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Simple model for hormone-activated adenylate cyclase systems

(glucagon/guanosine nucleotides/enzyme regulation)

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ABSTRACT **A simple model is developed to explain the activation of rat liver plasma membrane adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] by guanosine nucleotides and glucagon and the dependence of the catalytic rate on Mg^{2+}, H^+, and substrate concentrations. The basic model proposes that the adenylate cyclase system can exist in two states, A and B; that activating ligands bind preferentially to the B state; and that only the B state is active. Kinetic data are quantitatively fit to this model, and the binding constants for the interaction of the A and B states with glucagon, GTP, and guanyl-5′-ylimidodiphosphate are obtained. The substrates ATP and adenyl-5′-ylimidodiphosphate appear to show little preference between the A and B states, and simple Michaelis–Menten kinetics are sufficient to describe the dependence of the catalytic rate on substrate concentration under optimal conditions. The dependence of the rate on pH can be explained by postulating that one ionizable group in its acid form and one ionizable group in its basic form must be present at the active site in order for catalysis to occur. The activation and inhibition of the activity by Mg^{2+} can be explained by a similar mechanism with Mg^{2+} binding to activating and inhibiting sites. Glucagon and guanosine nucleotides appear to influence the dependence of the rate on Mg^{2+} and glucagon. The Mg^{2+} also may display some preference for the B state. A comparison of this model with others that have been proposed is given. The proposed model appears to provide a simple conceptual framework that is applicable to many adenylate cyclase systems.**

In this paper, a relatively simple model is developed to explain the kinetic behavior of rat liver plasma membrane adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] (cf. refs. 1 and 2 for reviews of adenylate cyclase systems). This model provides a conceptual framework which encompasses the known facts about the behavior of the liver plasma membrane adenylate cyclase and also appears to be consistent with many of the features of other adenylate cyclases. The absence of structural information about the adenylate cyclase enzyme and its associated proteins (e.g., hormone and nucleotide receptors) makes development of a detailed molecular model impossible. Instead, this work has the more modest goal of providing a conceptual framework for understanding the available information on liver membrane adenylate cyclase that is based on the principles utilized in interpreting the kinetics of well-characterized simple enzymes and allosteric enzymes.

The central facts to be explained are the activation of adenylate cyclase by guanosine nucleotides and hormones and the dependence of the catalytic rate on the substrate, H^+, and Mg^{2+} concentrations. The basic model proposes that the adenylate cyclase system (the enzyme and any associated proteins) can exist in two states, A and B; that activating ligands bind preferentially to the B state; and that only the B state is active. Furthermore, the activity of the B state is proposed to vary with substrate concentration, pH, Mg^{2+} concentration, and other variables analogously to the variations observed with simple enzyme systems. The observed rate of the enzymatic reaction, v, is assumed to be the product of three factors: the fraction of cyclase systems in the B state, f_B; the fraction of B in its active state, f_E; and the maximal velocity when f_B and f_E are equal to one, V_m:

$$v = f_B f_E V_m \qquad [1]$$

Activation by Glucagon and Guanosine Nucleotides. The fraction of molecules in the B state in the presence of i ligands, L_i, which bind to different sites on both the A and B states, can be written as:

$$f_B = \frac{K_0 \prod_i [1 + K_{Bi}(L_i)]}{\prod_i [1 + K_{Ai}(L_i)] + K_0 \prod_i [1 + K_{Bi}(L_i)]} \qquad [2]$$

where the constants are defined in Fig. 1, and each ligand has been assumed to bind independently to a different site. Note that if a ligand, L_1, is present at a constant concentration and the concentrations of other ligands are varied, the net effect will be to define a new constant, K_0 $[1 + K_{B1}(L_1)]/[1 + K_{A1}(L_1)]$, which replaces K_0 in Eq. 2. Thus, this treatment is valid when an unknown activator or inhibitor is present at a constant concentration. At saturating ligand concentrations, $f_B = K_0 \prod_i (K_{Bi}/K_{Ai})/[1 + K_0 \prod_i (K_{Bi}/K_{Ai})]$ and f_B is a constant. Finally, it should be noted that a ligand is an inhibitor if it binds preferentially to state A and an activator if it binds preferentially to state B.

This model can be compared directly to experimental results if the initial steady-state enzymatic velocity is determined under conditions where $f_E V_m$ is constant; in this case, $f_B = v/(f_E V_m)$. This appears to be true at optimal and constant values of substrate, Mg^{2+}, and hydrogen ion concentrations for the rat liver plasma membrane system. The initial steady-state velocity was determined at various concentrations of glucagon and GTP and of glucagon and guanyl-5′-ylimidodiphosphate [Gpp(NH)p], at pH 7.5 (25 mM Tris-Cl), 0.1% bovine-serum albumin, 10 mM Mg^{2+}, 0.1 mM ATP, 2 mM dithiothreitol, and 37°. The results obtained are summarized in Fig. 2. These data were fit to Eqs. 1 and 2 by a nonlinear least squares analysis, and the parameters obtained are presented in Table 1. The curves in Fig. 2 have been calculated with these parameters and Eqs. 1 and 2; the calculated curves and experimental points are in excellent agreement for all cases except for the data obtained with varying Gpp(NH)p and 0.1 μM glucagon, where a systematic deviation between the calculated curve and the experimental points occurs. This difference, 10–20%, has been observed in several different experiments. This may be due to the difficulty in correcting for the lag period observed in the presence of Gpp(NH)p (3) or to the presence of more than

Abbreviations: Gpp(NH)p, guanyl-5′-ylimidodiphosphate; App(NH)p, adenyl-5′-ylimidodiphosphate.

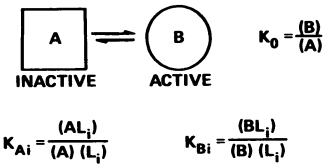

FIG. 1. The model used to explain the activation of adenylate cyclase by guanosine nucleotides and glucagon. In this figure, B is an active adenylate cyclase system, A is an inactive system, and K_{Ai} and K_{Bi} are binding constants for activator (L_i) binding to the A and B states, respectively.

one type of adenylate cyclase. However, in view of the lack of purity of the system, the agreement between theory and experiment is quite good.

Dependence of Catalytic Rate on Substrate. The variation of the steady-state initial velocity with substrate concentration can be described by the usual Michaelis–Menten equation:

$$v = \frac{V'_m}{1 + K_m'/(S)} \qquad [3]$$

where V_m' and K_m' are the observed maximal velocity and Michaelis constant and (S) is the substrate concentration. This equation has been found to be usually adequate with both ATP and adenyl-5′-ylimidodiphosphate [App(NH)p] as substrates, providing the Mg^{2+} concentration is sufficiently high and constant, the substrate concentration is not appreciably greater than 1 mM, and the pH is selected with care (4, 5). At substrate concentrations greater than 1 mM inhibition appears to occur, but this is complicated by the effects of Mg^{2+} which are considered below. The substrate Michaelis constants are not very different in the presence and absence of guanosine nucleotide and glucagon activators (5). Therefore, the substrate does not appear to show a marked preference between the A or B state so that f_B can be regarded essentially independent of the substrate concentration. Thus, the primary effect of guanosine nucleotides and glucagon is to alter the observed maximal velocity by changing f_B.

pH Dependence of Catalysis. Very little work has been done on the pH dependence of the adenylate cyclase reaction. However, the initial steady-state velocity has been measured as a function of pH with ATP and App(NH)p as substrates at substrate concentrations greater than K_m' in the presence and absence of saturating glucagon concentrations (4). In the absence of glucagon, the rate increases with increasing pH until a limiting value is reached, whereas in the presence of glucagon a bell-shaped curve is observed for both substrates. If the measured rate is assumed to approximate the maximal velocity, the observed pH dependence can be explained by postulating that two ionizable groups on the enzyme–substrate complex must be in the correct ionization state in order for catalysis to occur. This mechanism can be written as (cf. ref. 6):

$$\begin{array}{ccccc} E & & ES & & E \\ \updownarrow K_1 & & \updownarrow K_3 & & \updownarrow \\ EH + S & \rightleftharpoons & EHS & \rightleftharpoons & P + EH \\ \updownarrow K_2 & & \updownarrow K_4 & & \updownarrow \\ EH_2 & & EH_2S & & EH_2 \end{array} \qquad [4]$$

where E is the enzyme, S is the substrate, P represents the products, the K_i are ionization constants, and free protons have been omitted from the mechanism for the sake of simplicity. With this mechanism the observed steady-state parameters can be written as:

$$V'_m = \frac{V_m}{1 + (H^+)/K_4 + K_3/(H^+)}$$
$$K'_m = K_m \frac{1 + (H^+)/K_2 + K_1/(H^+)}{1 + (H^+)/K_4 + K_3/(H^+)} \qquad [5]$$

where V_m and K_m are the pH-independent maximal velocity and Michaelis constant, and the protolytic equilibria are assumed to be adjusted rapidly relative to the other steps in the mechanism. The following pK values can be estimated from the data (4): $pK_4 = 7.0$ and 7.3 and $pK_3 > 9$ and > 10 for ATP and App(NH)p, respectively, in the absence of glucagon; $pK_4 = 6.3$ and 7.3 and $pK_3 = 8.9$ and 9.5 for ATP and App(NH)p, respectively, in the presence of glucagon. The pK values can only be regarded as approximate because the pH dependence of the actual maximal velocity has not been measured. However, the principal point to be made is that the data are well accounted for by the mechanism of Eq. 4. Moreover, the pK values are clearly different for the two substrates and are altered by glucagon. The altered pH dependence in the presence of glucagon cannot be logically attributed to changes in the binding of glucagon because the pK values are different for the two substrates; neither is it likely that changes in K_m are a dominant factor. Thus, it appears that glucagon alters the pK values of ionizable groups at the active site. Changes in the pH, therefore, should alter the binding of glucagon, although this has not been looked for experimentally. This is analogous to the Bohr effect observed in the binding of ligands to hemoglobin (cf. ref. 7).

Dependence of Catalytic Rate on Mg^{2+}. The effect of Mg^{2+} on the adenylate cyclase system is very complex. At least two distinct effects are evident. Firstly, the substrate must be complexed with Mg^{2+} in order for the reaction to proceed (cf. refs. 4, 5, and 8). Moreover, the uncomplexed substrate appears to inhibit the reaction; the catalytic rate drops off rapidly when the ATP concentration exceeds the Mg^{2+} concentration. The rate also drops noticeably at high substrate concentrations (>1 mM) when the Mg^{2+} concentration is in the range 5–50 mM (5, 8); this can be attributed to the inhibition by uncomplexed ATP, but also could be due to MgATP inhibition. In any event, it is apparent that a major role of Mg^{2+} is to complex the substrate and that uncomplexed ATP probably is inhibitory. In addition to this role, Mg^{2+} appears to act independently as an activator and inhibitor of the adenylate cyclase system. Thus, for example, a bell-shaped curve is observed when the Mg^{2+} concentration is varied at constant ATP concentration (9), and the catalytic rate increases with increasing Mg^{2+} concentration with App(NH)p as substrate even when essentially all of the substrate has been complexed (5). Again, the dependence of the reaction rate on Mg^{2+} is different in the presence and absence of glucagon (5, 8). In the absence of glucagon, the rate increases with increasing Mg^{2+} concentration until a constant value is reached, whereas in the presence of glucagon a bell-shaped curve is observed for both ATP and App(NH)p. This behavior is highly reminiscent of the pH dependence of the adenylate cyclase reaction, and the data can be interpreted in terms of a mechanism identical to that of Eq. 4, except that Mg^{2+} is substituted for H^+. Similarly, the dependences of the Michaelis constant and maximum velocity of Mg^{2+} are given by Eq. 5 with Mg^{2+} substituted for H^+. With App(NH)p as substrate, the true maximal ve-

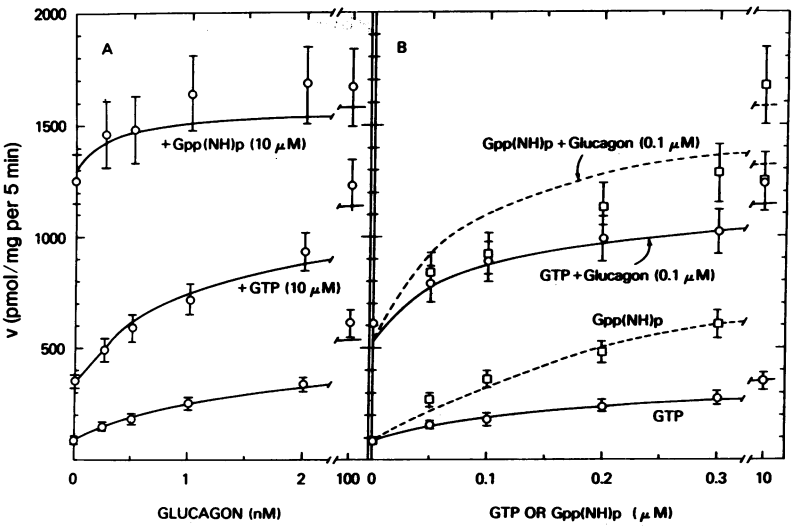

FIG. 2. Plots of the steady-state initial rate of adenylate cyclase system, v (pmol of cyclic AMP formed/mg per 5 min), in the presence of activators at pH 7.5 (25 mM Tris-Cl), 62.5 μg/ml of rat liver plasma membrane, 0.1% bovine-serum albumin, 0.1 mM ATP, 10 mM $MgCl_2$, 2 mM dithiothreitol, 37°. (A) Variation of v with glucagon in the absence of guanosine nucleotides, in the presence of 10 μM GTP, and in the presence of 10 μM Gpp(NH)p. (B) Variation of v with GTP (O, —) and Gpp(NH)p (□, - -) in the absence of glucagon and with 0.1 μM glucagon. The error bars indicate ±10% in v. The curves have been calculated with Eqs. 1 and 2 and the parameters in Table 1. The experimental procedure for carrying out the assays has been described elsewhere (10).

locity can be determined from available data in the absence of hormone and nucleotide activators and in the presence of saturating concentrations of glucagon and Gpp(NH)p (5). An analysis of the dependence of the maximum velocity on Mg^{2+} indicates that K_3 is 16 mM in the absence of the activators and 5 mM in their presence. Inhibition by Mg^{2+} is not observed so that $K_4 > 50$ mM. The ratio V_m'/K_m' is essentially independent of the Mg^{2+} concentration, which indicates $K_1 \ll 5$ mM. With ATP as substrate, K_3 can be estimated to be 2.5 mM from initial velocity data in the presence of activators (8). The available data are insufficient to interpret the dependence of the initial velocity on Mg^{2+} concentration in the presence of glucagon (8). Some of the results suggest that the binding of Mg^{2+} may be cooperative in nature, i.e., the rate depends on the Mg^{2+} concentration to a power greater than 1. However, it should be noted that the combination of the influence of Mg^{2+} on the substrate and enzyme can lead to apparent cooperativity, although actually two noncooperative processes are simply occurring simultaneously. The two principal conclusions to note are that a mechanism such as Eq. 4 quantitatively accounts for the data, and hormones and Gpp(NH)p influence the binding of Mg^{2+} to the enzyme–substrate complex (again implying the converse should be true). In addition to the above, Mg^{2+} also could influence the interconversion of the A state to the B state. In fact, the kinetic data with App(NH)p as substrate can be explained in terms of such a mechanism, but no solid evidence for or against such a hypothesis presently is available.

Table 1. Binding parameters for glucagon and guanosine nucleotides*

Activator	K_{Bi}	K_{Ai}	K_{Bi}/K_{Ai}
GTP	26.0 μM^{-1}	5.32 μM^{-1}	4.9
Gpp(NH)p	35.7 μM^{-1}	0.363 μM^{-1}	98
Glucagon	3.37 nM^{-1}	0.376 nM^{-1}	9.0

* pH 7.5 (25 mM Tris-Cl), 0.1% bovine-serum albumin, 0.1 mM ATP, 10 mM Mg^{2+}, 2 mM dithiothreitol, 37°. $K_0 = 0.0568$ as determined from the basal activity; $f_e V_m = 1618$ pmol/mg per 5 min. The parameters were obtained from a nonlinear least squares fit of the data according to Eq. 2.

The effects of metal ions on the adenylate cyclase system are obviously complex, and it will be difficult to distinguish between the various possibilities until the enzyme system can be obtained in a well-defined structural state.

Discussion

The proposed model adequately explains the available data for the rat liver plasma membrane adenylate cyclase within a simple conceptual framework: a two-state model with preferential ligand binding accounts for the activation by guanosine nucleotides and glucagon, and conventional enzyme mechanisms account for the dependency of the catalytic process on pH and Mg^{2+}. This type of model also is consistent with behavior observed with adenylate cyclase systems from other sources (cf. refs. 2 and 9–11), but a rather limited amount of data is available at this time. For example, the rat fat cell adenylate cyclase has a very high basal activity which is not greatly altered by hormones and guanosine nucleotides (12). This can be attributed to the enzyme's being predominantly in the B state. Moreover, the unusual inhibition of activity at short times by Gpp(NH)p can be explained by postulating the binding of the guanosine nucleotides to the B state is slow, whereas binding to the A state is rapid.

Undoubtedly this simple model will require amplification as the adenylate cyclase systems are purified and their structural properties are clarified. In particular, this model contains no cooperativity in ligand binding, although inclusion of this feature would be possible. At this time, a clear-cut demonstration that homotropic cooperative ligand binding occurs has not been made. In the case of Mg^{2+} (or other metal activators) this is particularly difficult because several different types of processes may be occurring simultaneously, which can lead to apparent cooperativity. A Hill plot with a slope of 3 has been reported for the activation of the turkey erythrocyte adenylate cyclase by Mg^{2+} (13), suggesting cooperative binding is occurring in this system; however, the underlying mechanism for this phenomenon remains to

be elucidated. In the case of guanosine nucleotide and glucagon activation, cooperativity does not appear to be a major factor, but the effect of a nucleotide activator on the enzymatic reaction is altered by glucagon and vice versa simply by mass action (Eq. 2, Fig. 2). Inclusion of cooperativity in the activator binding leads, of course, to the familiar models for allosteric enzymes (14, 15). A very difficult problem in assessing the occurrence of cooperativity in activator binding is that very often a good correspondence between activator binding isotherms and enzymatic velocity–activator isotherms is not observed. For example, GTP causes glucagon to dissociate from the system (16), whereas such an effect is not observed in the rate data. The question of what is *specific* binding to the enzyme system probably cannot be resolved until systems of higher purity are obtained, but the fact should not be overlooked that the lack of correspondence between equilibrium and kinetic isotherms may have important mechanistic implications. Some type of heterotropic allosterism is implied, of course, in the fact that glucagon appears to have a significant effect on H^+ and Mg^{2+} binding.

In discussing the experimental results in terms of Eq. 1, the implicit assumption has been made that variations in f_B and f_E can be considered independently. This is obviously an oversimplification inasmuch as it is clear that f_B and f_E both may depend on the same experimental parameter. For example, f_B is dependent on glucagon, which influences the binding of H^+ and Mg^{2+}, and f_E is dependent on both H^+ and Mg^{2+}. Also, both f_B and f_E are probably directly dependent on Mg^{2+}. This problem can be largely circumvented when comparing experimental results with the predictions of the model by not only holding the concentration of a particular component constant, but also working at an optimal concentration such that small changes in the binding of a particular constituent do not alter the reaction velocity appreciably. Thus, for example, if f_B is approximately unity, changes in f_E can be studied even though the concentrations of components are varied, which can also influence f_B. The possibility also should not be overlooked that ATP may compete for the guanosine nucleotide site (and vice versa) and that the metal ion also may play a role in the guanosine nucleotide activation (3, 5).

No attempt has been made to model the lag periods observed in activation by Gpp(NH)p (3). In fact, this can readily be done by postulating that either the ligand binding process or the interconversion of states A and B is slow. Rate constants then appear as additional parameters in the model.

Other models have been proposed for the adenylate cyclase system (cf. refs. 5 and 17). In particular, a three-state model can be used to fit the data for the rat liver plasma membrane system (5). In this model, the effects of pH and Mg^{2+} are explained as being due to a very strong inhibition of the enzymatic reaction by a protonated form of the substrate; this leads to inhibition constants in the nanomolar region so that the inhibitor dissociation constant is less than the substrate Michaelis constant by about a factor of 10^3. This model does not attempt to quantitatively explain the effects of guanosine nucleotides and glucagon, nor does it explain the occurrence of Mg^{2+} inhibition. The model of de Häen (17) is not directly applied to the rat liver membrane system, but very strong inhibition by free ATP is proposed rather than activation by Mg^{2+}. A recent kinetic study of detergent-dispersed cerebellar adenylate cyclase system is inconsistent with a mechanism involving strong inhibition by free ATP (18). (The rate law found in that work is a special case of that predicted by the mechanism of Eq. 4, with Mg^{2+} substituted for H^+.) Also, recent studies of the effects of different metal ions on the rat liver plasma membrane system cannot be explained by a mechanism involving free ATP inhibition, but are consistent with the general mechanism discussed here (C. Londos, personal communication). However, at the present time it is clear that many models can be developed which will adequately describe the behavior of adenylate cyclase systems; obviously, elucidation of the molecular mechanism will require clarification of the macromolecular components of the adenylate cyclase system. The virtue of the model proposed is its relative simplicity and the fact that it is based on mechanisms which have been found to be valid with simpler well-characterized enzymes.

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