PARTIAL PURIFICATION OF PHOSPHOLIPID EXCHANGE PROTEIN FROM BEEF HEART

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1. Introduction

The 105,000 *g* supernatant fraction of a 10% rat liver homogenate stimulates the exchange of phospholipids between mitochondria and microsomes [1–4], mitochondria and serum lipoproteins [2] and microsomes and serum lipoproteins.*** It was shown, that the stimulatory effect of the supernatant fraction was due to proteins [2]. Furthermore the exchange of phosphatidyl choline was stimulated to a greater extent than the exchange of phosphatidyl ethanolamine.

In the present paper preliminary work on the isolation of a phospholipid exchange protein from beef heart is reported. The partially purified fraction shows an 80 fold increase in specific activity. It accelerates the exchange of phosphatidyl choline and phosphatidyl inositol but has no effect on phosphatidyl ethanolamine.

2. Methods

The mitochondria and microsomes used in the assay of phospholipid exchange activity in the different protein fractions were prepared from rat liver as described previously [2]. Assay system: Phospholipid-^{32}P-labeled mitochondria (12.5 mg protein), unlabeled microsomes (5 mg protein) and variable amounts (0.5–20 mg) of the beef heart protein fractions were present in a total volume of 4 ml 0.25 M sucrose-1 mM EDTA (pH 7.4). Incubation was started by addition of the labeled mitochondria as the last component and was carried out for 20 min at 37°C. At the end of incubation the mitochondria were sedimented at 15000 *g* for 20 min in the high speed attachment of the International centrifuge (PR-2). The phospholipids of the mitochondrial supernatant were extracted with chloroform: methanol (2:1, v/v) and the phospholipid specific radioactivity was determined as described previously [2]. Controls without the beef heart protein fraction were carried through the whole procedure. The phospholipid specific radioactivity in the control incubations substracted from the specific radioactivity obtained after incubation in the presence of the beef heart protein fractions is designated as Δ specific radioactivity. The Δ specific radioactivity varied linearly in the range of protein concentrations assayed; it reflects the phospholipid-^{32}P transfer from mitochondria to microsomes due to the presence of the active protein. The Δ specific radioactivity per mg protein is the specific activity of the exchange protein (see table 1).

3. Purification

All manipulations during the isolation were performed at 4°C.

Step 1. The pH of a post-mitochondrial supernatant [5]† of a 25% beef heart homogenate was adjusted to

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*** Unpublished observations by B.Wallin.

† The mitochondrial supernatant was kindly provided by Dr. E.Racker.

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Table 1
Summary of purification procedures for beef heart phospholipid exchange protein.

For assay system see Methods.

Step	Volume (ml)	Protein (mg)	Specific activity*	Recovery** (%)	Purification factor***
1 pH Adjustment	4000	48898	1.6	100	–
2 First $(NH_4)_2SO_4$ fractionation	555	17760	4.3	96	3
3 Hydroxylapatite	88	2996	10.8	41	7
4 Sephadex G-100	856	488	83.3	51	51
5 Second $(NH_4)_2SO_4$ fractionation	12	213	133.3	36	82

* Δ specific radioactivity/mg protein added.
** Recovery is expressed as specific activity X mg of protein relative to step 1. The increase in recovery at step 4 was repeatable.
*** Purification factors are minimum values because they are based on the pH 5.1 supernatant instead of on the cytosol fraction. The advantage of the pH 5.1 adjustment was that it conveniently removed microsomes and soluble lipoproteins that would interfere with the assay of transferase activity. Adjustment to pH 5.1 removed 23% of the cytosol proteins.

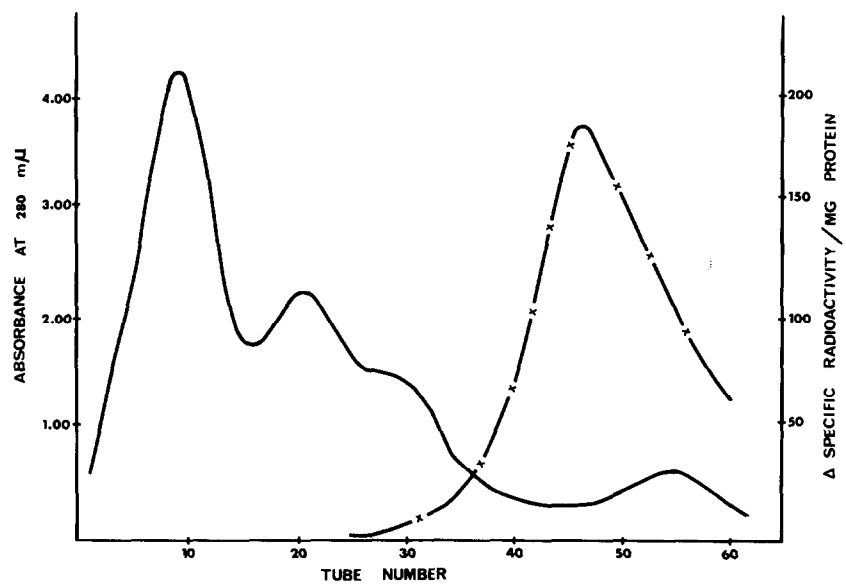

Fig. 1. 35 ml (20 mg protein/ml) of the active material of step 3 were applied to a Sephadex G-100 column (63 X 5 cm). Protein was eluted with 0.1 M phosphate (pH 7.2) at a flow rate of 60 ml/hr. 20 ml fractions were collected. The elution pattern was determined by measuring the absorbance at 280 mμ (——, scale at the left). Phospholipid exchange protein activity in the eluted protein fractions was assayed (see Methods, except that incubations were 40 min). x—x—x Δ specific radioactivity per mg protein, expressed in arbitrary units (scale at the right).

5.1 with 1 N HCl. The preparation was centrifuged at 15000 *g* for 10 min. The precipitate was discarded.

Step 2. The protein fraction that precipitated from the supernatant of step 1 between 35% and 70% saturation after addition of solid ammonium sulfate [6] was dissolved in a minimal amount of distilled water. The solution was dialyzed against 20 volumes of distilled water twice, each time for about 12 hours. The precipitate, formed during the dialysis, was removed by centrifugation.

Step 3. To each 100 ml of the supernatant of step 2 was added 100 ml of a hydroxylapatite (Bio Gel HTP, Bio Rad Laboratories) suspension in 10 mM phosphate buffer (pH 7.2) such that 3 g hydroxylapatite per g of protein were present in the final suspension. The hydroxylapatite, on which the active protein was adsorbed, was washed twice by resuspending it in 100 ml of the phosphate buffer. The active protein was eluted from the hydroxylapatite in 50 ml of 0.3 M phosphate buffer (pH 7.2). This elution process was repeated once. The active protein was precipitated from the combined supernatants with solid ammonium sulfate (70% saturation). The sedimented material was dissolved in a minimal volume of 0.1 M phosphate buffer (pH 7.2).

Step 4. Aliquots of the dissolved material of step 3 were applied to a Sephadex G-100 column (Pharmacia Fine Chemicals). The protein fractions were eluted from this column with 0.1 M phosphate (pH 7.2) and tested for phospholipid exchange activity. The eluates in tubes No. 35–58 (fig. 1) were combined.

Step 5. The protein fraction that precipitated from the combined eluates of step 4 between 51% and 68% saturation after addition of solid ammonium sulfate was dissolved in a small volume of 0.1 M phosphate (pH 7.2).

4. Results and discussion

The protein fractions which were isolated in the different steps of the purification procedure were tested for phospholipid exchange activity. The progress of the purification is summarized in table 1. At the end of the second ammonium sulfate fractionation the purification factor was 82 and the recovery of activity amounted to 36%.

Tests with liver mitochondria and microsomes shows that a crude rat liver pH 5.1 supernatant fraction accelerated the exchange of phosphatidyl choline, phosphatidyl inositol and phosphatidyl ethanolamine although the exchange of the latter phospholipid was considerably slower than that of the former two. The partially purified beef heart protein fraction stimulated only the exchange of phosphatidyl choline and phosphatidyl inositol. This suggests specificity of action for different tissues or animal species. It is also possible that separate proteins may accelerate the exchange of different classes of phospholipid.

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