DNA-binding sperm proteins with oligo-arginine clusters function as potent activators for egg CK-II

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Abstract The stimulatory effect of DNA-binding sperm proteins (histone and protamine) on the phosphorylation of p98 (ERp99/GRp94, one of the Hsp-90 family of proteins) by egg casein kinase II (CK-II) was investigated in vitro. It was found that (i) phosphorylation of p98 by egg CK-II in vitro is greatly stimulated by poly-Arg, but not by poly-Lys; and (ii) similar stimulation is observed with sperm histones $H2B_2$ and $H2B_3$ (sea urchin) and fish protamines, such as salmine A_1 (salmon) and protamine 3a (rainbow trout). These findings suggest that these DNA-binding sperm proteins function as potent activators for CK-II in fertilized eggs. All of these DNA-binding sperm proteins contain at least an oligo-Arg cluster as a common feature, which can interact with an acidic amino acid cluster of the regulatory β-subunit CK-II.

Key words: Casein kinase II; DNA-binding sperm protein; Protamine; Activation of CK-II; Heat shock protein-90; Sea urchin

1. Introduction

Casein kinase II (CK-II), a cAMP-, cGMP- and Ca^{2+}/phospholipid-independent serine/threonine protein kinase, is one of the key protein kinases responsible for cellular metabolic alteration through phosphorylation of more than fifty cellular polypeptides [1,2]. Recent reports have demonstrated that CK-II plays important roles in the initiation of DNA replication and the regulation of transcription: it modifies DNA binding proteins, such as DNA topoisomerases [3], DNA ligase [4] and SV-40 large T antigen [5], and transcriptional factors, such as Sp1 [6], serum response factor (SRF) [7], Ap1 [8] and upstream binding factor (UBF) [9]. Recently, we reported that (i) the biochemical properties of CK-II purified from unfertilized sea urchin eggs are similar to those of CK-IIs from various mammalian cells; (ii) CK-II is copurified with a 98 kDa polypeptide (p98) from a 1.5 M KCl extract of unfertilized sea urchin eggs; and (iii) p98 functions as an effective phosphate acceptor for CK-II in the presence of basic polypeptides, such as poly-Arg and protamine, in vitro [10].

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Abbreviations: CK-II, casein kinase II; DTT, dithiothreitol; ERp99, 99 kDa endoplasmic reticulum protein; GRp94, 94 kDa glucose regulated protein; HPLC, high performance liquid chromatography; Hsp-90, heat shock protein-90; p98, 98 kDa polypeptide; Arg, L-arginine; Lys, L-lysine, PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl-sulfate polyacrylamide gel electrophoresis.

Protamines of most vertebrate sperm have a high content of basic amino acids (45–80%) with plural oligo-Arg clusters [11,12]. However, the biological significance of the basic amino acid clusters of protamines as well as their physiological roles in fertilization remain to be elucidated. In order to understand the biological significance of CK-II in the regulation of transcription and DNA replication in fertilized eggs, it is important to identify the specific CK-II activators that the sperm bring into the egg. The present study has been performed to purify and biochemically characterize histones from spermatozoa of sea urchin and protamines from spermatozoa of fishes, and to investigate whether these proteins can act as potent CK-II activators using p98 (ERp99/GRp94) as a phosphate acceptor. Here, we describe (i) partial purification of CK-II copurified with p98 from unfertilized sea urchin eggs; (ii) purification and identification of histones from spermatozoa of sea urchin as potent CK-II activators; and (iii) the stimulatory effect of polypeptides with oligo-Arg clusters on the CK-II catalyzed phosphorylation of p98 in vitro. The data provided here show that DNA-binding sperm proteins with oligo-Arg clusters function as potent activators for egg CK-II and such activation is dependent on the number of Arg residues in the cluster.

2. Materials and methods

2.1. Materials

[γ-^{32}P]ATP (3,000 Ci/mmol) was obtained from Amersham (Arlington Heights, USA); DTT, PMSF, poly-Arg (5–15 kDa), poly-Lys (1–4 kDa), heparin, α-casein (bovine milk), salmine 3a, protamine A_1 and pepsin from Sigma Chemical (St. Louis, USA); calf thymus histones (whole histone, H1, H2A, H2B, H3 and H4) from Boehringer-Mannheim Biochemicals (Germany); DEAE-cellulose and phosphocellulose from Whatman (Maidstone, England); Sephacryl S300 and Mono Q HR5/5 columns from Pharmacia (Uppsala, Sweden); TSGgel-Phenyl 5PW column from Tosoh Co., Ltd. (Tokyo); and mouse monoclonal antibody to slime mold Hsp-90 from Funakoshi Biochemical Laboratory (Tokyo).

2.2. Preparation of histones from spermatozoa of sea urchin

The crude histone preparation from spermatozoa of the sea urchin, *Hemicentrotus pulcherrimus*, was obtained by a modification of the method originally described by Hill et al. [13]. Briefly, the spermatozoa (about 30 g) were homogenized, using a Potter type homogenizer, in 15 mM Tris-HCl buffer (pH 7.4) containing 60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 0.2 mM PMSF, 15 mM 2-mercaptoethanol and 0.34 M sucrose. The pellet obtained was washed five-times with the homogenizing buffer and the resulting precipitates were shaken for 60 min in 0.2 M H_2SO_4 in an ice bath. 25% trichloroacetic acid (TCA) (final 2.5%) were added to precipitate histones in the crude solution. The TCA-precipitate obtained was then suspended in absolute acetone containing 5 mM HCl and centrifuged at 3,500 × *g* for 15 min. The resulting precipitates were dissolved in 1 mM HCl and this crude histone preparation was used for further purification of histones as described below.

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2.3. Partial purification of egg CK-II

CK-II in a 1.5 M KCl extract of unfertilized sea urchin eggs was partially purified by means of successive phospho-cellulose column chromatography and gel filtration on a Sephacryl S300 column (HPLC) [10]. The Sephacryl S300 fraction (designated S300 fraction) was used as a partially purified CK-II fraction in the present study.

2.4. Detection of ^{32}P-labeled polypeptides

To detect ^{32}P-labeled polypeptides in the crude and purified CK-II fractions, these fractions were separately incubated for the indicated period at 30°C with 50 μM [γ-^{32}P]ATP (1,000 cpm/pmol) and 3 mM Mn^{2+} in the presence of CK-II activators (5 μg each), such as poly-Arg and DNA-binding sperm proteins (salmine A_1 (salmon), protamine 3a (rainbow trout) and histones $H2B_2$ and $H2B_3$ (sea urchin)). The ^{32}P-labeled polypeptides were detected by autoradiography after SDS-PAGE or two-dimensional gel electrophoresis (2DE), as reported previously [10].

3. Results

3.1. Purification and characterization of egg CK-II and its effective phosphate acceptor, p98

A cAMP-independent casein phosphorylating protein kinase in a 1.5 M KCl extract of unfertilized sea urchin eggs was partially purified by means of successive phospho-cellulose column chromatography (Fig. 1A) and gel filtration on Sephacryl S300 (Fig. 1B). The resulting kinase activity was then applied on a Mono Q column (HPLC) and detected in the fractions eluted between 0.50 M and 0.60 M NaCl as a single active peak, which exactly corresponded with a peak of absorbance at 280 nm (Fig. 1C). Phosphorylation of endogenous cellular polypeptides by the kinase was highly stimulated when poly-Arg (5 μg) was added to the reaction mixtures as an activator for the kinase (Fig. 1). The purified protein kinase (Mono Q fraction) was identified as CK-II, because its enzymatic properties were found to be similar to those of CK-IIs from various mammalian cells, as reported previously [10]: (i) p98 and casein function as effective in vitro phosphate acceptors for the kinase (Fig. 2A, lane 2); (ii) poly-Arg highly stimulates phosphorylation of p98 or casein by the kinase (Fig. 2A, lane 3); (iii) heparin (0.2 μg) completely inhibits this phosphorylation (Fig. 2A, lane 4); and (iv) CK-II in the Mono Q fraction is immunoprecipitated with anti-serum to *Drosophila* CK-IIβ (data not shown).

When the Mono Q fraction was incubated with 50 μM [γ-^{32}P]ATP (1,000 cpm/pmol) and 3 mM Mn^{2+}, the autoradiogram (Fig. 2A, lane 1) detected a 98 kDa polypeptide (p98) as a highly phosphorylated polypeptide. The N-terminal 23 residue sequence [GLFDEYGSKKSDYIKLYVRRVFI] of a 35 kDa poly-peptide (p35) formed from limited digestion of p98 with modified trypsin is identical to that at positions 396–418 of mouse 99 kDa endoplasmic reticulum protein (ERP-99) [14] and 94 kDa glucose regulated protein (GRp94) [15], which belong to the Hsp-90 family. A database analysis revealed that ERp99 and GRp94 are the same protein and each has at least 20 phosphorylation sites for CK-II. In addition, it was found that (i) p98 cross-reacted with anti-serum to slime mold Hsp-90 (Fig. 2B, lane 2) and it was a single polypeptide (pI 6.5) with a molecular weight of approximately 98 kDa (Fig. 2C). Moreover, two observations suggest that CK-II exists as a complex with p98 in unfertilized sea urchin eggs: (i) p98 is copurified with CK-II from a 1.5 M KCl extract of unfertilized eggs by using three different column chromatografies (Fig. 1); and (ii) p98 is detected as a mainly phosphorylated polypeptide in the anti-Hsp-90 immunoprecipitates of the Mono Q fraction after incubation with 50 μM [γ-^{32}P]ATP (1,000 cpm/pmol) in the presence of poly-Arg (5 μg) and 3 mM Mn^{2+} in vitro (Fig. 2C).

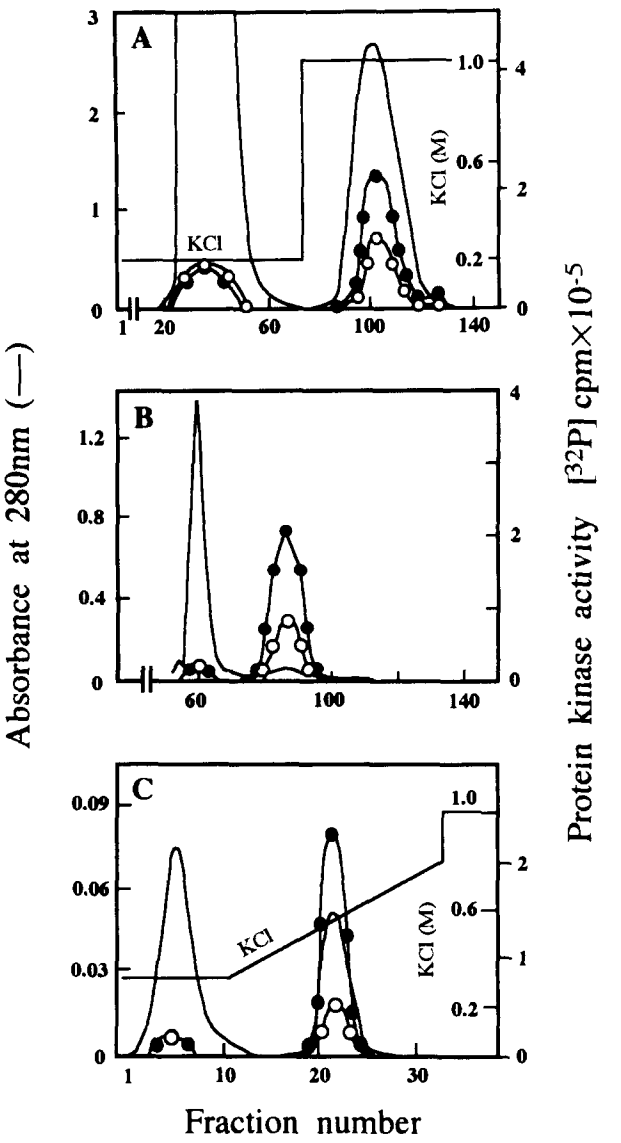

Fig. 1. Purification of CK-II in a 1.5 M KCl extract from unfertilized sea urchin eggs. (A) CK-II in a 1.5 M KCl extract from unfertilized sea urchin eggs was partially purified by phospho-cellulose column chromatography. The extract (about 550 mg protein) was applied on a phospho-cellulose column (2.4 × 15 cm), equilibrated with Buffer A (20 mM Tris-HCl (pH 7.6), 2 mM DTT, 0.1 mM PMSF, 1 mM $NaMnO_4$, 1 mM EDTA and 10% glycerol) containing 0.2 M NaCl. CK-II was eluted step-wise with Buffer A containing 1.0 M NaCl after washing the column with Buffer A containing 0.2 M NaCl. The CK-II activity was assayed in the presence (●) or absence (○) of poly-Arg (5 μg) without exogenous phosphate acceptors. Absorbance at 280 nm (—). (B) The phospho-cellulose CK-II active fraction (about 20 mg protein) was applied on a column (1.4 × 120 cm) of Sephacryl S300 (HPLC), equilibrated with Buffer B (20 mM Tris-HCl (pH 7.6), 2 mM DTT, 0.1 mM PMSF, 1 mM $NaMnO_4$, 1 mM EDTA and 10% glycerol) containing 1.0 M NaCl. Elution was carried out with the same buffer at the flow rate of 1.0 ml/min, and 2.0 ml fractions were collected. (C) The concentrated CK-II active S300 fraction was further purified by Mono Q column chromatography (HPLC). Elution was carried out with a linear gradient between 0.2 M and 0.8 M NaCl in Buffer A at the flow rate of 1.0 ml/min, collecting 1.0 ml fractions.

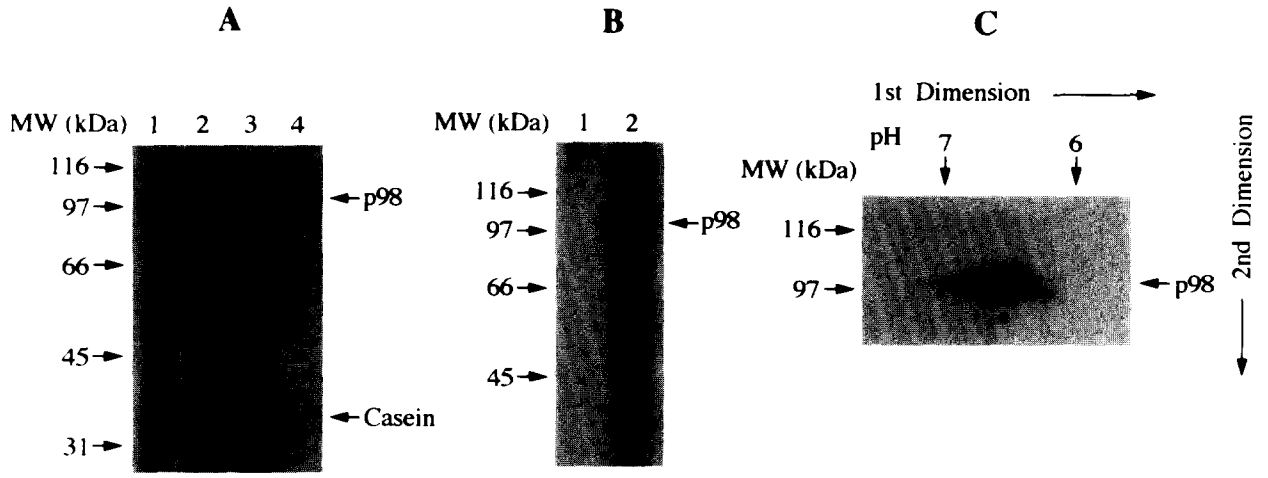

Fig. 2. Characterization of egg CK-II, and of p98 (as one of the effective phosphate acceptors for CK-II) in vitro. (A) To characterize a casein phosphorylating kinase purified from unfertilized sea urchin eggs, the Mono Q fraction (Fig. 1C, 0.1 μg) was incubated for 15 min at 30°C with 50 μM [γ-^{32}P]ATP (1,000 cpm/pmol) and 3 mM Mn^{2+} in the presence or absence of casein (an exogenous phosphate acceptor for CK-II, 10 μg), poly-Arg (5 μg) and heparin (0.2 μg). The ^{32}P-labeled polypeptides in the reaction mixtures were detected by autoradiography after SDS-PAGE. The Mono Q fraction was incubated with 50 μM [γ-^{32}P]ATP (1,000 cpm/pmol) and 3 mM Mn^{2+} in the absence of activators (lane 1), or presence of casein (lane 2), casein and poly-Arg (lane 3) or casein and heparin (lane 4). (B) To identify p98 in the Mono Q fraction as a polypeptide of the Hsp-90 family, the fraction (0.5 μg) was incubated with anti-serum to mouse Hsp-90 on Sepharose 6B, in the presence of protein-A. The resulting precipitates were washed five times with 20 mM sodium phosphate buffer (pH 7.2) containing 0.1 mM EDTA, 2 mM DTT and 0.5 M NaCl, and then incubated for 20 min at 30°C with 50 μM [γ-^{32}P]ATP and 3 mM Mn^{2+}, in the presence of poly-Arg (5 μg). The ^{32}P-labeled polypeptides were detected by autoradiography after SDS-PAGE. Normal rabbit serum (lane 1) and anti-Hsp-90 serum (lane 2). (C) The ^{32}P-labeled polypeptides in the immunoprecipitates (Fig. 2B, lane 2) were detected directly by autoradiography after two-dimensional gel electrophoresis (2DE).

3.2. *Characterization of sperm histones $H2B_2$ and $H2B_3$ as potent in vitro activators for egg CK-II*

Previously, we reported that phosphorylation of p98 by CK-II in the Mono Q fraction was greatly stimulated when the crude histone preparation from spermatozoa of *H. pulcherrimus* was present in the reaction mixtures, as compared with the stimulation determined with calf thymus histones (whole histone, H1, H2A, H2B, H3 and H4) [10]. Therefore, to identify a specific histone responsible for activation of CK-II in an in vitro assay system for phosphorylation of p98 by egg CK-II, the crude histone preparation was purified by HPLC on a TSKgel-phenyl PW50 RP column. At least six distinct polypeptides (**a–f**) were recognized (Fig. 3A) and polypeptide analysis by SDS-PAGE revealed a homogeneous polypeptide in each fraction (Fig. 3B). Polypeptides **a, b, d, e** and **f** were identified as histones H1, H2A, $H2B_3$, H3 and H4, respectively, since their partial N-terminal amino acid sequences (**a**: PGSPQKRAASPRKSPRKSPKKS; **b**: SGRGKGAKGKAKAKSRSSRA; **d**: PRSPAKTSPRKGSPRKGSPRKGSPSRKASP; **e**: ARTKQTARKSTGGKAPRKQL; and **f**: SGRGKGGKGLGKGGAKRHRK) corresponded exactly to the sequences of these five histones from gonads and spermatozoa of *Parechinus angulosus* [16,17] and *Psammechinus miliaris* [18]. In addition, the N-terminal 28 residue sequence (PRSPSKTSPRKGSPRKGSPSRKASPKRG) of polypeptide **c** purified from spermatozoa of *H. pulcherrimus* was 96.4% homologous to the analogous sequence of histone $H2B_2$ of another species, *Parechinus angulosus*: alanine at position 5 was substituted with serine in *H. pulcherrimus* [19].

The stimulatory effect of sperm histones $H2B_2$ and $H2B_3$ and other basic polypeptides (calf thymus histone H2B, salmine A_1 and poly-Arg) on the phosphorylation of p98 by egg CK-II was examined in vitro. It was found (Fig. 4) that (i) phosphorylation of p98 by CK-II was greatly stimulated by poly-Arg (lane 3), salmine A_1 (lane 4) and crude histone extract from sea urchin spermatozoa (lane 5); (ii) similar stimulation was observed with histones $H2B_2$ (lane 8) and $H2B_3$ (lane 9); and (iii) only slight stimulation was observed with sperm histones [H1 (lane 6), H2A (lane 7), H3 (lane 10) and H4 (lane 11)] and calf thymus histone H2B (lane 2), none of which have oligo-Arg clusters. The stimulatory effect of salmine A_1 was effectively substituted with other DNA-binding Arg-rich sperm proteins, such as protamine 3a and scombrine γ (spotted mackerel [12]) (data not shown). In addition, other cellular polypeptides (p70, p56, p52 and p34) were detected as highly phosphorylated polypeptides when the partially purified CK-II (Mono Q) fraction was incubated with 50 μM [γ-^{32}P]ATP (1,000 cpm/pmol) and 3 mM Mn^{2+} in the presence of either poly-Arg (Fig. 4, lane 3), salmine A_1 (lane 4), histone $H2B_2$ (lane 8) or histone $H2B_3$ (lane 9). This suggests that the activated egg CK-II can phosphorylate several cellular polypeptides (p70, p56, p52 and p34) copurified with p98 from unfertilized sea urchin eggs.

3.3. *Structural characteristics of DNA-binding sperm proteins as activators for egg CK-II in vitro*

There are oligo-Arg clusters at positions 2–7 and 24–29 of protamine 3a [12], positions 21–26 of salmine A_1 [12] and positions 45–57 of histones $H2B_2$ and $H2B_3$ (sea urchin [18,19]) as a common feature among these DNA-binding sperm proteins. To determine the specific domains of these polypeptides responsible for the stimulatory effect on CK-II activity (phosphorylation of p98) in vitro, synthetic oligo-Arg and oligo-Lys fragments, and polypeptide fragments with and without an oligo-Arg cluster (formed from limited digestion of histone $H2B_3$ with pepsin) were prepared. Fig. 5A shows that: (i) salmine A_1 greatly stimulates CK-II activity (lane 2); and

(ii) hexa-Arg fragment (lane 4), sperm histone $H2B_3$ (lane 6) and polypeptide fragments containing an oligo-Arg cluster prepared from histone $H2B_3$ (lane 8) significantly stimulate CK-II activity. However, there were no effects with oligo-peptides consisting of penta-Arg fragment (lane 3), hexa-Lys fragment (lane 5) or polypeptide fragments without oligo-Arg clusters prepared from histone $H2B_3$ (lane 7). Moreover, kinetic experiments (Fig. 5B) show that (i) the CK-II catalyzed phosphorylation of p98 is stimulated up to 22.0-, 10.5- or 5.1-fold by salmine A_1 containing plural oligo-Arg clusters, histone $H2B_3$ or hexa-Arg fragment, respectively; and (ii) this stimulation reaches a plateau above 0.1 μg of these polypeptides. Taken together, all these results suggest that the oligo-Arg sequence in sperm histone $H2B_3$ may be responsible for very high stimulation of the CK-II catalyzed phosphorylation of p98 in vitro and that this stimulatory effect may be dependent upon the number of arginine clusters composed of at least hexa-Arg in the DNA-binding sperm proteins.

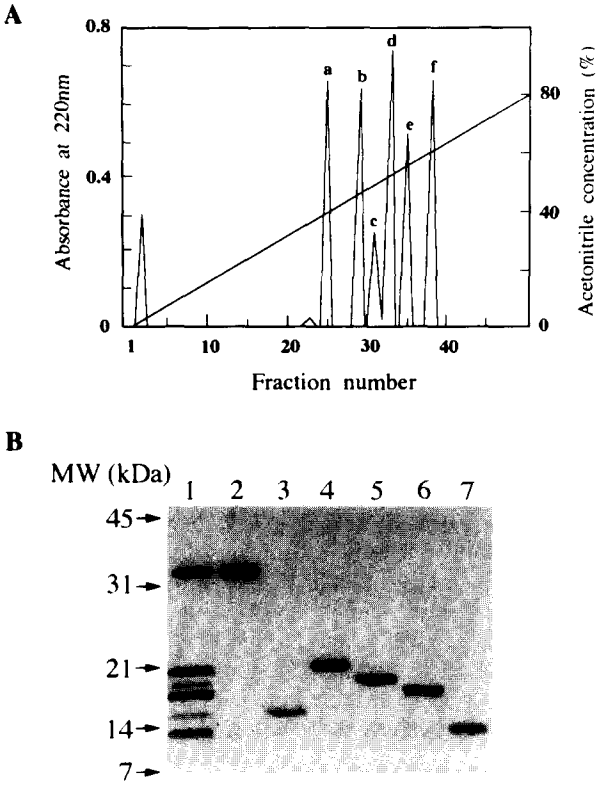

Fig. 3. Purification and homogeneity of histones from the crude preparation of sea urchin spermatozoa. (A) Histones in the crude sperm extract prepared from *H. pulcherrimus* were purified by TSGgel-Phenyl 5PW column chromatography (HPLC). Elution was carried out with a linear gradient between 0% and 80% acetonitrile at the flow rate of 1.0 ml/min, and 1.0 ml fractions were collected. Six major distinct protein peaks (**a** through **f**) were observed: (**a** (fraction 25), **b** (fraction 29), **c** (fraction 31), **d** (fraction 33), **e** (fraction 35) and **f** (fraction 38)). (B) Determination of the homogeneity of each histone by SDS-PAGE. Histones on the gel were detected by staining with Coomassie Brilliant Blue R250 (CBBR-250) after SDS-PAGE. Crude histone preparation from spermatozoa of sea urchin (lane 1); peak **a** (lane 2); peak **b** (lane 3); peak **c** (lane 4); peak **d** (lane 5); peak **e** (lane 6); and peak **f** (lane 7).

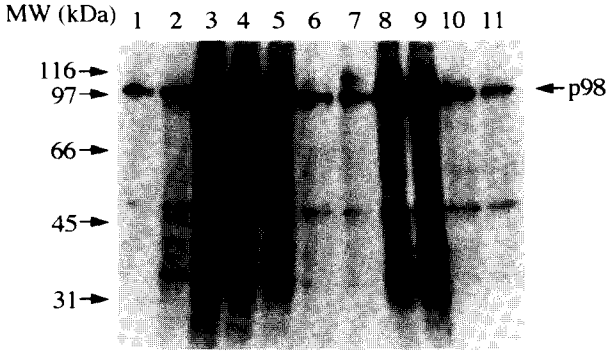

Fig. 4. Characterization of various DNA-binding sperm proteins as potent CK-II activators in vitro. The purified CK-II fraction (Mono Q fraction, approximately 0.1 μg) was incubated for 15 min at 30°C with 50 μM [γ-^{32}P]ATP (1,000 cpm/pmol) and 3 mM Mn^{2+} in the absence (lane 1) or presence of calf thymus H2B (lane 2), poly-Arg (lane 3), salmine A_1 (lane 4), crude sperm histone preparation (lane 5), purified sperm histone H1 (lane 6), histone H2A (lane 7), histone $H2B_2$ (lane 8), histone $H2B_3$ (lane 9), histone H3 (lane 10) or histone H4 (lane 11) at the concentration of 2 μg each. The ^{32}P-labeled polypeptides in the reaction mixtures were detected by autoradiography after SDS-PAGE.

4. Discussion

As previously reported [10], the enzymatic properties of the purified CK-II from unfertilized sea urchin eggs are similar to those reported for CK-IIs from various mammalian cells [2]. This egg CK-II was copurified with another polypeptide, p98, which functions as an effective phosphate acceptor for CK-II in the presence of either poly-Arg or salmine A_1 in vitro (Figs. 1 and 2).

Immunological results (Fig. 2B) and determination of the partial amino acid sequence of p98 revealed that it is identical to ERp99/ERp94, which belong to the Hsp-90 family. It has been reported that (i) CK-II is associated with Hsp-90; and (ii) this association prevents aggregation of CK-II and also stabilizes CK-II activity in mouse lymphoma L5178Y cells [20]. The direct interaction between α and β subunits of recombinant human CK-II (rhCK-II) and recombinant human Hsp-90 (rhHsp-90) in vitro has recently been demonstrated [21]. These two reports suggest a possibility that there is a protein–protein interaction between CK-II and ERp99/GRp94, since oligo-aspartic acid clusters at positions 313–318, 365–373 and 786–793 of the proteins [14,15] can interact with a cluster [KKKKIKR] of basic amino acid-residues at positions 72–80 of CK-IIα [2]. It seems likely that the association of CK-II with one of the Hsp-90 family of proteins may be physiologically important to recognize target proteins, e.g. steroid hormone receptors (DNA-binding transcriptional factors [22]) in transcriptional regulation [23] for their specific phosphorylation by activated CK-II. This possibility is strongly supported by our recent report [24], which shows that (i) p98 (ERp99/GRp94) is copurified with glucocorticoid hormone receptor (GR) and Hsp-70 from a 0.35 M KCl extract of mouse cells, using glycyrrhizin (GL)-affinity column chromatography (HPLC); and (ii) CK-II phosphorylates both p98 and GR in the presence of either poly-Arg or protamine 3a in vitro.

The DNA-binding sperm proteins with oligo-Arg clusters greatly stimulate CK-II catalyzed in vitro phosphorylation of

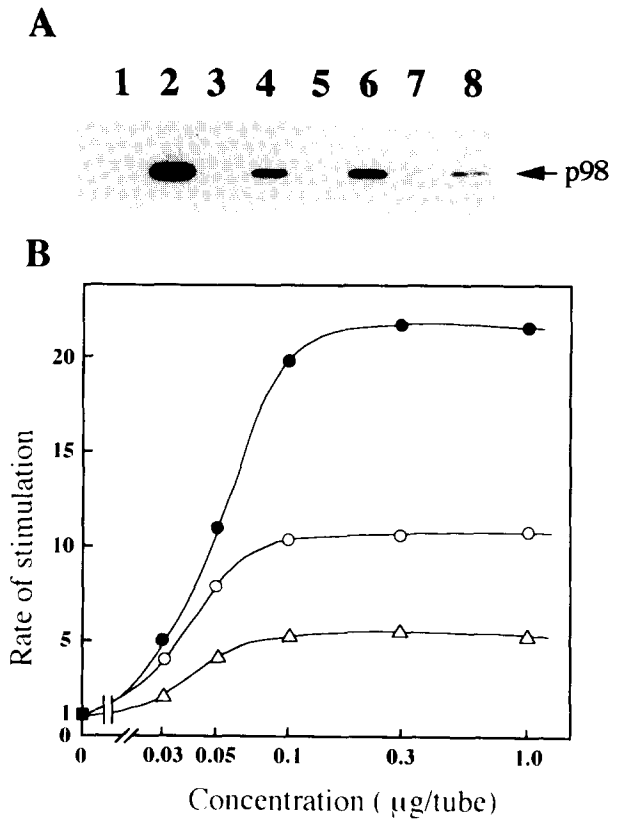

Fig. 5. (A) The stimulatory effects of various basic polypeptides, with and without an oligo-Arg cluster, on the CK-II catalyzed in vitro phosphorylation of p98. Polypeptides with and without an oligo-Arg cluster were fractionated by Mono S column chromatography (HPLC) after limited proteolysis of histone $H2B_3$ with pepsin. Both purified p98 (1 μg) and egg CK-II (approximately 50 ng) were incubated for 15 min at 30°C with 50 μM [γ-^{32}P]ATP (1,000 cpm/pmol) and 3 mM Mn^{2+} in the absence (lane 1) or presence of salmine A_1 (lane 2), penta-Arg fragment (lane 3), hexa-Arg fragment (lane 4), hexa-Lys fragment (lane 5), histone $H2B_3$ (lane 6), polypeptides without an oligo-Arg cluster prepared from histone $H2B_3$ (lane 7), or polypeptides with an oligo-Arg cluster prepared from histone $H2B_3$ (lane 8) at the presence of 0.3 μg of each polypeptide. After incubation (for 15 min at 30°C), the ^{32}P-labeled p98 in the reaction mixtures was detected directly by SDS-PAGE followed by autoradiography. (B) The dose-effects of salmine A_1, histone $H2B_3$ and hexa-Arg fragment on the CK-II catalyzed phosphorylation of p98 in vitro. The ^{32}P-labeled p98 in the reaction mixtures was detected by SDS-PAGE followed by autoradiography after incubation for 15 min at 30°C in the presence of the indicated concentrations of these three polypeptides. The autoradiogram were scanned with a spectrophotometer and the stimulation rates were estimated from the average values of three different experiments under the same experimental conditions. The stimulation rate 1 represents the phosphorylation of p98 determined in the absence of the CK-II activators. Salmine A_1 (●), histone $H2B_3$ (○) and hexa-Arg fragment (△).

p98. This is strongly supported by the following observations: (i) salmine A_1, histones $H2B_2$ and $H2B_3$ and oligo-Arg fragments composed of at least hexa-residues stimulate 22.0-, 10.5- and 5.1-fold, respectively, the CK-II catalyzed phosphorylation of p98 in vitro, but there are no effects with oligo-Lys fragments or poly-Lys (Fig. 5); and (ii) calf thymus histone H2B and other sperm histones (H1, H2A, H3 and H4), which lack oligo-Arg clusters, weakly stimulate CK-II activity (Fig. 5). Since there is a significant difference in the stimulatory effect on CK-II activity between histone $H2B_3$ and hexa-Arg fragments (Fig. 5B), we conclude that the primary sequences of the oligo-Arg cluster regions in histones $H2B_2$ and $H2B_3$ are important for exhibition of their stimulatory effects on CK-II activity. The protein–protein interaction between oligo-Arg clusters in these histones and a cluster (DLEPEDELED) of acidic amino acid residues at positions 55–64 of CK-IIβ [2] may be involved in activation of CK-II activity since their stimulatory effects are mediated by CK-IIβ [25]. However, the structural motif of the specific domains responsible for high stimulation of CK-II activity in the sperm histones and protamines remains to be elucidated.

Earlier reports have elucidated the following characteristics of DNA-binding sperm proteins (protamines) of mammals [11] and fishes [12]: (i) a high content of basic amino acids (45–80%) and the presence of plural oligo-Arg clusters; (ii) proline or alanine as the N-terminal amino acid; and (iii) the presence of at least four long oligo-Arg clusters separated by characteristic amino acid pairs (Pro–Ile, Gly–Gly, Val–Val or Val–Ser). It is further noted that there are two oligo-Arg clusters within the 12 C-terminal residues of protamine 3a and salmine A_1, which may be a specific domain interacting with the acidic amino acid-rich cluster of CK-IIβ. This possibility is supported by the following experimental results: (i) a synthetic oligo-Arg fragment composed of at least hexa-residues significantly stimulates CK-II activity (Fig. 5); and (ii) similar stimulation is observed with the conserved 12 C-terminal residues prepared from these two protamines (data not shown). In addition, the activated CK-II highly phosphorylates several cellular polypeptides, such as p70, p56, p52 and p34, in the partially purified Mono Q fraction from a 1.5 KCl extract of unfertilized sea urchin eggs (Fig. 4, lanes 3, 4, 5, 8 and 9). Determination of partial amino acid sequences of these three proteins revealed that p70, p56 and p34 had high homology with 70 kDa heat shock protein (Hsp-70), calreticulin (Ca^{2+}-binding protein) and elongation factor 2β, respectively. These observations suggest that (i) CK-II in unfertilized eggs is specifically activated by DNA-binding sperm proteins with oligo-Arg clusters, such as histones $H2B_2$ and $H2B_3$ (sea urchin) and protamines (fishes), which are brought into the egg by the sperm during fertilization; and (ii) the activated CK-II highly phosphorylates cellular polypeptides, such as p70, p56, p52 and p34.

Interestingly, a data-base analysis to identify polypeptides with oligo-Arg clusters (six or seven arginine-residues) included the following: DNA terminal protein (positions 366–371) of human adenovirus type 5 [26]; nuclear antigen 3C (positions 74–80) of Epstein-Barr virus (EBV) [27]; anti-repression transactivator protein (positions 45–51) of human immunodeficiency type 1 virus (HIV-1) [28]; and coat protein (positions 45–51) of Potato leafroll virus [29]. It is therefore possible to speculate that the protein–protein interaction between CK-IIβ and the oligo-Arg clusters of these viral polypeptides results in the specific activation of CK-II. Such activated CK-II predominantly phosphorylates regulatory mediators involved in cellular metabolic alteration and viral replication in the cells infected with adenovirus, EBV, HIV-1 and Potato leafroll virus.

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