TRANSFER OF PHOSPHOLIPIDS BETWEEN SUBCELLULAR FRACTIONS OF THE LUNG

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1. Introduction

Although it has been recognized in recent years that the lung is a multi-functional organ, its principal function remains that of gas exchange. In order to perform this function efficiently, the lungs produce a substance known as pulmonary alveolar surfactant which lowers the surface tension in the alveoli at end-respiratory volume thus preventing lung collapse or atelectasis [1,2]. It is generally accepted that dipalmitoylphosphatidylcholine is the major active component of pulmonary surfactant [3].

The alveolar type II epithelial cell of the lung is probably the sole source of surfactant (detailed [2,4]). This particular cell type is characterized by the presence of a unique subcellular organelle, the lamellar body. It is believed that surfactant is stored in the lamellar bodies prior to its excretion from the type II cell [5]. Autoradiography studies [6] have shown that the bulk of phosphatidylcholine (PC) is synthesized in the endoplasmic reticulum of the type II cell and thence transferred to the lamellar bodies. The absence of de novo synthesis of PC in lamellar bodies was supported recently by enzymatic studies on these organelles [7,8]. The mechanism by which phospholipids are transported from their site of synthesis to the lamellar bodies is unknown. Soluble proteins denoted as phospholipid-exchange proteins which catalyze the intracellular transfer of phospholipids have been described for several mammalian tissues such as liver, brain and intestine (reviewed [9,10]). The possibility exists that such proteins may be involved in the transfer of surfactant phospholipids from endoplasmic reticulum to the lamellar bodies.

The present study shows that the lung cytosol does contain protein(s) which catalyze the transfer of PC, not only between microsomes and mitochondria but also between microsomes and lamellar bodies. Interestingly, during the later stages of development of the fetal lung the profile of the specific activity of PC transfer runs parallel with that of cholinephosphotransferase which catalyzes the final step in the de novo synthesis of PC.

2. Materials and methods

2.1. *Preparation of subcellular fractions from adult rat and mouse lung and from the developing mouse lung*

Lung microsomes were isolated as in [11]. All microsomal pellets were washed by resuspension in 10 mM Tris–HCl (pH 8.6) then in 1 mM Tris–HCl (pH 8.6). The final pellets were suspended in 2 ml ice-cold 0.25 M sucrose/1 mM EDTA/10 mM Tris–HCl (pH 7.4) (SET). Radioactively-labelled microsomes from rat lung were prepared by intravenous injection of 40 μCi [*Me*-^{14}C] choline, 0.5 mCi [9,10-$^{3}H_2$] palmitic acid or 0.5 mCi $^{32}P_i$ into the tail vein; the lungs were removed after 4 h, 2 h or 20 h, respectively, rinsed in ice-cold SET and the microsomes were isolated. Labelled microsomes from mouse lung were prepared by injection of 10 μCi [*Me*-^{14}C]choline into the tail vein of each of a group of 10 mice and isolation of the microsomes after 2 h. Unlabelled mitochondria

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were isolated from rat or mouse lung by centrifuging a 10% homogenate at 600 × *g* for 10 min. The supernatant is then spun at 1400 × *g* for 10 min after which the mitochondria could be precipitated by spinning the 1400 × *g* supernatant at 5900 × *g* for 10 min. The mitochondrial pellets were washed 3 times by resuspension in SET. The final pellets were resuspended in 2 ml SET and always used on the day of preparation. Lamellar bodies were prepared from rat lung exactly as in [12]. The 100 000 × *g* supernatant fractions from adult rat or mouse lung were isolated as in [11]. These supernatants were adjusted to pH 5.1 with 3 M HCl. After standing in ice from 15–30 min with occasional stirring, the suspensions were centrifuged at 15 000 × *g* for 15 min. The supernatants were then adjusted to pH 7.4 with solid Tris. These pH 5.1 supernatants were always used on the day of preparation. The 100 000 × *g* supernatant fractions of developing mouse lungs were isolated from groups of 60–90 fetuses and from 40–50 day 3 newborns [13].

2.2. *Partial purification of the phospholipid-exchange protein from rat lung*

A 100 000 × *g* supernatant was prepared from a 25% (w/v) homogenate of 2 rat lungs [11]. The supernatant (6 ml) was applied to a Sephadex G-50 column (90 × 2.2 cm) and the protein eluted with 50 mM sodium chloride/10 mM potassium phosphate (pH 6.8) at a flow rate of 10 ml/h. Fractions of 2 ml were collected and assayed for PC transfer activity as in [14]. Activity appeared in the eluent at about 1.4-times the void volume (fig.1). Fractions 109–122 were pooled and dialyzed overnight against 100 vol. SET.

2.3. *Assay of phospholipid-transfer activity*

Radioactively labelled lung microsomes from rat (or mouse) lung were incubated with unlabelled lung mitochondria or lamellar bodies in the absence or presence of pH 5.1 supernatant or the partially purified exchange protein from rat lung. In some experiments, the partially purified exchange protein from rat lung was compared with a pure PC-exchange protein from bovine liver [15]. The detailed compositions of the incubations are included in the legends of fig.2 and tables 1,2. All incubations were performed in a shaking water-bath at 37°C for 30 min in total vol. 2 ml SET unless stated otherwise. The incubations were started by the addition of the labelled microsomes and terminated by immersion in ice. Mitochondria were isolated from the incubation medium by centrifugation at 15 000 × *g* for 5 min. The mitochondria were washed twice by resuspending the pellet in 1 ml SET. Microsomes were isolated from the first 15 000 × *g* supernatant by centrifugation at 100 000 × *g* for 1 h. Lamellar bodies were separated from microsomes and supernatant by centrifugation at 15 000 × *g* for 5 min. The pellets were washed twice by resuspension in 1 ml SET.

2.4. *Analyses*

Lipids were extracted from the various fractions by the method in [16] and subsequently chromatographed on silica-gel H thin-layer plates using chloroform/methanol/acetic acid/water (25:15:4:2, by vol.) as developing solvent. The various phospholipids were assayed for radioactivity via liquid-scintillation counting and the amounts of inorganic phosphorus estimated as in [17]. The various molecular classes of PC were resolved by argentation thin-layer chromatography after conversion into 1,2-diacyl-*sn*-glycerols [18]. Protein determinations were carried out by the method in [19].

3. Results

The pH 5.1 supernatant fraction of rat lung was found to catalyze the transfer of PC between ^{32}P-labelled rat-lung microsomes and unlabelled rat-lung mitochondria at a rate of 0.12 nmol/min/mg supernatant protein (for incubation conditions see legend to table 1). The transfer activity was linear with protein concentration over the range tested (0–4 mg protein) and transfer proceeded at almost linear rates up to 30 min. Partial purification of the phospholipid-exchange proteins from rat lung was achieved by gel filtration of the 100 000 × *g* supernatant on a Sephadex G-50 column (fig.1). The pooled fractions (109–122) transported PC at a rate of 1.46 nmol/min/mg protein.

Table 1 shows that this fraction does not only catalyze the transfer of PC between ^{32}P-labelled microsomes and unlabelled mitochondria but also that of phosphatidylinositol (PI); transfer of phos-

Table 1
Transfer of various phospholipids between rat-lung microsomes and mitochondria in the presence of either partially purified exchange protein from lung supernatant or pure phosphatidylcholine-exchange protein from bovine liver

Addition	PC	PI	PE
	(% transferred to mitochondria)		
Exchange protein(s) from lung	8.1 ± 0.7	10.8 ± 0.4	0.3 ± 0.2
Exchange protein from liver	20.0 ± 0.9	–	1.1 ± 0.2

The incubation mixture contained 0.58 mg microsomal protein, 0.62 mg mitochondrial protein and either 0.21 mg exchange protein from lung supernatant or 2.7 μg phosphatidylcholine-exchange protein from bovine liver. Results are expressed as % ^{32}P-labelled microsomal phospholipids transferred to mitochondria in 30 min incubation. The activities of the microsomal phospholipids were: PC, 6345 dpm; PI, 1089 dpm; PE, 3117 dpm. Incubations without addition of supernatant proteins served as controls

phatidylethanolamine (PE) was negligible. For comparison the PC-exchange protein from bovine liver was also incubated with the lung microsomes and mitochondria. In agreement with [15] this protein only catalyzes the transfer of PC.

Table 2 shows that not only mitochondria but also lamellar bodies from rat lung can function as acceptor membrane for PC donated by [*Me*-^{14}C]-choline-labelled microsomes. Under conditions where the incubation contained equal amounts of mitochondrial or lamellar body PC, the amount of microsomal PC taken up by the lamellar bodies was

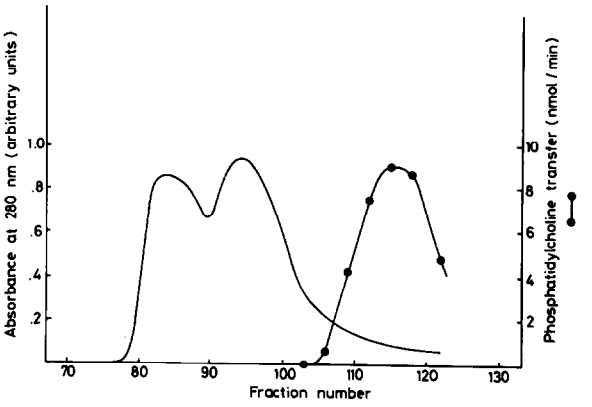

Fig.1. Fractionation of the 100 000 × *g* supernatant from rat lung on a Sephadex G-50 column (90 × 2.2 cm). For experimental details see section 2.

Table 2
Transfer of phosphatidylcholine between [*Me*-^{14}C]choline-labelled microsomes and either mitochondria or lamellar bodies from rat lung in the presence of partially purified exchange protein from rat lung supernatant

Acceptor organelles	PC transferred (nmol/min/mg prot)
Mitochondria	1.46
Lamellar bodies	0.22

The incubation mixture contained 0.46 mg microsomal protein (0.30 μmol PC), 0.59 mg mitochondrial protein (0.27 μmol PC) or 0.06 mg lamellar body protein (0.23 μmol PC) and 0.31 mg partially purified exchange protein. Incubations without exchange protein served as controls

Table 3
Exchange of various molecular classes of PC between [9,10-$^{3}H_2$]palmitate-labelled microsomes and unlabelled mitochondria in the presence of partially-purified exchange protein from rat lung supernatant

Classes of PC	Microsomal PC		PC transported to mitochondria	
	dpm	distribution (%)	dpm	distribution (%)
Disaturated	1440	67.9	195	44.8
Monoenoic	460	21.7	100	22.9
Dienoic	220	10.4	140	32.2

Incubations were carried out as described in the legend of table 2. The total radioactivity microsomal PC 2360 dpm

about 16% of that recovered in the mitochondria.

It was thought of interest to investigate whether the partially purified protein from rat lung shows a preference for any particular class of PC (table 3). In agreement with [20] it was found that after the administration of [9,10-$^3H_2$]palmitic acid, the majority of radioactivity of the microsomal PC was recovered in the disaturated class. Although the mono- and dienoic molecules became also significantly labelled, only very small amounts of radioactivity were measured in the polyenoic fractions (>Δ2). Comparison of the distribution of radioactivity among the various classes of transported PC with that of the original microsomal PC showed that the exchange protein from lung catalyzed the transfer of all classes of PC from microsomes to mitochondria, although there may be some preference for unsaturated PC molecules. A similar preference was observed for the pure PC-exchange protein from bovine liver (not shown).

Figure 2 shows the profile of the specific activity for the PC transfer of the lung supernatant during the development of this organ. For these experiments the mouse lung rather than rat lung was chosen since pregnant mice with an exactly timed gestation were available to us. The specific activity of the adult mouse-lung supernatant agreed with that observed for the rat lung. It can be seen in fig.2 that the specific activity for PC transfer increases on day 16 of gestation and reaches a maximum on day 17 (i.e., 2 days before birth) after which it declines again. A similar pattern had been observed [13] for the specific activity of cholinephosphotransferase, the enzyme that catalyzes the last step in the synthesis of PC [21].

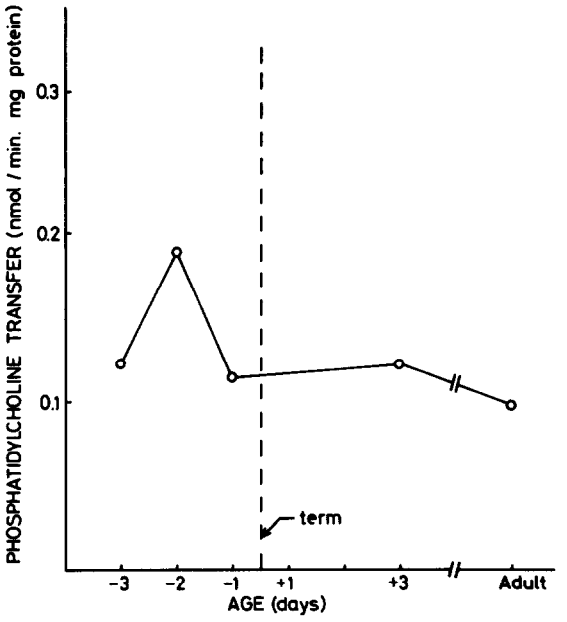

Fig.2. Specific activity of protein(s) that catalyze the transfer of PC in the 100 000 × *g* supernatant of the developing mouse lung. Aliquots of 2 mg supernatant protein, obtained from the lung at different stages of development, were incubated in the presence of 0.46 mg [*Me*-^{14}C]choline-labelled microsomes from adult mouse lung as donor membranes and 0.60 mg unlabelled mitochondria from adult mouse lung as acceptor membranes in total vol. 2.0 ml.

4. Discussion

As demonstrated for a variety of other tissues [9,10], the 100 000 × *g* supernatant of the lung contains protein(s) which can stimulate the transfer of PC and PI from their site of synthesis, the microsomes, to mitochondria. A protein fraction which catalyzes the transfer of these two phospholipids eluted from a Sephadex G-50 column with an elution volume that indicates an approx. mol. wt 15 000. This is in the same range as the phospholipid-exchange proteins purified from rat liver [22–24].

Of particular interest is the observation that lamellar bodies, the unique organelles of the type II cell, can also accept phospholipids from the exchange proteins. In interpreting the differences between mitochondria and lamellar bodies as acceptor organelles (table 2), it should be realized that, although the total amounts of mitochondrial and lamellar body PC in the incubation are equal, the pool of PC which is accessible for exchange with microsomal PC is much smaller for the lamellar body. Whereas the amount of PC in the outer mitochondrial membrane represents a considerable portion of the total mitochondrial PC, the amount of PC in the membrane surrounding the lamellar body is certainly much smaller than the amount of PC stored inside this organelle [5,12].

The results of table 3 show that the various classes of PC can be transferred from microsomes to mitochondria. The disaturated molecules are, however, exchanged to a somewhat smaller extent than the

unsaturated species which agrees with [25] where dipalmitoyl-PC was transferred by the PC-exchange protein from bovine liver provided this molecular species is present in a donor membrane containing unsaturated PC. Sonicated liposomes consisting of only dipalmitoyl-PC did not function as donor membranes [25], which agrees with [26].

It is well established that the synthesis of surfactant PC is turned on during the last period of fetal lung development [1,2,4]. Enzyme studies have shown that the specific activity of cholinephosphotransferase starts to increase on day 16 of gestation in the fetal mouse lung and reaches a maximum on day 17, after which it declines again [13]. Similar results have been reported for the developing rat lung [27]. It is intriguing to note that this pattern for cholinephosphotransferase is paralleled by similar changes in the specific activity of the protein(s) that catalyze the transfer of PC from microsomes to mitochondria (or lamellar bodies). This is the first indication that a relationship may exist between the biosynthetic capacity of a cell and its ability to redistribute lipids between its subcellular organelles.

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