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CHARACTERIZATION OF TWO ADENOSINE 3′:5′-PHOSPHATE-DEPENDENT PROTEIN KINASE SPECIES FROM CHINESE HAMSTER OVARY CELLS

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Summary

Chinese hamster ovary cells exhibit several characteristic morphological and physiological responses upon treatment with agents which increase the intracellular level of adenosine 3′:5′-phosphate (cyclic AMP). To better understand the mechanism of these cyclic AMP-mediated responses, we separated two cyclic AMP-dependent protein kinases (ATP:protein phosphotransferase, EC 2.7.1.37) (protein kinase I and protein kinase II) from the cytosol of Chinese hamster ovary cells by DEAE-cellulose chromatography and studied their properties. Protein kinase I is eluted at a lower salt concentration than protein kinase II and is stimulable to 10 times its basal catalytic activity, while protein kinase II is stimulable only 2-fold. Both kinases are completely dissociated by cyclic AMP and inhibited by specific cyclic AMP-dependent protein kinase inhibitor. They have similar K_m values for magnesium (approximately 1 mM), cyclic AMP (approximately 60 nM), and ATP (approximately 0.1 mM), and the dissociation constant (K_{dis}) for cyclic AMP (approximately 13 nM) is the same for both enzymes. However, they appear to have different substrate preferences and cyclic AMP-binding properties in that cyclic AMP bound to protein kinase II exchanges readily with free cyclic AMP, while that bound to protein kinase I is not exchangeable. The native enzymes have different sedimentation coefficients (6.4 S for protein kinase I and 4.8 S for protein kinase II), whereas those of the activated enzymes are the same (2.9—3.0 S). It appears that the two cyclic AMP-dependent protein kinases which differ from each other in their

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Abbreviation: CHO, Chinese hamster ovary.

regulatory subunits may play different roles in the mediation of cyclic AMP action in Chinese hamster ovary cells.

Introduction

Cyclic AMP-dependent protein kinase (ATP:protein phosphotransferase, EC 2.7.1.37) is found universally in mammalian tissues (for review see ref. 1). The enzyme is generally believed to be the molecular receptor for cyclic AMP and thus is thought to be responsible for the different physiological effects of this nucleotide [2,3]. Recent studies from several laboratories have established that the enzyme is composed of regulatory and catalytic subunits. Binding of cyclic AMP to the regulatory subunit releases the catalytic subunit, which then catalyzes the transfer of the γ-phosphate group from ATP to serine or threonine residues of the protein substrate [1,4—7].

Chinese hamster ovary (CHO) cells convert from an epithelial-like to a fibroblast-like morphology upon treatment with dibutyryl cyclic AMP [8—10] and cholera toxin [11], both agents that induce elevation of intracellular cyclic AMP levels [11—13] and activation of cyclic AMP-dependent protein kinase [14,15]. Accompanying the morphological changes are the disappearance of knob-like protrusions from the cell surface [8—10], a decrease in plant lectin agglutinability [8—10], an increase in collagen synthesis [8—10], and the polymerization of microtubules [16,17]. The morphological change appears to be cell-cycle specific: only cells in the G_1 phase of the cell cycle initiate the response to dibutyryl cyclic AMP [18]. Enucleated CHO cells can respond to dibutyryl cyclic AMP, indicating that nuclear elements per se are not required for the morphological conversion [19].

The positive temporal correlation between protein kinase activation in vivo and morphological changes mediated by dibutyryl cyclic AMP [14] and cholera toxin [15] strongly suggests the participation of the enzyme in mediating the effects of cyclic AMP. Characterization of the enzyme is thus necessary for further understanding of the mechanism of cyclic AMP action. Cyclic AMP-dependent protein kinase has been found to exist as two isozymic forms separable by ion-exchange chromatography [1,5,6,20]; however, the possible differences in enzymic properties between these two forms are not known. We report here the properties of the two species of the enzyme from the cytosol of CHO cells. They appear to differ in various enzymic parameters including molecular size, cyclic AMP-binding properties, and possibly regulatory subunits.

Materials and Methods

Cell culture, preparation of cell homogenate, and subcellular fractionation. CHO cell clone CHO-K_1, whose properties have been previously described [8], was employed. The cells were grown in medium F-12 supplemented with 10% heat-inactivated (56°C for 30 min), dialyzed fetal calf serum in plastic culture dishes (Falcon) under our standard tissue culture conditions of 5% CO_2 in air at 37°C in a 100% humidified incubator [8]. Cell extracts (27 000 × g supernatant)

were prepared from approx. 2.5×10^9 logarithmically growing cells grown in suspension and then homogenized in a buffer containing 10 mM Tris-HCl (pH 7.5) (Dounce homogenizer, 10 strokes) and centrifuged at 27 000 × *g* for 20 min.

Assay for cyclic AMP-dependent protein kinase. Standard reaction mixtures consisted of 50 mM Tris-HCl (pH 7.5)/3 mM magnesium chloride/0.1 mM [γ-^{32}P]ATP (approx. 10^6 cpm per reaction tube)/2 mg per ml histone, with or without 1 μM cyclic AMP/enzyme source, in a final volume of 100 μl. The reaction was carried out at 30°C. At different time intervals, 20-μl aliquots were taken from the reaction mixture and spotted onto Whatman 3 MM filter paper. The filter paper was immersed immediately in cold (4°C) 10% trichloroacetic acid/1% sodium pyrophosphate which was constantly stirred with a magnetic bar. After a total of four washes with cold trichloroacetic acid solution, the filter papers were washed in 95% ethanol, once in ether, dried in a 60°C oven, and counted in a Packard liquid scintillation counter in a scintillation cocktail consisting of 6 g PPO and 0.1 g POPOP per l toluene. Protein kinase activity is expressed as pmol $^{32}PO_4$ transferred to histone/min per mg cellular protein. Protein concentration was determined by the method of Lowry et al. [21] as previously described [14]. Walsh's protein kinase inhibitor [22], which specifically inhibits the activity of cyclic AMP-dependent protein kinase activity, was a gift from J.D. Corbin, Department of Physiology, Vanderbilt University.

Assay for cyclic AMP-binding protein. Cyclic AMP-binding activities of protein kinase fractions were assayed using Gilman's assay for binding protein as described previously [13], except that the crude CHO cell extract or the subcellular fractions were used. Binding was performed at 4°C for 60 or 75 min as specified, which yields maximum binding for cyclic AMP at either low (0.5 pmol) or high (10 pmol) concentrations. Activity is expressed as pmol cyclic AMP bound per mg protein.

Chromatography of cytosol protein kinase on DEAE-cellulose column. DEAE-cellulose chromatography was chosen based on the findings of many investigators, including Corbin et al. [23], that the two major species of cyclic AMP-dependent protein kinase can be separated from each other by use of this procedure. After three dialyses of the cytosol fraction in a 30× volume of a buffer (with three changes) containing 10 mM Tris-HCl (pH 7.5) and 14.8 mM β-mercaptoethanol, the cell extract (20 ml) was applied to a DEAE-cellulose (DE-52, Whatman) column (15 × 1.5 cm). The column was eluted with a salt gradient from 0 to 0.3 M NaCl (50 ml each) in 5 mM potassium phosphate buffer (pH 7.5) containing 10% glycerol, 1 mM EDTA, and 14.8 mM β-mercaptoethanol. Fraction (1 ml) were collected and assayed for cyclic AMP-binding protein and protein kinase activities as described above.

Sucrose gradient sedimentation. The pooled protein kinase fractions were concentrated 3-fold by ultrafiltration. An aliquot of the concentrated enzyme (0.2 ml) was layered onto a 12-ml linear 5—20% sucrose gradient containing 10 mM Tris-HCl (pH 7.5) and 14.8 mM β-mercaptoethanol, with or without cyclic AMP, and was centrifuged at 195 000 × *g* in an SW-41 rotor for 15 h using a Beckman model L-2 ultracentrifuge. Fractions (0.5 ml) were collected from the top with an Isco fractionator, and the protein kinase activity of each fraction

was assayed. Cytochrome *c* (1.71 S, 13 400), chymotrypsinogen (2.34 S, 23 200), ovalbumin (3.66 S, 43 500), mouse hemoglobin (4.19 S, 67 500), lactate dehydrogenase (6.82 S, 110 000), and catalase (11.3 S, 247 000) were used as molecular-weight markers and were monitored by absorption at 280 nm with a Zeiss spectrophotometer.

Results and Discussion

It has been reported that cyclic AMP-dependent protein kinase in most mammalian tissue exists as multiple forms separable by ion-exchange chromatography. Since more than 80% of cyclic AMP-binding and protein kinase activities are found in the cytosol of CHO cells [24], this fraction was chosen for further characterization of the enzyme activities. Dialyzed cytosol was applied onto a DEAE-cellulose column and eluted with an NaCl gradient as described in Materials and Methods. Two species of protein kinase activities, designated protein kinase I and protein kinase II, were separated by this procedure. There is not detectable ATPase activity in either species. Protein kinase I, which elutes at 50 mM NaCl, is stimulatable to 10 times the basal activity, while protein kinse II, which elutes at 150 mM NaCl, is stimulatable only 2-fold (Fig. 1C). No stimulation by 1 μM cyclic GMP was observed for either protein kinase species (Fig. 1D).

Cyclic AMP-binding activities coincide with the protein kinase activities (Fig. 1A). Cyclic GMP-binding activities fall at approximately the same fractions as

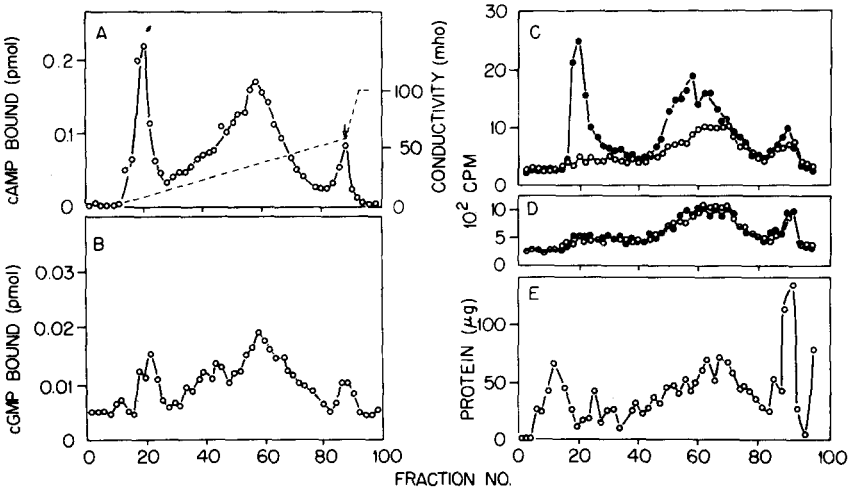
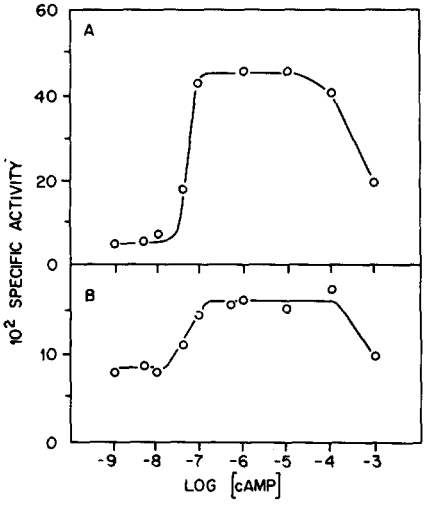


Fig. 1. Elution profile of CHO cytosol from DEAE-cellulose chromatography. A, cyclic AMP-binding activity (in pmol of ^{3}H-labeled cyclic AMP (cAMP) bound per 50-μl aliquot); 0.5 M NaCl wash indicated by arrow. B, cyclic GMP-binding activity (in pmol of ^{3}H-labeled cyclic GMP (cGMP) bound per 50-μl aliquot). The (8-^{3}H)-labeled cyclic AMP (Schwarz/Mann) used contains 28 Ci/mmol (approx. 12 000 cpm/pmol). The cyclic [G-^{3}H]GMP (New England Nuclear) used contains 9.92 Ci/mmol (approx. 4000 cpm/pmol). C, cyclic AMP-dependent protein kinase activity (in cpm of $^{32}PO_4$ tranferred to histone per 20-μl aliquot assayed in the presence (•) or absence (○) of 1 μM cyclic AMP). D, cyclic GMP-dependent protein kinase activity (in cpm of $^{32}PO_4$ transferred to histone per 20-μl aliquot assayed in the presence (•) or absence (○) of 1 μM cyclic GMP). E, protein profile (in μg protein per 100-μl aliquot). Chromatography was performed as described, and a 1-ml fraction was collected.

cyclic AMP-binding activities, but are only 10% as high, probably due to non-specific binding (Fig. 1B).

Fractions containing protein kinase I and protein kinase II activities were pooled individually, and their properties were compared. Over 80% of the protein kinase activity in the cytosol was consistently recovered after the DEAE-cellulose column chromatography. Since over 95% of the membrane-bound ($Na^+ + K^+$)-dependent ATPase has been removed through centrifugation and column chromatography, and since the optimum assay conditions used for protein kinase are not favorable for cyclic AMP phosphodiesterase, there were no measurable activities of ($Na^+ + K^+$)-dependent ATPase and cyclic AMP phosphodiesterase in either of the pooled fractions (data not shown) which contain protein kinase I and protein kinase II activities. Without such apparent contaminating activities, these partially purified fractions appear to be suitable for characterization of their enzymic properties. Based on the determination of specific activity in the presence of 1 μM cyclic AMP, protein kinase I and protein kinase II are 8—10 and 4—5-fold purified, respectively, as compared with protein kinase in the cytosol. Phosphorylation of histone increases linearly with time up to 15 min for both protein kinase species. Further increases, with a slight decline in phosphorylation rate, were observed up to 45 min (data not shown).

The dependence of enzyme activities on cyclic AMP concentrations was studied. As shown in Fig. 2, protein kinase I is stimulated maximally to 10 times the basal activity by 0.1 μM or higher concentration of cyclic AMP, wheras protein kinase II is activated only 2-fold. Inhibition of enzyme activities



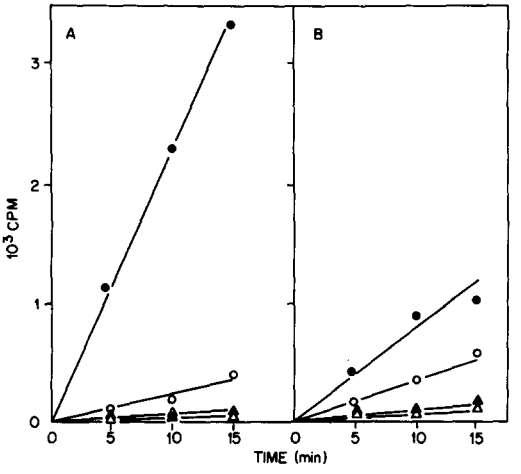


Fig. 2. Specific activity (pmol of $^{32}PO_4$ transferred/min per mg protein) of protein kinase I (A) and protein kinase II (B) as a function of cyclic AMP (cAMP) concentration. The reaction time is 10 min.

Fig. 3. Time course of phosphorylation of histone by protein kinase I (A) and protein kinase II (B) in the absence (○, ●) or presence (△, ▲) of Walsh's protein kinase inhibitor (0.5 units). The assays were performed in the presence (closed symbols) or absence (open symbols) of 1 μM cyclic AMP. 6 μg of protein kinase I or 8 μg protein kinase II was used for each assay. The activity is expressed as cpm of $^{32}PO_4$ transferred to histone.

of both protein kinase I and protein kinase II is observed when a high concentration of cyclic AMP (more than 0.1 mM) is present in the reaction mixture. K_{act} (concentration for 50% activation) values are estimated to be 55 nM for protein kinase I and 60 nM for protein kinase II. For routine assay 1 μM cyclic AMP was used to ensure maximal activation of the enzyme.

Walsh's protein kinase inhibitor [22] inhibits both protein kinase I and protein kinase II (Fig. 3), indicating that both enzyme species are similar to the cyclic AMP-dependent protein kinase reported in many mammalian tissues.

Both protein kinase I and protein kinase II have strict requirements for magnesium; calcium, manganese, or zinc cannot substitute for the divalent cation requirement. Apparent K_m (estimated as the concentration of magnesium required for half-maximal velocity) is estimated to be 1 mM for both protein kinase I and protein kinase II. Maximum activity is observed at 2.5 mM magnesium for both enzyme species (data not shown).

The two enzymes have a similar affnity for ATP. The values of apparent K_m for ATP estimated from the Lineweaver-Burk plot are 125 and 76 μM in the absence and presence of 1 μM cyclic AMP, respectively, for protein kinase I, and 167 and 88 μM ATP in the absence and presence of 1 μM cyclic AMP, respectively, for protein kinase II (Fig. 4).

The ability of protein kinase I and protein kinase II to bind cyclic AMP was studied by incubating protein kinase I and protein kinase II at 4°C for 75 min, a condition which yields maximum binding, with different concentrations of ^{3}H-labeled cyclic AMP and determining the amount of ^{3}H-labeled cyclic AMP bound to the enzymes. A reciprocal plot of the amount of bound cyclic AMP versus the reciprocal of the amount of free cyclic AMP is shown in Fig. 5. Both

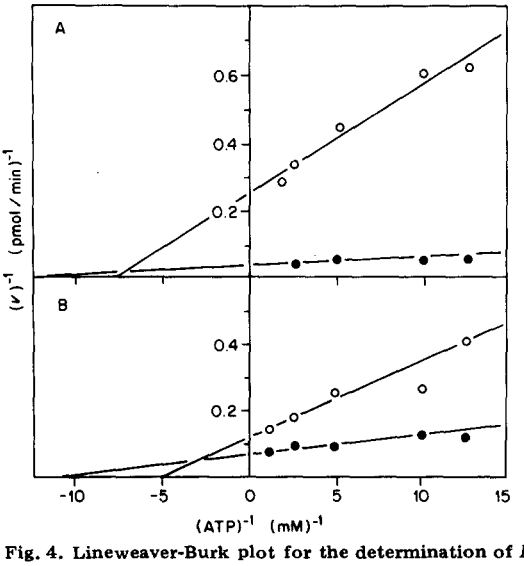
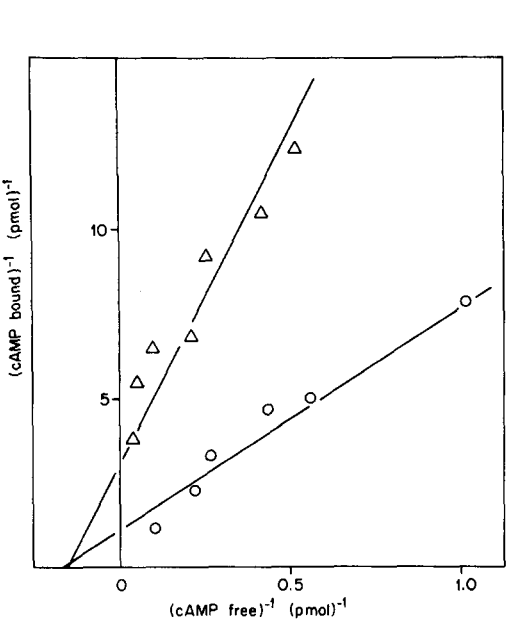


Fig. 4. Lineweaver-Burk plot for the determination of K_m for ATP. Protein kinase assays were performed with 6 μg protein of protein kinase I (A) and 8 μg protein of protein kinase II (B) in the absence (○) or presence (●) of cyclic AMP. The initial velocity (v) is expressed aa pmol $^{32}PO_4$ transferred to histone per min.



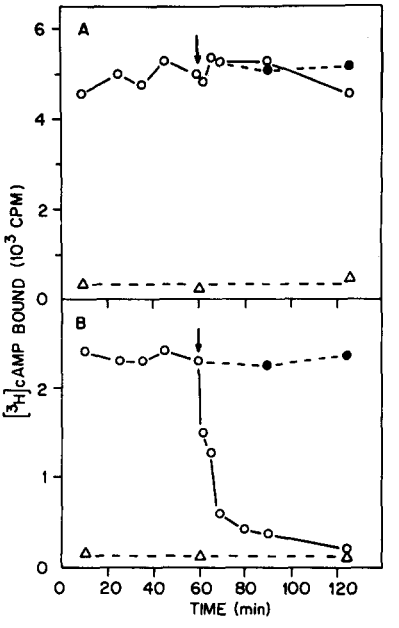


Fig. 5. Determination of the dissociation constant (K_{dis}) for cyclic AMP (cAMP) in protein kinase I (○) and protein kinase II (△) by plotting the reciprocal of the amount of cyclic AMP bound versus the reciprocal of the amount free. Binding assay was performed with 31 μg protein kinase I, 46 μg protein kinase II, and various amounts of ^{3}H-labeled cyclic AMP. Amount of free cyclic AMP is calculated by subtracting the amount retained in the Millipore filter from the total amount in the reaction mixture. All cyclic AMP concentrations are expressed as pmol.

Fig. 6. Exchange of bound ^{3}H-labeled cyclic AMP (cAMP) (10 pmol) by unlabeled cyclic AMP (100 pmol). Identical tubes containing 25 mM sodium acetate (pH 4.0), 32 μg protein kinase I (A) or 46 μg protein kinase II (B), and 10 pmol ^{3}H-labeled cyclic AMP were incubated at 4°C. Unlabeled cyclic AMP (100 pmol) was added after an incubation time of 60 min as indicated by the arrow. At different time intervals before or after the addition of unlabled cyclic AMP, the reaction was terminated to determine the amount of ^{3}H-labeled cyclic AMP bound (○). •, water (as a control) was added instead of unlabeled cyclic AMP at 60 min; △, unlabeled cyclic AMP and ^{3}H-labeled cyclic AMP were mixed before the addition of protein kinase I (A) or protein kinase II (B).

enzymes are estimated to have K_{dis} (dissociation constant) values of approximately 13 nM cyclic AMP.

The capacity to exchange bound cyclic AMP with unbound cyclic AMP is different for protein kinase I and protein kinase II. To determine cyclic AMP-binding activities, each enzyme species was incubated with 10 pmol ^{3}H-labeled cyclic AMP for 60 min, by which maximum binding is reached. Unlabeled cyclic AMP (100 pmol) was then added. The amount of ^{3}H-labeled cyclic AMP bound at time intervals before or after the addition of unlabeled cyclic AMP was determined. No exchange of labeled bound cyclic AMP with free unlabeled cyclic AMP is observed for protein kinase I (Fig. 6A). However, exchange of bound and unbound cyclic AMP, as indicated by the decrease in the amount of ^{3}H-labeled cyclic AMP bound, is observed as early as 2 min after the addition of unlabeled cyclic AMP to protein kinase II. At 60 min after adddition of the unlabeled cyclic AMP, the amount of ^{3}H-labeled cyclic AMP bound reaches the level observed when unlabeled and labeled cyclic AMP were premixed before

the addition of protein kinase II, indicating a complete exchange of bound cyclic AMP with free cyclic AMP (Fig. 6B). Since the decrease in the bound ^{3}H-labeled cyclic AMP occurs only in the presence of unlabeled cyclic AMP, it is unlikely that the decrease is due to degradation of ^{3}H-labeled cyclic AMP, because that would imply selective degradation of ^{3}H-labeled cyclic AMP in the presence of unlabeled cyclic AMP. The difference in exchangeability of the bound cyclic AMP suggests a difference in the cyclic AMP-binding portion, namely, the regulatory subunits of the two protein kinase species. The difference in regulatory subunits may also explain the difference in molecular size, since the enzyme species appear to have catalytic subunits of nearly identical molecular weight.

The molecular sizes of protein kinase I and protein kinase II were compared using a sucrose density gradient. Both protein kinase I and protein kinase II sediment as single peaks (Figs. 7 and 8). In the presence of 10 μM cyclic AMP in the gradient, the catalytic activities of both enzymes demonstrate a shift toward a lower molecular weight. Sedimentation values for native (not activated) and activated enzymes are estimated to be 6.4 and 3.0 S, respectively, for protein kinase I, and 4.8 and 2.9 S, respectively, for protein kinase II. Using as reference standards the linear plot of log molecular weight versus

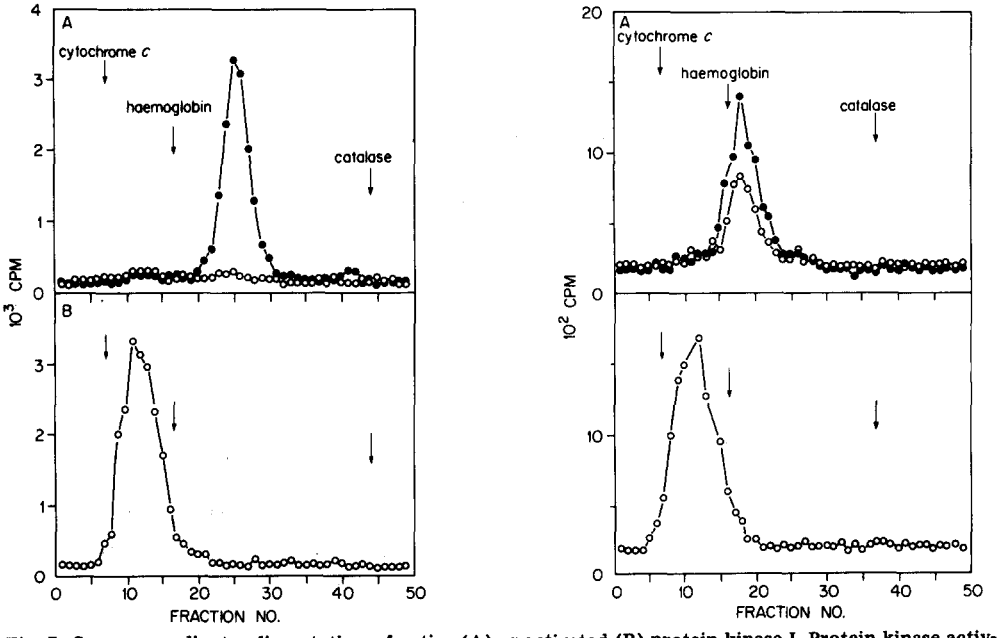


Fig. 7. Sucrose gradient sedimentation of native (A) or activated (B) protein kinase I. Protein kinase activity of each fraction (collected from the top) was assayed in the absence (○) or presence (●) of 1 μM cyclic AMP. Sucrose gradient with (A) or without (B) 10 μM cyclic AMP was prepared, and sedimentation was performed as described in Materials and Methods. The activity is expressed as $^{32}PO_4$ transferred to histone per 20-μl aliquot of each 0.25-ml fraction collected.

Fig. 8. Sucrose gradient sedimentation of native (A) and activated (B) protein kinase II. Sedimentation conditions were the same as described for Fig. 7. Enzyme assay was performed in the absence (○) or presence (●) of 1 μM cyclic AMP, and the activity is expressed as in Fig. 7.

log sedimentation coefficients obtained with the molecular-weight markers cytochrome, chymotrypsinogen, ovalbumin, mouse hemoglobin, lactate dehydrogenase, and catalase, the 'apparent' molecular weights for native and activated forms are estimated to be 105 000 and 34 000, respectively, for protein kinase I, 68 000 and 32 000, respectively, for protein kinase II (data not shown).

Substrate preferences of protein kinase I and protein kinase II appear to be different (Table I). In decreasing order of phosphorylation rates: for protein kinase I assayed without cyclic AMP, protamine > histone > phosvitin; for protein kinase I assayed with 1 μM cyclic AMP, histone > protamine > phosvitin; for protein kinase II assayed without cyclic AMP, phosvitin > protamine > histone; and for protein kinase II assayed with 1 μM cyclic AMP, phosvitin > histone > protamine. No significant phosphorylation of bovine serum albumin and casein is observed for either protein kinase I or protein kinase II. Properties of the two enzymes are summarized in Table I.

Different degrees of cyclic AMP stimulation are observed when different substrates are used. For both enzymes the highest degree of stimulation by cyclic AMP is seen when histone is used as substrate. Lower degrees of stimulation are observed when protamine and phosvitin are used. For protein kinase II, a 40% inhibition of phosphorylation rate was observed when phosvitin was used as substrate. The rates of phosphorylation of different substrates by protein kinase I and protein kinase II are shown in Table II.

It is known that phosvitin kinases are a group of cyclic AMP-independent protein kinases which are characterized by transferring phosphoryl groups preferentially to phosvitin or casein, but not to any appreciable extent to histone, and which are not stimulated by cyclic AMP [1,25—29]. The molecular weight of phosvitin kinase from calf brain [28] and chicken liver [29] has been estimated at 41 000 and 45 000, respectively. Although protein kinase II exhibits substrate preference for phosvitin over histone (Table II), protein

TABLE I

PROPERTIES OF PROTEIN KINASE I AND PROTEIN KINASE II IN THE CYTOSOL FRACTION OF CHO CELLS

Property	Protein kinase I	Protein kinase II
Elution in DEAE column	50 mM NaCl	150 mM NaCl
Sedimentation coefficient		
Holoenzyme	6.4 S	4.8 S
Catalytic subunit	3.0 S	2.9 S
"Apparent" molecular weight		
Holoenzyme	105 000	68 000
Catalytic subunit	34 000	32 000
K_m, ATP		
without cyclic AMP	$1.25 \cdot 10^{-4}$ M	$1.67 \cdot 10^{-4}$ M
with cyclic AMP	$0.76 \cdot 10^{-4}$ M	$0.88 \cdot 10^{-4}$ M
K_{act}, cyclic AMP (50% activation)	$5.5 \cdot 10^{-8}$ M	$6.0 \cdot 10^{-8}$ M
Activation by cyclic AMP (1 μM)	10X	2X
Substrate specificity (in decreasing order)	Histone, protamine, phosvitin	Phosvitin, histone, protamine
K_{dis}, cyclic AMP	$1.3 \cdot 10^{-8}$ M	$1.3 \cdot 10^{-8}$ M
Exchangeability of bound cyclic AMP	Not exchangeable	Readily exchangeable

TABLE II

RATE OF PHOSPHORYLATION OF DIFFERENT SUBSTRATES BY PROTEIN KINASE I AND PROTEIN KINASE II

The rate is expressed as pmol $^{32}PO_4$ transferred/min per mg protein. Reaction mixture contains 50 mM Tris-HCl (pH 7.5); 14 mM β-mercaptoethanol; 3 mM magnesium chloride; 0.1 mM ATP (γ-^{32}P), with or without 1 μM cyclic AMP; enzyme (6 μg protein for protein kinase I and 8 μg protein for protein kinase II); and 2 mg/ml of each protein substrate. Numbers in parentheses are percentage activities, with calf thymus histone as 100.

Protein substrate	Rate of phosphorylation					
	Protein kinase I			Protein kinase II		
	Without cyclic AMP	With cyclic AMP	Activity ratio * (with cyclic AMP/without cyclic AMP)	Without cyclic AMP	With cyclic AMP	Activity ratio * (with cyclic AMP/without cyclic AMP)
Calf thymus histone	222 (100)	3500 (100)	15.8	460 (100)	1300 (100)	2.8
Protamine	378 (170)	1702 (49)	4.8	812 (177)	1002 (77)	1.2
Phosvitin	95 (43)	284 (8)	3.0	3200 (696)	1800 (138)	0.6
Casein	—	—	—	80 (17.4)	50 (4)	0.6
Bovine serum	19 (9)	19 (0.5)	1.0	90 (20)	95 (7)	1.1

kinase II itself does not appear to represent phosvitin kinase activity because (a) protein kinase II activity is stimulatable by cyclic AMP, although to a lesser extent than that of protein kinase I (Figs. 1—3, Table II); (b) it is capable of phosphorylating histone with or without cyclic AMP to 72 and 14%, respectively, the value obtained with phosvitin as a substrate (Table II); (c) the 'apparent' molecular weights of the holoenzyme and the catalytic subunit are about 68 000 and 32 000 respectively. One cannot, however, completely rule out the unlikely possibility that CHO cells might contain bona fide phosvitin kinase activities co-chromatographed in the DEAE-cellulose column with protein kinase II as a mixture which exhibits unusual characteristics distinctly different from other reported phosvitin kinases [1,25—29] or that the presumed phosvitin kinase in CHO cells has a molecular weight identical to that of protein kinase II. The feasibility of pursuing this point further is limited by the small quantity of cultured cells available, in contrast to the relatively large quantity of animal organs and tissues obtainable.

The two protein kinase species may have different physiological roles and thus mediate different aspects of the cyclic AMP-induced changes. Cell membrane [30—36], microtubules [37], ribosomes [38—40], nuclear proteins [41, 42], and the enzyme itself [43] have been shown to be substrates for protein kinase. Both the cyclic AMP-binding and the protein kinase activities occurred in every subcellular fraction of CHO cells, e.g., nuclei, crude mitochondria, plasma membrane, microsome, ribosome, and cytosol [24]. That the phosphorylation of these elements by protein kinases is responsible for the different cyclic AMP-induced changes in CHO cells remains to be investigated.

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