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SPECIFIC TRANSFER OF DIPALMITOYL PHOSPHATIDYLCHOLINE IN RABBIT LUNG

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Summary

Liposomes were used as a tool to probe the specific transfer of dipalmitoyl phosphatidylcholine in rabbit lung. After incubation of liposomes with lung slices, lamellar bodies took up 30—50% more radioactively labeled dipalmitoyl phosphatidylcholine from egg yolk phosphatidylcholine liposomes than the labeled dioleoyl phosphatidylcholine, whereas mitochondria and microsomes took up both species at relatively equal rates. Furthermore, when 50% egg yolk phosphatidylcholine was replaced by dipalmitoyl phosphatidylcholine, the uptake of [1-^{14}C]dipalmitoyl phosphatidylcholine by lamellar bodies was greatly reduced due to the isotope-dilution effect of the unlabeled dipalmitoyl species. This competitive uptake was not observed in mitochondria and microsomes. Similar results were also observed when liposomes were incubated with the cell-free homogenate. On the other hand, distearoyl or dioleoyl phosphatidylcholine did not compete with the uptake of [1-^{14}C]dipalmitoyl phosphatidylcholine. These results suggest an intracellular specific transfer of dipalmitoyl phosphatidylcholine from liposomes to lamellar bodies, presumably by a specific transfer protein.

When liposomes were labeled with [1-^{14}C]dioleoyl phosphatidylcholine, the addition of unlabeled dioleoyl phosphatidylcholine up to 50% of total phosphatidylcholine concentration did not compete with the uptake of [1-^{14}C]-dioleoyl phosphatidylcholine in all fractions including lamellar bodies. This indicates that the intracellular uptake of liposomal dioleoyl phosphatidylcholine by lamellar bodies and other subcellular organelles was probably dominated by the fusion mechanism.

Introduction

In general, the mammalian lung surfactant system can be considered as a process of synthesis, storage, and secretion of the surfactant components [1]. The major surfactant component is phosphatidylcholine, of which dipalmitoyl phosphatidylcholine is the most abundant molecular species [2—4]. It is well known [5] that surfactant phosphatidylcholine is synthesized in the endoplasmic reticulum and stored in the lamellar bodies of pulmonary type II alveolar epithelial cells from where it is then secreted to the alveolar surface to prevent lung collapse during aspiration. However, the mechanisms of these sequential processes are far from understood.

The lamellar body is a unique subcellular organelle found in type II cells [1]. It is not exactly known how lamellar bodies form and mature. As has been suggested by electron-microscopic studies, lamellar bodies are probably initiated from membrane-bound granules in the cytoplasm in the developing fetal lung [6]. During development, phospholipids are added to the growing granule and the number of lamellae increases as the forming lamellar body enlarges. However, even in the mature lung in vitro [7,8] and in vivo [9,10] studies or in the alveolar type II cell adenoma [11], the newly synthesized surfactant phospholipids can be transferred from the synthetic site (endoplasmic reticulum) to the nascent lamellar bodies. This probably occurs through the Golgi apparatus by fusion of the lipids in the form of multivesicular bodies [7], or as a protein-phospholipid complex facilitated by phospholipid transfer (exchange) proteins [12,13]. To date, no evidence is available to explain how lamellar bodies could have a higher percentage of dipalmitoyl phosphatidylcholine than that in any other subcellular organelles in lung tissue [4,10,14]. Because of the lack of the phosphatidylcholine metabolic-pathway enzymes in lamellar bodies [5,15—17], a specific transfer of dipalmitoyl phosphatidylcholine from its synthetic site to lamellar bodies is postulated.

Earlier studies have shown that synthetic multilamellar lipid vesicles (liposomes) could be used as a tool to probe the phospholipid transfer process in lung [18]. By using this method, the surfactant-specific transfer mechanism was investigated. Results of this study show that dipalmitoyl phosphatidylcholine in the form of liposomes can be delivered to lamellar bodies in type II cells by a transfer mechanism highly specific for this lipid species.

Materials and Methods

Materials. Egg yolk phosphatidylcholine was purified from the lyophilized egg yolk and stored in benzene/ethanol (4 : 1, v/v) under N_2 at —20° C before use [18]. Dicetyl phosphate and dipalmitoyl phosphatidylcholine were purchased from Sigma Chemical Co., St. Louis, MO. Dioleoyl phosphatidylcholine, distearoyl phosphatidylcholine and cholesterol were the products of Supelco, Bellefonte, PA. Radioactive isotopes of [1-^{14}C]dipalmitoyl phosphatidylcholine, 2-[9,10-^{3}H]dipalmitoyl phosphatidylcholine, and [1-^{14}C]dioleoyl phosphatidylcholine were obtained from Applied Science Laboratories, State College, PA. [9,10-^{3}H]Palmitic acid was purchased from New England Nuclear, Boston, MA. All other chemicals were of reagent grade.

Preparation of lung slices and liposomes. Lung slices of 0.5 mm thickness were prepared from an adult male New Zealand rabbit lung after the lungs were isolated and perfused as described previously [18]. Multilamellar liposomes were prepared from egg phosphatidylcholine or a combination of egg phosphatidylcholine and other phosphatidylcholine species together with a trace amount of radioactively labeled phosphatidylcholine as specified, dicetyl phosphate, and cholesterol in a molar ratio of 7 : 2 : 1 [18]. Unless otherwise stated, the liposomal solution contained approx. 10 μmol phosphatidylcholine in 2 ml Krebs-Ringer bicarbonate buffer (pH 7.4). All liposomes used in these studies were prepared at room temperature (20—25°C) on the day of the experiment. The purity of all phosphatidylcholines (at least 96%) was confirmed by thin-layer chromatography, and their fatty acid composition was examined by gas-liquid chromatography.

Uptake of liposomes by lung slices. The standard reaction was carried out exactly as described before [18] by incubating 2 g of lung slices in 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) and 2 ml of liposomal solution at 37°C. Since the uptake of liposomes by lung tissue was a time-dependent process [18], for sensitivity and convenience the incubation was conducted for 1 h. At the end of the reaction, the incubation medium was removed and the lung slices were washed three times with 20 ml of ice-cold saline and homogenized in 6 ml of 0.33 M sucrose/0.01 M Tris-HCl buffer (pH 7.4). Subcellular fractions of lamellar bodies, mitochondria, microsomes and cytosol were isolated [17]. Radioactivity of phopshatidylcholine and the protein concentration in each fraction were determined [18]. For the comparative studies of the uptake of liposomes having different phosphatidylcholine species, the uptake of each group of liposomes was carried out in parallel by using the tissue slices obtained from the same animal lung to minimize biological and experimental errors.

Results

Stability of the absorbed liposomal phosphatidylcholine

Earlier studies have shown that, after the liposomes were incubated with the lung slices at 37°C for 1 h, the distribution of the radioactivity at the 1- and 2-positions of the absorbed liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine in lung slices and subcellular organelles was 1 to 1 [18]. This suggested that the degradation of the liposomal phosphatidylcholine in lung cells under the experimental conditions was unlikely. This observation was further confirmed by the present experiment. Liposomes contained doubly labeled 1-[1-^{14}C]-palmitoyl-2-[9,10-^{3}H]palmitoyl phosphatidylcholine prepared by using the method of Lands [19] were incubated with lung slices at 37°C for 1 h. Radioactivity of the ratio of ^{3}H : ^{14}C in the lung homogenate, lamellar bodies, mitochondria, microsomes and soluble fraction was determined. If the ratio of ^{3}H : ^{14}C in liposomes was designated as 1.00, then the average ratio of ^{3}H : ^{14}C in all fractions was 1.05 ± 0.02 obtained from three experiments.

Uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine and [1-^{14}C]-dioleoyl phosphatidylcholine by lung slices

A comparative study of the uptake of liposomes labeled with traces of

TABLE I

UPTAKE OF LIPOSOMAL [1-^{14}C]DIPALMITOYL AND [1-^{14}C]DIOLEOYL PHOSPHATIDYLCHOLINE BY LUNG SLICES AND SUBCELLULAR FRACTIONS

Liposomes labeled with [1-^{14}C]dipalmitoyl and [1-^{14}C]dioleoyl phosphatidylcholine contained 12.24 ± 5.99 and 11.17 ± 7.70 μmol phosphatidylcholine. Their specific radioactivities were 327.49 ± 101.13 and 388.53 ± 140.86 cpm/nmol phosphatidylcholine, respectively. Both liposomes were separately incubated with 2 g of lung slices at 37°C for 1 h. Results are means ± S.D. of five experiments.

Fractions	Uptake of phosphatidylcholine (nmol/h per mg protein)	
	[1-^{14}C]Dipalmitoyl phosphatidylcholine liposomes	[1-^{14}C]Dioleoyl phosphatidylcholine liposomes
Homogenate	12.22 ± 4.48	10.34 ± 3.10
Lamellar bodies	106.31 ± 36.93	45.51 ± 20.86 *
Mitochondria	6.11 ± 1.97	5.36 ± 1.19
Microsomes	7.16 ± 2.87	7.83 ± 1.63
Soluble fraction	3.31 ± 1.29	4.77 ± 1.59

* $P < 0.02$ (unpaired t-test) relative to dipalmitoyl phosphatidylcholine uptake.

[1-^{14}C]dipalmitoyl phosphatidylcholine and liposomes labeled with [1-^{14}C]-dioleoyl phosphatidylcholine by rabbit lung slices was conducted. The results of this study showed that the uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine and liposomal [1-^{14}C]dioleoyl phosphatidylcholine by lung slices were approximately the same (Table I). However, the rate of the intracellular uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine by lamellar bodies was about 2-fold greater ($P < 0.02$) than that of [1-^{14}C]dioleoyl phosphatidylcholine whereas the uptake of these two lipid species by mitochondria or microsomes was approximately equal after the nonspecific adsorbed liposomal phosphatidylcholine was substracted [18]. This observation was also supported by the experiment in which liposomes were doubly labeled with 2-[9,10-^{3}H]dipalmitoyl phosphatidylcholine and [1-^{14}C]dioleoyl phosphatidylcholine. This experiment would eliminate all possible experimental errors for the uptake of liposomal dipalmitoyl phosphatidylcholine and dioleoyl phosphatidylcholine, errors that could be generated from the experiments when conducted separately. Results of this study showed that the ratio of ^{3}H : ^{14}C in lamellar bodies was about 30% higher than that in mitochondria,

TABLE II

UPTAKE OF LIPOSOMES DOUBLY LABELED WITH 2-[9,10-^{3}H]DIPALMITOYL AND [1-^{14}C]DIOLEOYL PHOSPHATIDYLCHOLINE BY LUNG SLICES AND SUBCELLULAR FRACTIONS

Doubly labeled liposomes were incubated with 2 g of lung slices at 37°C for 1 h. The average concentration of liposomal phosphatidylcholine of three preparations was 10.91 ± 1.80 μmol. Results are means ± S.D. of eight experiments and were calculated based on the liposomal ^{2}H:^{14}C ratio equal to 1.00.

Fractions	Ratio of ^{3}H:^{14}C
Incubation medium	0.99 ± 0.03
Homogenate	1.08 ± 0.04
Lamellar bodies	1.38 ± 0.08
Mitochondria	1.08 ± 0.05
Microsomes	1.08 ± 0.04
Soluble fraction	1.09 ± 0.04

microsomes and homogenate, whereas the ratios of the latter three fractions were nearly the same as that of the incubation medium (Table II).

Effect of the concentration of dipalmitoyl phosphatidylcholine and dioleoyl phosphatidylcholine on the uptake of liposomal radioactively labeled phosphatidylcholine by lung slices

The molecular species of phosphatidylcholine of liposomes used in the above studies were those of egg yolk phosphatidylcholine with a trace amount of radioactively labeled dipalmitoyl or dioleoyl species. Although egg yolk phosphatidylcholine contains very small amounts of dipalmitoyl and dioleoyl species (0.7 and 3% of total phosphatidylcholine concentration, respectively) [20], this higher concentration of egg dioleoyl phosphatidylcholine might have some isotope-dilution effect on the uptake of [1-^{14}C]dioleoyl phosphatidylcholine by lamellar bodies. Therefore, in this experiment, the concentration of dipalmitoyl or dioleoyl phosphatidylcholine was brought up to 50% of total phosphatidylcholine in liposomes to eliminate the diverse iostope-dilution effect on these two species. In this experiment, it was assumed that slightly different final concentrations of these two lipid species (e.g., 50.7% of dipalmitoyl and 53% of dioleoyl) should not yield significant differences on each isotope dilution.

In this study, three groups of liposomes were prepared. Group A liposomal phosphatidylcholine was 100% from egg yolk; group B liposomes had 50% dipalmitoyl phosphatidylcholine and 50% egg phosphatidylcholine; group C liposomes contained 50% dioleoyl phosphatidylcholine and 50% egg phosphatidylcholine. All liposomes were prepared by the same procedure and labeled with traces of [1-^{14}C]dipalmitoyl phosphatidylcholine. The experiments of the study of the uptake of these three liposome groups by lung slices and subcellular organelles were carried out in parallel. The results of group A were used as control in comparison with those obtained from group B and group C. The uptake of [1-^{14}C]dipalmitoyl phosphatidylcholine by each fraction was calculated from the radioactivity and the specific radioactivity of liposomes which was derived from the total radioactivity and total concentration of liposomal phosphatidylcholine. The total radioactivity and total concentration of all three liposomal phosphatidylcholine were kept approximately the same. The nonspecific adsorbed liposomal phosphatidylcholine by subcellular organelles was also substracted in this and the following experiments [18]. The results of this study showed that the replacement of 50% egg phosphatidylcholine with dioleoyl species had no significant effect on the uptake of [1-^{14}C]dipalmitoyl phosphatidylcholine (Table III). In contrast, the uptake of [1-^{14}C]dipalmitoyl phosphatidylcholine from group B by lung slices was about 60% less than from group A (A : B = 2.42). Corresponding to this was the decrease in the uptake of [1-^{14}C]dipalmitoyl phosphatidylcholine by mitochondria and microsomes to the same extent (A : B = 2.60 and 2.01, respectively). Unlike these organelles, lamellar bodies took up about 3-fold less group B [1-^{14}C]-dipalmitoyl phosphatidylcholine than lung slices or mitochondria and microsomes, i.e., A : B of lamellar bodies was 7.88 (Table III).

The greatly reduced uptake of group B liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine by lamellar bodies could be due to the inhibiting effects of

TABLE III

EFFECT OF THE CONCENTRATION OF DIPALMITOYL AND DIOLEOYL PHOSPHATIDYLCHOLINE ON THE UPTAKE OF LIPOSOMAL [1-^{14}C]DIPALMITOYL PHOSPHATIDYLCHOLINE BY LUNG SLICES AND SUBCELLULAR ORGANELLES

Liposomes A contained 100% egg phosphatidylcholine; liposomes B contained 50% dipalmitoyl and 50% egg phosphatidylcholine; liposomes C contained 50% dioleoyl and 50% egg phosphatidylcholine. The concentrations of total phosphatidylcholine of liposomes A, B and C were 13.65 ± 1.87, 11.87 ± 0.97 and 11.73 ± 1.97 μmol, respectively. The specific radioactivities of liposomes A, B and C were 244.17 ± 90.08, 218.10 ± 34.50 and 266.57 ± 106.85 cpm/nmol phosphatidylcholine, respectively. Each group of liposomes was labeled with [1-^{14}C]dipalmitoyl phosphatidylcholine and incubated with 2 g of lung slices at 37°C for 1 h. Results are means ± S.D. of four experiments.

Fractions	Uptake of phosphatidylcholine (nmol/h per mg protein)			A:B	A:C
	A	B	C		
Homogenate	17.75 ± 2.38	7.33 ± 1.50	15.96 ± 1.75	2.42	1.11
Lamellar bodies	98.85 ± 10.25	12.55 ± 7.21	95.06 ± 22.29	7.88	1.04
Mitochondria	14.94 ± 3.41	5.74 ± 0.86	20.87 ± 2.81	2.60	0.72
Microsomes	16.58 ± 6.45	8.25 ± 3.54	20.33 ± 5.08	2.01	0.82

dipalmitoyl phosphatidylcholine. Two experiments were conducted to elucidate this possibility. In the first experiment, three groups of liposomes were prepared as those described above, except that each group of liposomes was labeled with [1-^{14}C]dioleoyl phosphatidylcholine. The results of this study showed that the replacement of 50% egg phosphatidylcholine by dipalmitoyl species only decreased the uptake of [1-^{14}C]dioleoyl phosphatidylcholine 30—50% by the lung slices and all subcellular fractions (Table IV). Hence, the uptake of [1-^{14}C]dioleoyl phosphatidylcholine by lamellar bodies has not been reduced greater than that by mitochondria or microsomes in group B. On the other hand, 50% dioleoyl phosphatidylcholine had no reduction on the uptake of [1-^{14}C]dioleoyl phosphatidylcholine either by lung slices or by subcellular organelles including lamellar bodies.

TABLE IV

EFFECT OF THE CONCENTRATION OF DIPALMITOYL AND DIOLEOYL PHOSPHATIDYLCHOLINE ON THE UPTAKE OF LIPOSOMAL [1-^{14}C]DIOLEOYL PHOSPHATIDYLCHOLINE BY LUNG SLICES AND SUBCELLULAR ORGANELLES

Liposomes A contained 100% egg phosphatidylcholine; liposomes B contained 50% dipalmitoyl and 50% egg phosphatidylcholine; liposomes C contained 50% dioleoyl and 50% egg phosphatidylcholine. The concentrations of total phosphatidylcholine of liposomes A, B and C were 13.24 ± 1.53, 12.57 ± 2.52 and 10.86 ± 1.82 μmol, respectively. The specific radioactivities of liposomes A, B and C were 257.33 ± 71.84, 199.69 ± 6.55 and 253.50 ± 77.26 cpm/nmol phosphatidylcholine, respectively. Each group of liposomes was labeled with [1-^{14}C]dioleoyl phosphatidylcholine and incubated with 2 g of lung slices at 37°C for 1 h. Results are means ± S.D. of three experiments.

Fractions	Uptake of phosphatidylcholine (nmol/h per mg protein)			A:B	A:C
	A	B	C		
Homogenate	19.01 ± 2.96	14.26 ± 1.39	16.20 ± 2.06	1.33	1.17
Lamellar bodies	52.12 ± 21.80	37.20 ± 2.88	55.41 ± 17.22	1.40	0.94
Mitochondria	18.40 ± 4.23	9.02 ± 1.71	19.59 ± 2.94	2.04	0.94
Microsomes	20.61 ± 4.07	12.83 ± 2.18	21.43 ± 3.10	1.61	0.96

TABLE V

EFFECT OF DIPALMITOYL PHOSPHATIDYLCHOLINE CONCENTRATION ON THE UPTAKE OF LIPOSOMAL [1-^{14}C]DIPALMITOYL PHOSPHATIDYLCHOLINE BY LUNG SLICES AND SUBCELLULAR ORGANELLES

The concentrations of total phosphatidylcholine of liposomes A, B and C were 32.12, 27.60 and 27.48 μmol, respectively. The specific radioactivities of liposomes A, B and C were 877.76, 1069.97 and 1088.54 cpm/nmol dipalmitoyl phosphatidylcholine, respectively. Each group of liposomes was labeled with [1-^{14}C]dipalmitoyl phosphatidylcholine and incubated with 2 g of lung slices at 37°C for 1 h. Results are the average of duplicates. Uptake of dipalmitoyl phosphatidylcholine is expressed as nmol/h per mg protein.

Fractions	% of diplamitoyl phosphatidylcholine in liposomes		
	A (10)	B (30)	C (50)
Homogenate	2.18	3.82	7.66
Lamellar bodies	14.64	30.26	51.36
Mitochondria	0.88	1.74	5.15
Microsomes	1.17	2.71	6.21
Soluble fraction	1.14	1.90	3.39

In the second experiment, liposomes containing 10, 30 and 50% dipalmitoyl phosphatidylcholine from total phosphatidylcholine were labeled with [1-^{14}C]-dipalmitoyl phosphatidylcholine and incubated with lung slices separately. The specific radioactivity (total radioactivity/total dipalmitoyl phosphatidylcholine concentration) of each group of liposomes was approximately the same. If the uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine by lung slices and subcellular organelles was calculated from this specific radioactivity, a steady increase of the uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine was observed in all fractions (Table V).

Effect of the concentration of distearoyl phosphatidylcholine on the uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine by lung slices

The purpose of this study was to elucidate whether the uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine by lamellar bodies could be affected by other disaturated phosphatidylcholines similar to that affected by dipalmitoyl phosphatidylcholine. We conducted a comparative study of the uptake by lung slices and subcellular organelles of liposomes containing 50% distearoyl and 50% egg phosphatidylcholine with the uptake of liposomes containing 100% egg phosphatidylcholine. Unlike dipalmitoyl phosphatidylcholine, this disaturated phosphatidylcholine had no isotope-dilution effect on the uptake of [1-^{14}C]dipalmitoyl phosphatidylcholine by lamellar bodies (Table VI). Howver, distearoyl phosphatidylcholine, similar to dipalmitoyl phosphatidylcholine, decreased by one-half the uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine by lung slices, hence reducing the amount of this liposomal phosphatidylcholine in mitochondria and microsomes to the same extent.

Effect of dipalmitoyl phosphatidylcholine on the uptake of liposomal [1-^{14}C]-dipalmitoyl phosphatidylcholine by the subcellular fractions in cell-free homogenate

The isotope-dilution effect on the uptake of liposomal [1-^{14}C]dipalmitoyl

TABLE VI

EFFECT OF DISTEAROYL PHOSPHATIDYLCHOLINE ON THE UPTAKE OF LIPOSOMAL [1-^{14}C]-DIPALMITOYL PHOSPHATIDYLCHOLINE BY LUNG SLICES AND SUBCELLULAR ORGANELLES

Liposomes A contained 100% egg phosphatidylcholine; liposomes B contained 50% distearoyl and 50% egg phosphatidylcholine. The concentrations of total phosphatidylcholine of liposomes A and B were 15.96 and 14.16 μmol, respectively. The specific radioactivities of liposomes A and B were 211.79 and 280.09 cpm/nmol phosphatidylcholine, respectively. Each group of liposomes was labeled with [1-^{14}C]-dipalmitoyl phosphatidylcholine and incubated with 2 g of lung slices at 37°C for 1 h. Results are means ± S.D. of triplicates.

Fractions	Uptake of phosphatidylcholine (nmol/h per mg protein)		A:B
	A	B	
Homogenate	13.99 ± 3.65	9.34 ± 1.01	1.50
Lamellar bodies	102.57 ± 22.22	100.29 ± 22.12	1.02
Mitochondria	13.24 ± 1.66	7.55 ± 2.87	1.75
Microsomes	19.21 ± 3.41	13.26 ± 2.67	1.45

phosphatidylcholine by lamellar bodies affected by the unlabeled dipalmitoyl phosphatidylcholine was also observed in the cell-free homogenate system. Group A liposomes contained 100% egg phosphatidylcholine and group B liposomes contained 50% dipalmitoyl and 50% egg phosphatidylcholine. Both groups of liposomes were labeled with traces of [1-^{14}C]dipalmitoyl phosphatidylcholine and separately incubated with 6 ml of homogenate prepared from 2 g of lung tissue at 37°C for 1 h. Each homogenate had approximately the same amount of liposomes, and the specific radioactivities of both groups of liposomes were relatively equal. The results of this study showed that a 50% dipalmitoyl phosphatidylcholine content in the total liposomal phosphatidylcholine lowered the uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine by lamellar bodies by 6.8-fold; lowered the uptake of this liposomal lipid by mitochondria by 3.5-fold and by microsomes by 4.7-fold when compared to the control values of the 100% egg phosphatidylcholine liposomes (Table VII).

TABLE VII

EFFECT OF DIPALMITOYL PHOSPHATIDYLCHOLINE ON THE UPTAKE OF LIPOSOMAL [1-^{14}C]-DIPALMITOYL PHOSPHATIDYLCHOLINE BY SUBCELLULAR FRACTIONS IN CELL-FREE HOMOGENATE

Liposomes A contained 100% egg phosphatidylcholine; liposomes B contained 50% dipalmitoyl and 50% egg phosphatidylcholine. All liposomes were labeled with [1-^{14}C]dipalmitoyl phosphatidylcholine. The specific radioactivities of liposomes A and B were 224.49 and 273.64 cpm/nmol phosphatidylcholine, respectively. Liposomes A contained 1.11 μmol phosphatidylcholine and liposomes B contained 0.92 μmol phosphatidylcholine and were incubated separately with 6 ml of homogenate prepared from 2 g of lung tissue at 37°C for 1 h. Results are means ± S.D. of triplicates.

Fractions	Uptake of phosphatidylcholine (nmol/h per mg protein)		A:B
	A	B	
Homogenate	10.63 ± 1.24	9.77 ± 0.11	1.09
Lamellar bodies	18.93 ± 4.16	2.77 ± 2.45	6.83
Mitochondria	24.60 ± 5.72	7.06 ± 3.46	3.48
Microsomes	21.73 ± 6.62	4.59 ± 1.12	4.73

Discussion

It is postulated that a specific transfer process favorable to dipalmitoyl phosphatidylcholine might exist in lung and could be responsible for the high concentrations of dipalmitoyl phosphatidylcholine in lamellar bodies. Although the phospholipid transfer proteins have recently been isolated from rat lung [21], sheep lung [22], and rabbit lung [23], the specificity of these proteins for dipalmitoyl phosphatidylcholine has not been observed. Failure to do so is probably a result of the reported assays not being sensitive enough to detect the specific transfer activity. In our study, liposomes serving as phospholipid donor and incorporated into the intact lung cells offer several advantages over the assays using biological membranes and isolated transfer proteins. These advantages are (1) the concentration and composition of liposomal phospholipids can be varied and controlled for experimental purposes, and (2) the transfer of liposomal phosphatidylcholine can be studied in the intact cells where the structures of subcellular organelles and the transfer proteins retain their original integrity.Furthermore, since the lamellar body is a unique organelle in Type II cells, transfer of liposomal phosphatidylcholine to lamellar bodies of type II cells in lung tissue can be considered to be similar to that occurring in the isolated type II cells.

In this study, the specific transfer activity in lung was determined by comparing the uptake of liposomal dipalmitoyl phosphatidylcholine and of dioleoyl or distearoyl phosphatidylcholine. The latter two lipid species were found to be either in trace amounts (about 2% of dioleoyl) or absent (distearoyl) in lung tissue [24,25]. The results shown in Tables I and II indicate that lamellar bodies took up liposomal dipalmitoyl phosphatidylcholine more readily than dioleoyl phosphatidylcholine. This was also substantiated by the isotope-dilution method. Theoretically, when 50% of egg phosphatidylcholine (approx. 1% dipalmitoyl) in liposomes labeled with traces of [1-^{14}C]dipalmitoyl phosphatidylcholine was replaced with unlabeled dipalmitoyl phosphatidylcholine, the number of molecules of dipalmitoyl phosphatidylcholine in liposomes should increase about 50-fold. Therefore, the chance of the transfer of [1-^{14}C]-dipalmitoyl phosphatidylcholine would be greatly reduced due to the competition of the transfer of unlabeled dipalmitoyl phosphatidylcholine if there is a specific transfer favoring this lipid species. In other words, the amount of the uptake of [1-^{14}C]dipalmitoyl phosphatidylcholine would decrease if it is calculated from the radioactivity taken up by the cells or subcellular organelles and the specific radioactivity of liposomes derived from the radioactivity of [1-^{14}C]dipalmitoyl phosphatidylcholine and the concentration of total liposomal phosphatidylcholine. As observed in Table III, although the uptake rates of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine by mitochondria and microsomes were reduced in the presence of 50% dipalmitoyl phosphatidylcholine (group B), the ratios of the uptake of liposomes of group A (100% egg phosphatidylcholine) : group B of these two subcellular fractions were nearly the same as that of the homogenate. In contrast, the uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine by lamellar bodies was reduced far more than by mitochondria and microsomes in the presence of 50% dipalmitoyl phosphatidylcholine. In other words, the ratio of groups A:B of lamellar

bodies was about 3 times higher than that of other fractions. These observations suggest several possible mechanisms of the uptake of liposomes by lung cells and subcellular organelles.

(1) In the presence of a high concentration of dipalmitoyl phosphatidylcholine, reduction of the rate of the uptake of liposomes by lung cells is probably due to the change of the physical state of liposomes because of the high gel-liquid crystalline phase transition temperature (T_c) of dipalmitoyl phosphatidylcholine (41°C) compared to T_c of egg phosphatidylcholine (−15°C) [26]. Even in a mixture of dipalmitoyl phosphatidylcholine and egg phosphatidylcholine at a ratio of 1 : 1, T_c of the mixed phosphatidylcholine would be still higher than that of 100% egg phosphatidylcholine. Therefore, the fluidity of the fatty acyl chains of phosphatidylcholine may affect the liposome-cell interactions (i.e., fusion or endocytosis of liposomes with plasma membranes).

(2) Since the ratios of the uptake of liposomal phosphatidylcholine of groups A:B in mitochondria and microsomes were nearly the same as that in the homogenate, I infer that the mechanism of the uptake of liposomes by these two subcellular organelles might be that of a secondary fusion. If there is any specific transfer of liposomal phosphatidylcholine to these two organelles in type II cells, it might not be detectable under these experimental conditions because of the complexity of these two subcellular fractions isolated from a mixture of all types of lung cells.

(3) Since dipalmitoyl phosphatidylcholine does not inhibit the uptake of liposomal phosphatidylcholine by lung cells and subcellular organelles to a great extent (Table IV and V), the much greater reduction of the uptake of group B liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine by lamellar bodies is obviously due to the isotope-dilution effect in the presence of unlabeled dipalmitoyl phosphatidylcholine. This suggests that dipalmitoyl phosphatidylcholine was selectively transferred to lamellar bodies. If lamellar bodies took up liposomal phosphatidylcholine only via the mechanism of fusion, we would expect to obtain a ratio of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine of groups A:B in lamellar bodies similar to that in other fractions.

In contrast to the effect of dipalmitoyl phosphatidylcholine concentration on the uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine, 50% dioleoyl phosphatidylcholine in liposomes (group C) had no effect on the uptake of liposomal [1-^{14}C]dipalmitoyl phosphatidylcholine by lung slices and subcellular organelles (Table IV). Two explanations are possible. First, this result is probably attributable to the fact that T_c of dioleoyl phosphatidylcholine (−22°C) is close to T_c of egg phosphatidylcholine [26], and hence, the unsaturated fatty acyl of dioleoyl phosphatidylcholine has no effect on the fusion mechanism. Second, since [1-^{14}C]dipalmitoyl phosphatidylcholine was specifically taken up by lamellar bodies, addition of unlabeled dioleoyl phosphatidylcholine provided no dilution effect on the uptake of [1-^{14}C]-dipalmitoyl phosphatidylcholine by this organelle.

The mechanism of the uptake of liposomal dioleoyl phosphatidylcholine by lung slices and subcellular organelles was studied with liposomes labeled with [1-^{14}C]dioleoyl phosphatidylcholine. In contrast to the results described above, there was no isotope-dilution effect for the uptake of liposomal [1-^{14}C]dioleoyl phosphatidylcholine by lamellar bodies after the liposomal

egg phosphatidylcholine (group A) was replaced with 50% unlabeled dioleoyl phosphatidylcholine (group C) (Table IV). Again, as seen previously, addition of 50% dioleoyl phosphatidylcholine to the liposomes (group C) had no effect on the uptake of liposomal phosphatidylcholine by lung slices, mitochondria and microsomes. However, for liposomes having 50% dipalmitoyl phosphatidylcholine (group B), the uptake of liposomal [1-^{14}C]dioleoyl phosphatidylcholine by lamellar bodies was only reduced to the same extent as those in other fractions, possibly due to the effect of T_c of dipalmitoyl phosphatidylcholine. Therefore, addition of dipalmitoyl phosphatidylcholine also had no dilution effect on the uptake of [1-^{14}C]dioleoyl phosphatidylcholine by lamellar bodies. These suggest that (1) lamellar bodies may take up liposomal [1-^{14}C]dioleoyl phosphatidylcholine by fusion, or that (2) transfer of [1-^{14}C]-dioleoyl phosphatidylcholine from liposomes to lamellar bodies could be a nonspecific process, i.e., it is probably for all mixed molecular species other than the dipalmitoyl phosphatidylcholine. Similarly, these two possible mechanisms could be applied as well as to the uptake of liposomal dioleoyl phosphatidylcholine by mitochondria and microsomes.

Since the dipalmitoyl phosphatidylcholine-specific transfer activity was observed in the homogenate (Table VII), and since the phospholipid transfer activity was also found in the 105 000 × g supernatant [18], it is suggested that a transfer protein which selectively binds dipalmitoyl phosphatidylcholine might exist in the lung cytosolic fraction. It is intriguing to note that in the intact cells the transfer of liposomal phosphatidylcholine was not only specific for dipalmitoyl species but also specific for transferring this lipid species to lamellar bodies. However, in the cell-free homogenate the transfer was less specific for the phospholipid receptors as shown by the results of the isotope-dilution effect observed in mitochondral and microsomal fractions (Table VII). It is noted that the uptake of liposomal phosphatidylcholine by lamellar bodies in the cell-free homogenate was much lower than that in tissue slices. This is probably due to the disruption of the integrity of lamellar body structure in the homogenate.

In conclusion, in the intact type II cells, phospholipids are probably added to lamellar bodies from their synthetic sites either by fusion of the lipids in the form of lipid vesicles or by the phospholipid transfer mechanism. The phospholipid transfer process is, however, highly, if not solely, specific for dipalmitoyl phosphatidylcholine which is essential for lowering the surface tension at the alveolar surface. Therefore, this specific transfer protein in lung may play an important role in lamellar body formation, and hence, in the lung maturation.

Finally, this study provides a method for liposomes to be used as a useful tool for probing the surfactant transfer mechanism in lung. In addition, the uptake of liposomes by lung in vivo might be of potential use clinically [27].

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