Characterization of phospholipids accumulated in pulmonary-surfactant compartments of rats intratracheally exposed to silica

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Phospholipids in the lung fractions, i.e. alveolar free cells, extracellular pulmonary surfactant, intracellular pulmonary surfactant (lamellar bodies) and microsomal fractions, of rats were examined 28 days after intratracheal injection of silica (40 mg/kg). Significant accumulations of phospholipids were observed in the extracellular- and intracellular-surfactant fractions of rats exposed to silica. The prominent phospholipid accumulated was phosphatidylcholine (PC), consisting mainly of the dipalmitoyl species. However, a compositional change in acidic phospholipids of surfactant fractions was produced by the silica treatment. The percentage of phosphatidylglycerol (PG) was significantly decreased; in contrast, that of phosphatidylinositol (PI) was increased. Thus the ratio PG/PI in the surfactant fractions was markedly decreased in response to silica. This compositional change in both acidic phospholipids occurred even in the early stages, i.e. before appreciable accumulations of alveolar phospholipids were noticed. The molecular-species profiles of both acidic phospholipids in the surfactant fractions were distinctly different from each other. The dipalmitoyl species accounted for more than 30 % of PG and less than 6 % of PI, respectively. These species patterns of PG and PI were similar in control and silica-treated rats. These findings suggest two possibilities that (1) PG and PI destined for pulmonary surfactant are synthesized from each specific CDP-diacylglycerol (DG) pool having different molecular species in the lung, or (2) individual enzymes responsible for synthesis of surfactant PG and PI have substrate specificities for molecular species of CDP-DG, thereby producing PG and PI having different molecular species in surfactant compartments.

INTRODUCTION

Previous studies on experimental animals exposed to silica have shown a marked stimulation of phospholipid biosynthesis and accumulation of phospholipids within the lungs (Heppleston *et al.*, 1974; Dethloff *et al.*, 1986). Although the total phospholipid content of the lungs has been shown to increase in response to silica (Grunspan *et al.*, 1973; Gabor *et al.*, 1978; Richards & Curtis, 1984), it was more recently shown that the major increase of phospholipids in the lung is associated with extracellular and intracellular pulmonary-surfactant compartments (Dethloff *et al.*, 1986). However, there have been only a few reports describing characteristics of phospholipids accumulated in the pulmonary-surfactant compartments of silica-treated rats. Several investigations showed that phospholipids normally present, as an indicator of the content of disaturated phosphatidylcholine (PC), in the lung are accumulated in response to silica (Dethloff *et al.*, 1986; Heppleston *et al.*, 1974; Gabor *et al.*, 1978; Richards & Curtis, 1984). On the other hand, more recently some changes in acidic phospholipids such as phosphatidylglycerol (PG) and phosphatidylinositol (PI) in bronchoalveolar-lavage fluids of patients with interstitial lung diseases have been noticed (Robinson *et al.*, 1988; Honda *et al.*, 1988). Since the pathological state in the lung produced by the exposure to silica seems to be associated with that of interstitial lung diseases, it is possible that some modifications may occur in phospholipids accumulated in the pulmonary-surfactant compartments in response to silica.

In the present study, characterization of phospholipids accumulated in the lungs of rats intratracheally exposed to silica was studied, with special reference to changes in acidic-phospholipid profiles.

EXPERIMENTAL

Materials

Phospholipase C (Type I from *Clostridium perfringens*, Type III from *Bacillus cereus*) was purchased from Sigma. 3,5-Dinitrobenzoyl chloride (DNB-Cl) was from Katakama Chemicals (Osaka, Japan). Silica-gel 60 plates were from Merck. All other chemicals were obtained commercially, of analytical grade.

Preparation of experimental animals intratracheally exposed to silica

The silica particles used were prepared by the method described by Dethloff *et al.* (1986). Briefly, 5 g of silica (SiO₂, approx. 99 % purity; particle size distribution 0.5–10 µm; approx. 80 % between 1–5 µm; Sigma) was boiled in 150 ml of 1 M-HCl. The silica was then washed four times with deionized water and sterilized in an oven at 200 °C for 2 h. The preparation was diluted to 400 mg/ml with sterile 0.9 % NaCl, and sonicated for 3 min at 30 W (Sonifier cell disruptor; Branson Sonic Power Co., Danbury, CT, U.S.A.) to prepare a suspension for intratracheal injection before use.

Wistar male rats, weighing 200–250 g, were anaesthetized by injecting intraperitoneally a mixture of atropine

Abbreviations used: PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; DG, diacylglycerol; DNB, dinitrobenzoyl.

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sulphate (5 µg/kg) and sodium pentobarbital (25 mg/kg). A curved cannula (26 gauge) was threaded into the main bronchus of the animals. The rats were held in a vertical position and injected with silica suspension (40 or 10 mg/kg body wt. in 0.1 ml of sterile saline) intratracheally. Rats injected with 0.1 ml of sterile saline intratracheally were used as controls. After the injection, the animals were returned immediately to their cages.

Isolation of lung fractions

At 28 days after a single intratracheal injection of silica, the rats were killed by bleeding through the abdominal aorta after intraperitoneal injection of sodium pentobarbital (25 mg/kg). Immediately after death, the chest of the animal was opened and the tracheae were cannulated with a polyethylene tube connected to a syringe. The lungs were filled with 10 ml of ice-cold saline containing 1 mM-phenylmethanesulphonyl fluoride, and the lavage effluents were then collected. This procedure was repeated four times. The combined effluents were centrifuged at 430 *g* for 10 min. The resulting pellet, containing sedimented cells, was termed 'alveolar free cells fraction'. The supernatant was centrifuged at 75000 *g* for 60 min to obtain extracellular pulmonary-surfactant fraction.

The intracellular lung fractions were isolated generally by the method described for isolation of rabbit lung fractions (Jobe, 1977). The lavaged lungs were washed with 0.32 M-sucrose/Tris/NaCl buffer (0.32 M-sucrose in 10 mM-Tris/HCl, 0.14 M-NaCl, 1 mM-CaCl₂, 1 mM-MgSO₄ and 0.1 mM-EDTA at pH 7.4) through the pulmonary artery, and then homogenized in the 0.32 M-sucrose/Tris/NaCl buffer. The crude homogenate was centrifuged at 1000 *g* for 5 min. The supernatant was filtered through nylon cloth and centrifuged at 7700 *g* for 20 min. The resultant pellet, containing lamellar bodies and mitochondria, was suspended in 0.32 M-sucrose/Tris/NaCl buffer, layered on a discontinuous gradient of 0.55 M-sucrose in Tris/NaCl buffer and 0.45 M-sucrose in Tris/NaCl buffer, and centrifuged at 90000 *g* for 3 h. The band obtained at the 0.45 M-/0.55 M-sucrose interface was aspirated. The fraction containing lamellar bodies was then diluted to 0.25 M-sucrose and centrifuged at 20000 *g* for 15 min. Thus the lamellar-body fraction, i.e. intracellular pulmonary-surfactant fraction, was obtained as the pellet. From the 7700 *g* supernatant, microsomes (microsomal fractions) were isolated as the pellet after centrifugation at 100000 *g* for 3 h through 0.8 M-sucrose/Tris/NaCl buffer.

In the experiments using a much smaller dose (10 mg/kg) of silica, the rats intratracheally exposed to silica were killed 4, 8, 12, 20 and 28 days after the injection. The extracellular pulmonary-surfactant fraction was isolated from bronchoalveolar lavage fluid of the rats as described above.

Lipid extraction and separation

Lipids of the lung fractions were extracted by the method of Bligh & Dyer (1959). For analysis of phospholipid composition, individual phospholipids were separated by two-dimensional t.l.c. on silica-gel 60 plates (Merck, Darmstadt, Germany). The solvent systems were chloroform/methanol/di-isobutyl ketone/acetic acid/boric acid (45:15:30:10:4, by vol.) for the first dimension, followed in the second dimension with chloroform/methanol/di-isobutyl ketone/pyridine/

water (30:25:25:35:8, by vol.), and then the plate developed with acetone twice in the first direction. After development, spots on the plates were detected by exposure to iodine vapour. The phospholipid content was determined by measuring phospholipid P (Bartlett, 1959).

Analysis of molecular species of phospholipids

PC, PG and PI were purified preparatively by using two-dimensional t.l.c. as described by Poorthuis *et al.* (1976), followed by the method of Arvidson (1968). The purified phospholipids were converted into 1,2-diacylglycerol (DG) by treatment with phospholipase C (Type I from *C. perfringens* for PC, and Type III from *B. cereus* for PG and PI) by the method described by Okano *et al.* (1978). Each phospholipid seemed to be hydrolysed completely, because after treatment with phospholipase C the substrate phospholipid spots were not detected on t.l.c. DNB derivatives of diacylglycerol (DNB-DG) were prepared and separated by h.p.l.c. by the methods described by Kito *et al.* (1985). The DG, dried under a flow of N₂, and DNB-Cl (25 mg) were dissolved in 0.5 ml of dry pyridine and heated in a sealed vial at 60 °C for 15 min. After being cooled in an ice bath, 0.1 M-HCl (2.0 ml) was added and the product was extracted with n-hexane (three times). The combined extract was dried under a flow of N₂. The residue was dissolved in n-hexane and washed with 0.1 M-HCl (three times), 0.1 M-NaHCO₃, 1 M-NaCl and water. The organic layer was dried under a flow of N₂ and the residue (DNB-DG) was dissolved in acetonitrile.

The h.p.l.c. separations were carried out on a Hitachi model 655 liquid chromatograph equipped with a variable-wavelength detector (model UVITEC-100; Nihon Bunko Co., Tokyo, Japan), which was operated at 254 nm in conjunction with an integrator (Shimadzu Chromatopac 51A). The molecular species were separated by chromatography on a 200 mm × 6 mm ERC-ODS-1161 column (Erma Optical Co., Tokyo, Japan). The eluting solvent system was an isocratic solvent, acetonitrile/propan-2-ol (4:1, v/v), pumped at a flow rate of 1.0 ml/min at room temperature. A 20–100 µl portion of the sample was injected into the liquid chromatograph. The molecular species contained in each peak were designated in accordance with the peak identification as described previously (Itoh *et al.*, 1985).

Other analytical methods

The content of protein was determined by the method of Lowry *et al.* (1951). Radioactivity was determined in a Beckman LS-9000 scintillation spectrometer with ACS II scintillator (Amersham International, Amersham, Bucks., U.K.).

RESULTS

At 28 days after a single tracheal injection of silica (40 mg/kg), the content of phospholipid increased remarkably in alveolar-space fractions, i.e. alveolar free cell fraction (8.4-fold) and extracellular pulmonary-surfactant fractions (2.3-fold), and lamellar bodies (7.8-fold) (Table 1). The phospholipid content of lung microsomes from silica-treated rats was the same as for control rats. It should be kept in mind that the microsomes originate from a variety of different cell types, whereas the surfactant fractions are derived from type II cells only.

Table 1. Phospholipid and protein contents of lung fractions isolated from rats intratracheally exposed to silica

Male rats weighing 200–250 g were injected intratracheally with a single dose (40 mg/kg) of silica and killed after the injection. The lung fractions were then isolated as described in the Experimental section. Values are means $\pm$ S.D. ($n = 6$); values in parentheses represent ratio of silica-treated to control.

	Phospholipid ($\mu\text{mol/rat}$)		Protein ($\mu\text{g/rat}$)	
	Control	Silica-treated	Control	Silica-treated
Alveolar free cell fraction	0.63 ± 0.48	5.29 ± 2.44 (8.4)	226 ± 73	944 ± 404 (4.2)
Extracellular pulmonary-surfactant fraction	1.74 ± 0.63	4.02 ± 1.63 (2.3)	199 ± 71	467 ± 253 (2.4)
Intracellular pulmonary-surfactant fraction (lamellar bodies)	0.99 ± 0.18	7.73 ± 4.28 (7.8)	479 ± 97	1405 ± 387 (2.9)
Microsomes	0.24 ± 0.15	0.31 ± 0.17 (1.3)	1222 ± 302	1968 ± 547 (1.6)

Table 2. Phospholipid composition of lung fractions isolated from rats intratracheally exposed to silica

Rats were injected intratracheally with a single dose (40 mg/kg) of silica and killed 28 days after the injection. The lung fractions were isolated and lipids were extracted from each fraction. The individual phospholipids were separated as described in the Experimental section. Values are means $\pm$ S.D. ($n = 6$) or means ($n = 2$): tr, trace (less than 0.1 %).

Phospholipid	Phospholipid composition (mol %)							
	Alveolar free cell fraction		Extracellular pulmonary-surfactant fraction		Intracellular pulmonary-surfactant fraction		Microsomes	
	Control ($n = 2$)	Silica-treated ($n = 6$)	Control ($n = 6$)	Silica-treated ($n = 6$)	Control ($n = 6$)	Silica-treated ($n = 6$)	Control ($n = 6$)	Silica-treated ($n = 6$)
PC	81.6	85.3 ± 4.5	82.3 ± 3.3	82.5 ± 6.0	72.9 ± 7.8	78.7 ± 7.7	48.7	48.0
Lyso-PC	tr	0.2 ± 0.4	0.3 ± 0.8	0.3 ± 0.7	0.5 ± 0.3	0.1 ± 0.1	1.5	2.0
Phosphatidylethanolamine	8.7	1.9 ± 1.2	6.1 ± 3.4	3.6 ± 2.5	8.0 ± 3.8	7.4 ± 2.8	19.7	18.9
Lysophosphatidylethanolamine	tr	tr	tr	tr	tr	tr	0.8	3.0
PI	1.1	6.7 ± 1.5	1.8 ± 0.9	6.7 ± 2.6	1.5 ± 0.9	5.5 ± 2.4	5.8	6.0
Lyso-PI	tr	0.3 ± 0.5	0.1 ± 0.2	0.2 ± 0.4	1.1 ± 1.1	0.3 ± 0.3	4.1	1.8
Phosphatidylserine	tr	tr	0.1 ± 0.3	tr	3.0 ± 1.2	2.1 ± 3.0	4.8	5.5
Bis(monoacylglycerol) phosphate	1.3	1.8 ± 1.1	1.1 ± 2.2	0.9 ± 1.0	0.9 ± 0.6	1.0 ± 1.7	2.5	3.6
PG	3.5	3.4 ± 0.6	7.5 ± 3.1	4.7 ± 2.1	6.8 ± 3.5	3.3 ± 1.3	3.9	3.3
Sphingomyelin	3.8	0.3 ± 0.3	0.8 ± 0.9	1.1 ± 0.9	5.3 ± 2.4	1.6 ± 2.5	8.2	7.9
PG/PI	3.18	0.51	4.17	0.70	4.53	0.60	0.67	0.55

However, this result indicates that the phospholipid accumulation in response to silica may be associated with pulmonary-surfactant system. The ratio of phospholipid to protein in extracellular pulmonary-surfactant fraction, lamellar bodies and microsomes was similar for the two animal groups, whereas that in alveolar free cell fraction of silica-treated rats was about twice that in the control rats.

As illustrated in Table 2, a predominant phospholipid in alveolar free cell fraction, extracellular pulmonary-surfactant fraction and lamellar bodies of the two animal groups was PC. However, the silica treatment produced a marked compositional change in acidic phospholipids in the extracellular- and intracellular-surfactant fractions. The percentage of PI in the surfactant fractions of silica-treated rats increased markedly; in contrast, that of PG decreased in response to silica, although the total amounts of PG and PI were not significantly altered. Thus, the ratio PG/PI was decreased from 4.2 in control rats to 0.7 in silica-treated rats in extracellular pulmonary-surfactant fraction, and from 5.2 to 0.60 in

intracellular pulmonary-surfactant fraction. The PI in alveolar free cell fraction also decreased in silica-treated rats, but PG was similar in both animal groups. On the other hand, the phospholipid compositions of microsomes from silica-treated rats were very similar to each other as well as the control values. This compositional change in PG and PI in extracellular pulmonary-surfactant fraction of silica-treated rats was found to occur even in the early stages of the treatment with a smaller dose (10 mg/kg) of silica (Fig. 1).

In Table 3, the relative amounts of individual molecular species of PC of the lung fractions from control rats are compared with those from silica-treated rats. The molecular-species compositions of PC in the lung fractions were very similar in control and silica-treated rats. The main species of PC in the surfactant fractions were, in decreasing order, $C_{16:0}/C_{16:0}$, $C_{16:0}/C_{18:2}$ and $C_{16:0}/C_{16:1}$ species. The molecular species compositions of PG and PI in each lung fraction were also very similar in both animal groups. However, it should be noted that, as shown in Table 4, the species patterns of the acidic

Fig. 1. Early changes in phospholipid composition of extracellular pulmonary-surfactant fraction by intratracheal injection of smaller dose (10 mg/kg) of silica to rats

Male rats were injected intratracheally with a smaller dose (10 mg/kg) of silica and killed 4, 8, 12, 20 or 28 days after the injection. The extracellular pulmonary-surfactant fraction was then isolated as described in the Experimental section. ●, PG; ○, PI.

phospholipids in the surfactant fractions were distinctly different. The main molecular species of PG in extracellular- and intracellular-surfactant fractions of control and silica-treated rats were $C_{16:0}/C_{16:0}$ (about 30–35%), $C_{16:0}/C_{18:2}$ and $C_{16:0}/C_{18:1}$ species in order, whereas those of PI in the surfactant fractions were $C_{18:0}/C_{20:4}$,

$C_{16:0}/C_{20:4}$ and $C_{16:0}/C_{18:1}$ species and the content of $C_{16:0}/C_{16:0}$ species was very low (less than 6%).

DISCUSSION

The present results clearly demonstrate that the phospholipid profiles of pulmonary surfactant are significantly altered in response to silica. The prominent changes in surfactant phospholipids observed after the silica treatment are (1) marked accumulation of surfactant phospholipids and (2) decrease in ratio of PG to PI. Dethloff *et al.* (1986) reported that the major increase in the phospholipid content of rat lung induced by silica is associated with the intracellular- and extracellular-surfactant compartments. We used a much lower dose of silica, 40 mg/kg, than that used by Dethloff *et al.* (1986), 200 mg/kg, but the present findings are generally in good agreement with those described by Dethloff *et al.* (1986), except for the compositional change in acidic phospholipids produced by the silica treatment. The results shown in Table 2 clearly indicate that, by the silica treatment, PI concentration, which was normally very low in pulmonary surfactant, was increased markedly, and concomitantly that of PG decreased. Thus the ratio of PG/PI in the intracellular- and extracellular-surfactant fractions decreased markedly in response to silica, although the total amounts of both acidic phospholipids were similar. This compositional change in PG and PI in pulmonary surfactant of silica-treated rats was found even in the early stages of the treatment with a smaller dose (10 mg/kg) of silica (Fig. 1). The decrease in PG/PI ratio in lung lavage fluids of rats treated with silica was noticed before appreciable accumulations of total phospholipids appeared in the alveoli. Richards *et al.* (1983) described that total surfactant phospholipid levels react very quickly to silica instillation for some types of quartz. However, the change in PG/PI ratio will be an alternative sign showing pathological state in the surfactant system produced by silica.

Table 3. Molecular-species composition of PC of lung fractions isolated from rats intratracheally exposed to silica

Abbreviation: tr, trace (< 0.1 %).

Main molecular species	Molecular species composition (%)							
	Alveolar free cell fraction		Extracellular pulmonary-surfactant fraction		Intracellular pulmonary-surfactant fraction		Microsomes	
	Control*	Silica-treated†	Control†	Silica-treated†	Control†	Silica-treated†	Control*	Silica-treated*
$C_{16:0}/C_{22:6}$	0.8	1.3	1.8	1.7	1.1	1.8	1.9	1.8
$C_{16:0}/C_{20:4}$	3.7	4.9	3.5	4.0	3.7	5.1	7.0	9.6
$C_{18:1}/C_{18:2}$	tr	0.8	0.8	1.0	0.7	0.7	2.4	2.0
$C_{16:0}/C_{18:2}$	21.6	14.4	23.4	18.6	17.8	16.7	20.7	32.0
$C_{16:0}/C_{16:1}$	9.5	13.4	10.0	11.7	16.3	12.6	5.2	3.7
$C_{18:0}/C_{20:4}$	0.4	1.0	1.3	0.8	0.6	1.1	3.9	1.7
$C_{16:0}/C_{18:1}$	6.7	6.7	6.3	6.6	8.2	7.7	18.1	21.2
$C_{16:0}/C_{16:0}$	54.9	53.9	49.3	51.1	47.4	51.6	35.8	24.0
$C_{18:0}/C_{18:2}$	1.3	1.8	1.7	1.7	1.7	1.6	2.4	2.1
Others	1.1	1.8	1.9	2.8	2.5	0.9	2.6	1.9

* Values represent means from two independent analyses, in which each sample was collected from three rats.

† Values represent means from two independent analyses, in which each sample was collected individually.

Table 4. Molecular species composition of PG and PI of lung fractions isolated from rats intratracheally exposed to silica

Abbreviation: tr, trace (< 0.1 %).

Main molecular species	Molecular species composition (%)					
	PG					
	Alveolar free cell fraction		Extracellular pulmonary-surfactant fraction		Intracellular pulmonary-surfactant fraction	
	Control*	Silica-treated†	Control†	Silica-treated‡	Control†	Silica-treated‡
C _{16:0} /C _{22:6}	3.4	2.5	3.7	3.9	2.5	3.5
C _{16:0} /C _{20:4}	10.7	6.0	4.4	3.5	2.7	5.7
C _{18:1} /C _{18:2}	2.8	1.9	2.1	4.0	2.5	5.5
C _{16:0} /C _{18:2}	23.8	21.0	39.5	28.3	22.4	24.7
C _{16:0} /C _{16:1}	tr	6.4	0.5	5.2	8.6	4.9
C _{18:0} /C _{20:4}	3.5	1.0	1.1	1.0	tr	1.0
C _{16:0} /C _{18:1}	15.7	19.7	13.9	16.3	20.8	19.9
C _{16:0} /C _{16:0}	36.7	38.6	32.3	32.9	37.8	30.3
C _{18:0} /C _{18:2}	2.1	2.1	0.8	1.8	tr	1.6
Others	1.3	0.8	1.7	3.1	2.7	2.9
	PI					
	Alveolar free cell fraction	Extra-cellular pulmonary-surfactant fraction	Intracellular pulmonary-surfactant fraction		Microsomes	
	Silica-treated	Silica-treated	Control	Silica-treated	Control	Silica-treated
C _{16:0} /C _{22:6}	6.2	10.9	11.8	11.4	10.6	11.7
C _{16:0} /C _{20:4}	13.0	15.2	12.9	12.6	8.7	9.6
C _{18:1} /C _{18:2}	9.7	10.3	13.4	10.5	10.5	11.6
C _{16:0} /C _{18:2}	13.1	9.3	9.4	10.0	13.3	14.7
C _{16:0} /C _{16:1}	3.3	1.0	3.2	3.8	3.2	3.5
C _{18:0} /C _{20:4}	27.7	29.8	31.5	25.4	39.5	33.2
C _{16:0} /C _{18:1}	17.4	15.7	14.6	17.4	6.3	7.0
C _{16:0} /C _{16:0}	3.4	5.2	2.2	3.0	4.6	5.1
C _{18:0} /C _{18:2}	2.8	1.5	tr	3.2	1.9	2.1
Others	3.4	1.1	1.0	2.8	1.4	1.5

* Values represent mean from two independent analyses, in which each sample was collected from three rats.

† Values represent mean from two independent analyses, in which each sample was collected individually.

‡ Each sample was collected from six rats.

Both acidic phospholipids, i.e. PG and PI, are synthesized from the same precursor CDP-DG in alveolar type II cells, stored in the lamellar bodies, and secreted into the alveolar space (Batenburg *et al.*, 1982, 1985). Normally the concentration of PG is much higher than that of PI in pulmonary surfactant of rat adult lung (Mason & Williams, 1980; Sato & Akino, 1982). However, the silica treatment produced a significant decrease in PG and a concomitant increase in PI in the surfactant compartments. This fact seems to indicate that a switch-over in the biosynthesis of both acidic phospholipids from CDP-DG in alveolar type II cells may occur in response to silica. Thus, in this pathological state produced by the silica treatment, PI synthesis from CDP-DG seems to be enhanced, but PG synthesis is suppressed. Similar switch-overs in the biosynthesis of both acidic

phospholipids have been shown in alveolar type II cells from rat adult lung (Batenburg *et al.*, 1982) and in rabbit perinatal lung (Hallman *et al.*, 1977, 1986). These previous studies suggested that PG and PI appearing in pulmonary surfactant are derived from the same CDP-DG precursor, which is probably located in microsomes of alveolar type II cells.

However, the present results demonstrated that the molecular-species profiles of PG in the surfactant fractions were distinctly different from those of PI (Table 4). The C_{16:0}/C_{16:0} species of PG was more than 30 % in the surfactant fractions of silica-treated and untreated rats, but that of PI was less than 6 % in these fractions. In interpreting these results, two possibilities may be considered, as follows. (1) PG and PI in surfactant compartments are derived from each specific pool of CDP-

DG in type II cells, i.e. there are different pools of CDP-DG directed for synthesis *de novo* of surfactant PG and PI. (2) Individual enzymes responsible for synthesis *de novo* of PG and PI have substrate specificities for the molecular species of CDP-DG, thereby producing PG and PI with different molecular species in surfactant fractions. However, such substrate specificities of the enzymes for different molecular species of CDP-DG have not been clarified in the lung. Furthermore, even if there are different pools of CDP-DG destined for synthesis of surfactant PG and PI in type II cells, it is still unknown whether these different pools of CDP-DG in type II cells are due to difference in the intracellular localization of both acidic phospholipids, i.e. microsomes for PI synthesis and mitochondria for PG synthesis (Liau *et al.*, 1984; Schlame *et al.*, 1986), or are due to different sites of PG and PI synthesis in microsomes, because the results of studies of the intracellular localization of PG synthesis in the lung are contradictory. Rüstow *et al.* (1988) reported that the species patterns, particularly the percentages of $C_{16:0}/C_{18:0}$ species, are very similar between surfactant PG and PI, i.e. the percentage of $C_{16:0}/C_{18:0}$ species was 14.4 % for PI and 16.9 % for PG in surfactant of rat adult lung. They concluded that both acidic surfactant phospholipids may be synthesized *de novo* from a common CDP-DG pool in lung microsomes. Our present results on the species patterns of PG and PI in surfactant of adult rat lung are definitely different from those described by Rüstow *et al.* (1988). Reasons for these differences between our data and the data of Rüstow *et al.* (1988) are not immediately obvious.

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