A UNIQUE PHOSPHATIDYLCHOLINE EXCHANGE PROTEIN ISOLATED FROM SHEEP LUNG

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Received 23 December 1977

1. Introduction

It has been established that disaturated phosphatidylcholine is the major surface-active agent in mammalian lung surfactant which lowers surface tension and prevents alveolar collapse at end-expiration [1,2]. Investigations of the synthesis, packaging and release of lung phosphatidylcholine (PC) have demonstrated that the endoplasmic reticulum of Type II pneumocytes is the major site of PC synthesis [3] and that lamellar bodies of these cells make up the primary intracellular storage organelle [4]. The mechanisms by which PC is transferred from the site of synthesis to the storage organelle or onto the alveolar surface are unknown. Phosphatidylcholine exchange proteins which catalyze the exchange of PC between membrane structures in vitro [5,6], are widely distributed [7] and have been well characterized in bovine liver [8,9], heart [10,11], brain [12] and in rat liver [13,14] and intestine [15].

We report here the purification of a novel phosphatidylcholine exchange protein from sheep lung with pI 7.1 and mol. wt 21 000 as determined by Sephadex G 75 chromatography and SDS–polyacrylamide gel electrophoresis.

A second phosphatidylcholine exchange protein also was isolated from sheep lung which is very similar to that purified from bovine liver [8]; pI 5.8, mol. wt 22 000. Both exchange proteins have been purified over 150-fold.

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2. Materials and methods

Adult sheep lungs were dissected free of bronchi, minced and 20% homogenates prepared in 10 mM Tris buffer (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA. The homogenates were centrifuged at 35 000 rev/min in a Beckman Rotor 35 for 2 h and the decanted supernatants designated the soluble fraction. The soluble fractions were frozen and stored at –40°C until used.

Transfer of [^{14}C]phosphatidylcholine from microsomes to liposomes was assayed as in [16], [^{3}H]-phosphatidylinositol transfer from microsomes to liposomes was assayed as in [12], and [^{14}C]phosphatidylethanolamine transfer assayed as in [13].

Protein was determined as in [17] using bovine serum albumin as a standard.

Polyacrylamide gel electrophoresis was carried out at pH 8.6 with 7.5% gels as in [18], and SDS–polyacrylamide gel electrophoresis on 7.5% gels at pH 7.2 in the presence of 0.1% SDS as in [19] using ribonuclease A, chymotrypsinogen A and ovalbumin as standards.

3. Results

Lung soluble fraction (1 liter) was thawed and dialysed against 20 liter 5 mM sodium phosphate buffer (pH 7.2) with two changes. This was applied to a DEAE-Cellulose (Whatman DE-52) column (5 × 37 cm). The column was eluted with 5 mM sodium phosphate buffer and the unbound protein

fractions which contained the PC transfer activity were pooled and dialysed against 10 mM citrate–20 mM phosphate buffer (pH 5.0).

This fraction was applied to a CM-cellulose (Whatman CM-52) column (5 X 28 cm). The column was washed with 10 mM citrate– 20 mM phosphate buffer (pH 5.0) and then eluted with a linear gradient (total vol. 5 liter) from 10 mM citrate–20 phosphate to 50 mM citrate–100 mM phosphate buffer at flow-rate 116 ml/h. The fractions were assayed for PC transfer activity and the active fractions which eluted at 30 mM citrate–60 mM phosphate were pooled. Solid ammonium sulfate was added to 95% saturation with stirring at 0°C and the pellet, obtained by centrifugation, was dissolved in and dialysed against 10 mM potassium phosphate buffer (pH 6.8) containing 50 mM NaCl and 30% sucrose.

The dialysate was then applied to a Sephadex G-75 column (2.5 X 55 cm) and eluted at flow rate 15 ml/h. The active fractions were pooled and dialysed against 40% sucrose containing 1% glycine.

Isoelectric focusing of these fractions was performed in an LKB 8101 electrofocusing column, 600 V, 72 h, with pH gradient 3.5–10. Two peaks of PC transfer activity were obtained, protein I with pI 5.8 and protein II with pI 7.1 (fig.1).

Pooled fractions of protein I and protein II were dialysed against 10 mM potassium phosphate buffer (pH 6.8) containing 50 mM NaCl and 40% sucrose and chromatographed on a calibrated Sephadex G-75 column (1.6 X 33 cm). The molecular weights of protein I (22 600) and protein II (20 600) were calculated from the elution volumes. 1% original activity with 152-fold final purification was obtained for protein I and 4% original activity with 267-fold final purification was obtained for protein II (table 1).

When protein II was analyzed by polyacrylamide gel electrophoresis two major bands with R_F 0.22 and R_F 0.30 and a very minor band with R_F 0.39 were observed. Protein I showed multiple bands by this procedure.

SDS–polyacrylamide gel electrophoresis of

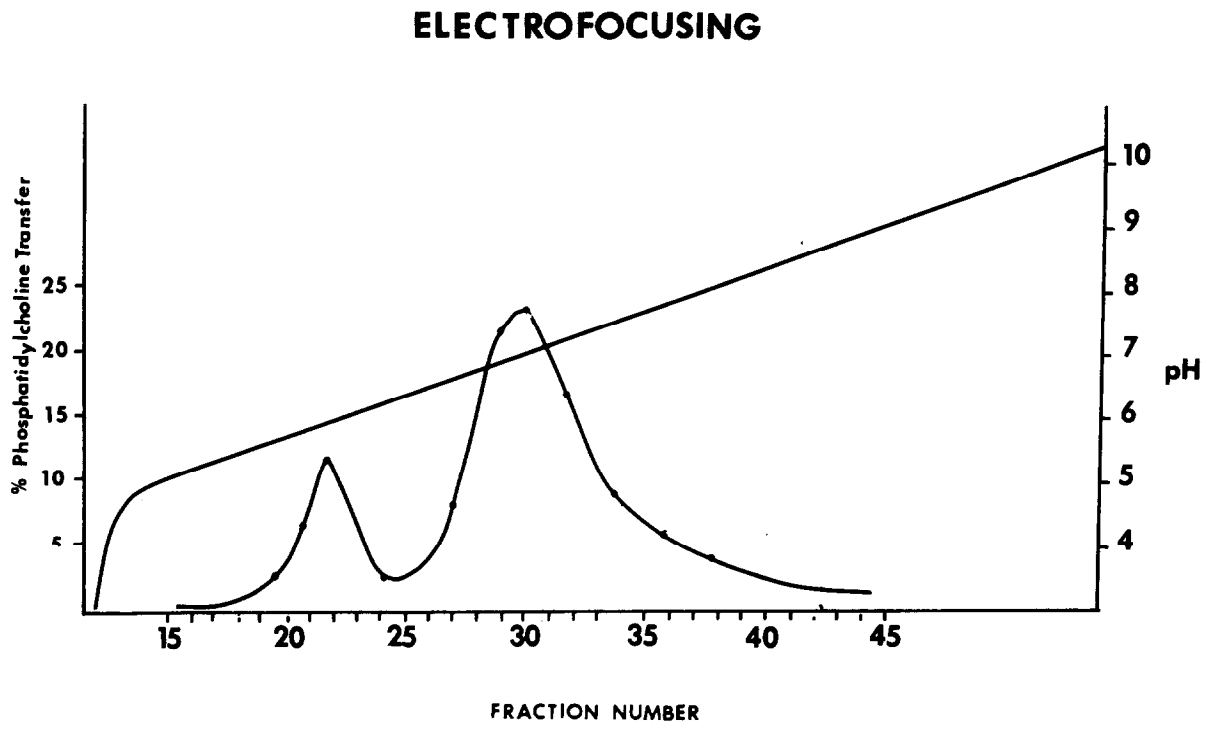

Fig.1. Isoelectric focusing was carried out as in [20] 600 V, 72 h, pH gradient 3.5–10. PC transfer activity (•——•) and pH (——) were measured.

Table 1
Partial purification of phosphatidylcholine exchange proteins from sheep lung

Purification step	Volume (ml)	Activity Total	% Recovered	Protein (mg)	Spec. act. (U/mg)	Purification factor
Soluble fraction	1008	23 231	100	20 825	1.1	–
DEAE-cellulose	1400	21 560	93	6300	3.4	3
CM-cellulose	726	7454	32	236	31.6	28
Sephadex G-75	128	3584	15	45	79.2	71
Electrofocusing Sephadex G-75						
Protein I	7	182	1	1	170.2	152
Protein II	8	986	4	3	298.8	267

PC transfer from microsomes to liposomes was assayed as in [9]. [^{14}C]PC-labeled rat liver microsomes (1.25 mg) were incubated with 1 μmol liposomal lipid (98 mol% egg PC, 2 mol% dicetylphosphate, trace [^{3}H]cholesterol ester) and lung-soluble protein. The assays were carried out at 37°C for 30 min in 10 mM Tris (pH 7.4) containing 0.25 M sucrose, and 1 mM EDTA in total vol. 2.5 ml; incubations were terminated by addition of 0.2 M sodium acetate (pH 5.0) containing 0.25 M sucrose. The aggregated microsomes were pelleted at 10 000 rev/min in a Sorvall SS 34 rotor; the liposomes were decanted, extracted and radioassayed. A unit of activity is equal to the % microsomal PC pool transferred to the liposomes in 30 min at 37°C

protein II showed a single major band with R_F 0.88 and a very minor band with R_F 0.73. Protein II was calculated to be mol. wt 21 000 by this procedure.

Both proteins showed a high specificity toward the transfer of PC and had little ability to catalyse the transfer of either phosphatidylinositol or phosphatidylethanolamine (table 2).

4. Discussion

Phosphatidylcholine biosynthesis has been studied extensively in lung tissue [4]. It has recently been shown that de novo synthesis of PC in the lung is associated entirely with the microsomal fraction and that lamellar bodies may play a role in subsequent modification of these PC molecules [3]. However, the intracellular mechanisms of PC transfer from the endoplasmic reticulum to lamellar bodies is unknown.

Table 2
Characteristics of phosphatidylcholine exchange proteins from sheep lung

	pI	Mol. wt	Specificity[a]		
			PC	PI	PE
Protein I	5.8	22 000	34.4	2.5	0.1
Protein II	7.1	21 000	60.0	0.7	0.6

[a] Specificity of transfer is expressed as nmol phospholipid transferred/min/mg protein. Assays were performed as in table 1 and section 2 with 29 μg protein I and 67 μg protein II

Protein II described here has mol. wt 21 000, pI 7.1 and accounts for the majority (85%) of the PC transfer activity recovered from the lung; such a PC exchange protein has not been reported previously. Protein I, with mol. wt 22 000, pI 5.8, is very similar to the PC exchange protein from bovine liver, purified and extensively characterized [8,9]. However, in lung tissue, this protein accounted for only about 15% of our recovered activity. Both proteins show a high degree of specificity toward PC.

We suggest that these proteins may be important in the intracellular transfer of PC in the mammalian lung surfactant system. Studies to determine the role of this unique protein are currently being conducted in our laboratories.

Acknowledgement

This investigation was supported by Public Health Service Grants No. HL 162448 and No. HL 21176 from the National Heart, Lung and Blood Institute.

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