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Superiority of interconvertible enzyme cascades in metabolic regulation: Analysis of monocyclic systems*

(cyclic cascade/covalent modification/allosteric effector/steady state)

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ABSTRACT **A theoretical analysis of monocyclic cascade models shows that the steady-state fraction of covalently modified interconvertible enzyme is a function of 10 different cascade parameters. Because each parameter can be varied independently, or several can be varied simultaneously, by single or multiple allosteric interactions of ligands with one or more of the cascade enzymes, interconvertible enzymes are exquisitely designed for the rigorous control of key metabolic steps. Compared with other reglatory enzymes, they can respond to a greater number of allosteric stimuli, they exhibit greater flexibility in overall control patterns, and they can generate a greatly amplified response to primary allosteric interactions of effectors with the converter enzymes. Contrary to earlier views, the decomposition of ATP associated with cyclic coupling of the covalent modification and demodification reactions is not a futile process. ATP decomposition supplies the energy needed to maintain concentrations of modified enzyme at steady-state levels that are in excess of those obtainable at true thermodynamic equilibrium.**

An important mechanism of cellular regulation involves the interconversion of active and inactive forms of key enzymes in metabolism by the cyclic coupling of covalent modification and demodification reactions. To date, three types of covalent modifications are known to be involved in enzyme regulation. They are: (*a*) the phosphorylation of seryl residues (2); (*b*) the nucleotidylation of tyrosyl residues (3); and (*c*) the ADP-ribosylation of arginyl (4) or other unidentified sites on the enzymes.

In each instance, covalent modification of the key enzyme is catalyzed by a "converter" enzyme; i.e., the modification involves the action of one enzyme upon another. Therefore, interconvertible enzyme systems are in effect bidirectional (cyclic) cascade systems.

Compared to other regulatory enzymes, interconvertible enzymes can respond to a greater number of allosteric stimuli, they exhibit greater flexibility in their control patterns, and they possess enormous amplification potential in their responses to variations in allosteric effector concentrations.

We present here the results of studies on monocyclic cascade systems, which are patterned after the phosphorylation–dephosphorylation cycles that are involved in the regulation of mammalian pyruvate dehydrogenase (5), phosphofructokinase (6), and tyrosine aminotransferase (7) activities.

A subsequent paper (8) is concerned with the results of studies on bicyclic cascades of the type involved in the regulation of *Escherichia coli* glutamine synthetase (3), mammalian glycogen phosphorylase (9), and glycogen synthase (10), and also of more extended, multicyclic cascade systems.

* Parts of this paper have been reported in ref. 1.

RESULTS

Monocyclic cascade

Fig. 1 depicts a monocyclic cascade in which it is assumed that the interconvertible enzyme, I, undergoes cyclic phosphorylation and dephosphorylation. The forward cascade is initiated by the binding of an allosteric effector e_1 to the inactive form (E_i) of the converter enzyme (E) (a protein kinase) and thereby converts it to a catalytically active configuration (E_a). The activated kinase catalyzes the phosphorylation of the inactive form (I_i) of the interconvertible enzyme and thereby converts it to the catalytically active form (I_a). This cascade is opposed by the regeneration cascade which is initiated by the binding of e_2 to the inactive form (R_i) of a phosphoprotein phosphatase, thus converting it to the active form (R_a), which catalyzes the conversion of I_a back to I_i.

Because in this model the interconversion of I_i and I_a is a cyclic process, it follows that, for any given metabolic state, the fraction of interconvertible enzyme in the form of I_a will be a function of the steady-state distribution between I_i and I_a. This in turn is a complex function of several parameters, including the concentrations of the allosteric effectors, e_1 and e_2, the dissociation constants, K_1 and K_2, for the effector–converter enzyme complexes, the specific activities of the converter enzymes, E_a and R_a, and the kinetic constants, α_f and α_r, for the forward and regeneration steps, respectively. The constants α_f and α_r are defined as $\alpha_f = k_f/K_f$ and $\alpha_r = k_r/K_r$, in which k_f and k_r are the specific rate constants and K_f and K_r are the dissociation constants for the $E_a \cdot I_i$ and the $R_a \cdot I_a$ complexes (not shown), respectively.

Fig. 1 shows also that with each complete interconversion cycle one equivalent of ATP is converted to ADP and P_i. This is the source of free energy needed to maintain a steady-state level of active interconvertible enzyme, I_a.

Steady-state equation

In deriving the steady-state equation for the monocyclic cascade, the ATP dependency of the interconversion of I_i and I_a has been ignored, because for any metabolic state the concentration of ATP is maintained at a constant level that is several orders of magnitude higher than the substrate enzymes. In addition it is assumed that: (*i*) there is rapid equilibrium in the formation of the enzyme–effector and enzyme–enzyme complexes; (*ii*) the concentrations of enzyme–enzyme complexes are negligibly low compared to the concentration of the active and inactive enzymes;† (*iii*) the concentrations of the allosteric

† When this condition is satisfied, $[E] \simeq [E_i] + [E_a]$, $[I] \simeq [I_i] + [I_a]$, $[R] \simeq [R_i] + [R_a]$. [E], [I], and [R] are total concentrations of E, I, and R, respectively.

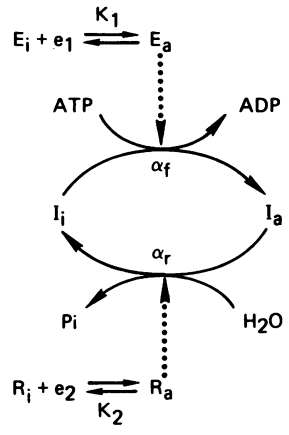


FIG. 1. Monocyclic cascade system. The parameters of α_f and α_r are defined as: $\alpha_f = (k_f/K_f)$, $\alpha_r = (k_r/K_r)$; k_f and k_r are specific rate constants for the forward and reverse reaction designated. K_1, K_2, K_r, and K_f are dissociation constants for E_a, R_a, E_aI_i, and R_aI_a, respectively. I_i and I_a are inactive and active interconvertible enzymes, respectively.

effectors e_1 and e_2 are maintained at constant levels for any given metabolic state. The extent to which these assumptions may yield results that are qualitatively at variance with those demanded by physiological constraints will be discussed later. With these assumptions, the reactions for the interconversion of I_i and I_a can be written as follows:

$$I_i + E_a \overset{K_f}{\rightleftharpoons} I_iE_a \overset{k_f}{\rightarrow} E_a + I_a \quad [1]$$

and

$$I_a + R_a \overset{K_r}{\rightleftharpoons} I_aR_a \overset{k_r}{\rightarrow} R_a + I_i. \quad [2]$$

When reactions 1 and 2 are at equilibrium,

$$\alpha_f[E_a][I_i] = \alpha_r[R_a][I_a] \quad [3]$$

with

$$[E_a] = \frac{[E][e_1]}{K_1 + [e_1]}, \quad [R_a] = \frac{[R][e_2]}{K_2 + [e_2]}, \quad \frac{k_f}{K_f} = \alpha_f,$$

and

$$\frac{k_r}{K_r} = \alpha_r.$$

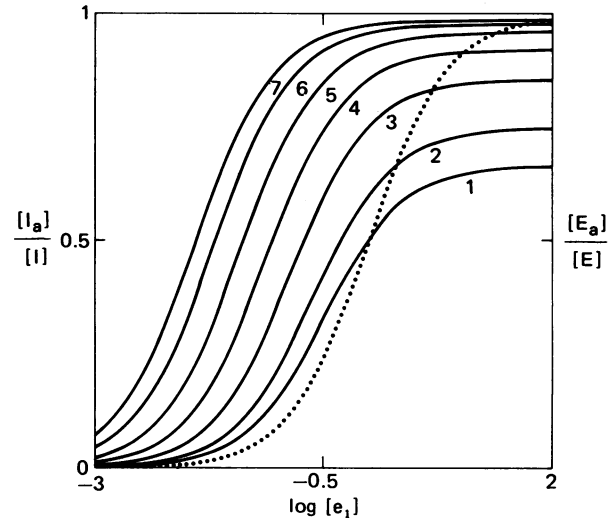
From Eq. 3 and the assumption that $[I] \simeq [I_a] + [I_i]$, a steady-state expression for the fraction of modified interconvertible enzyme is:

$$\frac{[I_a]}{[I]} = \left[\frac{\alpha_r[R][e_2](K_1 + [e_1])}{\alpha_f[E][e_1](K_2 + [e_2])} + 1\right]^{-1}, \quad [4]$$

in which [I], [E], and [R] are total concentrations of I, E, and R, respectively.

Predictions

The extraordinary capacity of an interconvertible enzyme to be regulated obtains from the fact that any one or all of the 10 parameters in Eq. 4 can be altered by allosteric interaction with one or more allosteric effectors. The curves in Fig. 2 show that when the binding of an effector, e_1, to E (shown by the dotted line) is linked to the interconvertible enzyme cascade, the indirect effect of e_1 concentration on the fractional activation of the interconvertible enzyme can vary enormously, depending on the magnitude and the number of parameters in the cascade cycle that are varied. In all instances, we assume that $K_1 = 1.0$, so the responses to e_1 concentration shown by curves 1–7 in Fig. 2 are not due to changes in the affinity of E for e_1, but rather



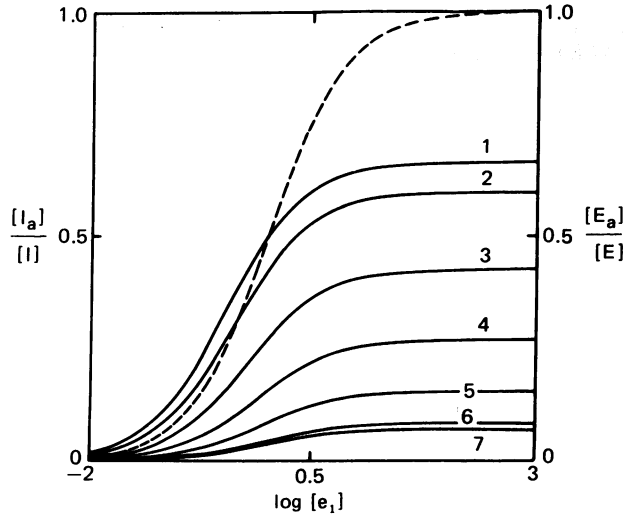
•• ••• Normal Saturation Curve

Curve	K_1	K_2	α_f	α_r	[E]	[R]	$[e_2]$
1	1	1	1	1	1	1	1
2	1	2	1	1	1	1	1
3	1	2	2	1	1	1	1
4	1	2	2	0.5	1	1	1
5	1	2	2	0.5	2	1	1
6	1	2	2	0.5	2	0.5	1
7	1	2	2	0.5	2	0.5	0.5

FIG. 2. Computer-simulated curves to show a stepwise variation of each parameter, except K_1, in a successively cumulative manner, by a factor of 2 in favor of the I_a formation. Computer-simulated curves were obtained with an interactive curve-fitting and graphic program, MLAB, developed at the National Institutes of Health (see ref. 16).

to alterations in the values of other parameters in the cascade system.

Variations of these parameters can have two effects on an interconvertible enzyme: (*i*) they may alter the $[e_1]_{0.5I}$ value, i.e., the concentration of e_1 required to produce 50% activation of the interconvertible enzyme; and/or (*ii*) they may alter the amplitude of activation, $([I_a]/[I])_{max}$, i.e., the fractional activation obtained with saturating levels of e_1. Curve 1 in Fig. 2 shows that when all parameters in Eq. 4 are assigned a value of 1.0, the $[e_1]_{0.5I}$ of the interconvertible enzyme is equal to the K_m value of e_1 for the activation of E, but the $([I_a]/[I])_{max}$ is only 0.67. Even under these conditions the concentrations of e_1 required to activate indirectly the interconvertible enzyme when $[I_a]/[I]$ is less than 0.5 are less than those required for comparable activations of the converter enzyme, E (compare curve 1 with the dotted curve in Fig. 2). Curves 2 to 7 in Fig. 2 show that when six parameters undergo 2-fold changes in magnitude in a successively cumulative manner there is a progressive downward shift in the $[e_1]_{0.5I}$ for the interconvertible enzyme and a progressive upward shift in the $([I_a]/[I])_{max}$ values. A comparison of curve 1 with curve 7 shows that with only 2-fold changes in each of the six parameters, the $[e_1]_{0.5I}$ value of the interconvertible enzyme undergoes an 80-fold change. Fig. 3 shows that with a 2-fold change in the opposite direction from those shown in Fig. 2, the $([I_a]/[I])_{max}$ is decreased from 0.67 for curve 1 (Fig. 3) to 0.07 for curve 7. It is noteworthy that under these conditions the concentration of e_1 required to obtain $0.5([I_a]/[I])_{max}$ approaches the value of K_1 as the $([I_a]/[I])_{max}$ decreases. Because 2-fold changes in the various parameters are well within the range of allosteric effects, these results illustrate the enormous control potential of the cyclic cascade system as compared to other types of enzyme regulation.



Curve	K_1	K_2	α_f	α_r	[E]	[R]	[e_2]
1	1	1	1	1	1	1	1
2	1	0.5	1	1	1	1	1
3	1	0.5	0.5	1	1	1	1
4	1	0.5	0.5	2	1	1	1
5	1	0.5	0.5	2	0.5	1	1
6	1	0.5	0.5	2	0.5	2	1
7	1	0.5	0.5	2	0.5	2	2

FIG. 3. Computer-simulated curves to show a stepwise variation of each parameter, except K_1, like those described in Fig. 2, by a factor of 2 in favor of the I_i formation.

Multiple patterns of monocyclic cascade control

The monocyclic cascade system discussed above is one of many patterns that are possible, depending upon the nature of the interactions between the allosteric effector e_1 and e_2 and the converter enzymes E and R. With the restriction that E must be activated by e_1, there are four unique patterns that can occur in the covalent modification systems. The steady-state expressions for these four cases are given in Table 1. In case I (depicted in Fig. 1) E and R are activated by e_1 and e_2, respectively. Case II is like case I except effector e_2 is an inhibitor rather than an activator for the converter enzyme R. In case III, the effector e_1 activates E and inhibits R. In case IV, e_1 activates both E and R enzymes. Figs. 4 and 5 illustrate the wide variation in the regulatory patterns elicited by these different types of monocyclic cascades. In these figures, the fractional activity of the interconvertible enzyme is expressed as a function of increasing concentration of e_1 in each of the four cases, when all parameters but one are held constant. Fig. 4 shows that the four cases differ markedly in their responses to variations in the value of K_1 with respect to both the $[e_1]_{0.5I}$ and $([I_a][I])_{max}$ values. In case IV, when $K_1 = K_2$, the value of $[I_a]/[I]$ is 0.5 at all concentrations of e_1.

Table 1. Steady-state expressions for four unique regulatory patterns obtained from the monocyclic cascade system*

Case	$E_i \rightarrow E_a$ by e_1	$E_i \rightarrow E_a$ by e_2	$R_i \rightarrow R_a$ by e_1	$R_i \rightarrow R_a$ by e_2	Steady-state expression for $[I_a]/[I]$
I	+	0	0	+	$\left[\left(\frac{K_1+[e_1]}{K_2+[e_2]}\right)\left(\frac{\alpha_r[R][e_2]}{\alpha_f[E][e_1]}\right)+1\right]^{-1}$
II	+	0	0	−	$\left[\left(\frac{K_1+[e_1]}{K_2+[e_2]}\right)\left(\frac{\alpha_r[R]K_2}{\alpha_f[E][e_1]}\right)+1\right]^{-1}$
III	+	0	−	0	$\left[\left(\frac{K_1+[e_1]}{K_2+[e_1]}\right)\left(\frac{\alpha_r[R]K_2}{\alpha_f[E][e_1]}\right)+1\right]^{-1}$
IV	+	0	+	0	$\left[\left(\frac{K_1+[e_1]}{K_2+[e_1]}\right)\left(\frac{\alpha_r[R]}{\alpha_f[E]}\right)+1\right]^{-1}$

* The notations +, −, and 0 represent activate, inactivate, and no effect, respectively.

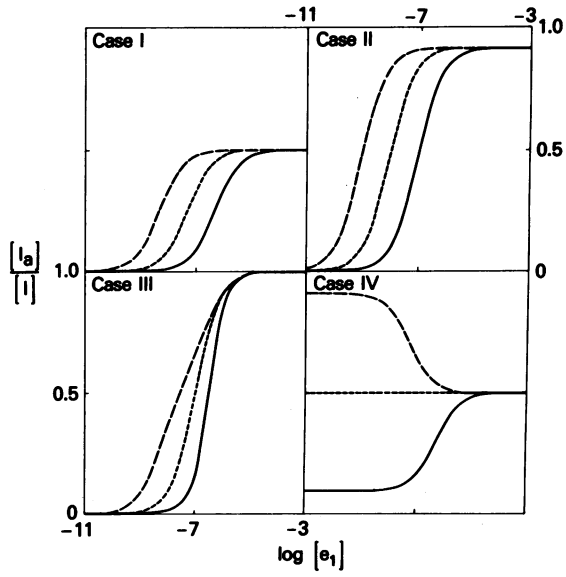


FIG. 4. Computer-simulated curves to demonstrate the effect of K_1 on the steady-state level of $[I_a]/[I]$ when all parameters in the monocyclic cascade system are being held constant, e.g., [E] = [R], $\alpha_f = \alpha_r$, $K_2 = 10^{-7}$ (except for case IV, in which $K_2 = 10^{-6}$), $[e_2] = 10^{-6}$, and varying $[e_1]$ and K_1. The curves ——, - - - -, and – – – are obtained with $K_1 = 10^{-6}$, 10^{-7}, and 10^{-8}, respectively. The four patterns derived from changing the role of e_1 and e_2 on either activation or inactivation of R_i or R_a.

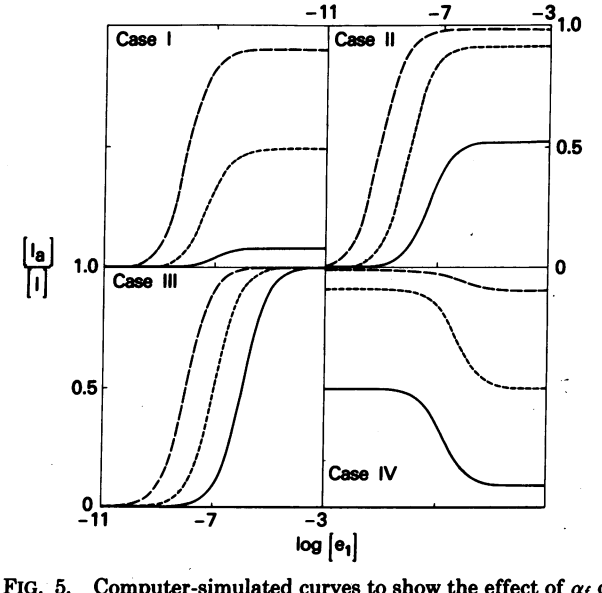


FIG. 5. Computer-simulated curves to show the effect of α_f on the steady-state level of $[I_a]/[I]$. The conditions are the same as in Fig. 4, except $K_1 = 10^{-7}$ and $\alpha_r = 10^5$. (Note again that $K_2 = 10^{-7}$, except for case IV, in which $K_2 = 10^{-6}$.) The curves ——, - - - -, and – – – are obtained with $\alpha_f = 10^4$, 10^5, and 10^6, respectively.

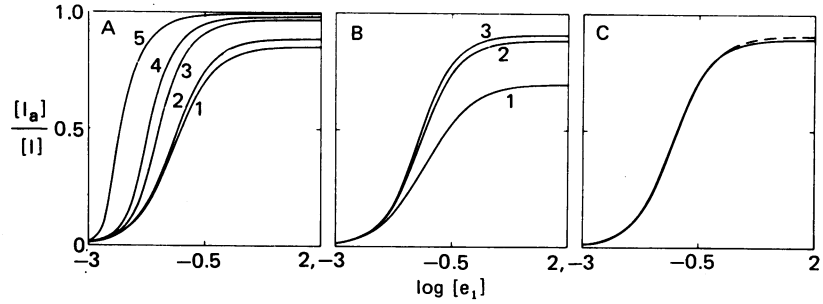


FIG. 6. Computer-simulated curves obtained with a quartic equation described in the *text*. (*A*) [I] is varied as follows: [I] = 1.0 (curve 1); 10 (curve 2); 100 (curve 3), 200 (curve 4), and 1000 (curve 5). Other variables are $K_1 = 1.0$, $K_2 = 2.0$, $K_f = 0.4$, $K_r = 0.2$, $k_f = 5$, $k_r = 2.5$, [E] = [R] = $[e_2]$ = 1.0. (*B*) Varying K_f and k_f. Curve 1 is obtained with $K_f = 0.5$, $k_f = 1$; curve 2 with $K_f = 2.5$, $k_f = 5$; and curve 3 with $K_f = 25$, $k_f = 50$. Other parameters are the same as those described in *A* except [I] = 10. (*C*) $[I_a]/[I]$ obtained with the quartic equation (——) and the simplified equation (- - -). The variables are: $K_1 = K_2$ = [E] = [R] = $[e_2]$ = 1.0, [I] = 10, $K_f = 10$, $K_r = 20$, $k_f = 25$, and $k_r = 10$.

Fig. 5 shows that variation in the value of α_f produces a different pattern of response with respect to both $[e_1]_{0.5I}$ and $([I_a]/[I])_{max}$ in each of the four cases, except for case III, where only $[e_1]_{0.5I}$ is affected.

The analysis of these four monocyclic cascade systems emphasizes further the great flexibility that is gained by the regulation of enzymes by the cyclic coupling of covalent modifications.

Validity of the simplifying assumptions

In deriving the steady-state functions shown in Table 1, it was assumed† that the concentrations of the complexes between the converter enzymes and the interconvertible enzyme are negligibly small compared to the concentration of either active or inactive forms of these enzymes. With this assumption the steady-state value of $[I_a]/[I]$ is independent of the total concentration I. However, from a quartic equation‡ based on the fact that $[I] = [I_a] + [I_i] + [I_aR_a] + [I_iE_a]$, it can be shown (Fig. 6) that the amplification factor,§ (defined as the ratio of $[e_1]_{0.5E}$ to $[e_1]_{0.5I}$) can increase as the concentration of I increases. Thus, for the conditions given in Fig. 6, $1/[e_1]_{0.5I}$ increases by a factor of 20 when [I] is varied from 10 to 1000. This increase in amplification factor is due to the addition of [I] as a variable in the $[I_a]/[I]$ expression. In addition, because α_f and α_r are defined as being equal to $k_fK_f^{-1}$ and $k_rK_r^{-1}$, respectively, it follows from the simplified expression (Table 1) that when all other parameters are held constant, the steady-state value of $[I_a]/[I]$ will be independent of the values of k_f and K_f or of k_r and K_r so long as the products of k_f and K_f^{-1} (or k_r and K_r^{-1}) are the same. However, this relationship is not valid for the quartic equation. Fig. 6*B* shows that when the quartic expression is used to calculate the dependence of $[I_a]/[I]$ on $[e_1]$, different saturation curves are obtained when k_f and K_f are varied simultaneously so that their product, α_f, is held constant. Nevertheless, Fig. 6*C* shows that when the conditions assumed for the derivation of Eq. 4 are approximately fulfilled, essentially identical saturation curves are obtained with both Eq. 4 and the quartic equation. Therefore, the assumptions made to simplify the derivation of the equations shown in Table 1 do not invalidate their usefulness in analyzing the fundamental characteristics of monocyclic cascade systems. To the contrary, the highly complicated and almost unmanageable quartic expressions derived by a more rigorous treatment of the steady-state functions confirm in principle most predictions of the simplified expressions and show additionally that the monocyclic cascade is potentially more sensitive to effector stimuli and can achieve an even greater amplification and flexibility of these stimuli than is predicted by the simplified expressions.

DISCUSSION

In the monocyclic cascade model described here, the covalent modification and demodification of an interconvertible enzyme is visualized as a *dynamic process* by means of which the specific activity of the enzyme is varied by shifting the steady-state equilibrium between active and inactive forms. This concept is more realistic and is certainly superior from the standpoint of regulation to earlier concepts in which interconvertible enzymes were considered to be "metabolic switches" which could be turned on or off in response to allosteric stimuli. The switch concept implies that the respective converter enzymes that catalyze the modification and demodification reactions are activated and inactivated, reciprocally, in an "all-or-none" manner. With the dynamic mechanism, however, activity of an interconvertible enzyme can vary smoothly, over a wide range, in response to changes in allosteric stimuli, and will become stabilized at a fixed level commensurate with the metabolic state of the cell.

Unlike the unidirectional cascades involved in blood clotting and complement fixation, where huge amplification leads to an explosive response to primary stimuli, the cyclic cascades can respond to fluctuations in the concentrations of many different metabolites, and are therefore more appropriately designed for controlled amplification of primary stimuli, as is needed in the regulation of key enzymes in metabolism.

It is noteworthy that, as a consequence of the multiplicative effects obtained when several cascade parameters are altered simultaneously, interconvertible enzymes can respond to effector concentrations that are well below the dissociation constants of the effector–allosteric enzyme complexes. For example, comparison of curve 7 with the dotted curve in Fig. 2 shows that if each of the six variables in Eq. 4 undergoes a 2-fold change, then a concentration of effector that causes only 2% activation of the kinase can produce 90% activation of the interconvertible enzyme.

Reed and his associates (11) have shown that the activity of the mammalian pyruvate dehydrogenase complex is regulated by phosphorylation and dephosphorylation of the α chain of the pyruvate dehydrogenase subunit of the complex. Mammalian pyruvate dehydrogenase is therefore a prototype for the

‡ The quartic equation so derived contains more than 200 terms. $[I_a]$ is calculated by Newton's method of successive approximation.

§ This factor refers to a time-independent signal amplification that reflects the relative concentration of the primary stimulus, e_1, required to obtain a 50% activation of E and I.

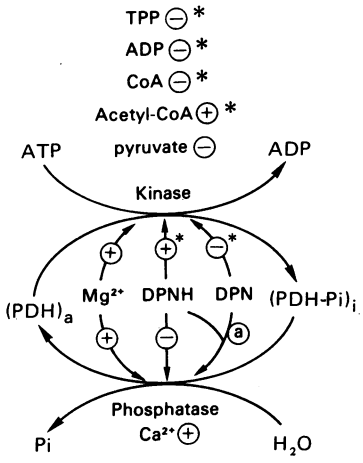


FIG. 7. The role of some effectors in covalent interconversion of mammalian pyruvate dehydrogenase (PDH). * Indicates that effect is observed only in the presence of K^+ or NH_4^+. ⓐ Indicates that DPN antagonizes the inhibition by DPNH. ⊕ And ⊖ indicate activation and inactivation, respectively.

kind of monocyclic cascade system analyzed here. It differs from our model only in that phosphorylation leads to inactivation rather than to activation of the interconvertible enzyme. Fig. 7 summarizes the results from several laboratories (11–13) showing that the highly specific kinase and the phosphoprotein phosphatase that catalyze the phosphorylation and dephosphorylation of pyruvate dehydrogenase, respectively, are subject to regulation by a large number of effectors, including K^+, NH_4^+, Ca^{2+}, pyruvate, acetyl-CoA, CoA, ADP, DPN, and DPNH. It is evident from Fig. 7 that three of the four mechanisms described in Table 1, and Figs. 4 and 5, are utilized in the regulation of the pyruvate dehydrogenase cascade. Acetyl-CoA and Ca^{2+} are positive modifiers of the kinase and phosphatase, respectively, and therefore correspond to effectors e_1 and e_2 in case I; whereas the roles of e_1 in cases III and IV are satisfied by DPNH and Mg^{2+}, respectively. To date no metabolite has been found to fill the role of e_2 in case II; i.e., an effector whose *only* ability is to inhibit the phosphatase.

Further, results of *in vivo* and *in vitro* experiments show that variations in the ratios of allosteric effectors lead to shifts in the steady-state level of pyruvate dehydrogenase phosphorylation (11–13) and of state of adenylylation in glutamine synthetase of *E. coli* (14, 15). These results support our assumption that the covalent modification of interconvertible enzymes is a dynamic process and that the specific activity of such enzymes is a function of the steady-state equilibrium between modified and unmodified forms of the enzyme.

Though not considered in the steady-state analysis, it should be emphasized that each complete cycle in a cyclic cascade leads to decomposition of ATP to ADP and P_i (Fig. 1). As noted previously, the role of ATP was disregarded in the present analysis because its concentration is maintained by metabolism at fairly constant levels that are several orders of magnitude greater than the concentrations of the interconvertible enzymes.

In earlier discussions, loss of ATP free energy associated with continual unrestrained coupling of phosphorylation and dephosphorylation of an interconvertible enzyme was viewed as a wasteful process. Indeed, cyclic cascades were referred to as "futile cycles" (17). The fact that energy could be conserved if continual cycling was prevented by all-or-none reciprocal controls of the opposing converter enzymes seemed reason enough for the notion that interconvertible enzymes function as on–off switches in the control of metabolism. As noted above, it seems more likely that enzyme interconversion is a dynamic process and as such it is endowed with a capacity for controlled amplification of effector stimuli unmatched by that obtained with discontinuous mechanisms of interconversions.

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