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PHOSPHOLIPID-TRANSFER ACTIVITY IN TYPE II CELLS ISOLATED FROM ADULT RAT LUNG

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Summary

The soluble fraction from adult rat lung type II cells stimulated the transfer of various phospholipids between either liposomes or rat lung microsomes and rat lung mitochondria. Compared to whole lung, type II cells are highly enriched in transfer activity suggesting that phospholipid-transfer proteins play a role in the transport of surfactant phospholipids. Sephadex chromatography of pH 5.1 supernatant from type II cells yielded only one fraction catalysing the transfer of phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine, while chromatography of pH 5.1 supernatant from whole lung yielded in addition a specific phosphatidylcholine-transfer and a specific phosphatidylglycerol-transfer protein.

Disaturated phosphatidylcholine and phosphatidylglycerol are the major lipid components of pulmonary surfactant which lowers surface tension in the alveoli and prevents lung collapse during expiration [1—3]. Alveolar epithelial type II cells are considered to be the site of surfactant synthesis [3—5]. These cells contain unique subcellular structures, the lamelar bodies, which store the surfactant prior to its secretion onto the alveolar surface [5]. The mechanism via which the surfactant lipids are transferred from their site of synthesis in the type II cell to the storage organelle is unknown. Several reports [6—10] suggest that phospholipid-transfer proteins are involved in the intracellular transport of surfactant phospholipids. Recently, we demonstrated the presence in whole rat lung of two specific transfer proteins, one catalysing the transfer of phosphatidylcholine and the other that of phosphatidylglycerol. In addition, a third protein or mixture of proteins was isolated from whole rat lung cytosol which catalysed the trans-

fer of phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol and phosphatidylethanolamine [10]. The suggestion was made that the specific phosphatidylcholine- and phosphatidylglycerol-transfer proteins may contribute to the biogenesis of lamellar bodies in the type II cell. However, all these studies [6—10] have been carried out with soluble proteins isolated from whole lung. Due to the heterogeneity of lung tissue, the results of these studies are not necessarily pertinent to type II cells, the producers of surfactant.

The present paper is the first report on the phospholipid-transfer activities of proteins in the soluble fraction of isolated type II cells from normal adult rat lung. In previous articles, these cells were shown to have the capability to synthesize phosphatidylcholine with a high percentage of disaturated species [11,12] and to produce a considerable percentage of phosphatidylglycerol [12], indicating that the cells retained these important functions during the isolation.

The isolation procedure involved trypsinization of lungs from male Wistar rats and density gradient centrifugation of the liberated cells [13,14] followed by differential adherence in primary monolayer culture [14,15]. The type II cells attached to the culture dishes were rinsed with medium [11,12] and scraped from the dishes with a rubber policeman into 2—3 ml 0.25 mM sucrose/1 mM EDTA/10 mM Tris-HCl (pH 7.4). Cells and medium were sonicated four times for 30 s at 0°C with an MSE ultrasonic disintegrator at maximal output and the sonicate was centrifuged at 105 000 × *g* for 60 min at 4°C. A pH 5.1 supernatant fraction was prepared from the 105 000 × *g* supernatant exactly as described earlier [6]. The 105 000 × *g* supernatant fraction from whole rat lung was isolated [16] and a pH 5.1 supernatant was prepared as described by Engle et al. [6].

Donor liposomes were prepared according to Kamp and Wirtz [17]. Liposomes consisting of egg yolk phosphatidylcholine and di[1-^{14}C]palmitoyl phosphatidylcholine (molar ratio 99:1) were used as donor membranes to measure phosphatidylcholine transfer; liposomes of phosphatidylcholine, phosphatidylglycerol and di[1-^{14}C]palmitoylphosphatidylglycerol (molar ratio 95:2.5:2.5) for phosphatidylglycerol transfer; and liposomes of phosphatidylcholine and phosphatidyl[2-^{14}C]ethanolamine (molar ratio 50:50) for phosphatidylethanolamine transfer.

Labelled microsomes were prepared by perfusion of isolated rat lung for 4 h with 0.5 mCi [1,3-^{3}H]glycerol as described previously [18]. After the perfusion, the microsomes were isolated according to Vereyken et al. [16], and suspended in 0.25 mM sucrose/1 mM EDTA/10 mM Tris-HCl (pH 7.4). Unlabelled mitochondria were isolated from rat lung by the procedure of Engle et al. [6].

The transfer of phospholipids from donor liposomes to rat lung mitochondria was measured as follows. Donor liposomes (0.05 μmol phospholipid) were incubated at 37°C for 1 h with rat lung mitochondria (0.75 mg protein) in the presence or absence of pH 5.1 supernatant from either type II cells or whole lung. The transfer reaction was terminated by centrifugation at 10 000 × *g* for 4 min. The mitochondrial pellet was washed once with 1 ml of ice-cold 0.25 mM sucrose/1 mM EDTA/10 mM Tris-HCl buffer. It was subsequent-

ly dissolved in 0.2 ml buffer, transferred to a scintillation vial, and assayed for radioactivity. The transfer of phospholipids from radioactively labelled lung microsomes to lung mitochondria was performed as described by Engle et al. [6]. Labelled microsomes (0.6 mg protein) were incubated at 37°C for 1 h with unlabelled lung mitochondria (0.6 mg protein) and pH 5.1 supernatant. Incubations without addition of supernatant served as controls. The assay was terminated by pelleting the mitochondria at 10 000 × *g* for 4 min. The pellets were washed once with 1 ml of 0.25 mM sucrose/1 mM EDTA/10 mM Tris-HCl buffer. Lipids were extracted from the mitochondria [19] and phospholipids were isolated by two-dimensional thin-layer chromatography as described before [20]. The various phospholipids were assayed for radioactivity by liquid scintillation counting. Protein was determined according to the method of Lowry et al. [21] with bovine serum albumin as standard.

Table I shows that pH 5.1 supernatant from type II cells catalysed the transfer of phosphatidylcholine and phosphatidylglycerol between liposomes and rat lung mitochondria. This transfer was linear with incubation time for

TABLE I

TRANSFER OF VARIOUS PHOSPHOLIPIDS BETWEEN LIPOSOMES OR RAT LUNG MICROSOMES AND RAT LUNG MITOCHONDRIA IN THE PRESENCE OF pH 5.1 SUPERNATANT FROM WHOLE RAT LUNG AND RAT LUNG TYPE II CELLS

The transfer of phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine was assayed using 18 and 300 μg of supernatant protein from type II cells and whole lung, respectively. Data are averages (± S.E.M.) of three experiments, each carried out in duplicate. PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine. n.d., not determined.

Donor membranes	Acceptor membranes	pH 5.1 Supernatant	Transfer of phospholipids (%/100 μg protein per h)		
			PC	PG	PE
Liposomes	mitochondria	type II cells	52.6 ± 1.9	35.5 ± 1.2	n.d.
		whole lung	3.1 ± 0.1	2.3 ± 0.6	n.d.
Microsomes	mitochondria	type II cells	18.3 ± 1.4	22.0 ± 3.3	19.8 ± 6.0
		whole lung	1.1 ± 0.1	1.6 ± 0.4	1.5 ± 0.4

at least 1 h and was proportional to protein concentration up to 20 μg of supernatant protein. The transfer of both phosphatidylcholine and phosphatidylglycerol between the liposomes and mitochondria was also stimulated by the pH 5.1 supernatant from whole lung. However, the transfer activities in supernatant from type II cells were at least an order of magnitude greater than those in whole lung supernatant. It can also be seen that pH 5.1 supernatant from type II cells catalysed the transfer of phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine from [1,3-^{3}H]glycerol-labelled lung microsomes to lung mitochondria. This transfer was proportional to protein concentration up to 20 μg and was linear with incubation time up to at least 1 h. The pH 5.1 supernatant from whole rat lung also enhanced the transfer of the various phospholipids between lung microsomes and mitochondria. With this assay system, we again observed that, compared to whole lung, type II cells are highly enriched in transfer activity. The transfer activities in supernatant from type II cells were about 16 times greater than those in whole lung supernatant. Although it seems unlikely it is possible that transfer activities in supernatant from whole lung were damaged by active protease(s) during the

isolation of supernatant. In that case, the enrichment in transfer activity of type II cells would be overestimated. Therefore, pH 5.1 supernatant from type II cells (50 μg) was preincubated with whole lung homogenate (120 μg) for 3 h to investigate the possibility of such an effect of protease activity on the transfer of phospholipids in type II cells. It was found, however, that the transfer activities of type II cells were not influenced by this preincubation.

In other experiments (not shown) it was found that rat lung lamellar bodies can also accept phospholipids from labelled microsomes in the presence of pH 5.1 supernatant from either whole lung or type II cells. Also, in this case the transfer activity of type II cells was far greater than that of whole lung.

In a recent study [10] we showed the presence of specific phosphatidylcholine- and phosphatidylglycerol-transfer proteins in whole rat lung by Sephadex chromatography of pH 5.1 supernatant. The suggestion was made that these proteins may contribute to the biogenesis of lamellar bodies. In order to examine the correctness of this assumption, the pH 5.1 supernatant from type II cells was applied to a Sephadex G-75 column and eluted with 50 mM NaCl/2 mM potassium phosphate, pH 7.0, at a flow rate of 4 ml per h (Fig.1). The phosphatidylcholine-, phosphatidylglycerol- and phosphatidylethanolamine-transfer activities emerged as a single peak. However, when pH 5.1 supernatant from whole lung was chromatographed on the same Sephadex G-75 column, a similar profile was observed as published earlier [10]: a non-specific fraction catalysing the transfer of phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine with the same elution volume as the transfer activity shown in Fig.1, was preceded by a specific phosphatidyl-

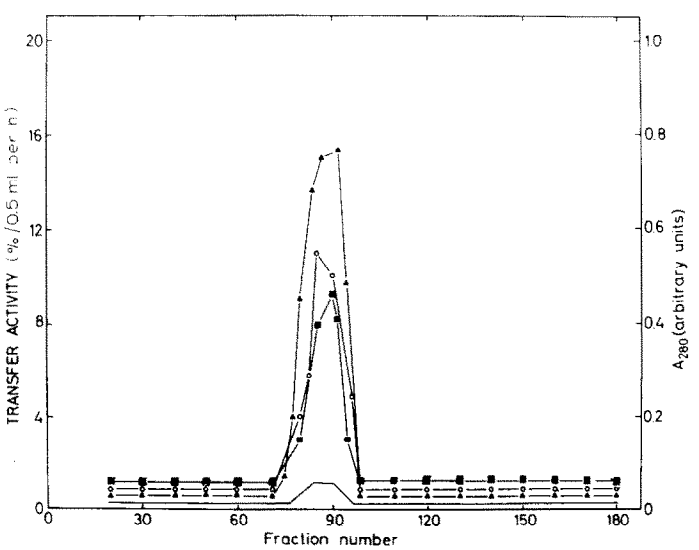

Fig.1. Chromatography of pH 5.1 supernatant from rat lung type II cells on Sephadex-G 75. The column (1 X 100 cm) was eluted with 0.002 M potassium phosphate buffer (pH 7.0) containing 0.05 M NaCl; fractions of 1 ml were collected. Protein (——) was measured by absorbance at 280 nm, and phosphatidylcholine (■——■), phosphatidylglycerol (○——○) and phosphatidylethanolamine (▲——▲) transfer activities were determined using the liposome/mitochondria assay. Aliquots of 0.5 ml were assayed.

choline-transfer protein and followed by a specific phosphatidylglycerol-transfer protein. The chromatography of pH 5.1 supernatant from type II cells on Sephadex G-75 suggests that in these cells only a non-specific transfer protein is present. It is tempting to speculate that this activity in type II cells (Fig.1) represents the non-specific transfer protein found in rat liver by Bloj and Zilversmit [22], although the present findings do not allow the conclusion that the phosphatidylcholine-, phosphatidylglycerol- and phosphatidylethanolamine-transfer activities reside in the same protein.

The far greater phospholipid-transfer activity in the type II cells as compared to whole lung endorses earlier suggestions [6—10] that transfer proteins play a role in the transport of surfactant phospholipids.

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References

1 King, R.J. and Clements, J.A. (1972) Am. J. Physiol. 223, 715—726
2 Hallman, M. and Gluck, L. (1975) Biochim. Biophys. Acta 409, 172—191
3 Goerke, J. (1974) Biochim. Biophys. Acta 344, 241—261
4 Batenburg, J.J. and van Golde, L.M.G. (1979) in Reviews in Perinatal Medicine (Scarpelli, E.M. and Cosmi, E.V., eds.), Vol. 3, pp. 73—114, Raven Press, New York
5 Askin, F.B. and Kuhn, C. (1971) Lab. Invest. 25, 260—268
6 Engle, M.J., van Golde, L.M.G. and Wirtz, K.W.A. (1978) FEBS Lett. 86, 277—281
7 Robinson, M.E., Wu, L.N.Y., Brumley, G.W. and Lumb, R.H. (1978) 87, 41—44
8 Spalding, J.W. and Hook, G.E.R. (1979) Lipids 14, 606—613
9 Whitlow, C.D., Pool, G.L., Brumley, G.W. and Lumb, R.H. (1980) FEBS Lett. 113, 221—224
10 Van Golde, L.M.G., Oldenburg, V., Post, M., Batenburg, J.J., Poorthuis, B.J.H.M. and Wirtz, K.W.A. (1980) J. Biol. Chem. 255, 6011—6013
11 Batenburg, J.J., Longmore, W.J. and van Golde, L.M.G. (1978) Biochim. Biophys. Acta 529, 160—170
12 Post, M., Batenburg, J.J. and van Golde, L.M.G. (1980) Biochim. Biophys. Acta 618, 308—317
13 Mason, R.J., Williams, M.C., Greenleaf, R.D. and Clements, J.A. (1977) Am. Rev. Respir. Dis. 115, 1015—1026
14 Mason, R.J., Williams, M.C. and Dobbs, L.G. (1977) in Pulmonary Macrophage and Epithelial Cells, Proc. Annu. Hanford Biol. Symp. 16th, Energy Res. Dev. Admin. Symp. Ser. 43 (Sanders, C.L., Schneider, R.P., Dagle, G.E. and Ragan, H., eds.), pp. 280—297, Technical Information Center, Energy Research and Development Administration, Springfield, VA
15 Mason, R.J. and Williams, M.C. (1977) Am. Rev. Respir. Dis. 115, 81—91
16 Vereyken, J.M., Montfoort, A. and van Golde, L.M.G. (1972) Biochim. Biophys. Acta 260, 70—81
17 Kamp, H.H. and Wirtz, K.W.A. (1974) in Methods in Enzymology (Fleischer, S. and Packer, L., eds.), Vol.32, pp. 140—146, Academic Press, New York
18 Batenburg, J.J., Post, M., Oldenborg, V. and van Golde, L.M.G. (1980) Exp. Lung Res. 1, 57—65
19 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911—917
20 Poorthuis, B.J.H.M., Yazaki, P.J. and Hostetler, K.Y. (1976) J. Lipid Res. 17, 433—437
21 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
22 Bloj, B. and Zilversmit, D.B. (1977) J. Biol. Chem. 252, 1613—1619