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A simple procedure for the purification of rat liver phosvitin kinase

Rat liver cytosol displays protein kinase activity toward both phosvitin and protamine. However, different enzymes are responsible for these two activities[1]: "phosvitin kinase" phosphorylates casein as well as phosvitin, but not protamine nor histones; while "protamine kinase", on the contrary, phosphorylates protamines and histones but not casein and phosvitin.

While the preparation of protamine kinase free of phosvitin kinase activity has been recently reported[1,2], no procedure has been described, up to now, to purify phosvitin kinase from any protamine kinase activity.

The present note describes a simple procedure for the isolation of purified phosvitin kinase completely free of protamine kinase from rat liver cytosol.

As can be seen in Table I, the partially purified preparations of rat liver phosvitin kinase obtained by the procedure commonly employed (precipitation at pH 4.7 followed by $(NH_4)_2SO_4$ fractionation)[3–5] exhibit a considerable protamine kinase activity which can not be completely removed even by subsequent DEAE-cellulose column chromatography.

TABLE I

COMPARISON BETWEEN PHOSVITIN KINASE AND PROTAMINE KINASE ACTIVITIES IN CRUDE CYTOSOL AND PARTIALLY PURIFIED PREPARATIONS OF PHOSVITIN KINASE

Crude cytosol was prepared by centrifuging the 0.25 M sucrose homogenate of rat liver for 60 min at 105 000 × *g* after nuclei and mitochondria had been previously discarded by differential centrifugations[6]. The purification of phosvitin kinase up to the $(NH_4)_2SO_4$ step was carried out following the procedure described by RODNIGHT AND LAVIN[4], with only one slight modification concerning the $(NH_4)_2SO_4$ concentration used to precipitate the enzyme, which was 0.55 instead of 0.50 saturation. The preparation of phosvitin kinase obtained by $(NH_4)_2SO_4$ precipitation was further purified by ion-exchange chromatography on DEAE-cellulose column (2 cm × 26 cm) equilibrated with 0.1 M Tris–HCl buffer (pH 9.0) and eluted with increasing concentrations of Tris (0.5, 1.0, 1.5 M) at the same pH. The whole phosvitin kinase activity was recovered in the fractions eluted with 1.5 M Tris. Phosvitin kinase was tested following the procedure already described[5], as ^{32}P transferred from [^{32}P]ATP to phosvitin by the crude cytosol and the partially purified preparations previously dialyzed against 0.05 M Tris–HCl (pH 7.0) containing 0.1 mM EDTA. Protamine kinase was assayed by adding 1 mg protamine (Salmine from British Drug Houses), instead of phosvitin, to the incubation medium. 0.25 ml of silicotungstic acid solution[7] was also added as well as trichloroacetic acid as precipitating agent at the end of incubation, since the protamine was not found to be completely precipitated by 10% trichloroacetic acid.

Purification step	*Protein (mg)*	*Enzyme activity*				*Ratio of phosvitin kinase to protamine kinase activity*
		Phosvitin kinase		*Protamine kinase*		
		*Counts/min in phosphoprotein**	*Purification factor*	*Counts/min in phosphoprotein**	*Purification factor*	
1. Crude cytosol	1577	802		1 304		0.615
2. pH 4.7 precipitation	300	2 824	3.5	2 686	2.05	1.051
3. $(NH_4)_2SO_4$ precipitation	71.95	9 633	12	5 381	4.12	1.79
4. DEAE-cellulose chromatography	1.55	384 245	479	76 849	59	5.00

* Counts/min as ^{32}P transferred to phosphoprotein per mg of enzyme protein per min.

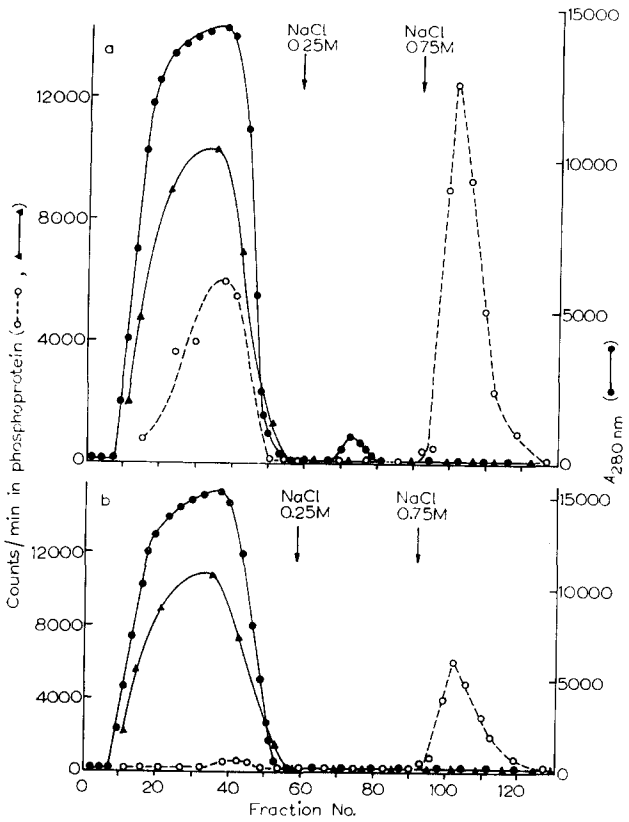

Fig. 1. Isolation of phosvitin kinase free of protamine kinase, from rat liver cytosol by P-cellulose column chromatography. 240 ml of crude cytosol prepared as described in Table I, containing 3.12 g protein, were dialyzed against several changes of 0.05 M Tris–HCl (pH 7.5) and filtered through a P-cellulose column (4.5 cm × 15 cm) equilibrated with the same buffer. The column was eluted with the same buffer containing increasing concentrations of NaCl, as indicated by the arrows. Phosvitin kinase and protamine kinase were tested directly in the single tubes by the procedures mentioned in Table I. ●—●, absorbance at 280 nm; ○—○, phosvitin kinase activity; ▲—▲, protamine kinase activity.

After many trials it has been found that a complete separation between the two protein kinases could be accomplished by simply filtering the crude cytosol through a phosphorylated cellulose (P-cellulose) column equilibrated with 0.05 M Tris–HCl buffer (pH 7.5) (Fig. 1a). The bulk of the proteins containing the whole protamine kinase activity are not retained on the column, while most of the phosvitin kinase binds to the resin and can be subsequently eluted by 0.75 M NaCl. It can be seen in Fig. 1a that detectable amounts of phosvitin kinase activity are still associated with the large protein fraction not retained by the resin: however, such a residual phosvitin kinase activity, unlike protamine kinase, can be easily removed from the bulk of the proteins upon rechromatography on P-cellulose under the same conditions (see Fig. 1b).

By the simple procedure just described it is possible to obtain from crude

cytosol, without any intermediate step, and with a yield near 100%, a phosvitin kinase purified more than one thousand times.

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