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CALF BRAIN PHOSVITIN KINASE

PURIFICATION OF THE KINASE ASSOCIATED WITH A PHOSPHATE-INCORPORATING PROTEIN

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SUMMARY

A phosvitin kinase was purified about 1100-fold from calf brain. The purification procedure entailed adsorption on calcium phosphate gel, $(NH_4)_2SO_4$ fractionation and chromatography on DEAE-Sephadex, hydroxylapatite and Sephadex G-200. A protein in the phosvitin kinase preparation was found to incorporate phosphoryl groups during incubation with [γ-^{32}P]ATP. This protein was firmly associated with the phosvitin kinase and could not be separated from it during the purification. After incubation of the purified phosvitin kinase preparation with [γ-^{32}P]ATP, [^{32}P]phosphorylserine and [^{32}P]phosphorylthreonine could be isolated from the ^{32}P-labelled protein in a ratio of about 5:1. The purified phosvitin kinase catalyzed the phosphorylation of phosvitin, casein and nuclear phosphoprotein purified from calf brain as well as rat liver. The enzyme was not stimulated by adenosine 3′,5′-monophosphate, nor did it catalyze the phosphorylation of histone to any appreciable extent.

INTRODUCTION

Protein kinases (EC 2.7.1.37) catalyze the transfer of phosphoryl groups from ATP to certain proteins. The phosphoryl groups are bound in ester linkages to serine and generally to threonine residues as well. The various groups of protein kinases may be distinguished by identifying the protein that is preferentially phosphorylated. One group consists of the phosvitin kinases, which show high specificity towards phosvitin and casein[1]. These kinases do not seem to be stimulated by adenosine 3′,5′-monophosphate (cyclic AMP). The histone kinases[2] represent another group of protein kinases, which preferentially phosphorylate histone in a reaction which is stimulated by cyclic AMP (ref. 3). A third group of protein kinases is made up of those which catalyze the phosphorylation of certain enzymes[4], such as phosphorylase, phosphorylase kinase and glycogen synthetase. Most of these kinases are stimulated by cyclic AMP and readily phosphorylate histone. There is consequently no clear distinction between this group of protein kinases and histone kinases.

The cyclic AMP stimulated protein kinases have been the subject of detailed investigations since they are probably involved in hormone action[5]. The protein kinases, which are not stimulated by cyclic AMP, *e.g.* the phosvitin kinases, have however not been studied to the same extent. The intracellular role of the phosvitin kinases is therefore only vaguely understood. However, phosvitin kinase has been shown to catalyze the phosphorylation of nuclear phosphoprotein[6] and of certain fractions of cell sap proteins[7].

The aim of the present investigation was to elucidate the relation between the phosvitin kinases and the intracellular phosphoproteins. For this purpose it was necessary to use highly purified systems. Phosvitin kinase has been extensively purified from brewer's yeast[1]. Partial purification procedures starting from other sources, such as ox brain[8], rat liver cell sap[9] and rat liver chromatin[10] have been described. Since we were interested in mammalian phosphoprotein metabolism it was necessary to prepare a pure mammalian phosvitin kinase. Calf brain was used as starting material since this organ was found to be rich in phosvitin kinase. The purification procedure is outlined in the present work. It is shown that the purification procedure for the phosvitin kinase also leads to an enrichment of a protein which becomes phosphorylated by [γ-^{32}P]ATP. This protein is firmly associated with the kinase and is assumed to be its physiological substrate. [^{32}P]Phosphorylserine ([^{32}P]-Ser-*P*) as well as [^{32}P]phosphorylthreonine ([^{32}P]Thr-*P*) could be isolated from the ^{32}P-labelled protein. Furthermore, the purified phosvitin kinase is shown to catalyze the incorporation of phosphoryl groups into nuclear phosphoprotein from calf brain or rat liver.

EXPERIMENTAL

Materials

DEAE-Sephadex (A-50), Sephadex G-200 and Sephadex G-50 were obtained from Pharmacia (Uppsala, Sweden). Dithiothreitol (Cleland's reagent) was a product of Calbiochem. Phosvitin and histone (Type II-A from calf thymus) were obtained from Sigma. Hammarsten casein was from Merck. Human serumalbumin was a product of AB Kabi, Stockholm, Sweden. [γ-^{32}P]ATP was prepared by the method of ENGSTRÖM[11]. Calcium phosphate gel was prepared as described by HUDSON AND TSUBOI[12] and hydroxylapatite by the method of TISELIUS *et al.*[13].

Preparation of nuclear phosphoprotein from rat liver and calf brain

Livers were obtained from white Sprague–Dawley rats. Calf brains were obtained from a local slaughterhouse, and were brought on ice to the laboratory within 45 min after killing the animals. Nuclei were prepared from both sources by the method of CHAUVEAU *et al.*[14] and phosphoprotein from these nuclei by the method of LANGAN[6], with the modifications suggested by GERSHEY AND KLEINSMITH[15]. The alkali-labile phosphate content of purified phosphoprotein was determined as orthophosphate[16] liberated after treatment with 1 M NaOH for 15 min at 100° (ref. 6). Purified phosphoprotein from both sources contained considerable phosvitin kinase activity, which could be destroyed by heating at 60° for 3 min (ref. 6).

Analytical methods

Radioactivity was measured on dried aliquots in aluminium cups as previously described[17]. Protein concentration was assayed according to LOWRY *et al.*[18] in the initial purification steps. Human serum albumin was used as standard. Protein concentration of the chromatographic steps was estimated from the ultraviolet absorbance at 280 nm and 260 nm measured using a Zeiss spectrophotometer Type PMQ II.

Ultrafiltration and dialysis

Pooled fractions from the chromatographic steps were concentrated by ultrafiltration according to EVERALL AND WRIGHT[19] with Union Carbide dialysis tubing (inflated diameter of 8/32 or 23/32 inch). Dialysis was performed in dialysis tubings of the same inflated diameter.

Assay of protein kinase activity

Before assay samples were carefully dialyzed against 0.05 M potassium phosphate buffer (pH 7.0) containing 10^{-4} M Cleland's reagent. Phosvitin kinase activity was then assayed essentially according to RABINOWITZ AND LIPMANN[1]. The 0.5-ml incubation mixture contained 1 mg of phosvitin, 25 μmoles of Tris–HCl* (pH 7.5), 2.5 μmoles of magnesium acetate, 0.5 μmole of [^{32}P]ATP and appropriate amounts of enzyme. Incubation was performed for 15 min at 37° and was interrupted by the addition of 0.1 ml of 50% trichloroacetic acid. Controls omitting phosvitin were processed similarly. Immediately before the addition of trichloroacetic acid to the control samples, 1 mg of phosvitin was added as a carrier. The precipitates were washed twice with 2 ml of 10% trichloroacetic acid and were dissolved in 0.5 ml of 0.1 M NaOH, which was plated in aluminium cups. Radioactivity was determined as described above. The specific radioactivity of the [^{32}P]ATP was 100–1000 counts/min per nmole. One unit of enzyme is defined as the amount of enzyme that catalyzes the transfer of 1 nmole of phosphoryl groups from ATP to 1 mg of phosvitin under the conditions used.

The substrate specificity of the purified enzyme was investigated using casein and histone as substrates, as well. The incubation conditions were the same as above, but the phosvitin was replaced by 1 mg of histone or casein. When protein kinase activity was measured in the presence of cyclic AMP ($5 \cdot 10^{-6}$ M), 0.75 μmole of theophyllin was added to the 0.5-ml incubation mixture.

Determination of [^{32}P]phosphate incorporated into protein during incubation with [^{32}P]ATP

Fractions to be analyzed were first dialyzed against 0.05 M potassium phosphate buffer (pH 7.0) containing 10^{-4} M Cleland's reagent. Appropriate samples were then diluted with dialysis buffer to 500 μl and 25 μl of 4 mM magnesium acetate and 475 μl of distilled water were added. They were placed in a waterbath at 37° and after allowing 3 min for temperature equilibration, the reaction was initiated by the addition of 25 μl of 0.4 mM [^{32}P]ATP (specific activity $2 \cdot 10^5$–$7 \cdot 10^5$ counts/min per nmole).

* The molarities of the buffers used are given with respect to added Tris and triethanolamine, respectively. All pH measurements were made at room temperature.

After incubation for 10 min 100 μl of 1 M HCl were added. 3 min later 100 μl of 10 mM unlabelled ATP were added followed at 30-sec intervals by 25 μl of 10% sodium dodecyl sulphate and 250 μl of 1 M triethanolamine–acetic acid buffer (pH 7.4). The incubation mixtures were chromatographed on columns (1.2 cm × 40 cm) of Sephadex G-50, which were eluted with 0.01 M triethanolamine–acetic acid buffer (pH 7.4) containing 0.25% sodium dodecyl sulphate. In this way ^{32}P-labelled protein was separated from excess [^{32}P]ATP. The total amount of [^{32}P]phosphate incorporated into the protein was estimated from its radioactivity, assuming the specific radioactivity to be the same as that of the [^{32}P]ATP used.

Assay of other enzyme activities

Phosphorylase and phosphorylase kinase activities were determined according to KREBS *et al.*[20]. Glycogen synthetase was assayed as described by LELOIR[21].

Methods for isolation and identification of [^{32}P]Ser-P and [^{32}P]Thr-P

Purified phosvitin kinase was incubated with [^{32}P]ATP essentially as described above. After inactivation and chromatography on Sephadex G-50 ^{32}P-labelled protein was precipitated by the addition of 3 vol. of acetone. The precipitate was dried under reduced pressure at room temperature. 30 μmoles of unlabelled Ser-*P* and 15 μmoles of unlabelled Thr-*P* were added in 2 M HCl and the mixture was hydrolyzed 20 h at 100°. [^{32}P]Ser-*P* and [^{32}P]Thr-*P* were isolated by chromatography on Dowex 50 and Dowex 1 as previously described[22]. The amounts of protein-bound [^{32}P]Ser-*P* and [^{32}P]Thr-*P* of the precipitates were calculated from the specific radioactivities of the isolated phosphoamino acids and total amounts of unlabelled phosphoamino acids added[22].

RESULTS

Purification of phosvitin kinase

The purification was carried out at 4°. The centrifugations were performed in a Sorvall model RC2-B centrifuge unless otherwise stated. An outline of the purification procedure and the result of a typical preparation is given in Table I.

Homogenization

5 kg of calf brain obtained from a local slaughter-house were frozen, thawed and homogenized portionwise in a Waring blendor with a total of 20 l of 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM EDTA for 3 min at 30-sec intervals. The homogenate was centrifuged in a refrigerated centrifuge at 3000 × *g* for 30 min. The precipitate was discarded.

Adsorption on calcium phosphate gel

Calcium phosphate gel (about 40 mg/ml) was added to the supernatant (0.2 l gel per l of supernatant). The mixture was stirred for 45–60 min and thereafter centrifuged for 15 min at 3000 × *g* in the refrigerated centrifuge. The gel was washed twice with 0.1 M potassium phosphate buffer (pH 7.0) containing 10^{-4} M Cleland's reagent and was centrifuged as above. The washing fluid which contained about 10% of the active material was discarded. The gel was eluted twice with 0.25 M potassium

TABLE I

PURIFICATION OF A CALF BRAIN PHOSVITIN KINASE

Calf brain, 5 kg, were used. Samples were dialyzed against 0.05 M potassium phosphate buffer (pH 7.0) containing 10^{-4} M Cleland's reagent before enzyme activity and protein concentration were determined. In the initial steps, protein was measured according to LOWRY *et al.*[18] and in the chromatographic steps by determining $A_{280\ nm}$ and $A_{260\ nm}$. Fractions within brackets were not further purified.

Purification step	*Total activity (units)*	*Total protein (mg)*	*Specific activity (units/mg)*	*Purification factor*	*Recovery (%)*
Homogenization	474 000	82 200	5.8	–	100
Calcium phosphate gel eluate	413 000	11 000	38	6.6	87
$(NH_4)_2SO_4$ precipitation	388 000	8 700	45	7.8	82
First DEAE-Sephadex					
[0.1 M NaCl	75 800	1 490	51	8.8	16]
0.5 M NaCl	132 000	1 080	120	21	28
Second DEAE-Sephadex					
[First peak	36 900	298	120	21	7.8]
Second peak	85 400	134	640	110	18
Hydroxylapatite	61 000	43.8	1400	240	13
First Sephadex G-200	38 000	12.8	3000	520	8.0
Second Sephadex G-200	33 700	5.3	6400	1100	7.1

phosphate buffer (pH 7.0) containing 10^{-4} M Cleland's reagent and was centrifuged as above. The precipitated gel was discarded.

$(NH_4)_2SO_4$ precipitation

The active material eluted from the gel was precipitated by adding $(NH_4)_2SO_4$ to 65% saturation. After intermittent stirring for about 30 min, centrifugation was performed at 4700 × *g* for 15 min. The precipitate was dissolved in 250 ml of 0.01 M triethanolamine–acetic acid buffer (pH 7.4) containing 0.1 M NaCl and 10^{-4} M Cleland's reagent and was carefully dialyzed against the same buffer.

First chromatography on DEAE-Sephadex

The dialyzed material was applied to two columns (each 7 cm × 35 cm) of DEAE-Sephadex, which were equilibrated with the aforementioned dialysis buffer. The columns were first eluted with this buffer and then stepwise with 0.3 and 0.5 M NaCl in 0.01 M triethanolamine–acetic acid buffer (pH 7.4) containing 10^{-4} M Cleland's reagent. As is shown in Table I about 30% of the phosvitin kinase activity recovered from the column was obtained in the run-off volume (0.1 M NaCl). This was not an effect of overloading the column since about 90% of the active material was again eluted with the run-off volume when rechromatographed under similar conditions.

Less than 10% of the activity was eluted with 0.3 M NaCl. The main part of the active material was instead displaced from the column with 0.5 M NaCl (Table I). $(NH_4)_2SO_4$ was added to this fraction to 65% saturation. The precipitate obtained after centrifugation at 4700 × *g* for 15 min was dissolved in 125 ml of 0.01 M triethanolamine–acetic acid buffer (pH 7.4) containing 0.3 M NaCl and 10^{-4} M Cleland's reagent and was carefully dialyzed against this buffer.

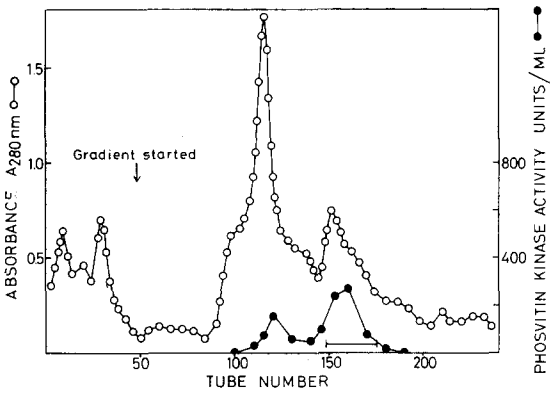

Fig. 1. Chromatography of phosvitin kinase on a column (4 cm × 39 cm) of DEAE-Sephadex. Elution was performed first with 0.3 M NaCl in 0.01 M triethanolamine–acetic acid buffer (pH 7.4) and then with a linear gradient, total volume 4 l, of 0.3 M NaCl to 0.7 M NaCl in 0.01 M triethanolamine–acetic acid buffer (pH 7.4). All buffers also contained 10^{-4} M Cleland's reagent. Fraction volume, 12–15 ml. Fractions were pooled as indicated, concentrated by ultrafiltration and chromatographed on hydroxylapatite.

Second chromatography on DEAE-Sephadex

After dialysis the material was applied to a DEAE-Sephadex column (4 cm × 39 cm) of DEAE-Sephadex, which was equilibrated with the last-mentioned dialysis buffer. Elution was performed as described in the legend of Fig. 1. As is clear from the figure two peaks containing enzyme activity were obtained. The first peak was not investigated further. Fractions comprising the second peak were pooled and concentrated by ultrafiltration to a volume of 140 ml.

Chromatography on hydroxylapatite

The concentrated material comprising the second peak of the DEAE-Sephadex

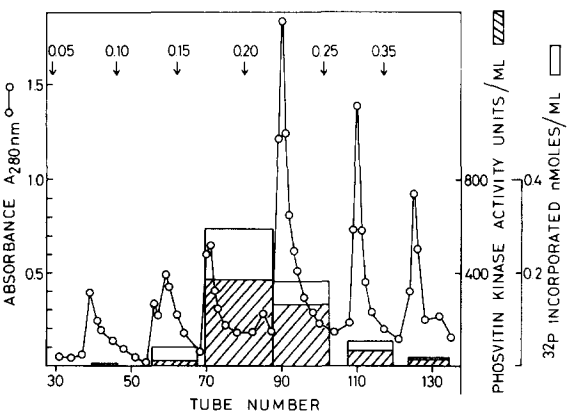

Fig. 2. Chromatography of phosvitin kinase on a column (3.0 cm × 5.3 cm) of hydroxylapatite. Elution was performed, as indicated in the figure with stepwise increments in the molarity of the potassium phosphate buffer (pH 7.0). The buffers contained 10^{-4} M Cleland's reagent. Fraction volume, 4 ml. Fractions eluted with 0.15 M and 0.20 M buffer were pooled, concentrated by ultrafiltration and chromatographed on Sephadex G-200.

chromatogram was applied to a column of hydroxylapatite, which was eluted stepwise as described in the legend of Fig. 2. Fractions were incubated with [^{32}P]ATP as described above and the amount of ^{32}P-labelled protein was determined. It was found that the fractions which contained phosvitin kinase also contained a protein which was phosphorylated by [^{32}P]ATP. Fractions eluted with 0.15 M and 0.20 M buffer were pooled and concentrated by ultrafiltration to a volume of 25 ml.

Chromatography on Sephadex G-200

The active material was applied to a column of Sephadex G-200 which was

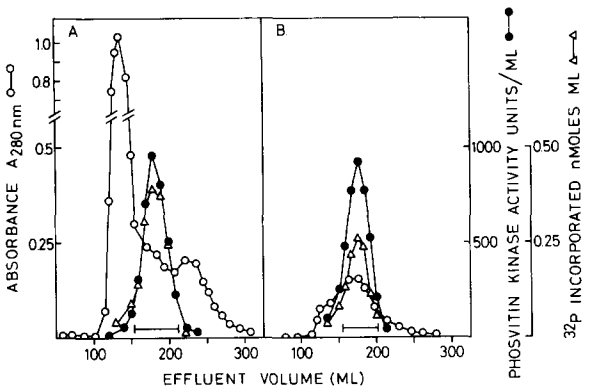

Fig. 3. A. Chromatography of phosvitin kinase on a column (2.15 cm × 103 cm) of Sephadex G-200. Elution was performed with 0.01 M triethanolamine–acetic acid buffer (pH 7.4) containing 1 M NaCl and 10^{-4} M Cleland's reagent. Fraction volume, 4.8 ml. Fractions were pooled as indicated, concentrated by ultrafiltration and rechromatographed on the same column. B. Rechromatography of phosvitin kinase from (A) under the same conditions as in (A).

eluted with 0.01 M triethanolamine–acetic acid buffer (pH 7.4) containing 1 M NaCl and 10^{-4} M Cleland's reagent. As can be seen from Fig. 3A the phosvitin kinase containing fractions still contained a protein which incorporated [^{32}P]phosphate after incubation with [^{32}P]ATP. The active fractions were pooled as indicated and concentrated by ultrafiltration to a volume of 9.4 ml. This material was rechromatographed on Sephadex G-200 under the same conditions (Fig. 3B). The phosphate-binding protein was still eluted in the same fractions as phosvitin kinase. Active fractions were pooled and concentrated by ultrafiltration to about 1 mg/ml. The enzyme could then be stored for several months at −20° without appreciable loss of activity.

Investigation of the purified phosvitin kinase fraction

Purity and protein character of the purified phosvitin kinase fraction

When the purified enzyme fraction was chromatographed on DEAE-Sephadex, the phosphate-binding protein was still eluted together with the phosvitin kinase (Fig. 4). The [^{32}P]phosphate-binding activity as well as the phosvitin kinase activity coincided with the protein peak indicating a high degree of purity.

The spectrum of purified phosvitin kinase is shown in Fig. 5. The ratio between the absorbance at 280 and 260 nm was 1.24. $E^{1\%}_{1\,cm}$ was found to be 15.7, using the

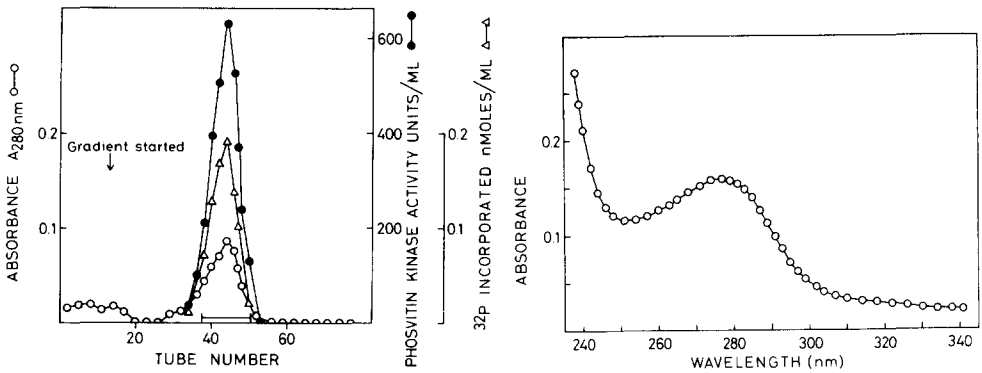

Fig. 4. Chromatography of phosvitin kinase on a column (1.3 cm × 12 cm) of DEAE-Sephadex. The column was eluted as described in the legend of Fig. 1, the total volume of the gradient was, however, 350 ml. Fraction volume, 3.5–4.0 ml. Fractions were pooled as indicated in the figure.

Fig. 5. Absorption spectra of purified phosvitin kinase fraction in 0.01 M potassium phosphate buffer (pH 7.25). Protein concentration, 0.100 mg/ml.

method of LOWRY *et al.*[18] for protein determination. These data indicate that the purified material consists mainly of protein but might contain a small amount of nucleic acid.

Amounts of [^{32}P]phosphate incorporated into the purified fraction. Isolation of [^{32}P]Ser-P and [^{32}P]Thr-P

The incorporation of [^{32}P]phosphate into the purified enzyme fraction was found to be about 3 nmoles per mg of protein at 10^{-5} M [^{32}P]ATP. [^{32}P]Ser-*P* and [^{32}P]Thr-*P* could be isolated from the ^{32}P-labelled enzyme fraction in a ratio of about 5:1.

Substrate specificity of the purified enzyme. Influence of cyclic AMP

The ability of the purified phosvitin kinase to phosphorylate other proteins than phosvitin is shown in Table II. The enzyme displayed high activity not only towards phosvitin but also towards casein. Histone was however, phosphorylated at a rate which was only about 1/100 that of phosvitin. The phosphorylation of casein or phosvitin was not enhanced by $5 \cdot 10^{-6}$ M cyclic AMP at 10^{-3} M [^{32}P]ATP. It was also investigated if cyclic AMP stimulated the phosphorylation of phosvitin at lower

TABLE II

SUBSTRATE SPECIFICITY OF PURIFIED PHOSVITIN KINASE

Protein kinase activity was measured as described under EXPERIMENTAL. The concentration of cyclic AMP was $5 \cdot 10^{-6}$ M.

Substrate	*nmoles of [^{32}P]phosphate transferred per mg of enzyme*	
	– cyclic AMP	*+ cyclic AMP*
Phosvitin	2600	2500
Casein	2000	2000
Histone	20	30

concentrations of [^{32}P]ATP. There was, however, no stimulation by $5 \cdot 10^{-6}$ M or $5 \cdot 10^{-5}$ M cyclic AMP at concentrations of ATP between $5 \cdot 10^{-6}$ M and $5 \cdot 10^{-4}$ M.

The possibility existed that the purified phosvitin kinase fraction represented a kinase and the corresponding phosphoryl enzyme. The activity of these phosphoryl enzymes is regulated by phosphorylation and dephosphorylation reactions. The best known examples of such enzymes are phosphorylase, phosphorylase kinase and glycogen synthetase[4]. None of these enzyme activities could, however, be demonstrated within the purified phosvitin kinase fraction.

TABLE III

PHOSPHORYLATION OF NUCLEAR PHOSPHOPROTEIN BY PURIFIED PHOSVITIN KINASE

Nuclear phosphoprotein was prepared from rat liver and calf brain as described under EXPERIMENTAL. The alkali-labile phosphate content of the purified protein was 0.44 and 0.27 μmole per mg of protein, respectively. 0.15 mg of rat liver phosphoprotein and 0.16 mg of calf brain phosphoprotein were separately incubated with 15 μmoles Tris–HCl buffer (pH 7.1), 11 μmoles NaCl, 2.5 μmoles $MgCl_2$ and 0.25 μmole [^{32}P]ATP (specific activity about 600 counts/min per nmole) in a final volume of 0.5 ml. Where indicated purified phosvitin kinase (0.036 mg) was added. In Expt. B the purified phosphoprotein was heated to 60° for 3 min to destroy endogenous phosvitin kinase activity. 3 min before addition of [^{32}P]ATP the samples were placed in a water-bath at 30°. Incubation was started by the addition of [^{32}P]ATP and was interrupted after 5 min by the addition of 100 μl of 50% trichloroacetic acid. 50 μl of phosvitin (20 mg/ml) were added as a carrier immediately before the trichloroacetic acid. The precipitates were washed 3 times with 10% trichloroacetic acid and were then dissolved in 0.1 M NaOH and plated in aluminium cups. The radioactivity was determined as described. The values have been corrected for phosphorylation of the purified phosvitin kinase preparation in the absence of nuclear phosphoprotein.

	nmoles of [^{32}P]phosphate incorporated per min per mg of phosphoprotein	
	Rat liver	*Calf brain*
(A) Phosphoprotein not heated		
− phosvitin kinase	0.88	1.34
+ phosvitin kinase	1.27	1.34
(B) Phosphoprotein heated		
− phosvitin kinase	0.03	0.03
+ phosvitin kinase	0.59	0.82

Phosphoprotein was prepared from calf brain and rat liver nuclei in order to investigate whether the nuclear phosphoprotein could serve as substrate for purified phosvitin kinase. The result is given in Table III. When purified phosphoprotein from either of the sources was incubated with [^{32}P]ATP, without added kinase, the phosphoprotein was phosphorylated by the endogenous phosvitin kinase. Addition of phosvitin kinase did not appreciably increase the incorporation of phosphoryl groups. It was thought that the low stimulation was due to the high endogenous phosvitin kinase activity, which concealed the effect of added phosvitin kinase. By heating the phosphoprotein for 3 min at 60° the endogenous kinase activity was destroyed (ref. 6). Addition of phosvitin kinase now resulted in a clear stimulation of the phosphorylation reaction.

DISCUSSION

A phosvitin kinase has been purified from calf brain to a high degree of purity. During purification it was found that the brain homogenate contained more than one kind of phosvitin kinase, which explains the rather low overall yield (7%). On fractionation on DEAE-Sephadex one phosvitin kinase containing fraction was eluted with 0.1 M NaCl and another with 0.5 M NaCl (Table I). Preliminary experiments show that these two fractions differ in several respects[23]. When the material eluted with 0.5 M NaCl was rechromatographed on a second DEAE-Sephadex column using gradient elution another two enzyme peaks were obtained (Fig. 1, Table I). Only the second one was purified further. Cleland's reagent seemed to stabilize the enzyme during pruification. When it was omitted there was a decrease in the overall yield.

The purified phosvitin kinase differs in several respects from the histone kinase purified from calf brain by MIYAMOTO *et al.*[24]. The phosvitin kinase preferentially phosphorylates phosvitin and casein (Table II) while the histone kinase preferentially phosphorylates histone. In contrast to the phosvitin kinase the histone kinase is stimulated by cyclic AMP.

The fractions containing phosvitin kinase were also found to contain a protein which was phosphorylated by [^{32}P]ATP. It was firmly associated with the kinase and could not be separated from it by any of the separation methods used. It is assumed that this phosphate-binding protein is a physiological substrate for phosvitin kinase. It is of interest that not only [^{32}P]Ser-*P* but also [^{32}P]Thr-*P* could be isolated from the ^{32}P-labelled protein.

The purified phosvitin kinase was shown to catalyze the phosphorylation of nuclear phosphoprotein purified from calf brain as well as rat liver. One important intracellular function of phosvitin kinase thus seems to be the phosphorylation of nuclear phosphoprotein. This is of considerable interest since it has been suggested that phosphorylation of nuclear phosphoprotein might be of importance in gene activation[6]. It is possible that the phosphate-binding protein that is associated with the phosvitin kinase is in fact a nuclear phosphoprotein. It is of interest in this context that purified nuclear phosphoprotein contains considerable amounts of phosvitin kinase, which is firmly bound to the phosphoprotein (Table III)[6]. Recently TAKEDA *et al.*[10] achieved a separation of the kinase from the phosphoprotein by chromatography on phosphorylcellulose. Experiments are in progress in our laboratory to separate the two components in the purified phosvitin kinase fraction by this method.

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