PROTEIN-CATALYZED TRANSFER OF PHOSPHATIDYGLYCEROL BY SHEEP LUNG SOLUBLE FRACTION

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1. Introduction

It is generally agreed that the phospholipids of lung surfactant lower the surface tension at the alveolar air-water interface and allow the alveolus to remain open at end-expiration [1]. While a monolayer of disaturated phosphatidylcholine is sufficient to adequately lower this surface tension [2], other phospholipids such as phosphatidyglycerol, the second most abundant surfactant phospholipid, may be required for monolayer formation under physiological conditions [3]. Phosphatidylglycerol is absent in the tracheal secretions of infants with Respiratory Distress Syndrome but appears during recovery from this disease [4]. Its dynamics are therefore of considerable interest. While a number of investigations have been concerned with the synthesis of phosphatidylglycerol [5,6], little is known about the mechanism by which it becomes a major constituent of lung surfactant.

The presence of phospholipid exchange proteins has been demonstrated in the soluble fraction of lung [7–9] and they are known to catalyze the transfer of phosphatidylcholine between membrane fractions of the lung [9,10]. These proteins may be important in providing a mechanism for packaging specific phospholipids into lamellar bodies, but thus far the transfer of phosphatidylglycerol has not been reported. We provide evidence here of protein catalyzed phosphatidylglycerol transfer by sheep lung soluble fraction; this activity chromatographs on Sephadex G75 with an elution volume corresponding to the 30 000 MW range.

2. Materials and methods

2.1. *Tissue preparations*

Adult sheep lung was washed, dissected free of bronchi and homogenized with a Potter–Elvehjem tissue grinder in 4 vol. of 0.25 M sucrose containing 1 mM EDTA and 10 mM Tris–HCl pH 7.4. The homogenate was filtered through a single layer of cheesecloth and centrifuged at 15 000 × *g* for 15 min. The supernatant was then centrifuged at 105 000 × *g* in a Beckman Rotor 50 Ti for 60 min. The supernatant was decanted and termed the soluble fraction. Protein was determined by the method of Lowry et al. [11].

2.2. *Chemicals*

Phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, dicetylphosphate, Tris buffer and EDTA were obtained from Sigma Chemical Company (St. Louis, MO). Sephadex G75 was obtained from Pharmacia Fine Chemicals (Piscataway, NJ) and [2-^{3}H]glycerol (7.9 Ci/mmol) was obtained from New England Nuclear (Boston, MA).

2.3. *Preparation of [^{3}H]phosphatidylglycerol*

Escherichia coli BB 20–14 (a glycerol auxotroph obtained as the generous gift of Dr Robert Bell, Duke University) was raised in Minimal Medium A [12] containing 0.2% glucose and 0.0002% [^{3}H]-glycerol (0.113 mCi/mmol) for 31 h. The bacteria were harvested by centrifugation, washed with distilled water, resuspended in methanol with sonication, and the lipids extracted by the method of Bligh and Dyer [13]. The phospholipid fraction was eluted from a silicic acid column and the [^{3}H]phosphatidylglycerol purified by thin-layer chromatography (TLC), using a solvent system of chloroform:methanol:HCl (87:13:0.2, v/v/v). The substrate was 95% pure on TLC by radioactivity and had a specific activity of 10 μCi/μmol. The [^{3}H]phosphatidylglycerol

was diluted to 0.27 μCi/μmol for incorporation into donor liposomes.

2.4. *Preparation of donor and acceptor vesicles*

Donor liposomes were prepared from phosphatidylethanolamine:phosphatidylcholine:[^{3}H]phosphatidylglycerol at a molar ratio of 80:10:10 in 50 mM Tris–HCl, 5 mM EDTA pH 7.4, which was sonicated for 5 min in an ice bath. This preparation was then centrifuged for 15 min at 105 000 × *g*, the supernatant decanted and used immediately as donor liposomes.

Acceptor multilamellar vesicles were prepared by gently vortexing 243 mg of crude phosphatidylcholine (Sigma type IX E), 11 mg of dicetylphosphate and a trace of [^{14}C]cholesteryl ether (as a nonexchangeable marker) in 20 ml of 50 mM Tris–HCl, 5 mM EDTA pH 7.4 buffer. The multilamellar vesicles were centrifuged at 15 000 × *g* for 30 min, the pellet gently resuspended in 20 ml of buffer and used immediately as acceptor multilamellar vesicles.

2.5. *Assay of phosphatidylglycerol exchange*

Lung fractions were dialyzed overnight against 50 mM Tris–HCl, 5 mM EDTA pH 7.4 buffer before assaying activity. The transfer of [^{3}H]phosphatidylglycerol from donor liposomes to acceptor multilamellar vesicles was assayed, using a procedure similar to that of Wirtz [14]. Donor liposomes (0.04 μmol phospholipid) and acceptor multilamellar vesicles (0.5 ml) were incubated with lung fractions in a final volume of 3 ml of 50 mM Tris–HCl, 5 mM EDTA pH 7.4 for 30 min at 37°C. The assay was terminated by immersing the tubes in an ice bath followed by pelleting the acceptor multilamellar vesicles at 15 000 × *g* for 30 min. The pellet was washed once with 1 ml of buffer, dissolved in chloroform:methanol (1:1, v/v), transferred to a scintillation vial, dried and radioactivity was determined. Recovery of multilamellar vesicles was routinely 70–80% as demonstrated by the nonexchangeable marker; transfer of phosphatidylglycerol was calculated from the ^{3}H/^{14}C ratio.

3. Results

Addition of sheep lung soluble fraction to the assay sytem described above resulted in the protein-catalyzed transfer of [^{3}H]phosphatidylglycerol from donor liposomes to acceptor multilamellar vesicles (table 1). Omission of soluble fraction or of acceptor multilamellar vesicles reduced the transfer to minimal values. The transfer activity of the soluble fraction was heat-labile and bovine serum albumin was not an effective substitute, ruling out nonspecific interactions. Addition of acceptor multilamellar vesicles after termination of the incubation resulted in the transfer of only small amounts of [^{3}H]phosphatidylglycerol to the acceptor membranes; this supports the evidence of protein-catalyzed transfer between the two membranes. Similar incubations were performed with both [^{3}H]phosphatidylglycerol and [^{14}C]cholesteryl ether (the nonexchangeable marker) in the donor liposomes and transfer calculated from the decrease in the ^{3}H/^{14}C ratio of the recovered liposomes. Values obtained with the system (data not shown) were comparable with those presented in table 1, providing additional confirmation of intermembrane phosphatidylglycerol transfer.

When donor liposomes, consisting of 90 mol/100 ml phosphatidylcholine and 10 mol/100 ml phosphatidylglycerol, were used as donor membranes, poor recovery of the [^{3}H]phosphatidylglycerol and inconsistent transfer values were obtained. This may have been the result of phosphatidylglycerol hydrolysis by sheep lung soluble fraction using these liposomes. However, using the donor liposomes described in Section 2

Table 1
Transfer of phosphatidylglycerol between membranes by sheep lung soluble fraction

Assay system	% Transfer
Complete[a]	20.0
Soluble fraction	3.2
Multilamellar vesicles	4.9
Heat-treated soluble fraction[b]	8.6
BSA[c]	5.1
Multilamellar vesicles last[d]	8.3

[a] Complete assay system contains 50 mM Tris–HCl, 5 mM EDTA pH 7.4 buffer, liposomes (0.04 μmol), multilamellar vesicles (0.5 ml) soluble fraction (2.3 mg protein) in a final volume of 3 ml, incubated at 37°C for 30 min

[b] Before incubation the soluble fraction was heated to 70°C for 15 min

[c] The soluble fraction in the complete assay system was replaced with bovine serum albumin (2 mg)

[d] The multilamellar vesicles were added to the complete assay system after the assay tubes were immersed in an ice bath following incubation

(and in table 1), greater than 93% of the [^{3}H]phosphatidylglycerol was recovered when both the donor liposomes and the acceptor multilamellar vesicles were extracted following separation. The [^{3}H]phosphatidylglycerol recovered from the acceptor multilamellar vesicles after transfer migrated as a single spot with authentic diacylphosphatidylglycerol on thin-layer chromatograms.

Chromatography of sheep lung soluble fraction on a Sephadex G75 column (fig.1) demonstrated that the phosphatidylglycerol transfer activity eluted as a single peak in a volume corresponding to a molecular weight in the 30 000 range. The pooled active fractions had a marked increase in specific activity, giving a purification of 13-fold as compared to the soluble fraction, while recovering 80% of the total activity (table 2). Transfer of [^{3}H]phosphatidylglycerol by the pooled fractions was dependent on both time and protein concentration (fig.2).

Table 2
Partial purification of phosphatidylglycerol transfer activity by Sephadex G75 chromatography[a]

	Soluble fraction	Pooled Sephadex G75
Volume (ml)	10	10
Total protein (mg)	113	10
Total activity (nmol/min)	1.53	1.23
% Recovery	–	80
Specific activity (nmol/mg/min)[b]	0.01	0.13
Purification factor	–	13

[a] Donor liposomes (0.04 μmol) and acceptor multilamellar vesicles (0.5 ml) in 50 mM Tris–HCl, 5 mM EDTA pH 7.4 and either sheep lung soluble fraction (1.14 mg) or pooled Sephadex G75 fraction (0.10 mg) in a total of 3 ml were incubated at 37°C for 30 min

[b] Specific activity was calculated as nmol of [^{3}H]phosphatidylglycerol transferred/mg protein/min of incubation

4. Discussion

Mammalian lung surfactant is a complex mixture of lipids and proteins [15]. High concentrations of disaturated phosphatidylcholine and of phosphatidylglycerol appear to be unique to this system. Bangham [2] has pointed out that disaturated phosphatidylcholine alone has sufficient tension lowering activity to play this role of surfactant, but that mixture of phosphatidylcholine with phosphatidylglycerol is necessary to permit spreading rapid monolayer formation at rates observed during normal ventilation [3]. Further, Hallman et al. [4] have observed that the appearance of phosphatidylglycerol in the alveolar

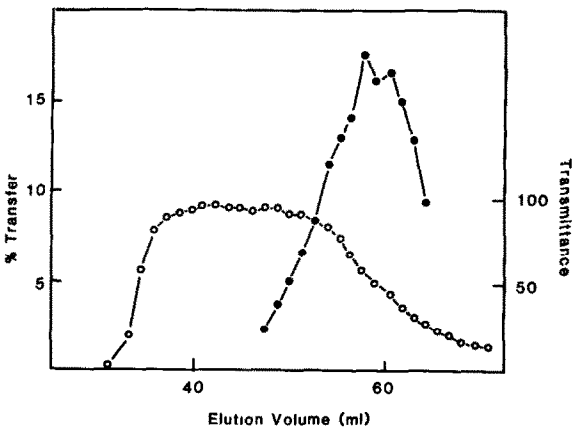

Fig.1. Chromatography of sheep lung soluble fraction on a Sephadex G75 column (1.4 cm × 62 cm), eluted with 10 mM sodium phosphate, 50 mM NaCl pH 6.8 at a flow rate of 10 ml/h at 4°C. Immediately following this elution the column was calibrated for MW by chromatographing chymotrypsinogen, ovalbumin and blue dextran standards. The peak of phosphatidylglycerol transfer activity corresponds to the 30 000 MW range. •——•, % [^{3}H]phosphatidylglycerol transfer; ○——○, transmittance at 280 nm.

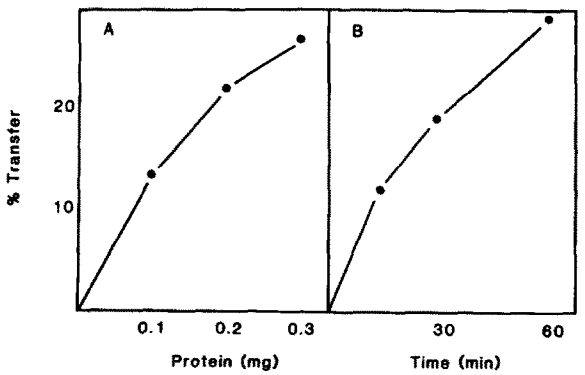

Fig.2. Sephadex G75 pooled fractions catalyzed transfer of [^{3}H]phosphatidylglycerol from donor liposomes (0.04 μmol) to acceptor multilamellar vesicles (0.5 ml). Values have been corrected by subtracting no protein blanks for corresponding times. (A) Effect of protein concentration on [^{3}H]phosphatidylglycerol transfer. Assay conditions were 37°C for 30 min. (B) [^{3}H]phosphatidylglycerol transfer as a function of time, incubation temperature 37°C.

subphase may herald recovery of infants suffering from Respiratory Distress Syndrome.

The mechanism by which quantities of specific phospholipids are packaged as lamellar bodies in the lung alveolar Type II cells is unknown. A class of proteins that demonstrate necessary phospholipid and membrane specificities are the phospholipid exchange proteins occurring in the cytosol of a variety of tissues [16,17]. Proteins catalyzing the transfer of lung phosphatidylcholine between membranes have been described in lung soluble fraction [7–10]. It is possible that these proteins transfer surfactant phosphatidylcholine in the alveolar Type II cell from its site of synthesis of the endoplasmic reticulum [18,19] to the developing lamellar bodies, thereby exerting a control over surfactant phospholipid quantity and/or quality. Phosphatidylglycerol also is synthesized by enzymes associated with the endoplasmic reticulum [5,6] and a protein capable of transferring phosphatidylglycerol may be required for its incorporation into lamellar bodies. Protein catalyzed transfer of phosphatidylglycerol has not been previously reported; this may be the result of rapid phosphatidylglycerol hydrolysis under certain conditions or it may be related to specific membrane requirements possessed by protein(s) catalyzing the transfer of phosphatidylglycerol. These features may serve to mask phosphatidylglycerol transfer activity.

In summary, we have demonstrated here that sheep lung soluble fraction contains protein(s) that catalyze the transfer of phosphatidylglycerol between membranes; these proteins may thus have an important role in the lung surfactant system. Characterization of these protein(s) is currently in progress in our laboratory.

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