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MULTIPLE FORMS OF PHOSVITIN KINASE FROM RAT LIVER CYTOSOL

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SUMMARY

Rat liver cytosol phosvitin kinase can exist in more than one active form, distinguishable by phosphorylated cellulose chromatography and Sephadex filtration

It is probable that they consist of different types of aggregates dissociable in solutions of high ionic strength

INTRODUCTION

In a previous paper a simple procedure was described for the purification from rat liver cytosol of phosvitin kinase which is completely free of protamine kinase[1]

The present paper shows that phosvitin kinase from rat liver cytosol can exist in more than one active form, distinguishable by means of *P*-cellulose chromatography and Sephadex filtration

MATERIALS AND METHODS

Purification of phosvitin kinase free of protamine kinase

The crude cytosol was prepared by centrifuging for 60 min at 105 000 × *g* the 25 000 × *g* post-mitochondrial supernatant of a 0.25 M sucrose homogenate of rat liver cells[2] The cytosol preparation (about 500 ml), containing about 6.50 g protein, was dialyzed against several changes of 0.05 M Tris–HCl buffer (pH 7.5) It was then subjected to chromatography on a phosphorylated cellulose (*P*-cellulose) column (4.5 cm × 15 cm) previously equilibrated with the same buffer used for the dialysis

The column was eluted with the same buffer containing increasing concentrations of NaCl, as indicated in the figures (continuous or stepwise gradient) The flow rate was 1.5 ml/min and 9-ml fractions were collected Phosvitin kinase activity was assayed in the single tubes as described later

Sephadex filtration

Each *P*-cellulose fraction, concentrated by Diaflo-membrane ultrafiltration, was dialyzed overnight against 0.05 M Tris–HCl buffer (pH 7.5) containing the required NaCl concentration (0.1 or 0.5 M NaCl) as indicated in the single experiment It was then submitted to molecular filtration on a Sephadex G-200 column (1.8 cm × 130 cm) previously equilibrated with the same buffered solution used in the previous dialysis Flow rates were about 4.5 ml/h, and 1.8-ml fractions were collected

Phosvitin kinase determination

Phosvitin kinase was determined by incubating the enzyme sample in 1 ml of a medium containing 1 mg of phosvitin, 6 μmoles $MgCl_2$, 200 μmoles of Tris buffer (pH 7 5) and 0 25 μmole of ATP containing 1–2 μC as [γ-^{32}P]ATP

The reaction was started by addition of the enzyme sample After 10 min, at 37°, the incubation was stopped by adding 0 3 ml 50% trichloroacetic acid and the precipitate washed 4 times with 5 ml of 10% trichloroacetic acid

Finally the precipitate was transferred to a stainless steel planchet and counted with a thin-window Geiger counter

[γ-^{32}P]ATP was prepared by the method of GLYNN AND CHAPPEL[3] Phosvitin was prepared from fresh egg yolk according the procedure of MECHAM AND OLCOTT[4]

RESULTS AND DISCUSSION

Fig 1 shows the profile obtained when crude rat liver cytosol was submitted to chromatography on a *P*-cellulose column, equilibrated with 0 05 M Tris–HCl buffer (pH 7 5), and eluted with a continuous linear gradient of NaCl

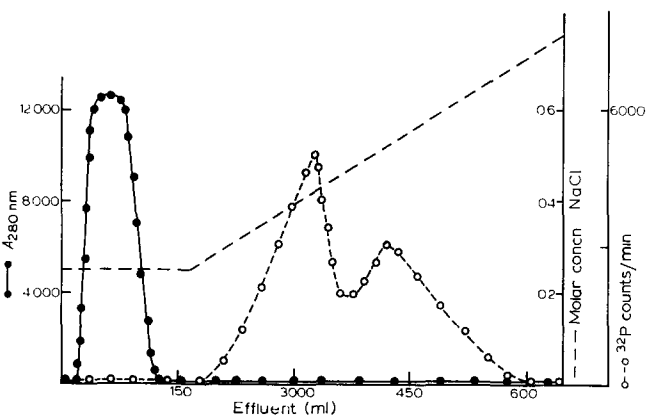

Fig 1 Chromatography profile for cytosol rat liver phosvitin kinase on *P*-cellulose column equilibrated with 0 05 M Tris–HCl buffer (pH 7 5) and eluted with 150 ml of the same buffer containing 0 25 M NaCl followed by a linear gradient ranging from 0 25 to 0 75 M NaCl (mixing chamber 0 25 M NaCl and reservoir 0 75 M NaCl, 250 ml each) ●—●, absorbance at 280 nm, ○---○, phosvitin kinase activity, expressed as ^{32}P transferred from [^{32}P]ATP to phosvitin

It can be seen that while the bulk of the cytosol proteins was not retained by the column, the phosvitin kinase activity was eluted at comparatively high ionic strength and resolved into two peaks The peaks were better separated when the ionic strength of the eluting buffer was increased in a stepwise manner (Fig 2)*

The two *P*-cellulose fractions, when submitted separately to molecular filtration on Sephadex G-200 at low ionic strength (0 1 M NaCl), exhibited different elution patterns (Fig 3a)

* Two peaks of phosvitin kinase activity were obtained also when the cytosol was chromatographed on DEAE cellulose column equilibrated with 0 05 M Tris buffer (pH 9 0) and eluted by increasing stepwise the concentration of the same buffer

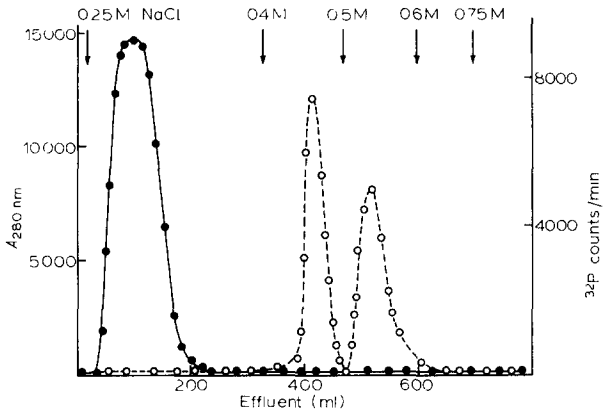

Fig 2 Chromatography profile for cytosol rat liver phosvitin kinase on *P*-cellulose column equilibrated with 0.05 M Tris–HCl buffer (pH 7.5) and eluted stepwise with the same buffer containing increasing concentrations of NaCl, as indicated by the arrows ●—●, absorbance at 280 nm This absorbance was not detectable in the single tubes of the peak A and B because of too low protein concentration The absorbance was detectable only when the two protein fractions were separately collected and concentrated by Diaflo-membrane ultrafiltration, ○---○, phosvitin kinase activity, expressed as ^{32}P transferred from [^{32}P]ATP to phosvitin The specific activity of the form A (5.9 mg protein) and B (1.5 mg protein) was about 927 and 3100 counts/min per μg protein, respectively

These different patterns indicate that the two enzyme fractions eluted from *P*-cellulose are not homogeneous However, when the two *P*-cellulose fractions were submitted separately or together to molecular filtration on Sephadex G-200 at high ionic strength (0.5 M NaCl), a single enzyme peak (Fig 3b), more retarded than those obtained at low ionic strength (Fig 3a), was obtained The enzyme fraction of this peak exhibited a specific activity much higher than that of the form A and B of Fig 2, thus indicating that during the Sephadex filtration at high ionic strength some other protein fractions devoid of phosvitin kinase activity were removed

These results suggest that the enzyme may exist in different forms depending on the ionic strength conditions The two enzyme forms separated by *P*-cellulose chromatography (Fig 2) may consist of two different types of aggregates of an enzyme protein (Fig 3b) with some other cytosol component (possibly proteins)

The aggregates differ in electrical charge and molecular weight and are dissociated by high ionic strength, thus giving rise to the enzyme form eluted as a single peak from Sephadex G-200 (Fig 3b) The enzyme form present in this Sephadex peak, when submitted to *P*-cellulose chromatography under the same conditions as in Fig 2, can be eluted with 0.7 M NaCl but not with lower NaCl concentrations as those used in the experiment of Fig 2

Moreover when the enzyme form eluted as a single peak from Sephadex with 0.5 M NaCl (see Fig 3b) is submitted again to molecular filtration on the same Sephadex column at low ionic strength (0.1 M NaCl), it is converted into some aggregate forms showing an elution pattern (Fig 3c), different from each one of those obtained by submitting directly the *P*-cellulose fractions (Fig 2) to Sephadex filtration at the same low ionic strength (compare Fig 3c with Fig 3a)

As appears from a comparison of the areas of peaks of Fig 3b and Fig 3c,

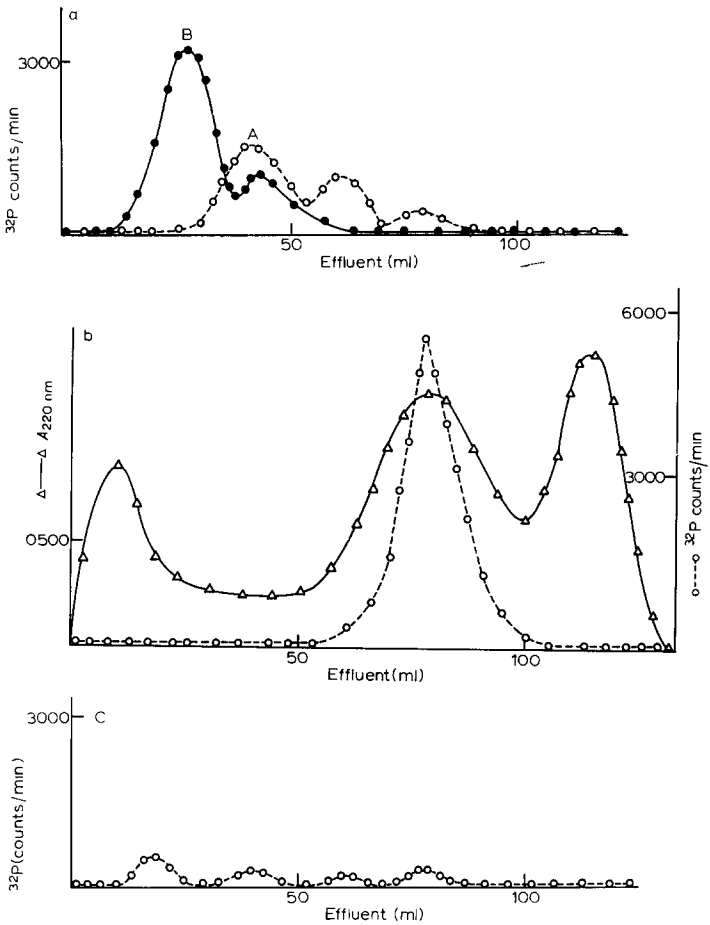

Fig 3 Molecular filtration on Sephadex G-200 column (1 8 cm × 130 cm) a Profiles obtained at low ionic strength (0 1 M NaCl) from the two *P*-cellulose fractions, previously dialyzed as described in MATERIALS AND METHODS (Curve A from 0 4 M NaCl peak, Curve B from 0 5 M NaCl peak) b profile obtained at high ionic strength (0 5 M NaCl) from a mixture of the two *P*-cellulose fractions (4 6 mg protein) The eluted enzyme form (1 1 mg of protein) exhibited a specific activity much higher than that of the initial mixture subjected to Sephadex filtration ○---○, phosvitin kinase activity, △—△, absorbance at 220 nm c Profile obtained from refiltration at low ionic strength (0 1 M NaCl) of the peak eluted from Sephadex at high ionic strength in the above experiment

aggregation is accompanied by a decrease of enzyme activity The loss of activity is dependent on the lowering of the ionic strength of the medium and not on the loss of some enzyme component during the last Sephadex filtration at low ionic strength Indeed the loss of activity does take place also when the enzyme is merely diluted with water to 0 1 M NaCl, while it does not take place if the dilution is performed at constant ionic strength, *i e* by using 0 5 M NaCl or 0 5 M potassium acetate Once the inactivation has occurred, it cannot be reversed by increasing the ionic strength of the medium

The crude enzyme preparation (original cytosol or the enzyme only partially

purified by $(NH_4)_2SO_4$ fractionation) once dissolved in 0 5 M NaCl does not undergo any inactivation by dilution to 0 1 M NaCl

This strengthens the idea that in the crude state the enzyme is bound to some other cytoplasm component (likely proteins) which could prevent such inactivation of the enzyme occurring when it is in a purified state

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