Comparison of Adenosine 3':5'-Monophosphate-dependent Protein Kinases from Rabbit Skeletal and Bovine Heart Muscle*

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Homogeneous preparations of adenosine 3':5'-monophosphate (cyclic AMP)-dependent protein kinase from rabbit skeletal (Peak I) and bovine heart muscle have been compared. Each enzyme has an $s_{20,w}$ value of 7.0. Each enzyme binds 2 mol of cyclic AMP per mol of enzyme and is dissociated in the presence of saturating concentrations of cyclic AMP into a dimeric regulatory subunit-cyclic AMP complex and two catalytic subunits. The isolated subunits recombine, resulting in the formation of the original holoenzyme in each case. Several differences between the two enzymes were found. Different salt concentrations are necessary for elution of the respective enzyme from DEAE-cellulose. Their regulatory subunits differ with respect to their sedimentation constants and mobility on sodium dodecyl sulfate gel electrophoresis. The regulatory subunit of the heart enzyme is rapidly phosphorylated by MgATP but this does not occur with the skeletal muscle enzyme. MgATP is bound with high affinity only to the skeletal muscle enzyme. The enzymes have different apparent dissociation constants and Hill coefficients for cyclic AMP binding. With the skeletal muscle enzyme MgATP increases the dissociation constant for cyclic AMP about 10-fold and decreases the Hill coefficient, while with the heart enzyme phosphorylation decreases the dissociation constant for cyclic AMP 5- to 6-fold and increases the Hill coefficient. Different concentrations of cyclic AMP are required to dissociate the skeletal and heart muscle enzymes. The presence of MgATP increases the concentration of cyclic AMP required to dissociate the skeletal muscle enzyme but decreases the concentration necessary to dissociate the heart enzyme.

Cyclic AMP exerts many diverse biological effects, a number of which are known to be due to the activation of protein kinase(s) (1). Evidence has been obtained that the mechanism through which cyclic AMP activates the enzyme involves the dissociation of an inactive holoenzyme form into a regulatory subunit-cyclic AMP complex and active catalytic subunits (2-5). The following equilibrium expression of the reversible dissociation of the skeletal muscle holoenzyme (R,C,) into a dimeric regulatory subunit-cyclic AMP complex (R,·cyclic AMP,) and two free catalytic subunits (C) has been established (6, 7).

$$R,C, + 2 \text{ cyclic AMP} \rightleftharpoons R, \text{·cyclic AMP,} + 2 \text{ C} \quad (1)$$

Early studies showed that cyclic AMP-dependent protein kinase activity in many tissue extracts can be separated into several fractions by ion exchange chromatography (8-13). Two major fractions have been identified and referred to as Peaks I and II. Corbin et al. (14) focused attention on the fact that these two types of enzyme can be distinguished by the ability of histones or high salt concentrations to alter their dependence on cyclic AMP. Presumably these effects are due to differences in the dissociability of the two kinds of protein kinase. The interpretation of some of these results has been difficult, however, because of the possibility that some of the observed peaks may have been due to partial proteolysis or because of other complications that can arise in dealing with enzymes in crude fractions.

Work with purified enzymes has also supported the idea that two major types of cyclic AMP-dependent protein kinases exist. The most intensively studied enzymes have been the rabbit skeletal muscle protein kinase isolated from Peak I separated on DEAE-cellulose (6, 7, 15) and the bovine heart protein kinase (16-20). The major distinguishing characteristics that have been reported for the purified heart and skeletal muscle enzymes are as follows. Rabbit skeletal muscle protein kinase* is easily dissociated into its subunits by cyclic AMP

*In this paper “skeletal muscle protein kinase” when used without qualification refers to the first peak of cyclic AMP-dependent protein kinase activity in many tissue extracts. The abbreviations used are: cyclic AMP or cAMP, adenosine 3':5'-monophosphate; Mes, 2-(N-morpholino)ethanesulfonic acid; $K_d$, apparent dissociation constant.
study of the two enzymes. The possible benefits to be derived from such a study were increased by indications that these enzymes from bovine heart are both available as homogeneous proteins, enzyme (6, 7, 17). The individual subunits of the rabbit muscle protein kinase isolated from the second DEAE-cellulose peak have also been purified and many of their properties determined (21). Preliminary studies carried out with the regulatory subunit of this enzyme show that it has many features in common with the regulatory subunit of the heart enzyme (21).

Because of the current interest in cyclic AMP-dependent protein kinases, and in view of the fact that rabbit skeletal muscle cyclic AMP-dependent protein kinase and the enzyme from bovine heart are both available as homogeneous proteins, it appeared worthwhile to do a simultaneous comparative study of the two enzymes. The possible benefits to be derived from such a study were increased by indications that these enzymes may serve as prototypes for two general isozymic forms of cyclic AMP-dependent protein kinase (21).

EXPERIMENTAL PROCEDURES

Materials

ATP, cyclic AMP, histone IIA mixture, 2(N-morpholino)ethane-sulfonic acid, and myoglobin type I were obtained from Sigma. Cyclic [3H]AMP and [3H]ATP were purchased from New England Nuclear. Cellulose-ester filters (HAWP) were from the Millipore Corp. DEAE-cellulose (Whatman DE52) was purchased from Reeve Angel, Inc. Horseradish peroxidase was obtained from Worthington. Phosphorylase b was prepared as described earlier (22). Histone IIA was coupled with CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals) as indicated by the manufacturer. The final concentration of histone IIA covalently bound was 10 mg/ml of resin. [γ-32P]ATP was prepared by a slight modification of the procedure of Glynn and Chappell (23).

Methods

Assays—Catalytic activity of the protein kinase was determined by a slight modification (13) of the filter paper method described by Reimann et al. (8). Nucleotide binding was measured by the Millipore filter technique (24) in a total volume of 0.1 ml containing 3 μmol of Mes (pH 6.9), 0.2 mg of bovine serum albumin, 10 μmol of NaCl, and cyclic [3H]AMP or [3H]ATP as indicated. Reactions were started by addition of aliquots of enzyme. After incubation at 23°, 80 μl of the reaction mixture were spotted on filters and washed twice with 5 ml of 5 mM Mes buffer (pH 6.9) containing 100 mM NaCl.

Density Gradient Centrifugation—Sucrose density gradient (5 to 15%) centrifugations were carried out according to Martin and Ames (25) in a Beckman SW 40 Ti rotor at 40,000 rpm for 17 hours at 20° using the following buffer: 5 mM Mes (pH 6.9), 100 mM NaCl, 0.2 mM EDTA, and 15 mM 2-mercaptoethanol. Samples containing 25 μg of the respective enzymes were preincubated at 23° for 20 min with or without nucleotides, as indicated, before layering on sucrose gradients.

enzyme activity eluted at a conductivity of 5 mmoles from DE59 chromatography of skeletal muscle extracts. This peak (Fig. 1A) represents approximately 80% of the total cyclic AMP-dependent protein kinase activity eluted during the chromatography procedure. Similarly bovine heart protein kinase refers to the second peak of cyclic AMP-dependent protein kinase eluted at a conductivity of 6 mmoles from DE59 chromatography of bovine heart extracts. This peak (Fig. 1B) represents approximately 90% of the total cyclic AMP-dependent activity eluted during the chromatography procedure.

Where indicated cyclic [3H]AMP, ATP, and magnesium acetate were present throughout the gradient. Gradients were divided into 70 fractions and aliquots were tested for catalytic activity and bound cyclic AMP. Phosphorylase b (s∞/v = 8.8), horseradish peroxidase (s∞/v = 3.8), and myoglobin (s∞/v = 2.05) were used as internal marker proteins (26).

Preparations—Rabbit skeletal muscle cyclic AMP-dependent protein kinase was isolated from the first peak separated by DEAE-cellulose chromatography as described previously (13).

Cyclic AMP-dependent protein kinase from bovine heart was prepared utilizing some steps taken from the method for the skeletal muscle enzyme (13) and one step taken from the procedure of Rubin et al. (16). Initial steps through the first DEAE-cellulose column were carried out, as described above, and four changes of 500 ml of buffer were used prior to the major peak of activity (see Fig. 1B) were pooled and solid ammonium sulfate was added (16.0 g/100 ml). The pH was kept between 7.0 and 7.5 by dropwise addition of ammonium hydroxide. After stirring for 1 hour at 4°, the precipitate was collected by centrifugation and discarded. Solid ammonium sulfate (19.9 g/100 ml) was added to the supernatant and the solution was stirred for 1 hour at 4°. The precipitate was collected by centrifugation, dissolved in 50 mM Tris/50 mM EDTA buffer (pH 8.0) containing 0.2 mM EDTA and 16 mM 2-mercaptoethanol, and dialyzed overnight against the same buffer.

CM-Sephadex C50 resin was added to the dialyzed fraction (13). After equilibration for 30 min the resin was separated by filtration and the filtrate was fractionated using alumina C, gel according to Rubin et al. (16). Active fractions were dialyzed overnight against 10 mM Mes buffer (pH 6.5), 0.2 mM EDTA, 185 mM NaCl, and 15 mM 2-mercaptoethanol. Dialyzed fractions were then applied to a histone A-Sepharose column in a ratio of 2 to 4 mg of protein/ml of resin. The column was washed extensively with the same buffer used for dialysis and the protein kinase was eluted with a linear NaCl gradient from 185 to 900 mM. Active fractions were pooled and dialyzed overnight against 5 mM Mes (pH 6.5) containing 0.2 mM EDTA and 15 mM 2-mercaptoethanol. After concentration on a small DEAE-cellulose column (13) the enzyme was applied to a Sepharose 6B column (2.5 × 170 cm) equilibrated with 5 mM Mes (pH 6.5), 100 mM NaCl, 0.2 mM EDTA, and 15 mM 2-mercaptoethanol. The peak fractions were pooled, concentrated, and reapplied to the same column. The procedure yielded an essentially homogeneous protein kinase preparation having a specific activity of 1.56 μmol of phosphate incorporated into histone mixture II per min per mg of enzyme protein at 30°. Total recovery was 10 to 15%. The original activity and sedimentation constant of the holoenzyme were the same as the activities of the subunits in sodium dodecyl sulfate gel electrophoresis were maintained for at least 4 weeks when the preparation was stored at 4°.

Catalytic subunits of bovine heart and rabbit skeletal muscle protein kinase were purified by a slight modification (27) of the previously described methods (13). Regulatory subunit of bovine heart protein kinase was prepared from purified holoenzyme by the addition of cyclic AMP and ATP. Cyclic AMP-dependent protein kinase was prepared from purified holoenzyme by the addition of cyclic AMP and ATP and was subsequently precipitated with CM-Sephadex C50 column (13). Small amounts of catalytic subunit still present in the effluent of the column were separated from the regulatory subunit-cyclic AMP complex on a 1-ml DEAE-cellulose column by stepwise elution with NaCl. The regulatory subunit of the rabbit skeletal muscle enzyme was obtained by a cyclic AMP affinity column procedure (28).

Preparation of Phosphorylated Heart Muscle Protein Kinase—Pure bovine heart muscle protein kinase (2 mg) was incubated at 30° in 0.7 ml of buffer containing 15 μmol of Mes (pH 6.9), 2.5 μmol of magnesium acetate, and 0.1 μmol of [γ-32P]ATP (663 cpm/pmol). The reaction was stopped after 10 min by addition of 14 μmol of EDTA. Under these conditions, in the absence of cyclic AMP, 1.4 mol of 32P were incorporated per mol of holoenzyme. The sample was then dialyzed at 4° against four changes of 500 ml of 5 mM Mes buffer (pH 6.9) that contained 100 mM NaCl, 0.2 mM EDTA, and 15 mM 2-mercaptoethanol. Radioactive phosphate incorporated into protein was determined on protein samples treated by incubation for 30 min at 75° in 17% trichloroacetic acid and on samples subjected to gel electrophoresis in the presence of sodium dodecyl sulfate. Protein was measured by the Lowry procedure using bovine serum albumin as the standard.

Miscellaneous Methods—Polycarboxylate gel electrophoresis (7.5% acrylamide) in the presence of 0.1% sodium dodecyl sulfate was carried out essentially as described by Weber and Osborn (29) except that in most cases a 25 mM Tris/192 mM glycine buffer system (pH 8.5) was used in place of the sodium phosphate.
RESULTS

Ion Exchange Chromatography—Crude extracts from rabbit skeletal or bovine heart muscle were applied to separate but otherwise identical DEAE-cellulose columns and eluted with a linear NaCl gradient. Under these conditions cyclic AMP-dependent protein kinase activity from each extract was resolved into two peaks (Fig. 1) which eluted at conductivities corresponding to about 20 mM (Peak I) and 165 mM NaCl (Peak II). However, 80% of the activity present in the rabbit skeletal muscle extract emerged at the lower salt concentration, whereas 90% of the activity present in the bovine heart extract eluted at the higher salt concentration, with the remaining activity in each case present in the other peak. The degree of asymmetry noted in the second peak of activity with the skeletal muscle extract was variable from one experiment to another. When homogeneous preparations of rabbit skeletal and bovine heart muscle cyclic AMP-dependent protein kinase were rechromatographed using this same type of column, they eluted in their original positions. This would indicate that the elution profile obtained with the crude extracts was probably not affected by nonspecific protein-protein interactions.

Sodium Dodecyl Sulfate Gel Electrophoresis—Purified rabbit skeletal muscle cyclic AMP-dependent protein kinase and the bovine heart enzyme were essentially homogeneous as judged by sodium dodecyl sulfate gel electrophoresis (Fig. 2). The catalytic subunits of both enzymes had the same relative mobility corresponding to a molecular weight of about 40,000. However, different mobilities were found for the regulatory subunits, corresponding to molecular weights of 48,000 and 55,000 for the skeletal muscle and heart enzymes, respectively. This difference in mobility was found when either the holoenzyme or the isolated regulatory subunits were used. Phosphorylation of the regulatory subunit of the heart enzyme resulted in a small but reproducible decrease in mobility to a position that corresponded to a molecular weight of 59,000 (not illustrated). This change was seen only when the Tris-glycine and not when the sodium phosphate buffer system was used.

Phosphorylation of Regulatory Subunit—In confirmation of the results of Erlichman et al. (19) it was found that the regulatory subunit of bovine heart cyclic AMP-dependent protein kinase was rapidly phosphorylated when the holoenzyme was incubated with [γ-32P]ATP. In the presence of cyclic AMP a maximal plateau value of 1.8 to 2.1 mol of 32P incorporated per mol of heart holoenzyme was reached within 1 min and maintained for at least 1 hour. However, essentially no radioactive phosphate was incorporated into the skeletal muscle enzyme at the early time points and less than 0.5 mol/mol of holoenzyme was found after 3 hours of incubation.

In order to exclude the possibility that the regulatory subunit of the heart enzyme was phosphorylated by a kinase missing from the skeletal muscle preparation, both enzymes were incubated together in the presence of [γ-32P]ATP and cyclic AMP. The reaction was terminated by addition of sodium dodecyl sulfate and the complete reaction mixture was applied to sodium dodecyl sulfate gels. Under these conditions radioactive phosphate was detected only in the slowest moving band corresponding to regulatory subunit of the heart enzyme (Fig. 3). This result would indicate that only the regulatory subunit of the heart enzyme is a substrate for the phosphoprotein kinase, and that the lack of phosphorylation of the regulatory subunit of the skeletal muscle enzyme was due to the lack of an appropriate kinase in the preparation.

High Affinity Binding of MgATP—Skeletal muscle cyclic AMP-dependent protein kinase is known to bind MgATP with high affinity (6, 7, 30). The catalytic subunit derived from the holoenzyme also binds MgATP, as evidenced by the fact that it utilizes the nucleotide-metal complex as a substrate in the phosphotransferase reaction, but the $K_a$ in this reaction is considerably higher than the apparent $K_a$ for the binding of MgATP to the holoenzyme (7, 8). In the present comparative study it was confirmed that MgATP binds to the skeletal muscle protein kinase ($K_a \approx 35$ mM) but no comparable high affinity site was found with the bovine heart enzyme (Fig. 4).
FIG. 3. Correlation between phosphate incorporated and protein stain after gel electrophoresis in the presence of sodium dodecyl sulfate. Skeletal muscle (3.0 µg) and heart holoenzymes (2.5 µg) were incubated together for 0.5 min at 30° in total volume of 0.04 ml containing 0.8 µmol of Mes (pH 6.9), 0.15 µmol of magnesium acetate, 0.01 µmol of ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA), 0.01 µmol of [γ-32P]ATP (262 cpm/pmol), and 1 nmol of cyclic AMP. Reaction was stopped by addition of sodium dodecyl sulfate (2%). After gel electrophoresis of the reaction mixture in the presence of sodium dodecyl sulfate, the gel was stained with Coomassie brilliant blue dye, destained, and intensity of stain was recorded at 550 nm. The solid line is Asso; the shaded area is counts per min of JT per slice. Bands corresponded to (from left to right): regulatory subunit of the heart protein kinase, regulatory subunit of skeletal muscle protein kinase, and catalytic subunits of the skeletal muscle and heart protein kinase.

FIG. 4. Binding of MgATP to skeletal muscle (○) or heart protein kinase (○). Holoenzymes from skeletal muscle or heart (23 nM each) were incubated with [3H]ATP for 30 min at 23° in the presence of 2 mM magnesium acetate. The amount of bound ATP was determined as described in the text.

Cyclic AMP Binding—In experiments carried out at protein kinase concentrations similar to those found in muscle (15, 27) it was found that the rabbit skeletal and heart muscle protein kinase each bound 2 mol of cyclic AMP per mol of holoenzyme (Fig. 5) with apparent $K_a$ of 0.1 µM for the skeletal muscle enzyme and 2.8 µM for the heart enzyme. The presence of MgATP did not change the total amount of cyclic AMP bound to either enzyme; however, the apparent $K_a$ for cyclic AMP were increased 10-fold to 1.0 µM for the skeletal muscle enzyme but decreased about 6-fold to 0.5 µM for the heart enzyme by MgATP. The same values were obtained when incubations were performed for 5, 15, or 30 min. Using phosphorylated heart enzyme that had been extensively dialyzed to remove MgATP (see "Methods"), an apparent $K_a$ for cyclic AMP of 0.4 µM was obtained, showing that phosphorylation of the regulatory subunit and not the presence of MgATP was responsible for the decrease in the apparent dissociation constant.

Scatchard or Lineweaver-Burk plots of the cyclic AMP binding data were nonlinear in the absence of MgATP but became nearly linear in its presence. Hill plots of the binding data gave $n$ values of 1.2 and 0.85 for the skeletal muscle and heart enzyme, respectively, in the absence of MgATP (Fig. 6). In the presence of MgATP an $n$ value of 1.0 was obtained for both enzymes. The same value was found when phosphorylated heart enzyme was used in the absence of MgATP. These studies would indicate that in the absence of MgATP or phosphorylation, cyclic AMP binds in an apparently positively cooperative manner to the skeletal muscle protein kinase ($n$ value >1) and in an apparently negatively cooperative manner to the nonphosphorylated heart enzyme ($n$ value <1). These apparent cooperative properties were no longer found when MgATP was present.

Sedimentation of Protein Kinases and Their Purified Subunits in Sucrose Density Gradients—The holoenzyme forms of the rabbit skeletal and heart muscle protein kinases sedimented as single species in the absence of cyclic AMP with an $s_{16,w}$ of 7.0 in each case as shown in Fig. 7A and B. The same result was obtained in the presence or absence of MgATP. The isolated catalytic subunits from either enzyme sedimented with an $s_{16,w}$ value of 3.4 and the isolated regulatory subunit-cyclic AMP complexes of the skeletal muscle and heart enzyme sedimented with $s_{16,w}$ values of 5.0 and 4.3, respectively. These latter values agree with those previously reported for dimeric regulatory subunit-cyclic AMP complexes (6, 7, 17). The $s_{16,w}$ values for the isolated subunits were not changed by MgATP or cyclic AMP.
skeletal muscle protein kinase and the bovine heart enzyme dissociated into their respective dimeric regulatory subunit-cyclic AMP complexes and catalytic subunits in the presence of high concentrations of cyclic AMP (Fig. 7, C and D). However, the concentration of cyclic AMP needed to effect dissociation was different for the two enzymes (Table I). In the absence of MgATP the skeletal muscle enzyme was completely dissociated at 0.15 μM cyclic AMP, whereas 10 μM cyclic AMP was required to dissociate the heart enzyme. In the experiment of Table I it was assumed that complete dissociation of the holoenzymes had occurred when the same s20,w value was obtained for the cyclic AMP binding peaks as for the isolated regulatory subunit-cyclic AMP complexes of the respective enzymes. At intermediate concentrations of cyclic AMP single symmetrical binding peaks having intermediate s20,w values were seen. In the presence of MgATP the concentration of cyclic AMP that was required to bring about complete dissociation of the skeletal muscle enzyme was increased to around 1.0 μM, but for the heart enzyme MgATP decreased the cyclic AMP requirement to about this same value. It will be noted that the concentrations of cyclic AMP required to dissociate the two protein kinases with or without MgATP were approximately the same as those required for the binding of 2 mol of cyclic AMP to the holoenzymes under the same conditions (refer to Fig. 5).

As in the cyclic AMP binding studies (see above), it was shown that the effect of MgATP on the dissociation of the heart protein kinase was due to phosphorylation of the enzyme, and not simply to the presence of MgATP. This was demonstrated by the experiment of Fig. 8 in which the apparent s20,w value of the cyclic AMP binding peaks plotted as a function of the cyclic AMP concentration for nonphosphorylated enzyme (upper curve) and phosphorylated enzyme from which MgATP had been removed by dialysis (lower curve).

Recombination of Isolated Subunits—In previous communications it was shown (6, 7) that MgATP facilitates recombination of skeletal muscle protein kinase subunits resulting in formation of the original cyclic AMP-dependent protein kinase. In the experiments shown in Fig. 7, E and F, the effect of MgATP on recombination of skeletal muscle and heart enzyme subunits was compared. Isolated subunits of skeletal muscle protein kinase mixed in a 1:1 ratio (based on the molecular weights of the monomer subunits) recombined in the presence of MgATP to yield a cyclic AMP-dependent enzyme with an s20,w value of 7.0 (Fig. 7E). When the skeletal muscle enzyme subunits were mixed in the same ratio but in the absence of MgATP no recombination occurred (data not shown). However, when the isolated regulatory and catalytic subunits of the heart protein kinase were incubated together in a 1:1 ratio complete recombination occurred in the absence (Fig. 7F) or presence of MgATP (data not shown) yielding a holoenzyme with an s20,w value of 7.0. The same result was obtained when isolated phosphorylated or nonphosphorylated heart regulatory subunits were used.

**DISCUSSION**

It is evident that the cyclic AMP-dependent protein kinases from rabbit skeletal or beef heart muscle have several properties in common, which can be summarized as follows. Each holoenzyme has an s20,w value of 7.0. Each enzyme binds 2 mol of cyclic AMP per mol of holoenzyme and dissociates into a regulatory subunit dimer containing 2 mol of bound cyclic AMP and two free catalytic subunits. The isolated subunits of each enzyme recombine stoichiometrically to form a cyclic AMP-dependent holoenzyme having a sedimentation constant that is the same as that of the original enzyme.

The skeletal muscle and heart protein kinases also differ in a number of ways. In the absence of MgATP, the apparent Ks...
TABLE I
Concentration of cyclic AMP necessary for dissociation of skeletal or heart muscle protein kinase in presence or absence of MgATP

Samples of each holoenzyme were preincubated with increasing concentrations of cyclic AMP in the presence or absence of ATP (0.1 mM) and magnesium acetate (2 mM) prior to layering on sucrose gradients containing these components (see "Methods"). Following centrifugation, fractions were assayed for cyclic AMP binding. Single symmetrical binding peaks were seen at all concentrations of cyclic AMP.

| Cyclic AMP | Skeletal muscle protein kinase | Heart protein kinase |
|------------|-------------------------------|---------------------|
| µM         | apparent \( K_{d_{g}} \) of cyclic AMP binding peak | \( K_{d_{g}} \) of cyclic AMP binding peak |
| 0          | 7.0                           | 7.0                 |
| 0.15       | 5.0                           | 6.4                 |
| 1.0        | 5.0                           | 4.9                 |
| 10.0       | 4.3                           | 4.4                 |

*Data from Fig. 7, A and B.

values and Hill coefficients for cyclic AMP binding are markedly different. In the presence of MgATP the apparent \( K_{d_{g}} \) for cyclic AMP binding increases for the skeletal muscle enzyme but decreases for the heart kinase. The concentration of cyclic AMP required for dissociation of the respective holoenzymes is different and is also shifted in the opposite direction by MgATP. Finally, the heart enzyme does not require MgATP for recombination in contrast to the skeletal muscle enzyme. The differences noted between the rabbit skeletal muscle and heart holoenzymes appear to be due largely to differences in their regulatory subunits as shown by differences in \( s_{30w} \), values, mobilities on sodium dodecyl sulfate gel electrophoresis, and their ability to serve as phosphate acceptors. It would appear that the catalytic subunits present in both enzymes are similar in all properties tested to date (21, 27).

The effect of MgATP on the apparent \( K_{d_{g}} \) for cyclic AMP binding when the skeletal muscle protein kinase was used is in good agreement with previously reported results (15, 30). However, the effects of the nucleotide on the dissociability of the heart enzyme reported in this study differ somewhat from those reported by Erlichman et al. (19). These investigators reported that phosphorylation was required for dissociation of the enzyme; whereas, the present study indicates that both the phospho and dephospho forms of the enzyme can be dissociated by cyclic AMP but that the dephospho form requires higher concentrations for dissociation to occur. The reasons for the discrepancy between these observations and those reported in the other study (19) are not known at present, but may reflect differences in the conditions employed or the preparations and concentrations of enzymes used. In particular, the higher temperatures (20°) employed in these studies might be expected to affect the binding of cyclic AMP and the interaction between the regulatory and catalytic subunits.

The physiological significance, if any, of the differences found between the heart and skeletal muscle enzymes is not clear. However, it seems possible that the difference in the apparent \( K_{d_{g}} \) values for cyclic AMP binding may be important in vivo. For the heart enzyme this property can be modified by phosphorylation, and one might expect that the state of the enzyme with respect to this parameter may be important in the control of its activity by cyclic AMP, especially if it is found that a mechanism exists for regulating phosphorylation-dephosphorylation. Changes in the activation properties of the skeletal muscle enzyme by cyclic AMP in vivo due to MgATP binding would seem less likely since MgATP is present in most tissues at concentrations greatly exceeding the dissociation constant for its binding. In any event, the results suggest that the activation of both enzymes is closely linked to the binding of cyclic AMP by the regulatory subunit, resulting in the release of an active catalytic subunit.

The dissociation constants* for cyclic AMP reported in this study are higher than those reported previously (15, 18, 20). This is probably due to the fact that both enzymes were studied at relatively high concentrations close to those determined for the enzymes in vivo (15, 27). An almost identical activation constant* for cyclic AMP has been reported (15) when physiological concentrations of the skeletal muscle enzyme were used. These higher values strongly suggest that in each tissue only a small part of the protein kinase is dissociated and activated when basal concentrations of cyclic AMP are present.

It is of interest that the differences between the heart and skeletal muscle protein kinases described in this communication may hold true for cyclic AMP-dependent protein kinase isoenzymes present in other tissues which have similar elution parameters on DEAE-cellulose (21).

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