alpha_{1f}\alpha_{2f}[I_1][E][e_1]^2(K_2 + [e_1])(K_3 + [e_3])} + \frac{\alpha_{2r}[R_2](K_4 + [e_1])K_3}{\alpha_{2f}[I_1][e_1](K_3 + [e_3])} + 1\right]^{-1} \quad [4]$$

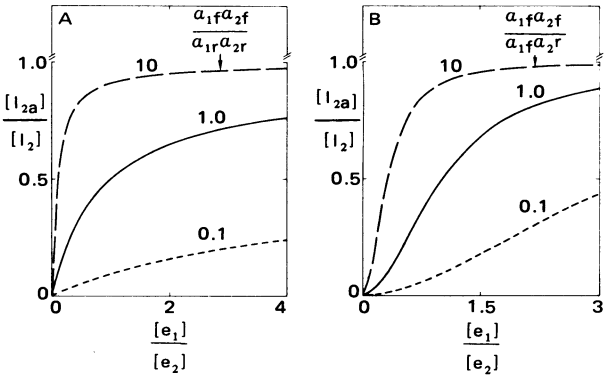


FIG. 4. Computer-simulated curves obtained with Eqs. **6** (*A*) and **7** (*B*) to demonstrate the fractional activity of I_2 as a function of [e_1]/[e_2] and $\alpha_{1f}\alpha_{2f}/\alpha_{1r}\alpha_{2r}$. The curves are generated with [R] = [E] = K_1 = K_2 = 1.0, K_3 = K_4 = 0.1, and [e_2] = 0.1.

$$\frac{[I_{2a}]}{[I_2]} = \left[\frac{\alpha_{1r}\alpha_{2r}[R_1][R_2](K_1 + [e_1])K_2K_3K_4}{\alpha_{1f}\alpha_{2f}[I_1][E][e_1]^2(K_2 + [e_1])(K_3 + [e_1])} + \frac{\alpha_{2r}[R_2](K_4 + [e_1])K_3}{\alpha_{2f}[I_1][e_1](K_3 + [e_1])} + 1\right]^{-1}. \quad [5]$$

Curves generated from these expressions are shown in Fig. 3. A comparison of these curves shows that the sensitivity to [e_1] [as measured by slopes of the Hill plots (n_H)] increases when e_1 becomes an effector at more than one step in the cascade. The values of n_H (calculated from the curves in Fig. 3*B*) are 1, 2, 2.5, and 3, respectively. It should be noted that the values of n_H obtained with Eqs. **4** and **5** can be different from those derived from Fig. 3*B* if K_2 and K_3 are given values different from those used here. In any case, n_H values greater than 1.0 are obtained when e_1 is utilized in more than one reaction in the cascade.

Closed bicyclic system

Fig. 1*B* shows a unique variation of the bicyclic cascade system in which both modified and unmodified forms of the interconvertible enzyme in the first cycle serve as converter enzymes catalyzing oppositional steps in interconversions of the enzyme in the second cycle. With this system, the ratio of [I_{1a}] to [I_{1i}] and not the total concentration of I_1 determines the steady-state level of the fractional activation of the target enzyme, I_2. With the same assumptions given above, the equation for fractional activation for the cascade depicted in Fig. 1*B* is

$$\frac{[I_{2a}]}{[I_2]} = \left[\frac{\alpha_{1r}\alpha_{2r}[R_1][e_2](K_1 + [e_1])}{\alpha_{1f}\alpha_{2f}[E][e_1](K_2 + [e_2])} + 1\right]^{-1}. \quad [6]$$

Fig. 4*A* shows that in a closed bicyclic cascade the steady-state value of [I_{2a}]/[I_2] increases as [e_1]/[e_2] increases, until it approaches a maximum that is determined by the ratio $\alpha_{1f}\alpha_{2f}/\alpha_{1r}\alpha_{2r}$. When [$e_1$]/[$e_2$] is maintained constant, the value of [I_{2a}]/[I_2] increases with increasing $\alpha_{1f}\alpha_{2f}/\alpha_{1r}\alpha_{2r}$. If e_1 is required for the activation of both E_i and I_{1a} in converting I_{2i} to I_{2a}, and e_2 is the activator for R_{1i} and I_{1i} in the conversion of I_{2a} to I_{2i}, the steady-state equation becomes

$$\frac{[I_{2a}]}{[I_2]} = \left[\frac{\alpha_{1r}\alpha_{2r}(K_1 + [e_1])K_3[R_1][e_2]^2}{\alpha_{1f}\alpha_{2f}(K_2 + [e_2])\,K_4[E][e_1]^2} + 1\right]^{-1} \quad [7]$$

in which K_3 and K_4 are dissociation constants for $I_{1a}e_1$, and $I_{1i}e_2$, respectively. Eq. **7** indicates that [I_{2a}]/[I_2] is a sigmoidal function of [e_1]/[e_2] ratio. Therefore, with this pattern of regulation, the activity of I_2 is more sensitive to the ratio [e_1]/[e_2] than it is for the situation described by Eq. **6**. The interrela-

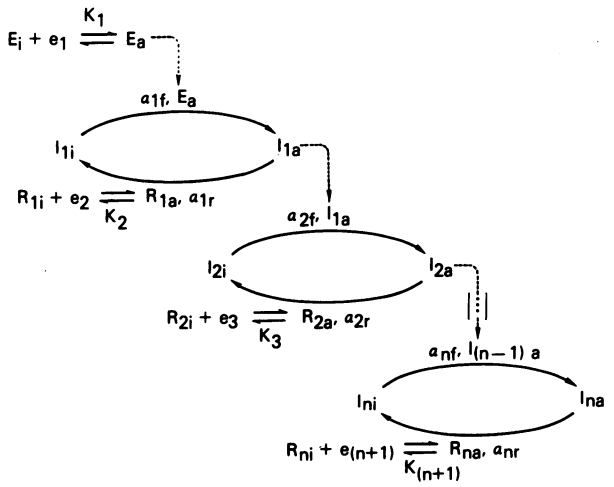


FIG. 5. Schematic representation of a multicyclic cascade system. See Fig. 1 for definition of notations.

tionship between $[I_{2a}]/[I_2]$ and $\alpha_{1f}\alpha_{2f}/\alpha_{1r}\alpha_{2r}$ is similar to that depicted in Fig. 4A.

Multicyclic system

A multicyclic cascade system that is initiated by an allosteric activator, e_1, followed by n successive covalent modification cycles is depicted in Fig. 5. The fractional activity of the target enzyme I_n in such a cascade is given by the general expression

$$\frac{[I_{na}]}{[I_n]} = \left[\frac{\alpha_{1r}\alpha_{2r}\alpha_{3r}\cdots\alpha_{nr}[R_1][R_2]\cdots[R_n][e_2][e_3]\cdots[e_{(n+1)}](K_1+[e_1])}{\alpha_{1f}\alpha_{2f}\alpha_{3f}\cdots\alpha_{nf}[E][I_1][I_2]\cdots[I_{(n-1)}][e_1](K_2+[e_2])(K_3+[e_3])\cdots(K_{(n+1)}+[e_{(n+1)}])} + \frac{\alpha_{2r}\alpha_{3r}\cdots\alpha_{nr}[R_2][R_3]\cdots[R_n][e_3][e_4]\cdots[e_{(n+1)}]}{\alpha_{2f}\alpha_{3f}\cdots\alpha_{nf}[I_1][I_2]\cdots[I_{(n-1)}](K_3+[e_3])(K_4+[e_4])\cdots(K_{(n+1)}+[e_{(n+1)}])} + \cdots + \frac{\alpha_{nr}[R_n][e_{(n+1)}]}{\alpha_{nf}[I_{(n-1)}](K_{(n+1)}+[e_{(n+1)}])} + 1\right]^{-1} \quad [8]$$

Eq. 8 was derived with the same assumptions described above. Fig. 5 shows that eight new variables are introduced with each additional cycle in the cascade.

Because the fractional activation of I_n is a multiplicative function of all these parameters, each additional cycle in the cascade increases enormously the amplification potential and capacity of the system to respond to multiple allosteric effectors. This is illustrated by the curves in Fig. 6. As a point of reference, curve $n = 0$ (Fig. 6) illustrates the fractional activation (saturation) of E alone as a function of the e_1 concentration when K_1 is 1.0. As is defined by K_1, the $[e_1]_{0.5}$ value for E is 1.0. An $[e_1]_{0.5I_n}$ value of 1.0 is also obtained for $[I_{na}]/[I_n]$ when all parameters in Eq. 8 are assigned values of 1.0. However, as is shown by curves $n = 1$ to 4, if K_1 is 1.0 and all other parameters in Eq. 8 that favor the forward steps are given values of 2 and all parameters that favor the regeneration steps are given values of 0.5, then the $[e_1]_{0.5I_n}$ value is decreased by increasing the number of cycles in the cascade. Thus, the $[e_1]_{0.5I_n}$ values calculated from curves 2, 3, 4, and 5 are 3.135×10^{-3}, 9.77×10^{-6}, 3.05×10^{-8}, and 9.54×10^{-11} concentration unit for one-, two-, three-, and four-cycle cascades, respectively. Thus, an amplification factor of about 10^{10} can be obtained in a four-cycle cascade by only 2-fold changes in each parameter. The *inset* in Fig. 6A shows that there is a linear relationship between the log of the amplification factor and the number of cycles in the cascade.

By neglecting the role of e_n where n is greater than 1.0, and by assuming that the ratio $\alpha_{1r}[R_1]/\alpha_{1f}[E]$ and $\alpha_{nr}[R_{nr}]/\alpha_{nf}[I_{(n-1)}]$ is the same and $= k_r'/k_f'$ for all values of n, Eq. 8 can

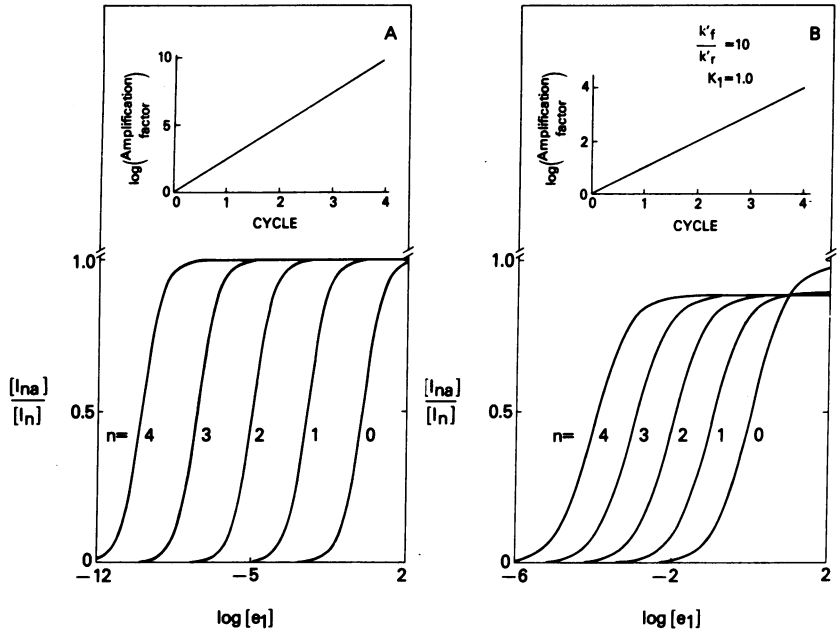


FIG. 6. (A) Computer-simulated curves obtained with Eq. 8 to show the dependence of $[I_{na}]/[I_n]$ on the number of cycles, n, as one varies $\log[e_1]$. The results are obtained with $\alpha_{nf} = 4$, $\alpha_{nr} = 0.25$, $[R_n] = [e_{(n+1)}] = 0.5$, $K_1 = 1$, $K_{(n+1)} = [E] = [I_{(n-1)}] = 2$. (B) Computer-simulated curves obtained with Eq. 9 and $K_1 = 1.0$, $k_f'/k_r' = 10$. The *insets* in A and B depict the linear relationship between $\log([e_1]_{0.5I_n})^{-1}$ and n.

be reduced to

$$\frac{[I_{na}]}{[I_n]} = \left[\left(\frac{K_1}{[e_1]}+1\right)\left(\frac{k_r'}{k_f'}\right)^n + \left(\frac{k_r'}{k_f'}\right)^{n-1} + \cdots + \left(\frac{k_r'}{k_f'}\right) + 1\right]^{-1}. \quad [9]$$

This equation illustrates more clearly the amplification capacity of multicyclic cascade systems, because the number of cycles, n, involved is expressed as an exponential function. Fig. 6*B* demonstrates the fractional activity of the target enzyme as described in Eq. 9 when k_f'/k_r' is 10, $K_1 = 1.0$, and $[e_1]$ is the variable. The calculated amplification factors are 9.1, 83, 833, and 8333 for one-, two-, three-, and four-cycle cascades, respectively.

DISCUSSION

The concept that the interconversion of enzymes is a dynamic process is supported by the following experimental observations: (*a*) the *in vivo* adenylylation of *E. coli* glutamine synthetase assumes different steady-state levels (between 0 and 12 equivalents per mole of enzyme) in response to various degrees of nitrogen starvation (6). *In vitro*, the steady-state level of adenylylation varies with variations in the concentrations of ATP, UTP, P_i, α-ketoglutarate, glutamine, Mg^{2+}, and Mn^{2+} (7, 8). (*b*) Variations in the ratios of different effectors that affect either phosphorylation or dephosphorylation of the mammalian pyruvate dehydrogenase lead to shifts in the steady-state level of enzymic activity both *in vitro* (9) and *in vivo* (10, 11). (*c*) In the presence of glucose 1-phosphate and glucose 6-phosphate, the interconversion of phosphorylase *a* and *b* leads to a mixture of the fully phosphorylated *a* and dephosphorylated *b* forms (12).

A remarkable flexibility of cyclic cascades obtains from the fact that a minimum of three enzymes and five additional parameters are involved in each interconvertible enzyme cycle; moreover, every enzyme in the cascade can be a separate target for one or more positive or negative metabolite effectors. Therefore, by means of allosteric and substrate interactions with the cascade enzymes, the concentrations of a multitude of regulatory signals can be sensed and their effects can be integrated to determine the steady-state distribution between active and inactive forms of the interconvertible enzymes, and hence their catalytic activities.

The great amplification potential stems from the fact that the response of the last interconvertible enzyme in a cascade to a primary stimulus, caused by allosteric interaction of an effector to the first converter enzyme in the cascade, is a multiplicative function of various parameters in the cascade. Because the total number of these parameters is directly proportional to the number of cycles in the cascade, a concerted effect of only 2-fold variations in the contributions of each parameter can yield a 3×10^2-, 10^5-, 3×10^7-, and 10^{10}-fold amplification of a primary stimulus, in a one-, two-, three-, and four-cycle cascade, respectively. However, unlike unidirectional cascades utilized in blood clotting and complement fixation, where high amplification leads to an explosive response to primary stimuli, the amplification capacity of cyclic cascades is susceptible to very fine regulation. This is because each parameter in the cascade can be independently regulated and the system is reversible by multiple reaction pathways.

It should be pointed out that, for each complete cycle, one equivalent of ATP is consumed. This is the price in free energy that the cell must pay to support such an effective mechanism of regulation. In the analysis of the model described here and in the previous paper, the role of ATP was neglected because ATP is present at a relatively constant level that is several orders of magnitude higher than that of the enzymes involved in the cascade system.

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