ISOLATION AND AMINO ACID ANALYSIS OF A NONSPECIFIC PHOSPHOLIPID TRANSFER PROTEIN FROM RAT LIVER

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1. Introduction

Proteins that accelerate the transfer of phospholipids between natural and artificial membranes have been characterized from several different sources [1]. In the few instances in which these proteins have been extensively purified they have been shown to be specific for phosphatidylcholine or phosphatidylcholine and phosphatidylinositol [2–5]. We have isolated, from the soluble fraction of rat liver, two protein fractions capable of transferring labeled phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidylserine, sphingomyelin and cholesterol from liposomes to mitochondria or erythrocyte ghosts [6] or from microsomes to mitochondria [7]. This report describes the purification to electrophoretic homogeneity of one of these proteins and its complete amino acid composition.

2. Materials and methods

[^{32}P]Phosphatidylethanolamine from rat liver and nonradioactive soy phosphatidylcholine were purified by chromatography on alumina as in [6]. Liposomes containing nonradioactive phosphatidylcholine, [^{32}P]phosphatidylethanolamine (molar ratio 1:1), 1% butylated hydroxytoluene and a trace of glycerol [9,10-^{3}H]trioleate were prepared in 250 mM sucrose, 1 mM Na_2EDTA, 50 mM Tris/HCl, 0.02% NaN_3, pH 7.4 (SET buffer), by sonication in a sonicating water bath under N_2 below 20°C [6]. Phosphatidylethanolamine transfer activity was determined by measuring the transfer of the labeled phospholipid from liposomes to beef heart mitochondria. Liposomes (5 μg phospholipid phosphorous) were incubated with mitochondria (34.1 μg phospholipid phosphorous) and an appropriate aliquot of the transfer protein in 1 ml SET buffer. After 2 h at 37°C, mitochondria were sedimented by centrifugation and aliquots of the supernatant were counted in a Packard Tri-Carb scintillation counter. The decrease in the ^{32}P/^{3}H ratio of the supernatant measured the transfer of phosphatidylethanolamine. One unit of activity is defined as the transfer of 1% of the labeled phosphatidylethanolamine/h. The preparation of partially-purified transfer protein CM_2 was detailed in [6]. Briefly, the procedure included the homogenization of fresh rat livers and preparation of a postmitochondrial supernatant. After a pH 5.1 precipitation, ammonium sulfate cut between 50% and 90% saturation and gel filtration through Sephadex G-75, the sample was subjected to ion exchange chromatography on CM cellulose equilibrated at pH 7.9. The second active peak was heated over 5 min at 90°C and the denatured protein was discarded by centrifugation. Polyacrylamide disc-gel electrophoresis was performed on 10% or 15% acrylamide gels in 0.188 M acetic acid, 0.188 M glycine (pH 3.6). Electrophoresis in the presence of sodium dodecylsulfate (SDS) was

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performed by the method in [8] on 15% polyacrylamide, at pH 7.2. Bio-Gel P-10 was obtained from Bio-Rad (Richmond, CA).

Protein samples were hydrolyzed at 110°C in 3 N mercaptoethane sulfonic acid for 20 h and 40 h in the presence of indole acetic acid to protect tryptophan. Performic acid oxidation of the protein was carried out as in [9]. Three 0.1 mg samples were analyzed: one hydrolyzed for 20 h, one for 40 h and one for 20 h after performic acid oxidation. The amino acid analyses were carried out on a Beckman Spinco Model 120C amino acid analyzer as in [10]. Sulfhydryl groups were determined by reaction of the protein with 5,5′-dithio bis (2,2′-nitrobenzoic acid) (DTNB) in the presence of 0.05% SDS to denature the protein [11]. The reaction mixture contained: 30 μmol Tris–HCl (pH 8.0), 3 μmol EDTA (pH 8.0), 0.1 μmol DTNB and 100 μg protein in final vol. 0.3 ml. The reaction was followed at A_{412} for 1 h at 22°C.

3. Results and discussion

Electrophoresis on polyacrylamide gels revealed that the CM_2 fraction after heat treatment was composed of a major protein band and two minor ones [6]. In an attempt to further purify the CM_2 fraction, a molecular filtration step through Bio-Gel P 10 was introduced. The sample, 3 ml, containing 3.1 mg protein and 4700 units phosphatidylethanolamine transfer activity, were loaded onto a Bio-Gel P 10 column (2.4 × 58 cm) equilibrated with 0.05 M Tris/acetate, 0.02 M NaCl (pH 7.4) and eluted with the same buffer at a 5 ml/h flow rate. Fractions, 2.1 ml, were collected and assayed for protein and transfer activity. Although the applied sample was spec. act. 1500 U/mg protein, the purest fractions ranged from spec. act. 968–1235 U/mg protein. The recovery of the transfer activity was 53% in this experiment but the activity was rapidly lost (within 48 h) upon storage at 4°C. Electrophoresis on polyacrylamide gels showed single protein bands, both at pH 3.6 (not shown) and at pH 7.2 in the presence of SDS (fig.1). It seems then that the removal of minor impurities present before the Bio-Gel step leads to an accelerated loss of the transfer activity. This rapid loss is probably associated with aggregation of the pure protein, as evidenced by the appearance of slow-moving bands on the polyacrylamide gels run at pH 3.6. Aggregation of the highly purified fractions was also found during the purification of a phosphatidylcholine exchange protein isolated from beef liver [2].

Fig.1. SDS–polyacrylamide gels of the fractions before and after gel chromatography on Bio-Gel P-10. For each sample, 20 μg protein was applied.

Table 1
Amino acid composition of purified phospholipid transfer protein

Amino acid	mol %	Nearest integer /mol protein
Lysine	15.7	19
Histidine	–	–
Arginine	–	–
Tryptophan	–	–
Aspartic acid and asparagine	10.4	12
Threonine	3.7	4
Serine	6.2	7
Glutamic acid and glutamine	11.1	13
Proline	3.6	4
Glycine	11.8	14
Alanine	9.1	11
Half cystine	1.7	2
Valine	4.6	5
Methionine	3.8	5
Isoleucine	4.4	5
Leucine	8.8	10
Tyrosine	–	–
Phenylalanine	5.1	6

The amino acid composition of the purified protein is shown in table 1. It is interesting that the protein lacks 4 amino acids, i.e., histidine, arginine, tryptophan and tyrosine and contains a very high proportion of lysine, in agreement with an isoelectric point > 8.5 [6]. It can be inferred that a significant portion of the aspartic and glutamic residues must take the form of asparagine and glutamine to account for the relatively high isoelectric point. The protein has a low content of half-cystine, only 2 mol/mol protein. Since no free sulfhydryl groups were found it is suggested that there is only one disulfide bond, or that sulfhydryl groups were not exposed under our denaturing conditions. The minimum molecular weight, which can be calculated assuming 1 disulfide bond/molecule, is 12 400, which is in good agreement with reported values of 12 500 and 13 500 by SDS–polyacrylamide gel electrophoresis and molecular filtration through Sephadex G-50, respectively [6].
A nonspecific phospholipid exchange protein with a low isoelectric point (pH 5.2) has been purified [12] from rat hepatoma; it contains the 4 amino acids missing from our protein. The partial purification of a phospholipid exchange protein from rat liver with a basic isoelectric point and mol. wt 17 000 has been reported [13]. The same protein was fully purified [5] and shown to be specific for the exchange of phosphatidylcholine. Its amino acid composition differs greatly from the composition of the transfer protein purified by us. Actually, this is the first time that the amino acid composition is reported for a phospholipid transfer protein with a high isoelectric point and capable of transferring most of the naturally found phospholipids in mammalian membranes.

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