Biochem. J. (1988) 253, 209–215 (Printed in Great Britain)

Molecular species of phosphatidylcholine and phosphatidylglycerol in rat lung surfactant and different pools of pneumocytes type II

Michael SCHLAME,*†§ Cristina CASALS,‡ Bernd RÜSTOW,* Harald RABE* and Dietrich KUNZE*

*Institute of Pathological and Clinical Biochemistry, and †Department of Anaesthesiology and Intensive Care Medicine, Charite Hospital, Humboldt University, Berlin, German Democratic Republic, and ‡Department of Biochemistry, Faculty of Chemistry, Complutense University, Madrid, Spain

It is not yet completely understood how a cell is able to export specific phospholipids, like dipalmitoylphosphatidylcholine (dipalmitoyl-PC), which is secreted by pneumocytes type II, into pulmonary surfactant. The acyl species composition of [^{3}H]PC which was synthesized in type II cells in the presence of [2-^{3}H]glycerol resembled the species composition of PC localized in intracellular pneumocyte membranes. This species pattern was different from the pattern of PC of lamellar bodies, i.e., intracellularly stored surfactant, by a higher proportion of dipalmitoyl-PC mainly at expense of 1-palmitoyl-2-oleoyl-PC. Lamellar body PC in turn showed the same species distribution as surfactant PC. The data suggest that subcellular compartmentation and/or intracellular transfer of PC destined to storage in lamellar bodies, but not secretion of lamellar bodies, involves an enrichment of dipalmitoyl-PC and a depletion of 1-palmitoyl-2-oleoyl-PC. In contrast, the acyl species pattern of phosphatidylglycerol does not seem to undergo gross changes on the path from synthesis to secretion.

INTRODUCTION

Dipalmitoylphosphatidylcholine (dipalmitoyl-PC) and phosphatidylglycerol (PG) are the most distinctive phospholipids of pulmonary surfactant (King, 1984), a surface tension-reducing lipoprotein, which are synthesized in and secreted by pneumocytes type II (Batenburg, 1984; Possmayer *et al.*, 1984; Rooney, 1985). This cell has proved to be an appropriate model to investigate intracellular mechanisms of lipid synthesis and lipid compartmentation because it has to realize synthesis and storage of a specific population of lipids which are destined for secretion into the alveolar space. One of the most intriguing questions is how pneumocytes type II are able to enrich a unique phospholipid species, like dipalmitoyl-PC, to become the major molecule of surfactant.

Recent studies have demonstrated that dipalmitoyl-PC is synthesized *de novo* in the intracellular membranes of pneumocytes type II but the percentage of dipalmitoyl species is further increased by a species-selective deacylation and reacylation cycle (Mason & Dobbs, 1980; Post *et al.*, 1983; Mason & Nellenbogen, 1984). The next step of surfactant assembly represents the formation and maturation of lamellar bodies where the surface-active material is stored until secretion (Possmayer *et al.*, 1984). There are mainly two possibilities to consider in the PC transfer from intracellular membranes to lamellar bodies: transfer via Golgi complex, or via phospholipid transfer proteins (Rooney, 1985). It is not known if the transfer of PC as well as its storage in lamellar bodies is accompanied by a further enrichment of dipalmitoyl species. This poses the problem of whether PC, which is produced for assembly of intracellular pneumocyte membranes, has the same acyl species pattern as PC destined to surfactant.

In this paper we report the almost complete acyl species composition of PC in surfactant (PC after secretion), in lamellar bodies (PC of intracellular surfactant, i.e., PC before secretion) and in whole pneumocytes type II (mixed PC of intracellular membranes and intracellular surfactant). [2-^{3}H]Glycerol was incorporated into PC of pneumocytes type II in order to compare the acyl species composition of newly generated [^{3}H]PC with the composition of both PC of intracellular surfactant (lamellar bodies) and PC of intracellular membranes which were isolated from type II cells. Parallel investigation of the acyl species pattern of PG, the second characteristic phospholipid of surfactant, is also reported.

MATERIALS AND METHODS

Materials

The present work was carried out with male Wistar rats (180–250 g) which were fed *ad libitum*. Special care was taken that only lungs appearing completely white after perfusion, without visible pathological alterations, were processed for further experiments. Trypsin, NADPH, sodium succinate, cytochrome *c* from horse heart, *Bacillus cereus* phospholipase C and Tris were obtained from Boehringer (Mannheim, Germany). [2-^{3}H]Glycerol (sp. radioactivity 1 Ci/mmol) was purchased from The Radiochemical Centre (Amersham, U.K.). Silica gel HR was from Merck (Darmstadt, Germany), florisil and Hepes from Serva (Heidelberg,

Abbreviations used: PC, phosphatidylcholine; PG, phosphatidylglycerol; fatty acyls are indicated as number of carbon atoms:number of double bonds.

§ To whom correspondence should be addressed at: Institut für Pathologische und Klinische Biochemie, Bereich Medizin (Charite) der Humboldt Universität, Schumannstrasse 20/21, 1040 Berlin, German Democratic Republic.

Germany), percoll (silica-polyvinylpyrrolidinone) was from Pharmacia (Uppsala, Sweden), *Clostridium welchii* phospholipase C from Sigma (St. Louis, MO, U.S.A.), soya-bean trypsin inhibitor from Reanal (Budapest, Hungary), 2,2′-*p*-phenyl-bis(5-phenyloxazole) from Riedel-de-Haen AG Seelze (Hannover, Germany) and 2,5-diphenyloxazole from Packard–Becker B.V. Chemical Co. (Groningen, Germany). Minimal essential medium and fetal-calf serum were purchased from a local supplier (Staatliches Institut für Immunpräparate und Nährmedien, Berlin, G.D.R.). α-Naphthyl isocyanate was purified by distillation *in vacuo*. All other chemicals were commercially obtained in analytical grade.

Isolation of surfactant and lamellar bodies

Surfactant was obtained from rat lung lavage fluid as described previously (Dethloff *et al.*, 1986). Post-lavaged lung tissue was gently homogenized in NaCl buffer (0.15 M-NaCl/10 mM-Tris (pH 7.4)/0.1 mM-EDTA) using a Teflon/glass Potter–Elvehjem. The homogenate was spun at 1000 $g_{av.}$ for 20 min and the remaining supernatant at 19000 $g_{av.}$ for 30 min. The 19000 g pellet was resuspended in 10 ml of NaCl buffer and layered over 10 ml of NaCl buffer containing 0.75-M-sucrose. Centrifugation at 12000 $g_{av.}$ for 10 min yielded a crude lamellar body fraction at the interface. Crude lamellar bodies were sedimented and resuspended in 2 ml of 1 M-sucrose and were further purified by upward-flotation density-gradient ultracentrifugation exactly as described by Chander *et al.* (1983).

Isolation of pneumocytes type II

Type II pneumocytes were dissociated from two rat lungs according to Mason *et al.* (1977) and purified by percoll density-gradient centrifugation as described by Skillrud & Martin (1984). Cells were incubated in 5 ml of Dulbecco's modified minimal essential medium containing 20 mM-Hepes (pH 7.4) and 10% (v/v) fetal-calf serum to remove macrophages by differential adhesion on plastic. After 1 h non-adherent cells were used for further procedures. By this method we collected 4–12 million cells (determined with a haemacytometer). More than 90% of them were viable according to both Trypan Blue exclusion (Mason *et al.*, 1977) and lactic dehydrogenase latency (Bergmeyer, 1970).

Histochemical staining of alkaline phosphatase provided an appropriate tool for current control of pneumocyte preparations, because pneumocytes type II are distinguished from other lung cells by this histological marker (Eckert, 1982). Medium for phosphatase reaction containing phosphorylated naphthol AS was prepared according to Lojda *et al.* (1964) but pH was shifted to 8. The solution was supplemented by 1 mg of Fast Blue BB/ml, filtered and layered over an acetone-fixed smear of the cell preparation. After 30 min incubation at 37 °C pneumocytes type II were marked by alkaline phosphatase reaction appearing with red cytoplasm and visible inclusion bodies. Other cells, in particular macrophages and lymphocytes, which account for most of the contaminating cells (Mason *et al.*, 1977) remained pale yellow. Employing this method the purity of pneumocyte type II preparations ranged from 70 to 85%. This result could be confirmed by electron microscopy as well as the modified Papanicolaou stain (Kikkawa & Yoneda, 1974), the most established procedure to judge pneumocyte preparations. The type II pneumocyte content was somewhat lower than given by other laboratories purifying the cells in primary culture. However, culturing may lead to alterations of the pneumocyte lipid composition (Mason & Dobbs, 1980).

Isolation of heavy pneumocyte membranes

For isolation of intracellular pneumocyte membranes enriched in microsomes and mitochondria but depleted of low-density material such as lamellar bodies, cells of three pooled preparations, frozen in hypotonic sucrose (50 mM-sucrose/10 mM-Hepes (pH 7.4)/0.25 mM-EDTA), were destroyed by two thawing-freezing cycles with intermediate homogenization (20 strokes by a Teflon/glass Potter–Elvehjem). Isotonic condition was re-established by 1:1 dilution with 0.5 M-sucrose and the homogenate was spun at 170 $g_{av.}$ for 5 min. The pellet was resuspended in hypotonic sucrose and was again subjected to the described procedure. Heavy membranes were sedimented (80000 $g_{av.}$, 30 min) from the combined 170 g supernatants after the sucrose concentration had been adjusted to 0.6 M. It is evident that these centrifugation conditions were far from allowing sedimentation of lamellar bodies or surfactant-related material due to their low density (see e.g. Chander *et al.*, 1983; King, 1984).

Lipid analysis

Lipids were extracted according to Bligh & Dyer (1959) and separated by two-dimensional t.l.c. (Schlame *et al.*, 1986) for phosphate determination (Hallermayer & Neupert, 1974). For analysis of the acyl species pattern, PC and PG were isolated by one-dimensional t.l.c. on silica gel HR + 1% florisil (w/w) developed with chloroform/acetone/methanol/acetic acid/water (10:4:2:2:1, by vol.). Phospholipids were extracted from silica gel spots using chloroform/methanol (2:1, v/v) and the extracts were washed with 0.5 M-Tris (pH 7.2) to neutralize traces of acetic acid. After evaporation under a stream of nitrogen, phospholipids were redissolved in ether/ethanol (98:2, v/v). Complete hydrolysis of PC to diacylglcyerol using phospholipase C from *Clostridium welchii* (Kates, 1972) or of PG by means of phospholipase C from *Baccillus cereus* (Schlame *et al.*, 1986) was described. Diacylglycerols were derivatized with α-naphthyl isocyanate and separated on RP 18 (5 μm) h.p.l.c. columns using a Hewlett Packard model 1084B equipped with a fluorescence detector. The details of derivatization and h.p.l.c. procedure (Krüger *et al.*, 1984; Rüstow *et al.*, 1987) and the specific applications to either PC (Rüstow *et al.*, 1985, 1987) or PG (Schlame *et al.*, 1986) were given in preceding papers. Examination of the elution characteristics of the h.p.l.c. column used for this study revealed the localization of three minor components (18:2–20:4, 18:1–18:2 and 18:1–18:1) in other peaks as indicated in our earlier reports (Rüstow *et al.*, 1985; Schlame *et al.*, 1986). Isomerization of 1,2-diacylglycerols to 1,3-diacylglycerols, whose urethanes are eluted with lower retention time, was determined to be lower than 5% (for methodology see Krüger *et al.*, 1984; Rüstow *et al.*, 1987).

To determine the pattern of [2-^{3}H]glycerol-labelled species, individual peaks were collected and then were processed for counting of radioactivity by evaporation of the h.p.l.c. solvent with subsequent addition of 8 ml of

Table 1. Characterization of preparations from rat lung

Isolated fractions were subjected to lipid extraction for determination of total phospholipid content and percentage of PC, PG, PE (phosphatidylethanolamine), SM (sphingomyelin) and DPG (diphosphatidylglycerol). Other minor phospholipids are not listed. Aliquots of the preparations were used for measurement of protein concentration (Lowry *et al.*, 1951) and activity of acid phosphatase with the substrate 4-nitrophenyl phosphate (Bergmeyer, 1970). All values represent single determinations. n.d., not determined.

Source	Acid phosphatase (nmol/min·mg of protein)	Phospholipid/protein ratio (μmol/mg of protein)	Phospholipid composition (%)					
			PC	PE	PG	SM	DPG	Others
Surfactant	1.7	5.1	82	3	9	2	0	4
Lamellar bodies	9.2	5.4	74	5	13	2	0	5
Pneumocytes type II	n.d.	0.2	58	16	7	9	1	8

toluol/ethanol (99:1, v/v) containing diphenyloxazole (4 g/l) and 2,2′-*p*-phenylbis-(5-phenyloxazole) (40 mg/l). Radioactivity was measured with a Philips liquid-scintillation counter (model PW4700). Due to the low yield of PG in the pneumocyte preparations, [^{3}H]PG-derived species were supplemented with unlabelled tracer diacylglycerol (obtained by hydrolysis of rat lung PC) to facilitate the detection of h.p.l.c. peaks during sample collection.

In order to determine the percentage of PC radioactivity which was associated with the acyl moiety, isolated PC was subjected to alkaline hydrolysis by heating (60 °C, 2 h) with 1 M-KOH in ethanol/water (95:5, v/v). The saponified fatty acids were extracted with n-hexane and radioactivity was counted in the n-hexane and ethanol/water-phase respectively.

Fatty acid pattern was analysed by gas chromatography of fatty acid methyl esters, produced according to Morrison & Smith (1964), by means of a Varian 2100 using a coiled glass column with 10% EGSS-X (Krüger *et al.*, 1984).

RESULTS

Characterization of preparations

According to the data given in Table 1 the preparations met the characteristics reported of purified surfactant and lamellar bodies, marked by their high phospholipid/protein ratio and the typical phospholipid composition (more than 70% PC, about 10% PG) (Chander *et al.*, 1983; Crecelius & Longmore, 1983; King, 1984). To demonstrate that surfactant and lamellar bodies are really different fractions we determined acid phosphatase which is present in lamellar bodies (in accordance with Chander *et al.*, 1983) but has much lower activity in the surfactant preparation (Table 1).

The preparation of pneumocytes type II was characterized by different cytological methods (see under 'Materials and methods'). The considerable purity of this preparation could be confirmed by the high PG content (Table 1) and the high percentage of dipalmitoyl species in PC (see Table 2), both of which were close to the respective values in surfactant and lamellar bodies. However, type II cells were distinguished from lamellar bodies by their lower phospholipid/protein ratio and their different phospholipid pattern (Table 1 in accordance with Crecelius & Longmore, 1983).

Molecular species in surfactant, lamellar bodies and pneumocytes type II

As listed in Table 2 the species compositions of PC from lamellar bodies and surfactant were very similar containing mainly 16:0 species (16:0–16:0 > 16:0–16:1 > 16:0–18:2 > 16:0–18:1). PC extracted from pneumocytes type II resembled the PC of lamellar bodies and surfactant with the exception of some differences in polyunsaturated species, i.e. 16:1-containing species and 18:0–18:1 species. The fatty acid pattern calculated from the molecular species composition of one surfactant preparation was controlled by parallel gas chromatographic examination (Table 3). Both methods provided identical distribution of the three major fatty acids. Approximately, the pattern paralleled recently published data of PC from rat surfactant (Shelley *et al.*, 1984) or lamellar bodies (Chander *et al.*, 1983). The remarkable proportion of 16:1, which proved to be the second major fatty acid after 16:0, was also described (Chander *et al.*, 1983; Shelley *et al.*, 1984). Table 2 shows that this fatty acid was mainly recovered in the 16:0–16:1 species.

The acyl species pattern of PG has already been published by our laboratory (Schlame *et al.*, 1986). The new series of experiments (Table 2) resolved a further species, 16:0–16:1, which may incidentally be retained by either neighbouring fraction. However, despite some minor differences from the earlier published data it could be confirmed that 16:0–16:0 and 16:0–18:1 are the major molecular species of surfactant PG. PG from surfactant and lamellar bodies showed almost identical species compositions but were clearly distinguished from PC, essentially by a lower ratio of 16:0–16:0 to 16:0–18:1.

Molecular species in heavy pneumocyte membranes

Heavy intracellular membranes were sedimented from homogenized type II pneumocytes by a procedure designed to remove light lamellar body material. Table 4 indicates that PC of these membranes, which were enriched in the marker enzymes of endoplasmic reticulum (NADPH:cytochrome *c* reductase) and mitochondria (succinate:cytochrome *c* reductase), did not meet the species composition of PC of whole pneumocytes from which the membranes were derived. The most striking

Table 2. Molecular species of PC and PG from surfactant, lamellar bodies and pneumocytes type II

Molecular acyl species (with minor components in parentheses) are arranged by increasing retention times of their respective diacylnaphthylurethanes upon h.p.l.c. separation. Percentage distribution is given as mean of n experiments with S.E.M. in parentheses.

Acyl species	PC surfactant ($n = 4$)	PC lamellar bodies ($n = 4$)	PC pneumocytes type II ($n = 3$)	PG surfactant ($n = 2$)	PG lamellar bodies ($n = 4$)
Not identified	1.4 (0.4)	2.1 (0.5)	0.1 (0.1)	0 (0)	1.9 (0.8)
14:0–16:1, 16:0–20:5	0.5 (0.2)	1.0 (0.5)	8.4 (1.5)	4.1 (0.5)	2.6 (1.7)
18:2–22:6, 18:2–20:4					
18:2–18:2 (18:1–16:1)	10.8 (0.5)	9.0 (1.5)	17.5 (3.7)	3.1 (2.5)	2.1 (2.1)
16:0–16:1	15.8 (3.0)	22.1 (2.1)		10.5 (0.1)	11.1 (1.5)
16:0–22:6, 16:0–20:4 (18:1–22:6)	3.2 (0.3)	3.7 (0.4)	14.0 (0.4)	6.1 (1.4)	7.3 (2.0)
16:0–18:2	10.1 (1.9)	11.4 (2.9)		8.6 (1.3)	16.2 (7.3)
16:0–22.5, 18:1–18:2	0.8 (0.3)	1.0 (0.3)	1.0 (0.3)	2.5 (0.1)	2.4 (0.8)
16:0–20:3	0.8 (0.8)	1.6 (1.2)	3.0 (1.8)	1.5 (0.6)	1.5 (0.5)
16:0–16:0	39.1 (4.3)	35.0 (5.3)	34.6 (2.7)	25.9 (6.7)	24.2 (3.7)
16:0–18:1	8.2 (1.2)	9.6 (0.9)	10.7 (1.7)	18.0 (0.4)	17.2 (2.8)
18:0–22:6, 18:1–18:1	0.4 (0.1)	0.3 (0.2)	0.2 (0.1)	1.8 (0.6)	1.7 (0.4)
18:0–20:4	1.4 (0.4)	1.2 (0.8)	3.7 (1.5)	3.4 (1.6)	2.1 (0.7)
18:0–18:2	0.7 (0.5)	0.6 (0.5)		1.8 (1.6)	0.6 (0.4)
18:0–22:5	1.5 (1.4)	0.2 (0.1)	0.6 (0.3)	1.5 (1.4)	0.5 (0.3)
18:0–203 (16:0–22:2)	1.6 (1.2)	0.6 (0.4)	0.8 (0.1)	2.4 (0.4)	0.9 (0.6)
18:0–16:0	0.8 (0.6)	1.2 (0.5)	3.5 (1.8)	3.9 (1.0)	2.2 (0.9)
18:0–18:1	1.6 (1.3)	0.5 (0.3)	5.2 (3.3)	4.3 (4.1)	4.7 (4.4)

Table 3. Major fatty acids of PC from rat lung surfactant

PC was isolated from one preparation of pulmonary surfactant. An aliquot was analysed by gas chromatography for fatty acid methyl esters, the other aliquot was processed for h.p.l.c. analysis of the diacylnaphthylurethanes in order to calculate the fatty acid composition from the acyl species pattern. All other fatty acids than those indicated accounted for less than 5% according to the gas chromatographic determination.

	Percentage distribution according to:	
Fatty acids	Gas chromatography of fatty acids	H.p.l.c. analysis of acyl species
16:0	63.0	63.3
16:1	10.9	9.4
18:1	5.9	5.0

difference was the higher proportion of 16:0–18:1 at the expense of 16:0–16:0. Hence, membrane PC seemed to be distinguished from surfactant PC due to a shift between these major species.

Incorporation of [2-^{3}H]glycerol into different molecular species

In order to analyse if [^{3}H]PC, which was newly generated in type II pneumocytes, was more similar to membrane PC or to surfactant PC, the cells were incubated with [2-^{3}H]glycerol for 2 h. PC incorporated radioactivity exclusively in the glycerol backbone because only 0.7% of [^{3}H] was found in the acyl moieties. The [^{3}H] radioactivity which was distributed among the individual PC species (Table 5) approached the PC acylation pattern of heavy pneumocyte membranes (Table 4) rather than of surfactant or lamellar bodies (Table 2). PG incorporated [2-^{3}H]glycerol into molecular species with similar patterns to PC with the exception of some 16:0/polyunsaturated species (Table 5). But in contrast with PC there is no gross difference between the species composition of ^{3}H-labelled PG and PG localized in surfactant (Table 2).

DISCUSSION

To our knowledge we are the first to report an almost complete, h.p.l.c. resolved, acyl species pattern of surfactant PC (Table 2). Surprisingly, the content of the major species, 16:0–16:0, was somewhat lower than the percentage of disaturation determined in other laboratories (for review see King, 1984). The degree of disaturation was mostly obtained by OsO_4 oxidation of unsaturated lipids (Mason *et al.*, 1976), a method which may lead to overestimation of disaturation due to recovery of monounsaturated species in the disaturated fraction (Chan *et al.*, 1983). However, even the separation of diacylglycerol species on $AgNO_3$-impregnated thin layers reveals somewhat higher proportions of saturated PC. Until now there has been no direct comparison of species separations by h.p.l.c. and t.l.c. in the same laboratory. Despite the higher resolution and sensitivity

Table 4. Molecular PC species in pneumocytes type II and heavy pneumocyte membranes

Three preparations of type II pneumocytes were pooled and about 70% were used for isolation of high-density pneumocyte membranes (see under 'Materials and methods'). The percentage distribution of PC acyl species was analysed in the pooled pneumocyte preparation and in the heavy membrane fraction respectively. Data of one from two independent experiments are listed. For measurement of cytochrome reductases in the whole type II cell homogenate and in the heavy membrane fraction the assay of Sottocasa *et al.* (1967) was followed using either 0.1 mM-NADPH or 3 mM-succinate as substrate.

Acyl species (%)	Whole pneumocytes type II	Heavy pneumocyte membranes
Not identified	0.1	0
14:0–16:1, 16:0–20:5, 18:2–22:6, 18:2–20:4	9.4	9.8
18:2–18.2 (18:1–16:1), 16:0–16:1	17.3	17.9
16:0–22:6, 16:0–20:4 (18:1–22:6), 16:0–18:2	14.4	14.9
16:0–22:5, 18:1–18:2	0.7	1.0
16:0–20:3	4.8	0.4
16:0–16:0	36.0	28.0
16:0–18:1	9.3	18.4
18:0–22:6, 18:1–18:1	0.2	0.1
18:0–20:4	1.1	2.9
18:0–18:2, 18:0–22:5	1.5	2.0
18:0–20:3 (16:0–22:2)	0.6	0.4
18:0–20:3 (16:0–22:2)	0.6	0.4
18:0:16:0	1.7	2.2
18:0–18:1	1.9	2.0
Enzyme activities (μmol/min·mg of protein)		
NADPH:cytochrome *c* reductase	2.4	12.8
Succinate:cytochrome *c* reductase	3.6	32.3

Table 5. Incorporation of [2-^{3}H]glycerol into molecular species of PC and PG in type II pneumocytes

Type II pneumocytes from two rats were incubated in 5 ml of minimial essential medium containing 20 mM-Hepes (pH 7.4)/10% (v/v) fetal-calf serum/0.05 mM-[2-^{3}H]glycerol (sp. radioactivity 500 d.p.m./pmol) for 2 h. During this time the cells incorporated 100–200 pmol of [2-^{3}H]glycerol into PC and 20–40 pmol of [2-^{3}H]glycerol into PG. After incubation cells were sedimented and lipids were extracted in order to analyse the percentage distribution of radioactivity among the acyl species of PC and PG. For PC S.E.M. (n = 3) is given in parentheses; values of PG represent a single experiment.

Acyl species	[^{3}H]PC (% distribution)	[^{3}H]PG (% distribution)
Not identified	1.5 (2.1)	5.5
14:0–16:1, 16:0–20:5, 18:2–22:6, 18:2–20:4	2.4 (0.9)	3.2
18:2–18:2 (18:1–16:1), 16:0–16:1	6.9 (2.7)	6.2
16:0–22:6, 16:0–20:4 (18:1–22:6), 16:0–18:2	22.6 (1.1)	10.7
16:0–22:5, 18:1–18:2	3.4 (1.6)	4.1
16:0–20:3	1.9 (1.4)	3.2
16:0–16:0	18.3 (5.0)	23.8
16:0–18:1	23.3 (1.8)	27.5
18:0–22:6, 18:1–18:1	5.6 (2.4)	3.3
18:0–20:4		1.4
18:0–18:2		2.8
18:0–22:5	0.5 (0.4)	0
18:0–20:3 (16:0–22:2)	0.4 (0.4)	0
18:0–16:0	3.5 (2.1)	5.5
18:0–18:1	5.0 (2.9)	2.9

of the h.p.l.c. method employed here, the data obtained by t.l.c. cannot be neglected *per se*. Furthermore, it must be kept in mind that surfactant acyl composition depends on animal species (Shelley *et al.*, 1984) and, perhaps, feeding conditions, both of which may influence the degree of disaturation.

The observation that the dipalmitoyl proportion of PC from pneumocytes type II approached the dipalmitoyl content of surfactant PC (Table 2) led to the following alternatives. Firstly, PC of intracellular pneumocyte membranes could have a surfactant-like configuration, or secondly, the data may reflect a large pool of intracellularly stored surfactant, i.e. lamellar bodies. The latter conclusion seemed to be valid because the PC species pattern of pneumocyte membranes, which were enriched in mitochondria and microsomes (referred to as heavy pneumocyte membranes, Table 4), could be distinguished from surfactant PC, mainly by a shift from 16:0–16:0 to 16:0–18:1. However, the difference was not extreme and the membrane PC seemed to be adapted to a considerable dipalmitoyl percentage. It must be kept in mind that, due to the heterogeneity of the heavy membrane fraction, PC of different membrane compartments may be more or less close to the species pattern of surfactant PC.

In the presence of [2-^{3}H]glycerol pneumocytes type II incorporated radioactivity into different phospholipids, e.g. PC and PG, as a function of time (data not shown). In order to analyse the molecular species of ^{3}H-labelled PC the cells were incubated for 2 h because Mason & Nellenbogen (1984) demonstrated that this incubation time allows the bulk of newly synthesized PC to undergo acyl chain remodelling. Hence we assumed the ^{3}H-species pattern to define a specific pool of PC molecules whose fate was synthesis *de novo* and subsequent acyl tail remodelling. In our experiments we found the ^{3}H radioactivity completely associated with the glycerol backbone of PC. Therefore no radioactivity could be incorporated via acylation of endogenous lyso-PC.

The species pattern of newly synthesized [^{3}H]PC was closer to the species pattern of PC in heavy intracellular membranes (Tables 4 and 5) than to surfactant PC, suggesting that pneumocytes mainly synthesize a membrane-like PC which does not exactly fit the high disaturation of surfactant PC. This implies that the subcellular processing (including compartmentation) of PC, which is destined to secretion into the alveolar space, must assert a slight selectivity preferring 16:0–16:0 over 16:0–18:1.

The ratio 16:0–16:0 to 16:0–18:1 provided a sensitive

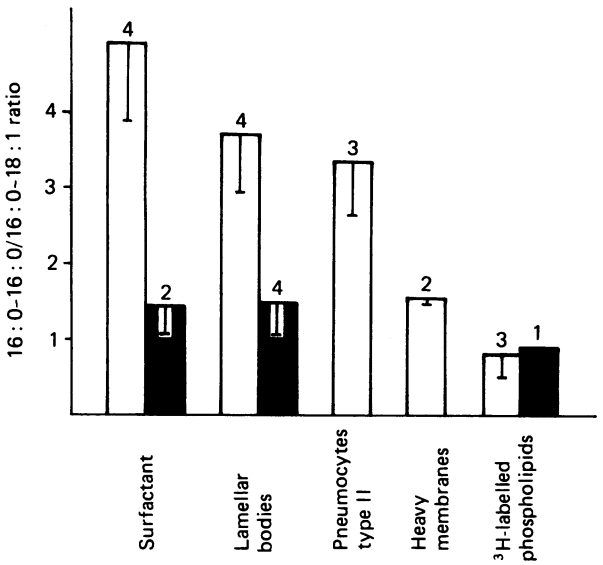

Fig. 1. Ratio of 16:0–16:0 to 16:0–18:1 species of PC and PG

The columns represent means with S.E.M. of the ratio of 16:0–16:0 species to 16:0–18:0 species in PC (open columns) and PG (closed columns) from the indicated fractions. Number of determinations is given at the top of each column.

parameter to follow the change of the PC species pattern from synthesis to secretion (Fig. 1). The stepwise rise of this ratio from newly synthesized PC toward extracellular surfactant PC was in line with a dipalmitoyl enrichment parallel to the subcellular processing. The most striking drop of the ratio was seen between lamellar bodies and heavy pneumocyte membranes (Fig. 1). Therefore the subcellular processing resulting in assembly of lamellar bodies, but not secretion of lamellar bodies, was thought to realize the species selection in order to enrich 16:0–16:0 at the expense of 16:0–18:1.

Because we do not completely understand how PC is transferred from intracellular membranes to lamellar bodies, there remain numerous possibilities to explain the mechanism of this species selection. One principal possibility of selection *a priori* is that surfactant phospholipids are synthesized in a separate membrane compartment and do not exchange with other cellular phospholipids on any step of their intracellular path. Other possibilities are, e.g., dipalmitoyl enrichment by PC movement through a series of particles with decreasing density (Jobe *et al.*, 1981) or by protein-facilitated PC transfer in the cytosol (Funkhouser & Read, 1985). Finally, the lamellar body assembly itself may interfere in trimming the acyl species pattern: isolated lamellar body proteins were demonstrated to reconstitute selectively with disaturated PC (Nijssen *et al.*, 1987). Lecerf *et al.* (1987) reported the localization of a PC:ceramide phosphocholine transferring activity on lamellar bodies. The enzyme seems to have a substrate preference of 16:0–18:1-PC over 16:0–16:0-PC. Thus, during lamellar-body maturation this enzyme might serve as regulator for reduction of the 16:0–18:1 portion in favour of dipalmitoyl-PC (Lecerf *et al.*, 1987).

In contrast with PC, PG showed the same species composition in lung mitochondria, lung microsomes and pulmonary surfactant (Schlame *et al.*, 1986). It was only logical to find the PG acyl species of surfactant similar to those of lamellar bodies (Table 2, Fig. 1). The emerging view is that PG is synthesized *de novo*, undergoes only minor remodelling (Mason & Nellenbogen, 1984) and is then transferred to become a surfactant constituent without further changes of the acyl species composition (see also Fig. 1).

We are grateful to Dr. H. Eckert (Institute of Lung Diseases and Tuberculosis, Berlin, G.D.R.) for his invaluable advice and help in histochemical staining of the pneumocyte preparations. Electron microscopy was performed in the laboratory of Professor H. David (Department of Pathology at Charite Hospital).

REFERENCES

Batenburg, J. J. (1984) in Pulmonary Surfactant (Robertson, B., VanGolde, L. M. G. & Batenburg, J. J., eds.), pp. 237–270, Elsevier Science Publishers, Amsterdam

Bergmeyer, H. U. (1970) Methoden der enzymatischen Analyse, vol. 1, 2nd edn., Akademie-Verlag, Berlin

Bligh, E. G. & Dyer, W. J. (1959) Can. J. Biochem. **37**, 911–917

Chan, F., Harding, P. G. R., Wong, T., Fellows, G. F. & Possmayer, F. (1983) Can. J. Biochem. Cell Biol. **61**, 107–114

Chander, A., Dodia, C. R., Gil, J. & Fisher, A. B. (1983) Biochim. Biophys. Acta **753**, 119–129

Crecelius, C. A. & Longmore, W. J. (1983) Biochim. Biophys. Acta **750**, 447–456

Dethloff, L. A., Gilmore, L. B., Brody, A. R. & Hook, G. E. R. (1986) Biochem. J. **233**, 111–118

Eckert, H. (1982) Angewandte Histo- und Zytochemie in der Diagnostik Intrathorakaler Tumoren, p. 12, Akademie-Verlag, Berlin

Funkhouser, J. D. & Read, R. J. (1985) Chem. Phys. Lipids **38**, 17–27

Hallermayer, G. & Neupert, W. (1974) Hoppe-Seyler's Z. Physiol. Chemie **355**, 279–288

Jobe, A., Ikegami, M., Sarton-Miller, I., Jones, S. & Yu, G. (1981) Biochim. Biophys. Acta **666**, 47–57

Kates, M. (1972) in Techniques of Lipidology (Work, T. S. & Work, E., eds.), p. 569, North-Holland Publishing Company, Amsterdam

Kikkawa, Y. & Yoneda, K. (1974) Lab. Invest. **30**, 76–84

King, R. J. (1984) in Pulmonary Surfactant (Robertson, B., VanGolde, L. M. G. & Batenburg, J. J., eds.), pp. 1–15, Elsevier Science Publishers, Amsterdam

Krüger, J., Rabe, H., Reichmann, G. & Rüstow, B. (1984) J. Chromatogr. **307**, 387–392

Lecerf, J., Foilland, L. & Gagniarre, J. (1987) Biochim. Biophys. Acta **918**, 48–59

Lojda, Z., Vecerek, B. & Pelichova, H. (1964) Histochemistry **3**, 428–454

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275

Mason, R. J. & Dobbs, L. G. (1980) J. Biol. Chem. **255**, 5101–5107

Mason, R. J. & Nellenbogen, J. (1984) Biochim. Biophys. Acta **794**, 392–402

Mason, R. J., Nellenbogen, J. & Clements, J. A. (1976) J. Lipid Res. **17**, 281–284

Mason, R. J., Williams, M. C., Greenleaf, R. D. & Clements, J. A. (1977) Am. Rev. Respir. Dis. **115**, 1015–1026

Morrison, W. R. & Smith, M. J. (1964) J. Lipid Res. **5**, 600–605
Nijssen, J. G., Promes, L. W., Hardeman, D. & Van den Bosch, H. (1987) Biochim. Biophys. Acta **917**, 140–147
Possmayer, F., Yu, S.-H., Weber, J. M. & Harding, P. G. R. (1984) Can. J. Biochem. Cell Biol. **62**, 1121–1133
Post, M., Schuurmans, E. A. J. M., Batenburg, J. J. & Van-Golde, L. M. G. (1983) Biochim. Biophys. Acta **750**, 68–77
Rooney, S. A. (1985) Am. Rev. Respir. Dis. **131**, 439–460
Rüstow, B., Kunze, D., Rabe, H. & Reichmann, G. (1985) Biochim. Biophys. Acta **835**, 465–476
Rüstow, B., Rabe, H. & Kunze, D. (1987) in Chromatography of Lipids in Biomedical Research and Clinical Diagnosis (Kuksis, A., ed.), pp. 191–224, Elsevier Science Publishers, Amsterdam
Schlame, M., Rüstow, B., Kunze, D., Rabe, H. & Reichmann, G. (1986) Biochem. J. **240**, 247–252
Shelley, S. A., Paciga, J. E. & Balis, J. U. (1984) Lipids **19**, 857–862
Skillrud, D. M. & Martin, W. J. (1984) Lung **162**, 245–252
Sottocasa, G. L., Kuylenstierna, B., Ernster, L. & Bergstrand, A. (1967) J. Cell Biol. **32**, 415–438

Received 16 September 1987/23 December 1987; accepted 29 January 1988