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PROTAMINE KINASE FROM YEAST

MIEKO NAKASHIMA, YOSHIMI TAKAI, HIROHEI YAMAMURA and YASUTOMI NISHIZUKA

Department of Biochemistry, Kobe University School of Medicine, Kobe 650 (Japan)

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Summary

A protein kinase (ATP:protein phosphotransferase, EC 2.7.1.37) which preferentially phosphorylates protamine is purified about 250-fold from the soluble fraction of baker's yeast (*Saccharomyces cerevisiae*). This enzyme is not sensitive to activation by cyclic nucleotides. Histone is about 5% as active as protamine in the reaction rate. Neither casein, phosvitin nor glycogen phosphorylase is active as substrate. The enzyme is distinguishable from casein kinase of the classical type (Rabinowitz, M. and Lipmann, F. (1960) J. Biol. Chem. 235, 1043—1050) and from adenosine 3′,5′-monophosphate-dependent protein kinase described earlier (Takai, Y., Yamamura, H. and Nishizuka, Y. (1974) J. Biol. Chem. 249, 530—535).

Introduction

Yeast casein kinase (ATP:protein phosphotransferase, EC 2.7.1.37), which transfers the terminal phosphate of ATP to seryl and threonyl residues of bovine casein and egg yolk phosvitin, has been described first by Rabinowitz and Lipmann [1]. Adenosine 3′,5′-monophosphate (cyclic AMP)-dependent protein kinase has recently been isolated from the organism in this laboratory [2], and evidence has been presented that the enzyme may play roles similar to those described for mammalian tissues [3]. During the course of this study another type of protein kinase has been found which preferentially phosphorylates protamine rather than histone. Cyclic nucleotides show no effect. This enzyme is clearly distinguishable from casein kinase and will be tentatively referred to hereafter as protamine kinase. Partial purification and properties of this enzyme are described briefly in the present article.

Materials and Methods

Baker's yeast (*Saccharomyces cerevisiae*) in pressed cake was obtained from Oriental Yeast Co. Cyclic AMP-dependent protein kinase, its catalytic and regulatory units and casein kinase were partially purified from this organism as described previously [2]. Calf thymus whole histone was prepared by the method of Johns [4]. Salmon sperm protamine (Lot 26B 8060), bovine serum albumin and horse heart cytochrome *c* (type III) were obtained from Sigma. Bovine casein (Hammarsten) was purchased from Merck AG-Darmstadt. Egg yolk phosvitin was obtained from Mann. Human γ-globulin (Fraction II) and ovalbumin (twice recrystallized) were obtained from Nutritional Biochemicals. Rabbit muscle glycogen phosphorylase *b* was obtained from Boehringer Mannheim. [γ-^{32}P] ATP was prepared by the method of Glynn and Chappel [5]. Cyclic [^{3}H] AMP (4.47 Ci per mmole) was a product of New England Nuclear. Other chemicals were obtained from commercial sources.

Protein kinase was assayed in the reaction mixture (0.25 ml) which contained 2.5 μmoles of Tris · HCl at pH 7.5, 2.5 μmoles of magnesium acetate, 2.5 nmoles of [γ-^{32}P] ATP (3–8 · 10^4 cpm per nmole), 100 μg of salmon sperm protamine as phosphate acceptor protein and enzyme preparation. Cyclic AMP (0.4 μM) was added where indicated. The reaction was carried out for 10 min at 30° C and terminated by the addition of 5 ml of ice-cold 10% trichloroacetic acid. Acid-precipitable material was collected on a Millipore filter (pore size, 0.45 μm) and radioactivity determined using a Nuclear Chicago Geiger-Muller gas flow counter, model 4338, as described previously [6]. One unit of protein kinase activity was defined as that amount of enzyme which incorporated one pmol of phosphate from [γ-^{32}P] ATP into acid-precipitable material per min under the standard assay conditions. Cyclic AMP-binding activity was assayed by measuring the binding of cyclic [^{3}H] AMP under the conditions specified earlier [7].

Acid hydrolysis and isoelectrofocusing electrophoresis were carried out under the conditions described previously [7,8]. Molecular weight of the enzyme was estimated by gel filtration on a Sephadex G-75 column (93 × 2.5 cm) with human γ-globulin, bovine serum albumin, ovalbumin and horse heart cytochrome *c* as standard proteins under the conditions specified earlier [2,7]. Protein was determined by the method of Lowry et al. [9] with bovine serum albumin as a standard.

Results

Partial purification of the enzyme

Baker's yeast (40 g wet weight) was suspended in 5 vol. of 50 mM Tris · HCl at pH 7.5 containing 1 mM EDTA and 6 mM 2-mercaptoethanol (buffer A) and was crushed in a French press cell at 20 000 p.s.i. All manipulations were carried out at 0—4° C. The homogenate was centrifuged for 20 min at 20 000 × *g*. To the supernatant solution (200 ml, 4400 mg of protein), 10.5 ml of 20% streptomycin sulfate was added, and the resulting precipitate was removed by centrifugation. Ammonium sulfate (60 g) was added to the supernatant (50% saturation), and the precipitate was collected by centrifugation and dissolved in

30 ml of buffer A. The solution (660 mg of protein) was dialyzed overnight against a large volume of buffer A, and then applied to a DEAE-cellulose column (15 × 3 cm) equilibrated with buffer A. After the column was washed with 200 ml of the same buffer, elution was carried out with an application of a linear concentration gradient of NaCl (0- 0.4 M, 1200 ml) in buffer A. Fractions (12 ml each) were collected. When each fraction was assayed for protein kinase with protamine and histone as substrate, one major and one minor peak appeared in the fractions 35 through 55 and fractions 60 through 80, respectively, as shown in Fig. 1A and B; both peaks did not respond to cyclic AMP and reacted with protamine much faster than with histone. When cyclic AMP (0.4 μM) was added to each assay mixture, an additional peak appeared in the fractions 16 through 30 (Fig. 1A and B). This enzyme was identified as cyclic AMP-dependent protein kinase which was described in a previous paper [2]. When each fraction was assayed for protein kinase with casein as substrate, again two peaks, major and minor, appeared in the fractions 16 through 30 and fractions 60 through 80, respectively, as shown in Fig. 1C; both kinases were independent of cyclic AMP and reacted with phosvitin as well as casein. The

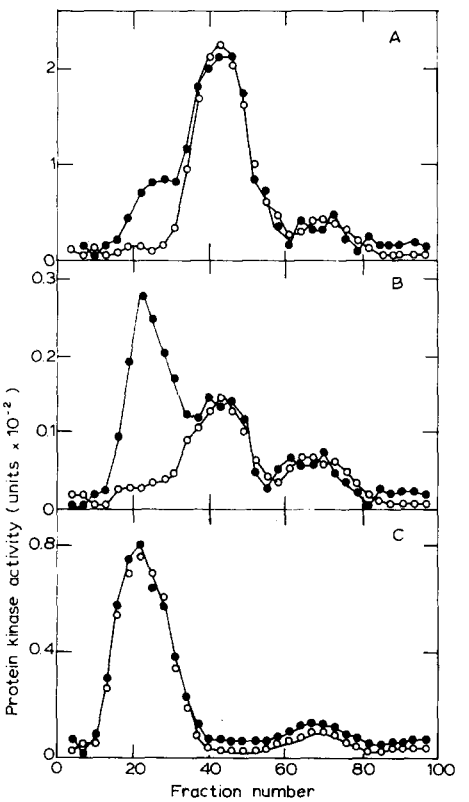

Fig. 1. DEAE-cellulose column chromatography of various protein kinases from yeast. Detailed experimental conditions are described in the text. Unit of protein kinase activity is defined in the text. Closed and open circles represent protein kinase activity in the presence and absence of cyclic AMP (0.4 μM), respectively. Protein kinase activity in each fraction was assayed with protamine (A), histone (B) and with casein (C) as substrate.

TABLE I

SUMMARY OF PURIFICATION OF YEAST PROTAMINE KINASE

Detailed experimental conditions are described in the text. Unit of protein kinase activity is defined in the text.

Fraction	Volume (ml)	Protein (mg)	Total activity (units)	Spec. act. units $\times 10^{-3}$/mg
Crude supernatant	200	4400	440	0.1
Streptomycin sulfate	190	2660	266	0.1
Ammonium sulfate	30	660	264	0.4
DEAE-cellulose*	252	348	600	1.7
Ammonium sulfate	20	150	525	3.5
Hydroxylapatite	4	70	420	6.1
Sephadex G-75	5	14	360	25.7

* At this step some unknown inhibitor factors might be removed.

protein kinase in the fractions 16 through 30 (Fig. 1C) was separable from cyclic AMP-dependent protein kinase eluted in the same fractions by isoelectrofocusing electrophoresis, and was shown to be identical with casein kinase originally described by Rabinowitz and Lipmann [1]. In contrast, a small amount of enzyme eluted in the fractions 60 through 80 (Fig. 1C) appeared to phosphorylate protamine and, to a lesser extent, histone and casein. The properties of this protein kinase are not defined in the present studies.

In order to purify further protamine kinase fractions (35 through 55) were pooled and 98 g of ammonium sulfate was added (60% saturation). The precipitate was dissolved in 20 ml of Buffer A and dialyzed overnight against a large volume of buffer A. The solution (150 mg of protein) was then applied to a hydroxylapatite column (4 × 2 cm) equilibrated with buffer A, and protamine kinase eluted with 60 ml of 30 mM potassium phosphate at pH 7.5 in buffer A. Under these conditions cyclic AMP-dependent protein kinase and casein kinase, which slightly contaminated the preparation, remained adsorbed on the column. The eluate was concentrated to about 4 ml (70 mg of protein) using a Saltorius membrane filter apparatus equipped with an SM 13 200 filter, and was subjected to a Sephadex G-75 column (93 × 2.5 cm) equilibrated with buffer A. Fractions (4.5 ml each) were collected. Protamine kinase appeared as a single symmetrical peak in the fractions 42 through 50. Table I summarizes a typical result of the purification procedures. Protamine kinase was purified about 250-fold starting from the crude extract and with an overall recovery of more than 80%. This preparation was practically free of endogenous substrate under the standard assay conditions, and could be stored at −20° C for at least 2 months without loss of activity.

Properties of protamine kinase

Table II shows relative effectiveness of substrate proteins in comparison with cyclic AMP-dependent protein kinase and casein kinase obtained from the same organism. Protamine kinase preferentially phosphorylated salmon sperm protamine. Calf thymus whole histone was about 5% as active as protamine in the reaction rate, whereas histone was about 40% as active as protamine for cyclic AMP-dependent protein kinase. Bovine casein and egg yolk phosvitin

TABLE II

SUBSTRATE SPECIFICITY OF PROTAMINE KINASE, CYCLIC AMP-DEPENDENT PROTEIN KINASE AND CASEIN KINASE OBTAINED FROM YEAST

Enzyme activity was assayed under the standard assay conditions except that 100 μg each of the acceptor protein indicated was employed. The numbers indicate units of protein kinase activity, and those in parentheses represent percentage activities with salmon sperm protamine as 100 for protamine kinase and cyclic AMP-dependent protein kinase, and with bovine casein as 100 for casein kinase.

Substrate	Protamine kinase		Cyclic AMP-dependent protein kinase		Casein kinase	
	Without cyclic AMP	With cyclic AMP	Without cyclic AMP	With cyclic AMP	Without cyclic AMP	With cyclic AMP
Salmon sperm protamine	16.3 (100)	16.1 (100)	7.1 (100)	16.5 (100)	0 (0)	0 (0)
Calf thymus histone	0.9 (5.5)	0.8 (5.0)	1.8 (25)	7.0 (42)	0 (0)	0 (0)
Bovine casein	0 (0)	0 (0)	0 (0)	0 (0)	16.4 (100)	16.5 (100)
Egg yolk phosvitin	0 (0)	0 (0)	0 (0)	0 (0)	11.3 (69)	11.0 (67)
Bovine serum albumin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Human γ-globulin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Glycogen phosphorylase *b*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

were active for casein kinase, but completely inactive for protamine kinase. Bovine serum albumin, human γ-globulin and rabbit muscle glycogen phosphorylase *b* were totally inactive as substrates for these enzymes. Protamine kinase obtained from trout testis by Jergil and Dixon [10] was stimulated by cyclic AMP, while the enzyme described here was not activated by cyclic AMP nor did it bind the cyclic nucleotide. Other cyclic nucleotides also showed no

TABLE III

COMPARATIVE PROPERTIES OF PROTAMINE KINASE AND CYCLIC AMP-DEPENDENT PROTEIN KINASE OBTAINED FROM YEAST

Property	Protamine kinase	Cyclic AMP-dependent protein kinase*
Km for ATP**	$5 \cdot 10^{-6}$ M	$1.2 \cdot 10^{-5}$ M
Optimum Mg^{2+} **	10—20 mM	5 mM
Optimum pH**	6.0—8.0	7.5
Molecular weight	30000	58000 (30000)
Isoelectric point	pH 5.6	pH 7.7 (pH 6.9)
Half life at 50°C at pH 7.5***	10 min	2 min (30 s)

* The data are taken from ref. 2. The numbers represent the values of the holoenzyme, and those in the parentheses indicate the values of the catalytic unit.

** The enzyme activity was assayed under the standard assay conditions except that each parameter indicated was varied.

*** Each enzyme (45 μg protein/ml) was heated in buffer A under the same conditions, and an aliquot was assayed for protein kinase under the standard conditions described in the text.

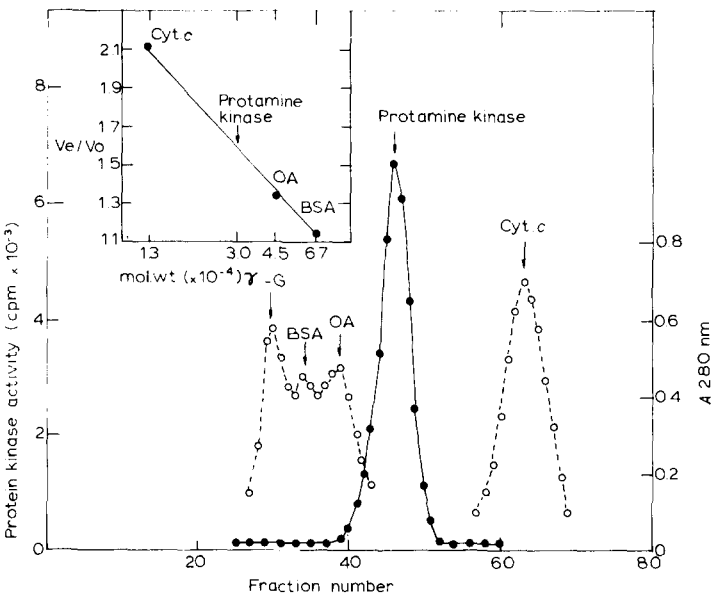

Fig. 2. Estimation of the molecular weight of protamine kinase by gel filtration on Sephadex G-75. Closed and open circles represent protamine kinase activity and absorbance at 280 nm, respectively. In the inset, V_e/V_o of the enzyme and three marker proteins (bovine serum albumin (BSA), ovalbumin (OA) and horse heart cytochrome *c* (Cyt *c*) with molecular weights of 67 000, 45 000 and 13 000, respectively) were plotted against the molecular weight. V_o was estimated with human γ-globulin (γ-G).

effect. When protamine was fully phosphorylated and then subjected to acid hydrolysis followed by paper electrophoresis, a major radioactive spot was found to be associated with phosphoserine. Table III summarizes some kinetic and physical properties of the enzyme in comparison with cyclic AMP-dependent protein kinase from the same organism. A divalent cation, either Mg^{2+}, Mn^{2+} or Co^{2+}, was needed for the enzyme; the maximum activity was obtained at 10—20 mM for Mg^{2+} and at 1—10 mM for Mn^{2+} and Co^{2+}. These cations were inhibitory at concentrations higher than 30 mM. The enzyme was active at pH 6—8 with potassium phosphate (20 mM) in the presence of 10 mM Mg^{2+}.

Protamine kinase showed a symmetrical peak upon gel filtration on a Sephadex G-75 column as shown in Fig. 2, and the molecular weight was calculated to be about 30 000; the molecular weight was identical with that of the catalytic unit of cyclic AMP-dependent protein kinase (Table III). Nevertheless, protamine kinase described here was clearly distinguishable from the catalytic unit: (a) the catalytic unit was inhibited by the regulatory unit of cyclic AMP-dependent form [2], whereas protamine kinase was not inhibited by the regulatory unit; (b) the catalytic unit reacted with rabbit skeletal muscle glycogen phosphorylase kinase resulting in the activation of this enzyme [3], whereas protamine kinase was inactive in this capacity; and (c) protamine kinase exhibited different kinetic and physical properties from those of the catalytic unit as shown in Table III.

Discussion

A protein kinase which is capable of phosphorylating protamine rather than histone has been described recently in rat uterus [11], brain [12] and liver (Nishiyama, K. and Takai, Y., unpublished). Such protein kinase is independent of cyclic AMP and clearly distinguishable from both cyclic AMP-dependent protein kinase and casein kinase. The yeast protein kinase briefly described in this paper shows closely similar properties to the mammalian enzyme. Nevertheless, the phosphate acceptor proteins occurring naturally in yeast remain unexplored.

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