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Note

Application of fast protein liquid chromatography for the isolation of vertebrate casein kinase-1

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The purification of casein kinase-1 from various sources has received considerable attention during the last decade due to the pleiotropic effect of this enzyme on metabolic processes[1,2]. Traditional methods of isolation include several chromatographic steps[3–5], which made the whole procedure laborious and time-consuming and reduced either the yield of the enzyme or its activity. This communication presents a simplified method for the isolation of casein kinase-1 from animal sources which uses the advantages of high-performance liquid chromatography (HPLC) of proteins.

EXPERIMENTAL

Apparatus

A fast protein liquid chromatography (FPLC) system (Pharmacia, Uppsala, Sweden) was used, consisting of two P-500 pumps, an MV-7 injection valve with 10- and 50-ml Superloops for sample injection, an LCC-500 chromatography controller, an UV-1 monitor with an HR-10 flow cell (optical path 10 mm), equipped for detection at 280 nm, a FRAC-100 fraction collector and REC-482 dual pen recorder. Pre-packed chromatography columns of Mono Q HR 5/5 (5 cm × 5 mm I.D.) and Mono S HR 5/5 (Pharmacia) were used for HPLC.

Reagents

Triethanolamine hydrochloride (TEA–HCl), phenylmethylsulphonyl fluoride (PMSF) and sodium dodecyl sulphate (SDS) were obtained from Serva, [γ-^{32}P]ATP from Isotop (Tashkent, U.S.S.R.), casein from Calbiochem, 2-mercaptoethanol and Coomassie Blue R-250 from Loba-Chemie; other salts and reagents were of analytical purity.

Protein in crude extracts and chromatographic fractions was assayed by the method of Bradford[6].

Enzyme test

Casein kinase activity was assayed in 20-μl aliquots of each fraction by the transfer of radiolabelled phosphate from ATP to dephosphorylated casein in 0.1 ml of a standard incubation mixture containing 20 m*M* TEA–HCl, pH 7.8, 100 m*M* sodium

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chloride, 5 m*M* magnesium chloride, 6 m*M* 2-mercaptoethanol, 1 m*M* EDTA, 0.1 m*M* PMSF, 10% glycerol, 0.1 m*M* [γ-^{32}P]ATP (specific activity 1000 cpm/pmol), 60 μg of casein and enzyme. Reactions were initiated by the addition of enzyme and incubated for 30 min at 34°C. Measurement of ^{32}P incorporation is described in refs. 3 and 4. One unit of enzyme activity is defined as the amount of kinase that catalyzes the transfer of 1 pmol of phosphate from ATP to casein in 1 min under the conditions described above.

Isolation of enzyme

Fresh rabbit liver and ripening oocytes of *Rana temporaria* frog were quick-frozen and stored in liquid nitrogen. Samples of 10 g were homogenized in 100 ml of buffer A containing 20 m*M* TEA–HCl, pH 7.8, 100 m*M* sodium chloride, 5 m*M* magnesium chloride, 6 m*M* 2-mercaptoethanol, 1 m*M* EDTA, 0.5 m*M* PMSF, 10% glycerol in a rotary blendor for 5 min at 1000 rpm and additionally in a Potter Elvehjem tissue grinder (20 strokes). Cell debris was removed by centrifugation at 15000 *g* for 20 min. To the crude extracts obtained, finely powdered ammonium sulphate was slowly added to 45% saturation at 4°C with constant stirring. After 1 h the precipitates formed were collected by centrifugation at 20000 *g* for 1 h and dissolved in sodium chloride-free buffer A so that the final ammonium sulphate concentration was 0.2 *M*. This solution was then centrifuged at 150000 *g* for 1 h and the precipitate was removed. The supernatants were diluted by addition of an equal volume of buffer A without sodium chloride and each was batch-adsorbed to 20 ml of phosphocellulose P_{11} equilibrated in buffer A. After 1 h the resin was collected by filtration, washed four times with 50-ml aliquots of buffer A, transferred to a column (20 × 1.5 cm) and eluted with a 100-ml linear gradient of 0.1–1.2 *M* sodium chloride in buffer A. Casein kinase-1 from both sources was eluted at 0.44–0.5 *M* sodium chloride in a volume of 20 ml. The casein kinase-I peak from phosphocellulose was collected, diluted to 0.05 *M* sodium chloride and was applied to a Mono Q HR 5/5 column, equilibrated with buffer A containing 0.05 *M* sodium chloride at a flow-rate of 1 ml/min. The enzyme was eluted as an individual peak in a volume of 5 ml by a linear gradient of sodium chloride (0.05–0.6 *M*) in buffer A at a flow-rate of 1 ml/min. The casein kinase-1 peak from Mono Q was collected and applied to a Mono S HR 5/5 column. The enzyme was eluted as an individual peak in a volume of 1–2 ml by a linear gradient of 0.1–0.6 *M* sodium chloride at the same flow-rate. The casein kinase-1 peak from Mono S was collected, diluted to 0.2 *M* sodium chloride and applied to a 0.5-ml column of Blue-Sepharose (Pharmacia), equilibrated with buffer A at a flow-rate of 3 ml/h. The column was washed with 2 ml of buffer A containing 0.2 *M* sodium chloride and the enzyme was eluted in a volume of 1 ml by buffer A containing 1.5 *M* sodium chloride and 10 m*M* ATP, then glycerol was added to 50% and the enzyme stored at −18°C without any loss of activity for at least 4 months.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli[7] in 10% gels with a Pharmacia GE-4 electrophoresis apparatus and an EPS 400/500 power supply. Gels were stained with 0.05% Coomassie Blue R-250 in 50% trichloroacetic acid.

RESULTS AND DISCUSSION

The first stage of the enzyme purification from extracts is a modification of the procedure developed for the isolation of casein kinase-1 from calf thymus[4]. Enzyme is recovered by batch adsorption to phosphocellulose from ribosome-free supernatants and is separated from casein kinase-2 by gradient elution (Fig. 1).

Further purification of casein kinase-1 was achieved by chromatography on Mono Q and Mono S. FPLC on Mono Beads ion exchangers provides the major purification steps in the procedure described (Table I). The enzyme binds quantitatively to the Mono Q at *ca.* 0.05 *M* sodium chloride and is eluted at *ca.* 0.12 *M* sodium chloride (Fig. 2a,b). The peak of casein kinase activity which elutes at *ca.* 0.38 *M* sodium chloride reflects the presence of trace amounts of casein kinase-2 in the preparation. The next step in the purification is chromatography on Mono S. Casein kinase activity is eluted as a single narrow peak at *ca.* 0.28 *M* sodium chloride coincident with the protein peak (Fig. 2c and d).

The final stage of the procedure is chromatography on Blue-Sepharose which serves to concentrate and to purify the enzyme (Table I). Both an high salt concentration and ATP are required to elute casein kinase-1 from resin.

As is seen from the present data, the chromatographic behaviour of enzymes from both sources on ion exchangers used is almost identical. The final preparations contain casein kinase-1 with a specific activity of 570–650 units/μg which is homogeneous according to SDS-PAGE (Fig. 3). The M_r values of the enzymes from oocytes and liver are 34 000 and 37 000 respectively. The total yield of the enzyme from the both sources is about 20% (Table I).

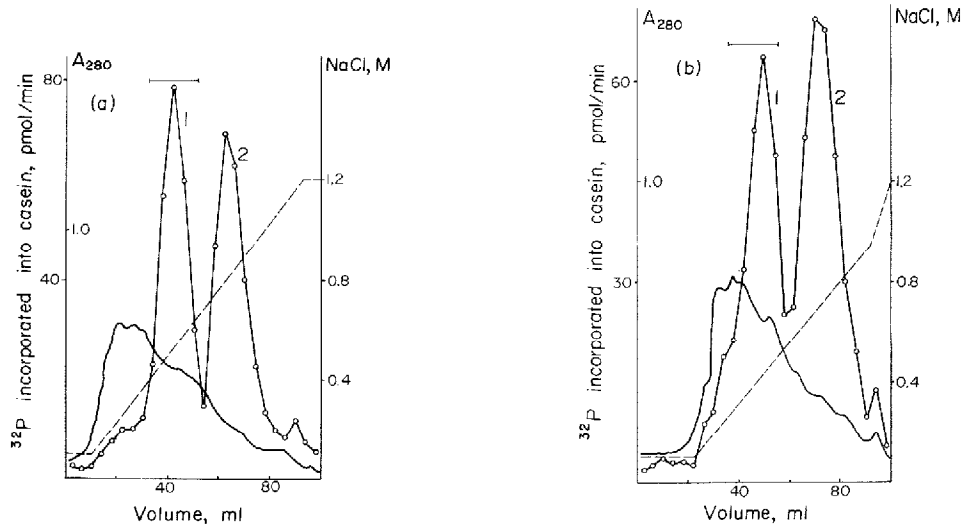

Fig. 1. Chromatography of ribosome-free extracts from rabbit liver (a) and frog oocytes (b) on phosphocellulose columns. The fractions were assayed for casein kinase activity as described in Experimental (○—○). Absorbance was monitored at 280 nm, 2.0 absorbance units full scale (a.u.f.s.). The sodium chloride concentration is indicated by the dashed line. Pooled fractions are indicated by the bar. Peaks: 1 = casein kinase-1; 2 = casein kinase-2.

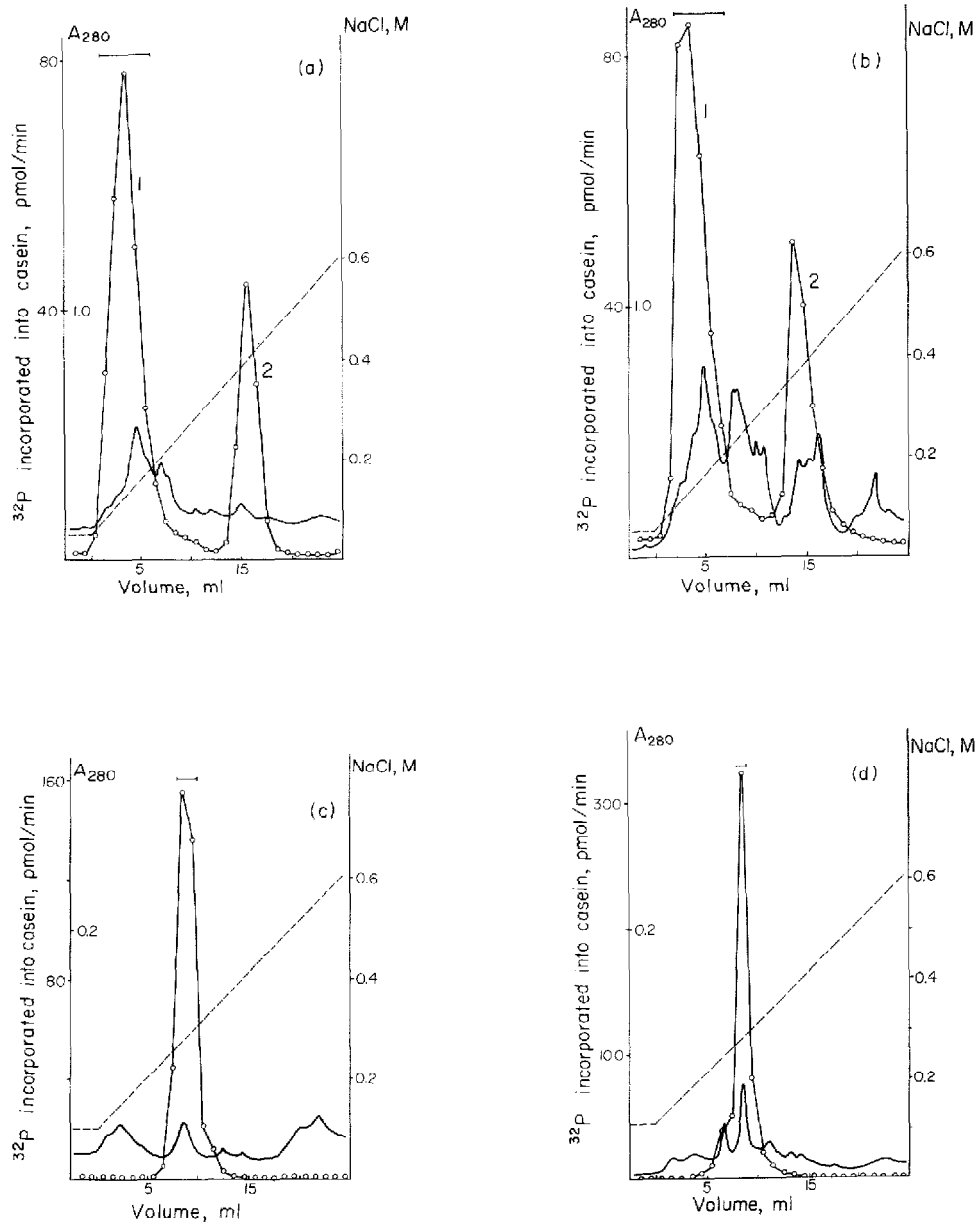

Fig. 2. Chromatography of casein kinase-1 from rabbit liver on Mono Q (a) and Mono S (c) HR 5/5 columns and casein kinase-1 from frog oocytes on Mono Q (b) and Mono S (d) HR 5/5 columns (see Experimental). The fractions were assayed for casein kinase activity as described in Experimental (○—○). The sodium chloride concentration is indicated by the dashed line. Absorbance was monitored at 280 nm, 0.5 a.u.f.s. Pooled fractions are indicated by the bar. Peaks as in Fig. 1.

TABLE I
PURIFICATION OF CASEIN KINASE-1 FROM ANIMAL EXTRACTS

Source	*Step*	*Total protein (mg)*	*Total activity (units)*	*Specific activity (units/mg)*
Rabbit liver	Ribosome-free extract	570.4	139 750[a]	240
	Phosphocellulose P_{11}	8.25	47 440	5750
	Mono Q column	0.47	14 650	31 170
	Mono S column	0.046	14 730	320 220
	Blue-Sepharose	0.021	13 650	650 000
Frog oocytes	Ribosome-free extract	427.5	150 300[a]	350
	Phosphocellulose P_{11}	13.2	36 600	2770
	Mono Q column	0.63	17 250	27 380
	Mono S column	0.055	16 930	307 820
	Blue-Sepharose	0.027	15 340	568 150

[a] This value represents a summary activity of mixed casein kinases in the extract.

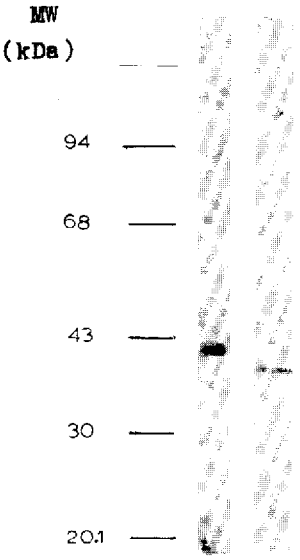

Fig. 3. SDS-PAGE of casein kinase-1 from rabbit liver (left), 0.5 μg, and from frog oocytes (right), 0.4 μg.

CONCLUSION

The procedure developed uses the advantages of Pharmacia Mono Beads ion exchangers Mono Q, Mono S and the FPLC system and takes two working days to obtain highly active homogeneous casein kinase-I in good yield starting from animal tissue. Purification of the enzyme, according to the conventional procedure of Dahmus[4], in my hands resulted in half the yield and takes 4 days to obtain pure enzyme. Analytical scale Mono Q and Mono S HR 5/5 columns gave good results also

on a semi-preparative scale and in combination with phosphocellulose and Blue-Sepharose are suitable for the purification of hundreds of micrograms of casein kinase-1 from animal tissues.

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