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An Adenosine 3':5'-Monophosphate-Adenosine Binding Protein from Mouse Liver

Factors Affecting the Activation of the Binding Protein by Adenosine 5'-Triphosphate[1]

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A cyclic AMP-adenosine binding protein, whose binding sites are activated by preincubation in the presence of Mg^+-ATP, has been purified to apparent homogeneity from mouse liver (P.M. Ueland and S.O. Døskeland, 1977, *J. Biol. Chem.* **252**, 677-686). The degree of activation of both the cyclic AMP binding site and a high-affinity site for adenosine depends on the concentration of ATP during the preincubation. The velocity and the degree of activation are dependent on the temperature and the presence of Mg^{2+} and K^+. The NH_4^+ ion can be substituted for K^+, whereas Na^+ is inefficient. Low pH promotes the conversion from the inactive to the active form. The apparent affinity for adenosine to the high-affinity site for this adenine derivative and the affinity for cyclic AMP to the site specific for this nucleotide are independent of the degree of activation as judged from the slope of Scatchard plots. The activation of the cyclic AMP binding site by ATP (6 mM) was determined at pH 7 in the presence of 10 μM cyclic AMP, AMP, ADP, or adenosine. Adenosine specifically inhibits the activation and does not promote the inactivation of the binding protein. The possibility that the apparent inhibition of activation was effected by interference with cyclic AMP binding by adenosine was ruled out.

Two types of cyclic AMP binding proteins have been described in mouse liver. One type has a high affinity for cyclic AMP, is associated with cyclic AMP-dependent protein kinase (1), and has been characterized from a wide variety of tissues (2-5). We have purified to apparent homogeneity a cyclic AMP binding protein not related to protein kinase (6). This protein has, in addition, sites which preferentially interact with adenosine. The binding protein not exposed to Mg^{2+}-ATP exists in an inactive form defined by a low binding capacity for cyclic AMP relative to the adenosine binding capacity. By treatment of this protein with Mg^{2+}-ATP, the binding capacity for cyclic AMP increases severalfold and a high-affinity site for adenosine appears (6). The nature of the activation process has been investigated to some extent. Neither a change in sedimentation behavior nor phosphorylation accompanies the activation (6). In this paper, factors influencing the activation of the binding protein by ATP are reported.

MATERIALS AND METHODS

ATP (disodium salt) from equine muscle, ADP, AMP, cyclic AMP, adenosine, and Hepes[3] buffer were from Sigma Chemical Co., St. Louis, Missouri.

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[3] Abbreviations used: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; buffer A, 20 mM Hepes, pH 8.0, containing 20% glycerol and 10 mM 2-mercaptoethanol; buffer B, buffer A at pH 7.0.

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Cyclic [8-^{3}H]AMP (27 Ci/mmol), [U-^{14}C]adenosine (0.5 Ci/mmol), and [2-^{3}H]adenosine (21 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, and the purity was checked by thin-layer chromatography (6). Cellulose ester filters (HAWP; 0.45 μm) were purchased from Millipore Corp. Bedford, Massachusetts. Other reagents were obtained from the sources given previously (6).

Preparation of cyclic AMP-adenosine binding protein. Details of the procedure have been given previously (6). Briefly, cytosol from 400 g of liver was purified through the following steps: polyethylene glycol fractionation, DEAE (DE-52)-cellulose chromatography, ammonium sulfate fractionation, Sephadex G-150 chromatography, isoelectric focusing, and finally sucrose gradient centrifugation. The purity was checked by polyacrylamide gel electrophoresis, which showed a single band in the absence and presence of sodium dodecyl sulfate.

Measurement of cyclic [^{3}H]AMP and [^{3}H]adenosine binding. Cyclic [^{3}H]AMP (0.54 Ci/mmol) or [^{3}H]adenosine (1.42 Ci/mmol) and binding protein were mixed in the appropriate buffer containing 20% glycerol and 10 mM 2-mercaptoethanol and incubated at 0°C for 18 h in a total volume of 90 μl. The measurement of [^{3}H]adenosine binding was routinely performed in the presence of 100 μM unlabeled cyclic AMP to inhibit the binding of [^{3}H]adenosine to the cyclic AMP binding site (6). The incubations were terminated by adding 2 ml of ice-cold 80% saturated ammonium sulfate containing 100 μM unlabeled cyclic AMP and adenosine. The protein was allowed to precipitate for 1 h and was collected by suction through Millipore filters and washed three times with 3 ml of 65% ammonium sulfate. The filters were put into scintillation vials containing 1 ml of 2% sodium dodecyl sulfate, and, after shaking for 30 min, 7 ml of Diluene (Packard) was added. Experimental data suggesting that the ligand was neither bound to nor released from the binding protein during or after ammonium sulfate precipitation have been published (6).

RESULTS

Incubation conditions. Figures 1A and 1B show the pH dependence of the apparent affinity of the binding protein for cyclic AMP and adenosine, respectively. The Scatchard (7) plot for cylic AMP binding showed its steepest slope at alkaline pH (8.0). The apparent affinity for adenosine was less dependent upon pH. On the basis of these results, the binding of cyclic AMP was measured at pH 8 and the binding of adenosine at pH 7 throughout this study.

Concentration of ATP. It has been reported previously that the cyclic AMP binding site is activated by preincubation in the presence of 0.5 to 10 mM ATP (1, 6). To study the activation of the high-affinity site for adenosine at various concentrations of ATP, the binding of [^{3}H]adenosine was measured over a 100-fold concentration range and the data were plotted according to Scatchard (7). Measurement of the binding capacity for adenosine does not give information regarding the degree

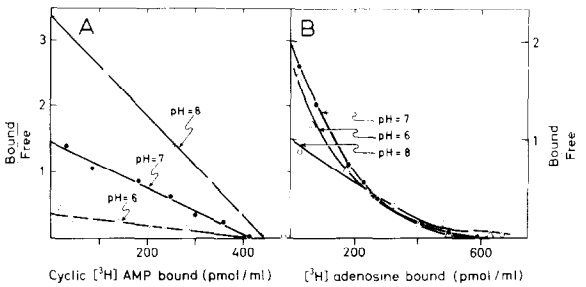

FIG. 1. Binding of cyclic [^{3}H]AMP and [^{3}H]adenosine to the activated binding protein at different pH. Binding protein (4 mg/ml) was preincubated in the presence of 9 mM ATP, 10 mM Mg^{2+}-acetate, and 150 mM KCl in 15 mM Hepes buffer, pH 7.0. The incubation was conducted at 30°C for 60 min. Samples of 30 μl were subjected to gel chromatography on Sephadex G-25 columns (0.45 × 6 cm) equilibrated with 20% glycerol and 10 mM 2-mercaptoethanol in distilled water. The temperature was 0-2°C. The binding protein (50 μg/ml) was incubated in the presence of various concentrations of cyclic [^{3}H]AMP or [^{3}H]adenosine in the following buffers containing 20% glycerol and 10 mM 2-mercaptoethanol: 20 mM Mes buffer, pH 6.0 (△——△); 20 mM Hepes buffer, pH 7.0 (●——●) and pH 8.0 (○——○). A, the data for cyclic [^{3}H]AMP binding plotted according to Scatchard; B, Scatchard plot for [^{3}H]adenosine binding.

of activation of the high-affinity site for adenosine because the binding capacity for this adenine derivative does not increase upon activation (6). Figure 2 shows that the activation of the high-affinity site for adenosine had nearly the same ATP requirement as the activation of the cyclic AMP binding site. The affinities for cyclic AMP and adenosine were independent of the degree of activation as judged from the slope of the bound/free versus bound graph.

Effect of pH on the activation. The activation process was highly dependent upon pH. Both the cyclic AMP site and the adenosine binding site were activated to a higher degree at pH 6 than at pH 7. Only a small degree of activation was observed at pH 8 under conditions otherwise the same (Figs. 3A and 3B). The affinities for both ligands were independent of the degree of activation.

Cation requirements. The binding protein was preincubated for 30 min in the presence of 6 mM ATP and 150 mM KCl, whereas the concentration of Mg^{2+} varied in the range of 0–40 mM (Fig. 4). The activation of the cyclic AMP binding site was almost totally dependent upon the presence of Mg^{2+} (Fig. 4A). The high-affinity site for adenosine was activated to some extent in the absence of this cation (Fig. 4B).

Figure 5 shows the effect of KCl. As for magnesium, the activation of the cyclic AMP binding site seemed to be more dependent upon K^+ than the activation of the high-affinity site for adenosine. The results presented in Table I suggest a certain specificity in the requirement for monovalent cation, i.e., NH_4^+ but not Na^+ could replace K^+ as judged by the activation of the cyclic AMP binding site.

Time dependency. The time course of activation was determined at pH 7 for both the cyclic AMP site (6) and the adenosine site (data not shown). The activation seemed to proceed at a somewhat higher velocity for the adenosine binding site than for the cyclic AMP site. The effect of

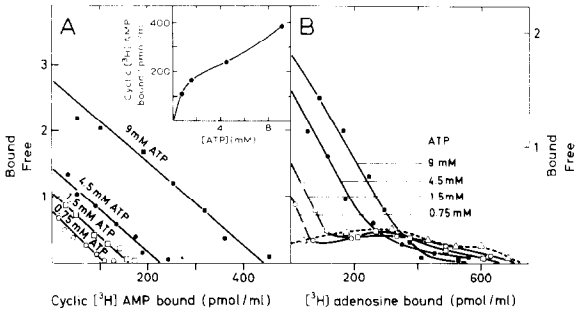

FIG. 2. The effect of the concentration of ATP on the activation of the cyclic AMP and adenosine binding sites. Binding protein (4 mg/ml) was preincubated in the presence of 0.75 mM (○——○), 1.5 mM (□——□), 4.5 mM (●——●), or 9 mM (■——■) ATP in 20 mM Hepes buffer, pH 7.0, containing 150 mM KCl and 10 mM Mg^{2+}-acetate. The preincubation was run for 30 min at 30°C. Samples of 30 μl were subjected to gel chromatography as described in the legend to Fig. 1, and the protein was eluted quantitatively. The protein excluded from the column was incubated (50 μg of protein/ml) either in the presence of various concentrations of cyclic [^{3}H]AMP (0.05 to 10 μM) in 20 mM Hepes buffer, pH 8.0, containing 20% glycerol and 10 mM 2-mercaptoethanol (buffer A) or in the presence of various concentrations (0.05 to 10 μM) of [^{3}H]adenosine in 20 mM Hepes buffer, pH 7.0, containing 20% glycerol and 10 mM 2-mercaptoethanol (buffer B). The measurement of adenosine binding was performed in the presence of 100 μM unlabeled cyclic AMP. The incubation and the determination of protein bound ligand are described under Materials and Methods. A, Scatchard plots for the binding of cyclic [^{3}H]AMP to the binding protein preincubated in the presence of increasing concentrations of ATP. Inset shows the binding capacity for cyclic AMP as a function of the concentration of ATP. B, Scatchard plots for the binding of [^{3}H]adenosine to the binding protein treated as above. △----△ indicates the binding to the nonactivated protein.

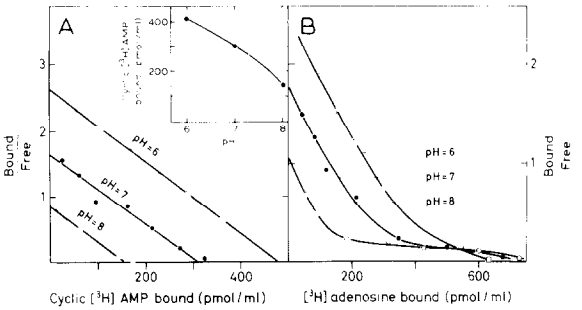

FIG. 3. The effect of pH on the activation of the cyclic AMP and adenosine binding sites by ATP. Binding protein (4 mg/ml) was preincubated for 30 min at 30°C in the presence of 6 mM ATP, 10 mM Mg^{2+}-acetate, and 150 mM KCl in 20 mM Mes buffer, pH 6.0 (□——□), or in 20 mM Hepes buffer, pH 7.0 (●——●) or pH 8.0 (○——○). Gel filtration and incubation were performed as described in the legend to Fig. 2. A, Scatchard plot for the binding of cyclic [³H]AMP to the binding protein activated at pH 6, 7, and 8. Inset shows the binding capacity for cyclic AMP as a function of pH. B, Scatchard plot for the binding of [³H]adenosine to the protein activated under the same conditions.

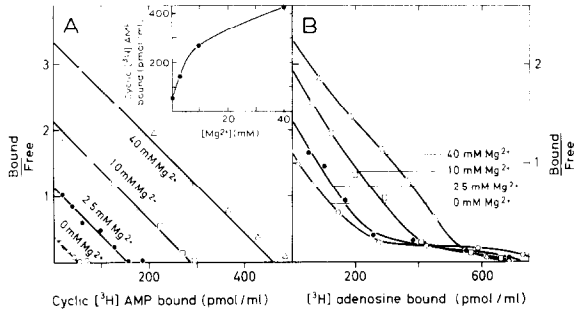

FIG. 4. The effect of magnesium on the activation of the cyclic AMP and adenosine binding sites by ATP. Binding protein (4 mg/ml) was preincubated in the presence of 6 mM ATP and 150 mM KCl in 20 mM Hepes buffer pH 7.0 containing 0 mM (○——○), 2.5 mM (●——●), 10 mM (□——□), or 40 mM Mg^{2+}-acetate (△——△). The preincubation was allowed to proceed for 30 min at 30°C. Gel filtration and incubation (50 μg of protein/ml) were performed as described in the legend to Fig. 2. A, Scatchard plot for the binding of cyclic [³H]AMP to the binding protein activated at various concentrations of magnesium. Inset shows plot of the binding capacity for cyclic AMP versus concentration of Mg^{2+}. B, Scatchard plot for the binding of [³H]adenosine to the binding protein activated at the same concentrations of magnesium.

pH on the velocity of activation of the cyclic AMP site was determined at pH 6, 7, and 8. At low pH both the velocity and the degree of activation were increased (Fig. 6A). Similar results were obtained for the adenosine binding site (data not shown). Both K^+ and Mg^{2+} increased the velocity and the degree of activation (Figs. 6B and 6C).

In conclusion, low pH, K^+, and Mg^{2+} increase the degree of activation of the cyclic AMP binding site as a function of time. Qualitatively similar results were obtained for the activation of the adenosine binding site, but this process seemed to proceed somewhat more rapidly and was less dependent upon K^+ and Mg^{2+}. The magnitude of this difference was difficult to estimate as the bound/free versus bound graph for adenosine binding was nonlinear, probably because of heterogeneity of the binding sites. Even after prolonged preincubation (90 min) the same degree of activation was not obtained when low pH (6) and high pH (8) and two concentrations of K^+ or Mg^{2+} were com-

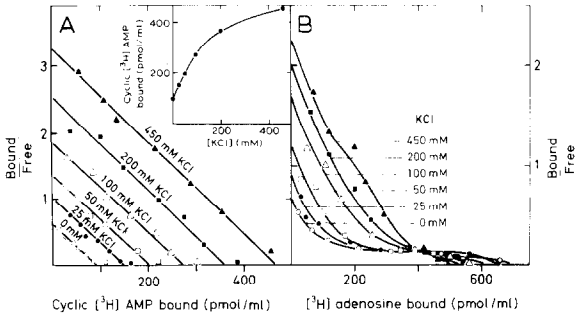

FIG. 5. The effect of KCl on the activation of the cyclic AMP and adenosine binding sites by ATP. Binding protein (4 mg/ml) was preincubated in the presence of 6 mM ATP and 10 mM Mg^{2+}-acetate in 20 mM Hepes buffer, pH 7.0, containing no KCl (○——○) or 25 mM (●——●), 50 mM (□——□), 100 mM (△——△), 200 mM (■——■) or 450 mM KCl (▲——▲). The preincubation was run for 30 min at 30°C. Gel filtration and incubation (50 μg of protein/ml) were performed as described in the legend to Fig. 2. A, Scatchard plot for the binding of cyclic [^{3}H]AMP to the binding protein activated at various concentrations of KCl. Inset shows plot of binding capacity for cyclic AMP versus concentration of KCl. B, Scatchard plot for the binding of [^{3}H]adenosine to the binding protein activated at the same concentrations of KCl.

TABLE I

SPECIFICITY IN THE REQUIREMENT FOR MONOVALENT CATION[a]

Addition	Concentration (mM)	Picomoles of cyclic [^{3}H]AMP bound per milliliter of incubation mixture
Control	–	75
KCL	150	310
	450	412
NH_4Cl	150	230
	450	296
NaCl	150	102
	450	115

[a] Binding protein (4 mg/ml) was preincubated in the presence of 6 mM ATP, 10 mM Mg^{2+}-acetate, and the concentration and type of salt indicated. The preincubation was performed for 30 min at 30°C in 20 mM Hepes buffer, pH 7.0. Gel filtration and incubation in the presence of cyclic [^{3}H]AMP were performed as described in the legend to Fig. 2.

pared. Thus, before the final conclusion on the mode of action of these factors can be made, detailed information regarding the stability of the binding protein during prolonged incubations at different ionic strengths and pH must be available. Because the binding properties change widely, no parameter giving information on the intactness of the protein is available at the moment.

Temperature. The experiments presented have been conducted at a preincubation temperature of 30°C. When the binding protein was preincubated at 0°C for 6 h in the presence of ATP (6 mM), Mg^{2+} (10 mM), and KCl (150 mM) at pH 7.0, no activation was observed. By increasing the temperature from 30 to 37°C, a twofold increase in the velocity of activation was observed (data not shown). Thus, the activation process is a temperature-dependent phenomenon. In contrast, the binding of adenine derivatives to the activated protein is less dependent on this factor (6).

The effect of adenosine on the activation. Adenosine seems to bind to a site different from that of cyclic AMP (6). It was of interest to investigate whether binding of adenosine would affect the activation process. The binding protein was activated for increasing periods of time by ATP (6 mM) alone and by ATP in the presence of 10 μM cyclic AMP, AMP, ADP, and adenosine.

AMP and ADP competitively inhibit the binding of cyclic AMP and probably bind to the protein (6). Nucleotide carried over from the preincubation mixture could inhibit cyclic AMP binding in spite of being partially removed by gel filtration. How-

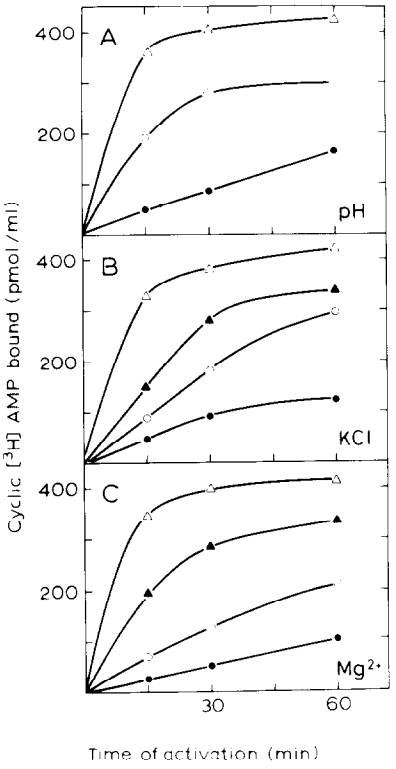

FIG. 6. Time course of activation. Effect of pH, KCl, and Mg^{2+}. A, Binding protein (4 mg/ml) was preincubated in the presence of 6 mM ATP, 10 mM Mg^{2+}-acetate, and 150 mM KCl in 20 mM Mes buffer, pH 6 (△——△), or 20 mM Hepes buffer, pH 7.0 (○——○) or pH 8.0 (●——●). The incubation was allowed to proceed at 30°C for the time indicated. Gel filtration and incubation (50 μg of protein/ml) were performed as described in the legend to Fig. 2. Binding capacity for cyclic AMP is plotted against time of activation. B, Binding protein (4 mg/ml) was preincubated in the presence of 6 mM ATP and 10 mM Mg^{2+}-acetate in 20 mM Hepes buffer, pH 7.0, containing 0 mM (●——●), 50 mM (○——○), 150 mM (▲——▲), or 450 mM KCl (△——△). The preincubation was run for the time indicated at 30°C. Gel filtration and incubation (50 μg of protein/ml) were performed as described in the legend to Fig. 2. Binding capacity for cyclic AMP is plotted against time of activation. C, Binding protein (4 mg/ml) was preincubated in the presence of 6 mM ATP and 150 mM KCl in 20 mM Hepes buffer, pH 7, containing 0 mM (●——●), 2.5 mM (○——○), 10 mM (▲——▲), or 40 mM Mg^{2+}-acetate (△——△). The preincubation was run at 30°C for the time indicated. Gel filtration and incubation (50 μg of protein/ml) were performed as described in the legend to Fig. 2. Binding capacity for cyclic amp is plotted against time of activation.

ever, if the binding to the cyclic AMP site obeys Michaelis–Menten kinetics, the determination of the cyclic AMP binding capacity in the presence of a competitive inhibitor (AMP or ADP) could be accomplished by increasing the concentration of cyclic [^{3}H]AMP until a plateau was obtained. Therefore, the degree of activation was determined as cyclic AMP binding capacity by incubating at two concentrations of cyclic [^{3}H]AMP (5 and 10 μM). The experimental details are given in the legend to Fig. 7. Even when the preincubation was performed in the presence of cyclic AMP, no reduction in the binding capacity for cyclic [^{3}H]AMP was observed. This could perhaps be explained by partial

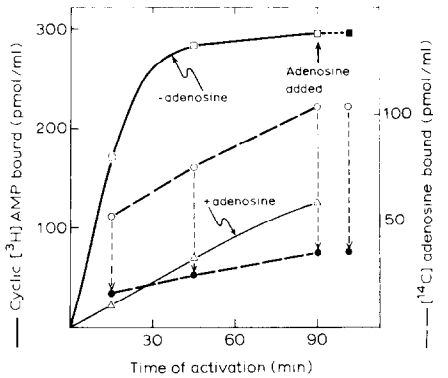

FIG. 7. Inhibition of activation by adenosine. Binding protein (2 mg/ml) was preincubated in the presence of 6 mM ATP, 10 mM Mg^{2+}-acetate, and 150 mM KCl in 20 mM Hepes buffer, pH 7.0, with and without [^{14}C]adenosine (10 μM). One fraction of the incubation mixture not containing adenosine was made 10 μM in [^{14}C]adenosine after being incubated for 90 min (−adenosine). Samples of 30 μl were taken at 15, 45, and 90 min and at 90 plus 15 min (from the fraction made 10 μM in adenosine at time = 90 min) and applied to Sephadex G-25 columns (0.45 × 6 cm) equilibrated with buffer A. The protein was quantitatively eluted in 200 μl, and aliquots of 15 μl were counted to determine the amount of [^{14}C]adenosine bound to the protein prior to incubation in the presence of cyclic [^{3}H]AMP (○- - -○). To determine the binding capacity for cyclic AMP (△, □ ■) the binding protein was incubated for 18 h at 0°C with 5 and 10 μM cyclic [^{3}H]AMP in buffer A. The amount of [^{14}C]adenosine bound to the protein after 18 h of incubation was determined simultaneously (●- - -●). The dashed arrows indicate the dissociation of [^{14}C]adenosine during the incubation.

displacement of cyclic AMP by the 600-fold concentration of ATP present during the excess and efficient removal of cyclic AMP by the Sephadex G-25 filtration. The activation was not affected by AMP or ADP (10 μM). The results obtained for adenosine are presented in Fig. 7. Adenosine inhibited the activation, and the percentage inhibition was highest after a short activation period. The binding of [^{14}C]adenosine was measured simultaneously, before and after the completion of the incubation (18 h at 0°C) in the presence of cyclic [^{3}H]AMP (Fig. 7). In the absence of adenosine, maximal activation was obtained within 45 min of preincubation under the conditions used. To assure that bound adenosine carried over from the preincubation mixture did not affect the determination of the binding capacity for cyclic [^{3}H]AMP, the following experiment was conducted. An incubation mixture containing binding protein activated (preincubated) for 90 min was made 10 μM in [^{14}C]adenosine and incubated for a further 15 min. The amount of [^{14}C]adenosine bound was the same as the amount bound to the protein during 90 min of preincubation in the presence of adenosine. The binding capacity for cyclic [^{3}H]AMP was not decreased (Fig. 7). Figure 7 further shows that about 60% of the [^{14}C]-adenosine bound was released from the binding protein after 18 h of incubation at 0°C (indicated by arrows).

No activation by adenosine. Binding protein was preincubated for 30 min under the conditions described in the legend to Fig. 7 with 10 μM adenosine and without ATP. As expected, no activation of the cyclic AMP site was detected (data not shown).

Preliminary studies on the inactivation: No effect of adenosine. Preliminary studies on the inactivation of the activated binding protein was performed to exclude the possibility that adenosine promoted inactivation rather than inhibited activation. Binding protein (4 mg/ml) was preincubated in the presence of 6 mM ATP, 40 mM Mg^{2+}, and 450 mM KCl for 30 min at 30°C and then subjected to gel filtration through a Sephadex G-25 column equilibrated with 20 mM Hepes buffer, pH 7.0, and eluted in the same buffer. Binding protein was diluted to 0.5 mg/ml and KCl and Mg^{2+} were added to final concentrations of 300 and 20 mM, respectively. Incubation was performed at 30°C for 0, 15, and 60 min in the absence and presence of 15 μM adenosine and was terminated by applying samples of 30 μl to Sephadex G-25 columns (0.45 × 6 cm) equilibrated with buffer A. The temperature was 0–2°C. The binding capacity for cyclic [^{3}H]AMP was determined at two concentrations of cyclic [^{3}H]AMP (5 and 10 μM) as described in the preceeding paragraph and under Materials and Methods. The binding capacities for cyclic [^{3}H]AMP were 100, 80, and 65% after 0, 15, and 60 min of incubation, respectively. The results were the same in the absence and presence of adenosine (data not shown).

These experiments have been conducted several times. For reasons not apparent to us yet, the degree of inactivation as a function of time varied from one experiment to another. Any effect of adenosine on the inactivation was, however, never observed.

Summary. The essential points concerning the binding properties of the nonactivated and activated forms of the cyclic AMP–adenosine binding protein and the factors promoting or inhibiting the activation are given in Table II.

DISCUSSION

Both the homogeneous binding protein and the crude binding protein purified through one step [i.e., DEAE-cellulose chromatography or sucrose gradient centrifugation (6)] exist in the inactive form when not exposed to Mg^{2+}–ATP. Measurement of the degree of activation in fresh crude liver extract is hampered by the presence of adenosine deaminase and cyclic AMP binding protein associated with the cyclic AMP-dependent protein kinase. In this paper factors affecting the conversion from the inactive to the active form are reported. The data convey little information regarding their mode of action. Thus, the information presented here is a survey of the factors influencing the

TABLE II

BINDING PROPERTIES OF THE NONACTIVATED AND ACTIVATED BINDING PROTEIN AND FACTORS AFFECTING THE CONVERSION FROM THE NONACTIVATED TO THE ACTIVATED FORM

	Nonactivated binding protein	Factors affecting the activation process	Activated binding protein
Cyclic AMP binding	Low capacity; apparent $K_d = 1.5 \cdot 10^{-7}$ M; one class of binding sites		High capacity; $K_d = 1.5 \cdot 10^{-7}$ M; one class of binding sites
Adenosine binding	High capacity; low affinity		High capacity; $K_d = 2 \cdot 10^{-7}$ M; heterogeneity of binding site
Factors promoting the activation		ATP, Mg^{2+}, KCl, low pH; high temperatures (30°C)	
Factors inhibiting the activation		Adenosine; low temperature (0°C)	

activation process and may be an introduction to further studies on the molecular mechanism of activation. The data reported may be of value when disclosing the existence of similar proteins from other tissues. We have purified a cyclic AMP-adenosine binding protein from bovine adrenal cortex which has similar requirements for activation (8).

A cation specificity was observed showing a superficial resemblance to that reported for the activation of other proteins. Pyruvate kinase (9), phosphofructokinase (10), and aspartokinase I homoserine dehydrogenase (11, 12) require K^+ for activity. With respect to pyruvate kinase and phosphofructokinase, NH_4^+ could replace K^+, whereas Na^+ was inefficient. Thus, the ionic requirements for activation of the cyclic AMP-adenosine binding protein seem to fit with the generalization made by Evans and Sorger (13). Those enzymes activated by the K^+ ion are also usually activated by NH_4^+ but little by Na^+.

The potassium ion may exert its role either by complexing with ATP (14, 15) or by changing the conformation of the protein. The binding protein possibly has binding sites for K^+ or NH_4^+, as suggested for phosphofructokinase from yeast (16). Ionic effects on protein hydration have recently been proposed as a mechanism of ionic activation of enzymes (17). Similarly, a change in pH probably alters the conformation of the protein such that at low pH the binding protein is more prone to undergo a change in activation. The apparent low affinity for cyclic AMP at low pH (Fig. 1A) could thus be explained by a change in the degree of activation after incubation in the presence of cyclic [^{3}H]-AMP.

Two possibilities exist as to the mode of action of ATP. This nucleotide may serve as a chelator to remove a metal-ion complex which stabilizes the protein in an inactive configuration and/or binding of ATP (6) may alter the structure of the binding protein. ATP effects on phosphoprotein phosphatase have been explained by the chelating effect of ATP (18). However, the fact that no activation was observed after preincubation in the presence of EDTA (1) supports the possibility that a change in conformation through ATP binding is the way that ATP effects the activation.

Kinetic binding studies indicate that adenosine specifically interacts with a site different from that of cyclic AMP. Cyclic AMP and ADP probably do not bind to this site as judged from no inhibition of adenosine binding. AMP was a poor inhibitor (6). The observation that only adenosine among these adenine derivatives was a potent inhibitor of activation further emphasizes the difference between the cyclic AMP site and the adenosine binding

site. It may be a step toward understanding the functional properties of the cyclic AMP-adenosine binding protein, as the inhibition of activation is a measurable effect of adenosine binding. A detailed study on the correlation between adenosine binding and this effect would be of interest.

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REFERENCES

1. DØSKELAND, S. O., AND UELAND, P. M. (1975) *Biochem. Biophys. Res. Commun.* **66**, 606–613.
2. GILL, G. N., AND GARREN, L. D. (1970) *Biochem. Biophys. Res. Commun.* **39**, 335–343.
3. KUMON, A., YAMAMURA, H., AND NISHIZUKA, Y. (1970) *Biochem. Biophys. Res. Commun.* **41**, 1290–1297.
4. REIMANN, E. M., BROSTROM, C. O., CORBIN, J. D., KING, C. A., AND KREBS, E. G. (1971) *Biochem. Biophys. Res. Commun.* **42**, 187–194.
5. ERLICHMAN, J., HIRSCH, A. H., AND ROSEN, O. M. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 731–735.
6. UELAND, P. M., AND DØSKELAND, S. O. (1977) *J. Biol. Chem.* **252**, 677–686.
7. SCATCHARD, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672.
8. DØSKELAND, S. O., AND UELAND, P. M. (1977) *Biochem. J.* **165**, 561–573.
9. KARCHMAR, J. F., AND BOYER, P. D. (1953) *J. Biol. Chem.* **200**, 669–682.
10. LARDY, H. (1962) *The Enzymes* **6**, 67–74.
11. OGILVIE, J. W., SIGHTLER, J. H., AND CLARK, R. B. (1969) *Biochemistry* **8**, 3557–3567.
12. PATTE, J. C., LEBRAS, G., LOVINY, T., AND COHEN, G. N. (1963) *Biochim. Biophys. Acta* **67**, 16–30.
13. EVANS, H. J., AND SORGER, G. J. (1966) *Annu. Rev. Plant Physiol.* **17**, 47–76.
14. LOWENSTEIN, J. M. (1960) *Biochem. J.* **75**, 269–274.
15. MELCHIOR, N. C. (1954) *J. Biol. Chem.* **208**, 615–627.
16. MAVIS, R. D., AND STELLWAGEN, E. (1970) *J. Biol. Chem.* **245**, 674–680.
17 LOW, P. S., AND SOMERO, G. N. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 3305–3309.
18. KATO, K., KOBAYASHI, M., AND SATO, S. (1975) *J. Biochem.* **77**, 811–815.