Biochimica et Biophysica Acta, 318 (1973) 313–325

BBA 76427

SOME PROPERTIES OF PHOSPHATIDYLCHOLINE EXCHANGE PROTEIN PURIFIED FROM BEEF LIVER

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(Received March 29th, 1973)

SUMMARY

A phospholipid exchange protein has been purified 2680-fold from beef liver. The assay of the exchange activity of the protein was based on the transfer of [^{14}C]-phosphatidylcholine from microsomes labeled with [^{14}C]phosphatidylcholine to liposomes. The homogeneity of the protein has been established by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, immunoelectrophoresis and isoelectric focusing. The protein has a molecular weight of approximately 22000 and an isoelectric point of 5.8. The amino acid composition has been determined. The protein contains one disulfide bridge and has glutamic acid as the N-terminal amino acid. Phospholipid, tentatively identified as phosphatidylcholine, was found to be present in the protein preparation. The protein stimulated specifically the exchange of phosphatidylcholine between mitochondria and microsomes from rat liver.

INTRODUCTION

In a previous study[1] we reported the isolation of a phospholipid exchange protein from beef liver which specifically stimulated the exchange of phosphatidylcholine. The specificity of the protein was apparent in the phospholipid exchange between mitochondria and microsomes from rat liver as well as between these membranes and liposomes. Recently Ehnholm and Zilversmit[2] reported the isolation of two phospholipid exchange protein fractions from beef heart. Both fractions stimulated the exchange of phosphatidylcholine and, to a lesser extent, sphingomyelin between antigen-sensitized and nonsensitized liposomes. Apparently the phosphatidylcholine exchange protein is widely distributed; its activity has been detected, for example, in beef liver[1] and heart[2,3], in rat liver, heart, kidney and brain[4], and in guinea pig brain[5].

The purification described in the present study has been substantially modified to give a better yield of phosphatidylcholine exchange protein of a higher specific activity. Moreover, the assay of exchange activity has been simplified. Some of the chemical properties of the phosphatidylcholine exchange protein such as amino acid composition and lipid content, are presented.

MATERIALS AND METHODS

Preparation of membrane fractions

Microsomes from rat liver labeled with [^{14}C]phosphatidylcholine, and liposomes were prepared as described previously[1]. Liposomes consisted of phosphatidylcholine (98 mole%), phosphatidic acid (2 mole%) and a trace of [7α-^{3}H]cholesteryloleate (0.01% by weight). Phosphatidylcholine was isolated from egg yolks according to the method of Papahadjopoulos and Miller[6]. Phosphatidic acid was prepared from egg yolk phosphatidylcholine by degradation with phospholipase D extracted from Savoy cabbage[7]. [7α-^{3}H]Cholesteryloleate (150 μCi/mg) was purchased from New England Nuclear. The protein content of the membrane fractions was determined by the method of Lowry *et al.*[8].

Assay

Exchange activity was determined by measuring the transfer of [^{14}C]phosphatidylcholine from microsomes labeled with [^{14}C]phosphatidylcholine to liposomes which contained a trace of [^{3}H]cholesteryloleate. The [^{3}H]cholesterol ester, which does not redistribute between liposomes and microsomes under the conditions of incubation, serves as an internal standard of liposomal phospholipid.

^{14}C-Labeled microsomes (2.5 mg protein) were incubated with ^{3}H-labeled liposomes (1 μmole phospholipid-P) and the beef liver protein fractions in a total volume of 2.5 ml 0.25 M sucrose–0.001 M EDTA–0.01 M Tris (pH 7.4). Incubation was carried out for 15 min at 25 °C. At the end of the incubation the pH of the medium was quickly adjusted to 5.1 by addition of 0.5 ml 0.2 M sodium acetate–acetic acid (pH 5.0) in 0.25 M sucrose. Adjustment of pH caused an aggregation of microsomes. Microsomes were sedimented at 9000 rev./min for 10 min in the SS 34 rotor of the Sorvall centrifuge in Sorvall tubes (No. 250) with adapters. The supernatant containing the liposomes was collected and the lipids were extracted according to Bligh and Dyer[9].

The extract was concentrated to dryness *in vacuo*, dissolved in 0.3 ml methanol and mixed with 15 ml of toluene (0.5% PPO, 0.03% POPOP). Radioactivity was measured with a Packard Tricarb liquid scintillation spectrometer and corrected to 100% relative efficiency by external standard. The ^{14}C/^{3}H ratio of the liposomal lipid extract was calculated. Controls without beef liver protein were carried through the entire procedure. The ^{14}C/^{3}H ratio corrected for the control reflects the transfer of [^{14}C]phosphatidylcholine from microsomes to liposomes in the presence of phosphatidylcholine exchange protein. The ^{3}H radioactivity recovered in the lipid extract is an index for liposomal phospholipid remaining in the supernatant after precipitation of the microsomes. In general 70–80% of the liposomal phospholipid was recovered. Loss of liposomal phospholipid was due to coprecipitation with microsomes.

The purification factor of a particular phosphatidylcholine exchange protein fraction was calculated from a ^{14}C/^{3}H standard curve. This ^{14}C/^{3}H curve was routinely determined for the exchange activity present in the pH 5.1 supernatant (starting material in the purification). Fig. 1 shows the ^{14}C/^{3}H ratio as a function of the amount of pH 5.1 supernatant protein at different times of incubation.

A unit of phosphatidylcholine exchange protein activity is defined as the amount of protein which stimulates the transfer of 1 nmole phosphatidylcholine per min

from microsome to liposome at 25 °C. Units of activity have been determined as described before[1].

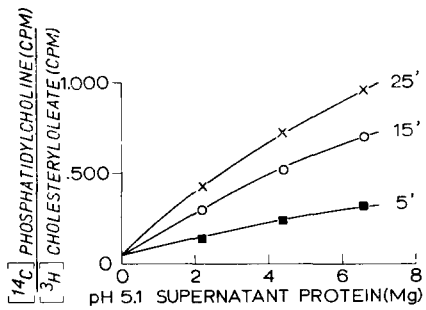


Fig. 1. Transfer of [^{14}C]phosphatidylcholine from microsomes labeled with [^{14}C]phosphatidylcholine to liposomes labeled with [^{3}H]cholesteryloleate as a function of the amount of pH 5.1 supernatant protein and time of incubation. For incubation conditions, see assay in Materials and Methods. ■—■, ○—○, ×—×; 5, 15 and 25 min of incubation, respectively.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

Electrophoresis was performed according to the method of Weber and Osborn[10]. Molecular weight was calculated from molecular weight *versus* migration plots constructed with proteins of known molecular weight. The proteins used were bovine serum albumin (Armour Pharmaceutical Company, England), pepsin (Serva, Germany), trypsin (Fluka, Switzerland), and sperm whale myoglobin (Mann Laboratories).

Isoelectric focusing

A LKB 8101 electrofocusing column and LKB ampholine solutions (LKB Productor AB, Stockholm) were used according to Vesterberg and Svensson[11]. Electrofocusing was carried out with a pH gradient from 5 to 8 during 48 h at 5 °C with a final voltage of 600 V.

Immunological studies

Antibodies against phosphatidylcholine exchange protein were induced in rabbits. About 1 mg of purified protein in 1 ml of 0.9% NaCl mixed with an equal volume of Freunds adjuvant was injected in the foot pads of male albino rabbits. After 3 weeks another 0.5 mg of protein in the absence of Freund's adjuvant was injected subcutaneously. The animals were bled two weeks after the last injection and the antisera were prepared. Antigen–antibody reaction was tested by immunoelectrophoresis in 1% agar, at pH 8.6 [12].

Amino acid analysis

Protein was extensively dialysed against distilled water, lyophilized and then hydrolysed in 6 M HCl *in vacuo* at 110 °C for 24, 48 and 72 h. Analyses were carried out on a Beckmann Unichrom amino acid analyzer. The amino acid composition was calculated by taking the average of the amino acid composition of the 24-, 48- and 72-h hydrolysates. Loss of serine due to hydrolysis was taken into consideration by extrapolation to zero time. Tryptophan was determined after hydrolysis in 6 M HCl

in the presence of thioglycolic acid for 24 h according to the method of Gruen and Nicholis[13]. The sulfhydryl content was determined with Ellman's reagent in the presence of sodium dodecyl sulfate as described by Habeeb[14]. Disulfide bridges were analyzed for after reduction with sodium borohydride following the method of Cavallini *et al.*[15].

Amino-terminal analysis

The N-terminal amino acid was characterized by dansylation as described by Gros and Labouesse[16]. The dansylated amino acids were detected after chromatography on polyamide layers (Schleicher and Schüll, Germany) according to the method of Woods *et al.*[17]. Reference dansyl amino acids were purchased from Sigma (U.S.A.). The N-terminal amino acid was determined also by Edman degradation[18]. The phenylthiohydantoin amino acids were separated by chromatography on polyamide sheets using the method of Wang *et al.*[19]. Spots were detected by immersion of the sheets in 0.5% starch, followed by spraying with a 0.01 M iodine solution. The iodine was dissolved in 0.5 M potassium iodide–0.5 M sodium azide.

Protein determination

Protein was routinely determined by the method of Lowry *et al.*[8] with bovine serum albumin as standard. Protein content of fractions containing pure protein was also determined by measurement of the absorbance difference between 215 and 225 nm[20].

Lipid determination

Lipids were extracted from lyophilized protein according to the method of Folch *et al.*[21]. Total phospholipid phosphorus was determined by the method of Chen *et al.*[22] after ashing of the sample according to the procedure of Ames and Dubin[23]. Phospholipids were separated on silica gel H plates with a solvent system consisting of chloroform–methanol–acetic acid–water (25:15:4:2, by vol). Egg yolk phosphatidylcholine and a total lipid extract of rat liver were used as references. Lipid-containing spots were detected after spraying with 20% sulfuric acid or Dragendorff's reagent.

Determination of exchange specificity

In order to determine which class of phospholipids was stimulated by a particular beef liver protein fraction in the phospholipid exchange, the protein fraction was incubated with ^{32}P-labeled microsomes and unlabeled mitochondria as described before[1].

RESULTS

Purification of phosphatidylcholine exchange protein

All manipulations during the isolation were performed at temperatures between 0–6 °C. The different steps of the purification are summarized in Table I.

Step 1. Fresh beef liver (10000 g) obtained from the local slaughterhouse was cut into pieces and washed with 10 l of 0.25 M sucrose. A 30% homogenate in 0.25 M sucrose was prepared with a Waring Blendor (1 min at high speed). Nuclei and cell

TABLE I

PURIFICATION OF PHOSPHATIDYLCHOLINE EXCHANGE PROTEIN FROM BEEF LIVER

Step	*Volume (ml)*	*Protein (mg)*	*Specific activity**	*Recovery (%)*	*Purification factor***
1. First pH adjustment	16 700	407 000	0.5	100	—
2. Second pH adjustment	14 800	131 000	2	135	4
3. $(NH_4)_2SO_4$ precipitation	2 590	66 000	3	104	6
4. DEAE-cellulose	5 600	4 600	28	63	56
5. CM-cellulose	340	235	430	50	860
6. Sephadex G-50	670	39	1340	26	2680

* Specific activity is expressed as units of phosphatidylcholine exchange protein activity per mg protein.

** Purification factors are based on the activity of the original pH 5.1 supernatant.

debris were removed by centrifugation in an International PR-6 centrifuge (15 min at 600 $\times g$). The supernatant was centrifuged in the GSA rotor of a Sorvall centrifuge to sediment mitochondria and lysosomes (20 min at 15000 $\times g$). The pH of the supernatant was adjusted to 5.1 with 3 M HCl. Within 2 h after pH adjustment the precipitate was sedimented by centrifugation in the Sorvall centrifuge (15 min at 15000 $\times g$) and discarded.

Step 2. The pH of the resulting supernatant was adjusted to 3 with 3 M HCl. Next the solution was dialysed overnight against an equal volume of 0.25 M sucrose–0.05 M Tris (pH 10.5). As a result the pH of the dialysate increased to about 6.5 while a heavy precipitate formed. The precipitate was sedimented by centrifugation in the Sorvall centrifuge (10 min at 15000 $\times g$) and discarded. This treatment resulted in an increase of the exchange activity since 135% of the activity was recovered in the supernatant. The purification was 4-fold.

Step 3. Solid $(NH_4)_2SO_4$ (45 g/100 ml) was added to precipitate the active protein. The precipitate was dissolved in 0.01 M sodium phosphate–0.01 M β-mercaptoethanol (pH 7.2) and dialysed against the same buffer. Yield of activity was 104% while a 6-fold purification was obtained.

Step 4. The dialysate was applied to a DEAE-cellulose column (Whatman DE-52 microgranular). Elution was begun with 0.01 M sodium phosphate–0.01 M β-mercaptoethanol (pH 7.2). The ionic strength of the buffer was increased stepwise by addition of NaCl. Exchange activity appeared in the effluent with 0.005 M NaCl in the buffer. A typical elution pattern is given in Fig. 2. In general effluents with a specific activity above 10 were combined. Yield of activity was 63%.

Step 5. Solid $(NH_4)_2SO_4$ (65 g/100 ml) was added to 90% saturation in order to precipitate all protein. The precipitate was dissolved in a minimal volume of 0.01 M citric acid–0.02 M Na_2HPO_4–0.01 M β-mercaptoethanol (pH 5.0). After dialysis against the same buffer the solution was applied to a CM-cellulose column (Whatman, CM-52 microgranular).

Elution was started with the citric acid–phosphate buffer followed by a linear gradient between 0.01 M citric acid–0.02 M Na_2HPO_4–0.01 M β-mercaptoethanol

(pH 5.0) and 0.05 M citric acid–0.1 M Na_2HPO_4–0.01 M β-mercaptoethanol (pH 5.0). Exchange activity appeared in the effluent with about 0.025 M citric acid–0.05 M phosphate. A typical elution pattern is given in Fig. 3. The fractions with a specific activity above 200 were combined. The combined fraction was purified 860-fold; the yield was 50%.

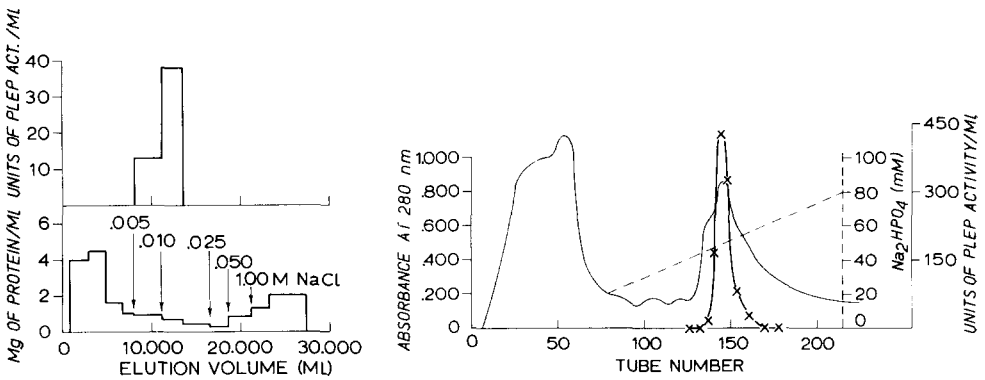


Fig. 2. Elution pattern of phosphatidylcholine exchange protein (PLEP) from a DEAE-cellulose column. 66 g protein were applied to column (6 cm × 55 cm). Eluting buffer was 0.01 M sodium phosphate–0.01 M β-mercaptoethanol (pH 7.2). Arrows in the figure indicate where the ionic strength of the buffer was increased with sodium chloride. Flow rate was 150 ml/h. Protein was determined according to the method of Lowry *et al.*[8].

Fig. 3. Elution pattern of phosphatidylcholine exchange protein (PLEP) from a CM-cellulose column. 4.6 g protein were applied to column (3 cm × 37 cm). Eluting buffer was 0.01 M citric acid–0.02 M Na_2HPO_4–0.01 M β-mercaptoethanol (pH 5.0). Linear gradient was formed with 1500 ml eluting buffer and 1500 ml 0.05 M citric acid–0.1 M Na_2HPO_4–0.01 M β-mercaptoethanol (pH 5.0). Flow rate was 100 ml/h. 28-ml fractions were collected. Elution of protein was determined by measuring absorbance at 280 nm. ×—×, units of phosphatidylcholine exchange protein activity per ml effluent.

Step 6. Then the protein was applied to a Sephadex G-50 column (Pharmacia Fine Chemicals) and eluated with 0.05 M citric acid–0.1 M Na_2HPO_4–0.01 M β-mercaptoethanol (pH 5.0). The fractions which possessed a constant specific activity were combined (Tubes no. 52–58, see Fig. 4). This final step resulted in a 2680-fold purified protein. The recovery amounted to 26%. The phosphatidylcholine exchange protein was stored at −20 °C in 50% glycerol. Phosphatidylcholine exchange protein was homogeneous by several criteria. Disc gel electrophoresis on sodium dodecyl sulfate–polyacrylamide gave one single band (Fig. 5). Immunoelectrophoresis against anti-phosphatidylcholine exchange protein gave a single precipitin line (Fig. 6).

Properties of phosphatidylcholine exchange protein

Protein part. The amino acid composition of the protein is given in Table II. A striking feature is the low content of half-cystine, *i.e.* 2 moles half-cystine per mole protein. The absence of free sulfhydryl groups was shown with Ellman's reagent in the presence of sodium dodecyl sulfate. After reduction with sodium borohydride

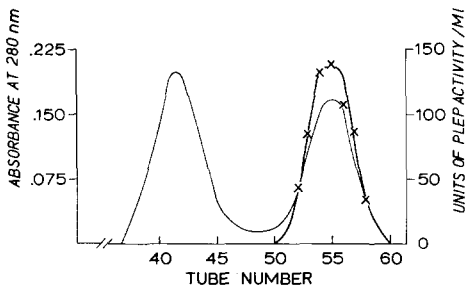


Fig. 4. Elution pattern of phosphatidylcholine exchange protein (PLEP) from a Sephadex G-50 column. 30 mg protein in 45 ml buffer were applied to column (5 cm × 72 cm). Eluting buffer was 0.05 M citric acid–0.1 M Na_2HPO_4–0.01 M β-mercaptoethanol (pH 5.0). Flow rate was 15 ml/h. 15-ml fractions were collected. Elution of protein was determined by measuring absorbance at 280 nm. × — ×, units of phosphatidylcholine exchange protein activity per ml effluent.

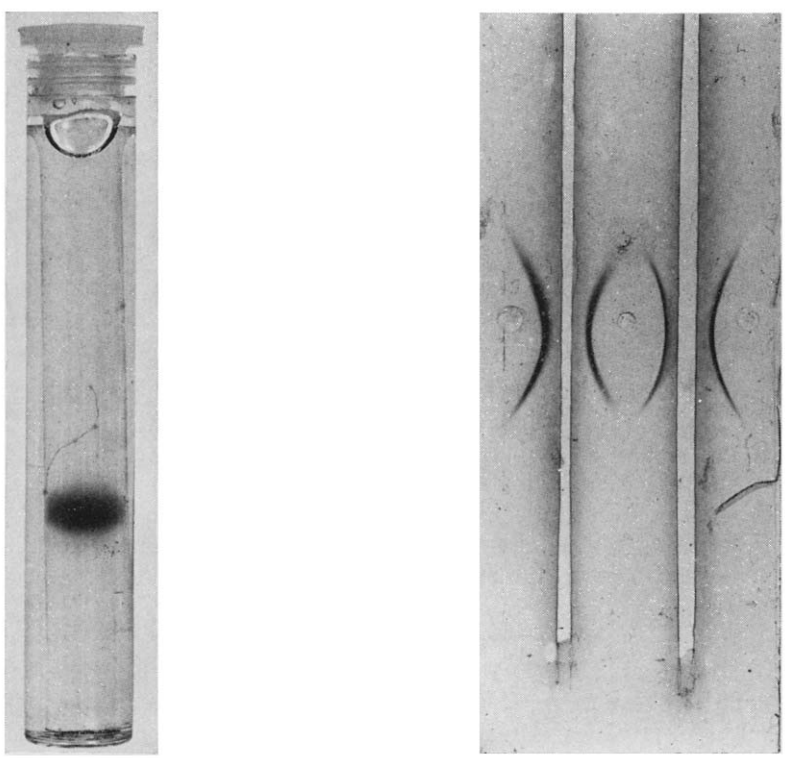


Fig. 5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of phosphatidylcholine exchange protein. For experimental details, see Materials and Methods.

Fig. 6. Immunoelectrophoresis of phosphatidylcholine exchange protein. For experimental details, see Materials and Methods. The wells contained phosphatidylcholine exchange protein and the troughs contained antiserum.

TABLE II

AMINO ACID COMPOSITION OF PHOSPHATIDYLCHOLINE EXCHANGE PROTEIN

Amino acid	*Moles residue per 10 moles of aspartic acid after hydrolysis for*			*Nearest integer per mole protein*	*Integral number X residue mol. wt*
	24 h	*48 h*	*72 h*		
Asp*	10 0	10.0	10.0	16	1840
Thr	3.5	2.6	3.0	5	505
Ser	6.4	4.7	4.9	11	957
Glu*	17.8	18.3	19.0	29	3741
Pro	6.9	5.5	5.5	10	970
Gly	10.0	9.6	9.9	16	912
Ala	8.5	8.6	8.4	14	994
Val	9.2	10.4	10.1	16	1584
Cys ($^1/_2$)	1.4	1.7	1.3	2	206
Met	2.5	2.4	2.4	4	524
Ile	3.2	3.6	3.6	6	678
Leu	8.9	9.5	9.5	15	1695
Tyr	6.4	5.0	5.1	9	1467
Phe	4.6	4.8	4.8	8	1176
Lys	9.4	9.9	10.0	16	2048
His	1.8	1.5	1.9	3	411
Arg	5.2	5.2	5.1	8	1248
Trp**	—	—	—	2	372
Total				190	21328

* Amide content has not been determined.

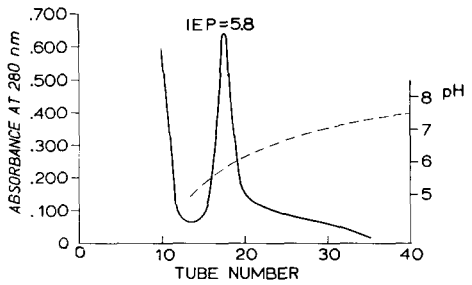
** Determined after hydrolysis in the presence of thioglycolic acid according to the method of Gruen and Nicholis[13].

free SH groups were demonstrated. From these results it is concluded that one disulphide bond is present in the protein molecule.

It follows from the amino acid composition that the content of acidic amino acids exceeds the content of basic amino acids (45 moles glutamic *plus* aspartic acid *versus* 24 moles lysine *plus* arginine). This is reflected in the isoelectric point of the protein. Isoelectric focusing gave a single peak with an isoelectric point at pH 5.8 (Fig. 7). By both the Edman procedure and dansylation the N-terminal amino acid was glutamic acid.

Lipid part. Lipids present in the pure protein fraction were extracted according to Folch *et al.*[21]. It was found that 250 μmoles of protein (5.5 mg) contained 100–150 μmoles of phospholipid phosphorus. The extracted lipids were separated by chromatography on silica gel H plates. After charring with sulfuric acid one lipid-containing spot was visible. The R_F value of this spot was identical to that of egg yolk and liver microsomal phosphatidylcholine. The lipid-containing spot reacted positively to Dragendorff's reagent. It may be concluded that it is very probably phosphatidylcholine which is bound to the protein.

Molecular weight. In the previous study a molecular weight of 22000 was calculated from the elution and void volumes after chromatography on Sephadex



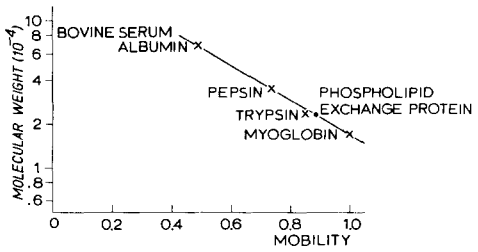


Fig. 7. Isoelectric focusing of phosphatidylcholine exchange protein. For experimental details see Materials and Methods.

Fig. 8. Determination of molecular weight of phosphatidylcholine exchange protein by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The method of Weber and Osborn[10] was used.

TABLE III

EFFECT OF VARIOUS STEPS OF THE PURIFICATION ON THE EXCHANGE OF INDIVIDUAL PHOSPHOLIPIDS

Unlabeled mitochondria (10 mg protein) and ^{32}P-labeled microsomes (2.0 mg protein) were incubated with the active protein fractions of Step 1 (9.8 mg protein), Step 2 (3.6 mg protein), Step 4 (2.4 mg protein) and Step 6 (0.006 mg protein) in a total volume of 3 ml 0.25 M sucrose–0.001 M EDTA–0.01 M Tris (pH 7.4) for 30 min at 37 °C. The microsomes before incubation contained [^{32}P]phosphatidylcholine (34310 cpm), [^{32}P]phosphatidylinositol (4490 cpm) and [^{32}P]phosphatidylethanolamine (15330 cpm). The figures in the table give the radioactivity transferred to the mitochondria.

Protein fraction	*Distribution of total ^{32}P in mitochondrial phospholipids* (cpm)*		
	Phosphatidyl-choline	*Phosphatidyl-inositol*	*Phosphatidyl-ethanolamine*
Step 1 (pH 5.1 supernatant)	11 447 (29.2%)	1139 (20.5%)	1343 (6.0%)
Step 2	6 989 (16.1%)	228 (0%)	513 (0%)
Step 4	12 723 (32.9%)	231 (0%)	421 (0%)
Step 6 (exchange protein)	8 030 (23.4%)	210 (0%)	450 (0%)
control	1 440	220	500

* The figures in parentheses are the percent of microsomal ^{32}P radioactivity transferred to the mitochondria, corrected for the control incubation.

G-75[24]. Electrophoresis on sodium dodecyl sulfate–polyacrylamide yielded a single band with an estimated molecular weight of 23000 (Fig. 8). From the amino acid analysis a molecular weight of 21320 can be computed (Table II).

Specificity. The various steps of the purification have been assayed for their ability to stimulate exchange of phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine between ^{32}P-labeled microsomes and mitochondria. It follows from Table III that the pH 5.1 supernatant protein fraction stimulates the exchange of all three phospholipids. However, the ability to stimulate exchange of

phosphatidylinositol and phosphatidylethanolamine has disappeared after the pH 5.1 supernatant has gone through a pH 3 adjustment (Step 2).

From Step 2 onwards the isolated protein fractions stimulate only the exchange of phosphatidylcholine.

In some experiments the transfer of [^{32}P]sphingomyelin from ^{32}P-labeled microsomes to mitochondria was determined in the presence of the purified exchange protein. Redistribution of [^{32}P]sphingomyelin between the two subcellular membrane fractions was not observed.

DISCUSSION

The purification procedure presented in this study resulted in a 2700-fold purified phosphatidylcholine exchange protein. This protein was homogeneous by the following criteria: sodium dodecyl sulfate–polyacrylamide gel electrophoresis, immunoelectrophoresis, isolectric focusing and N-terminal amino acid analysis.

A distinctive feature of the purification procedure is the apparent activation of the exchange protein after pH 3 treatment (Step 2). It is possible that the protein is modified at a low pH, resulting in an increased activity. On the other hand it could be that an inhibitor or regulating factor of the phosphatidylcholine exchange protein has been removed. In this respect it should be mentioned that the protein fraction of Step 2 lacked the ability to stimulate the exchange of phosphatidylinositol and phosphatidylethanolamine between mitochondria and microsomes (see Table III). With regard to previous observations[1] this protein fraction will also not transfer phosphatidylethanolamine and phosphatidylinositol from microsome to liposome. As a result the surface charge of the liposome will not change during incubation. The pH 5.1 supernatant, however, is capable of transferring those phospholipids, thereby introducing a negative surface charge on the liposome. It is suggested that the activation of the phosphatidylcholine exchange protein, observed in Step 2, could be partially due to a less negative surface charge of the liposome. This would agree with observations that an increased negative surface charge of the liposome inhibits the transfer of phosphatidylcholine between mitochondrion and liposome[25] and between liposome and liposome (Hellings, J. and Wirtz, K. W. A., unpublished).

It appears that the present purification yielded a 9-fold higher purified protein than was obtained in the previously published purification. This was unexpected since the electrophoresis gels of both preparations were comparable. At this moment we have no explanation for this discrepancy.

A complicating factor in the purification is the tendency of the exchange protein to aggregate, particularly at the later stages of the purification. Aggregates in the form of long filaments appear if the concentration of exchange protein is as low as 0.1 mg/ml. Protein which is dialysed against distilled water and subsequently lyophilized is insoluble. It is thought that these properties may reflect the hydrophobic character of the protien.

The exchange protein has an average hydrophobicity of 1109 calories per amino acid residue, calculated from the amino acid composition according to the method of Bigelow[26]. From the study of this author it follows that the exchange protein has a high average hydrophobicity relative to its molecular weight of 22 000. This suggests an excess of non-polar residues which leads to intermolecular hydrophobic bonding

i.e. formation of aggregates[27]. In this respect the exchange protein and, for example, Fractions III and IV from human serum high density lipoprotein[28,29] show a similar behavior. The latter proteins have also high average hydrophobicities (1106 and 1165 cal., respectively) compared to their molecular weights of 27000 and 17000, respectively.

Fractions III and IV, main constituents of the high density lipoprotein, take part in the extracellular transfer of phosphatidylcholine whereas the exchange protein is involved in the intracellular transfer of this phospholipid. It is known that serum lipoproteins do not stimulate exchange of phospholipids between membranes[30]. Fractions III and IV were also found to be inactive in the exchange of phosphatidylcholine between mitochondria and microsomes (Moleman, P., unpublished). Recent observations[31,32] that the carboxyl-terminal cyanogen bromide fragments of Fractions III and IV bind phosphatidylcholine, might provide a clue as to whether the interactions of phosphatidylcholine with those proteins and the exchange protein compare.

Analysis of lipids bound to the exchange protein demonstrated that about 0.5 mole phospholipid phosphorus was present per mole of protein. The phospholipid was tentatively identified as phosphatidylcholine. To what extent the phosphatidylcholine content of the protein is affected by the purification procedure is not known. Studies are currently in progress to determine whether or not the bound phosphatidylcholine is involved in the exchange, and what number of binding sites for phosphatidylcholine are present on the protein.

Since phosphatidylcholine was found to be bound to the exchange protein, it is attractive to assume that the protein functions as an intracellular carrier of phosphatidylcholine. Studies with the antibody against phosphatidylcholine exchange protein suggest that several phospholipid exchange proteins are present in beef liver (Harvey, M. S. and Wirtz, K. W. A., unpublished). Recently Ehnholm and Zilversmit[2] have isolated two phospholipid exchange protein fractions from beef heart which differed in molecular weight and isoelectric point. A wide distribution of the exchange proteins may be inferred from the fact that phospholipid exchange protein activity has been demonstrated in mammalian tissues[1–5], plants[33] and probably in unicellular organisms such as tetrahymena[34].

Apart from the phospholipid exchange proteins, other proteins have been described which are involved in the intracellular transfer of various lipid-like components. The sterol carrier protein functions as a carrier of water-insoluble precursor products in the biosynthesis of cholesterol[35,36]. It is hypothesized that next the carrier protein–cholesterol complex is assembled into low density lipoprotein[37]. A receptor protein for fatty acids denoted as Z-protein, has been isolated from rat liver[38]. The mechanism by which the phospholipid exchange proteins operate in an environment of intracellular membranes which contain lipids, remains to be solved[39].

Recent studies of Kagawa *et al.*[40] have provided evidence that a phospholipid exchange protein from beef heart was capable of net transfer of phosphatidylcholine. The exchange protein activated $^{32}P_i$–ATP exchange in phosphorylating particles which contained phosphatidylethanolamine and cardiolipin but lacked phosphatidylcholine, by introducing the latter phospholipid. In the instance where the particle was complete except for phosphatidylethanolamine, activation of $^{32}P_i$–ATP exchange failed since the exchange protein did not transfer phosphatidylethanolamine into the particle. The latter observation suggests that an effective interaction of the phospholipid ex-

change protein from beef heart with phosphatidylethanolamine does not take place.

The present as well as the previous study[1] demonstrates that the exchange protein from beef liver specifically stimulates the exchange of phosphatidylcholine. Activities which stimulate the exchange of phosphatidylethanolamine and phosphatidylinositol have been removed during purification. The specificity of the purified protein argues in favour of an electrostatic interaction between the zwitterionic phosphorylcholine group of the phosphatidylcholine molecule and the protein. The protein probably also contains hydrophobic sites to accommodate the hydrocarbon chains. Exchange of sphingomyelin which has the same polar head group as phosphatidylcholine, was negligible between mitochondria and microsomes. Ehnholm and Zilversmit[2] observed that exchange proteins isolated from beef heart stimulated the exchange of phosphatidylcholine six times faster than the exchange of sphingomyelin between liposomes which contained equal amounts of those phospholipids. This could mean that the hydrophobic interaction of sphingomyelin with the phosphatidylcholine exchange protein is less effective. On the other hand sphingomyelin incorporated into liposomes or natural membranes might be more tightly bound than phosphatidylcholine in those structures and, therefore, less available for exchange.

If the initial interaction of the purified exchange protein with the phospholipid is only a matter of charge it is surprising that the protein does not stimulate the exchange of phosphatidylethanolamine between mitochondria and microsomes. Similar observations have been made for the exchange of phospholipids between liposomes[2]. Studies of Phillips *et al.*[41] indicate that in a bilayer the polar head group of phosphatidylcholine is extended normal to the bilayer causing repulsion between the head groups. In contrast the polar head group of phosphatidylethanolamine is tangential to the plane of the bilayer resulting in a net neutralization of charge. Thus, an effective electrostatic interaction of phosphatidylethanolamine with the exchange protein may not be possible.

ACKNOWLEDGEMENTS

The authors wish to thank Dr F. C. Reman (Gaubius Institute, T.N.O., Leiden) for help in the amino acid analysis. The authors are grateful to Dr B. J. M. Zegers (University Hospital, Utrecht) for preparing the antisera and performing the immunoelectrophoresis. Dr J. Bours (Laboratory of Medical Anatomy and Embryology, State University, Utrecht) is gratefully acknowledged for help and advice in isoelectric focusing.

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