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Interaction of phospholipid liposomes with plasma membrane isolated from alveolar type II cells: effect of pulmonary surfactant protein A

Yoshio Kuroki *, Masanori Shiratori, Yoshinori Ogasawara, Akiko Hattori, Wataru Tsunezawa, Toshio Honma, Toyoaki Akino

Department of Biochemistry, Sapporo Medical University School of Medicine, South-1 West-17, Chuo-ku, Sapporo 060, Japan

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Abstract

Pulmonary surfactant protein A (SP-A) augments the uptake of phospholipid liposomes containing dipalmitoylphosphatidylcholine (DPPC) by alveolar type II cells. The SP-A-mediated uptake process of lipids by type II cells have not been well understood. In the present study we investigated the SP-A-mediated interaction of phospholipids with plasma membrane isolated from alveolar type II cells. SP-A increased the amount of liposomes containing radiolabeled DPPC associated with type II cell plasma membrane by 4-fold compared to the control without SP-A when analyzed by sucrose density gradient centrifugation. This effect is dependent upon the SP-A concentration. The enhancement was inhibited by anti-SP-A antibody and EGTA. When type II cell plasma membrane and liposomes containing [^{14}C]DPPC and [^{3}H]triolein were coincubated with or without SP-A, analysis on sucrose density gradients revealed that the profiles of [^{14}C]DPPC and [^{3}H]triolein in each fraction were almost identical with or without SP-A, indicating that SP-A mediates the binding of liposomes to plasma membrane but not transfer of DPPC. SP-A increased the association of liposomes containing DPPC with the membrane by 2-fold more than that containing 1-palmitoyl-2-linoleoyl-phosphatidylcholine (PLPC). SP-A induced aggregation of phospholipid liposomes containing PLPC as well as those containing DPPC, but the final turbidity of DPPC liposomes aggregated by SP-A was only by 15% greater than that of PLPC liposomes. The amount of DPPC liposomes associated with the plasma membrane derived from type II cells was 2-fold greater than that from liver. We speculate that the SP-A-mediated interaction of lipids with type II cell plasma membrane may contribute, in part, to the lipid uptake process by type II cells.

Keywords: Phospholipid liposome–plasma membrane interaction; Liposome; Plasma membrane; Pulmonary surfactant protein A; SP-A; (Alveolar type II cell)

1. Introduction

Alveolar type II cells produce and secrete pulmonary surfactant that plays important roles in preventing the alveoli from collapsing and in host defence mechanism of the lung. The major surfactant-associated protein SP-A with reduced and denatured molecular masses of 26–38 kDa contributes to the regulation of phospholipid homeostasis in the alveolar space. This protein interacts with phospholipids. It binds to phosphatidylcholine, especially strongly to dipalmitoylphosphatidylcholine (DPPC) [1], an essential lipid component of the surfactant. It causes aggregation of the phospholipid vesicles containing DPPC in a Ca^{2+}-dependent manner [2]. SP-A also interacts with alveolar type II cells. It inhibits secretion of phosphatidylcholine by type II cells [3,4] via a putative receptor expressed on alveolar type II cells [5,6].

Several evidences that surfactant lipids are taken up and recycled by type II cells have been accumulated [7–12]. Claypool et al. [13] reported that hydrophobic surfactant proteins enhanced the uptake of surfactant-like lipids by primary cultures of type II cells. In vivo study by Wright et al. [14] showed that the surfactant subfraction that contained SP-A was taken up into rabbit lungs to a greater extent than that without SP-A. They demonstrated that SP-A augments the uptake of phospholipids by freshly isolated type II cells [15]. The SP-A-mediated uptake of phosphatidylcholine by type II cells is preferential for DPPC [16]. Although these experimental approaches have

Abbreviations: SP-A, pulmonary surfactant protein A; DPPC, dipalmitoylphosphatidylcholine; PLPC, 1-palmitoyl-2-linoleoyl-phosphatidylcholine.

* Corresponding author. Fax: +81 11 6125861.

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provided the hypothesis that SP-A may direct lipids to type II cells in the alveolar space, the uptake process of lipids by type II cells have not been well understood. We investigated the SP-A-mediated interaction of phospholipids with plasma membrane isolated from alveolar type II cells, since this may be an important first step to initiate uptake process of lipids into these cells.

2. Materials and methods

2.1. Lipids

1,2-Dipalmitoyl-L-3-phosphatidylcholine (DPPC), 1-palmitoyl-2-linoleoyl-L-3-phosphatidylcholine (PLPC), egg phosphatidylcholine (PC), phosphatidylglycerol (PG) and cholesterol were purchased from Sigma. 1-Palmitoyl-2-[^{14}C]palmitoyl-L-3-phosphatidylcholine ([^{14}C]DPPC), dipalmitoylphosphatidyl[*N-methyl*-^{3}H]choline ([^{3}H]DPPC) and [9,10(n)-^{3}H]triolein ([^{3}H]triolein) were obtained from New England Nuclear Research. 1-Palmitoyl-2-[^{14}C]linoleoyl-L-3-phosphatidylcholine ([^{14}C]PLPC) was purchased from Amersham.

2.2. Liposome preparation

A phospholipid mixture (60% of DPPC or PLPC, 20% egg PC, 15% PG and 5% cholesterol, by weight) in chloroform/methanol (2:1, v/v) was dried under nitrogen and hydrated in 5 mM Tris buffer (pH 7.4) containing 0.15 M NaCl (1.0 mg phospholipid/ml). This mixture was then probe-sonicated with a Sonifier cell disruptor (Heat System-Ultrasonics). The liposomes was radiolabeled with a trace amount of [^{3}H]DPPC, [^{14}C]DPPC, [^{14}C]PLPC or [^{3}H]triolein.

2.3. Purification of SP-A

Surfactant was isolated from Sprague–Dawley rats given intratracheal instillation of 10 mg of silica in saline 4 weeks before lung lavage [17]. The surfactant was purified by the method of Hawgood et al. [2], and then delipidated by extraction with 1-butanol (98 ml of butanol per 2 ml pellet). The lipids were separated from the protein precipitate by centrifugation and SP-A was purified from the protein precipitate as described previously [5]. Briefly, the protein was suspended in 5 mM Tris buffer (pH 7.4) and dialyzed against the same buffer. The protein suspension was centrifuged at $150\,000 \times g_{av}$ for 1 h and the supernatant was applied to affinity chromatography on mannose-Sepharose 6-B [18] followed by gel filtration over Bio-Gel A-5m (Bio-Rad). SP-D was also purified from rat lung lavage fluids as described previously [19], based on the method reported Persson et al. [20]. The protein content was estimated by the method of Lowry et al. [21] using bovine serum albumin as the standard.

2.4. Isolation of plasma membrane from alveolar type II cells

Alveolar type II cells were isolated from adult male Sprague–Dawley rats by tissue dissociation with elastase (Elastin Product) and metrizamide density gradients by the method of Dobbs and Mason [22]. Freshly isolated type II cells (approx. $100 \cdot 10^6$ cells) were suspended in 1.5 to 2.0 ml of 5 mM Tris buffer (pH 7.4) and left on ice for 10 min. The resulting cell suspension was homogenized with a Dounce homogenizer. The homogenate was quickly adjusted to 0.9 M sucrose using 2 M sucrose and layered appropriately on a gradient consisting of 1 ml each of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 M sucrose and 3 ml each of 0.9 M and 1.5 M sucrose. This gradient system is a modification of that described by Duck–Chong [23]. The gradients were centrifuged at $90\,000 \times g_{av}$ for 4 h and the bands between 0.9 and 1.5 M sucrose were collected as a plasma membrane fraction. The plasma membrane isolated from $100 \cdot 10^6$ type II cells contained 367 ± 115 nmol (mean $\pm$ S.D., $n = 3$) of phospholipids and 450 ± 177 μg of proteins. Alkaline phosphatase was measured as a marker of plasma membrane by the method of Edelson et al. [24]. Alkaline phosphatase has been shown to be localized in the plasma membrane of alveolar type II cells [24]. To isolate the plasma membrane derived from liver, rat liver was minced and homogenized with Potter homogenizer, and the plasma membrane fraction was isolated by sucrose density gradient centrifugation as described above.

2.5. Interaction of phospholipid liposomes with plasma membrane

40 μg protein of plasma membrane, which contained 33.5 ± 3.5 nmol (mean $\pm$ S.D., $n = 3$) of phospholipids, was incubated with 1 ml of 10 mM Tris buffer (pH 7.4) containing 0.15 M NaCl, 1 mM $CaCl_2$, phospholipid liposome (40 nmol as DPPC or PLPC) with a trace amount of [^{14}C]DPPC, [^{3}H]DPPC or [^{14}C]PLPC in the absence or the presence of 10 μg/ml SP-A at 37°C for 60 min. The incubation mixture was immediately processed for sucrose density gradient centrifugation to separate the liposomes that were associated with the plasma membrane from unbound liposomes. After the incubation 1 ml of Tris buffer (pH 7.4) containing 0.15 M NaCl and 1 mM $CaCl_2$ was added into the reaction mixture and layered on a top of a gradient consisting of 2 ml each of 0.2, 0.4, 0.6 M sucrose and 4 ml of 0.8 M sucrose. The gradients were centrifuged at $93\,000 \times g_{av}$ at 4°C for 4 h. 2 ml each from the top was collected, and the radioactivity in each fraction was counted. The final 2 ml was collected with the pellet. Each fraction was also assayed for alkaline phosphatase activity [24]. In some experiments, the plasma membrane was incubated with liposome containing [^{14}C]DPPC and a trace amount of [^{3}H]triolein, non-exchangeable marker, and processed for sucrose density gradient centrifugation.

2.6. Liposome aggregation

Liposome aggregation was performed by the method based on that described by Hawgood et al. [2]. SP-A (20 μg/ml) in 20 mM Tris buffer (pH 7.4) containing 0.15 M NaCl was preincubated for 3 min with 100 μg/ml unilamellar liposomes containing DPPC or PLPC prepared as described above. After equilibration, turbidity was measured at 400 nm using a Hitachi U2000 spectrophotometer at room temperature. Following the initial absorbance readings, $CaCl_2$ was added to a final concentration of 5 mM at a time of 30 s and turbidity was further measured until a time of 10 min.

3. Results

Phospholipid liposomes containing [^{3}H]DPPC were incubated with the plasma membrane isolated from type II cells in the absence or the presence of SP-A, and processed for sucrose density gradient centrifugation. Since SP-A induces liposome aggregation [2], control experiment without plasma membrane was performed (Fig. 1A). More than 90% of radioactivities were found in the fractions cosedimented with 0 and 0.2 M sucrose in the absence of SP-A, while the profile of radioactivities associated with the gradient fractions shifted to the right in the presence of SP-A; this is likely to be due to vesicle aggregation induced by SP-A. However, the radioactivities associated with the pellet remained less than 1% of total radioactivities even in the presence of SP-A. When the plasma membrane was incubated with liposomes, significant amounts of radioactivities were recovered in the pellet (Fig. 1B). The percent of radioactivity in the pellet fraction in the absence or the presence of SP-A was 2.83 ± 0.7% (mean ± S.D., $n = 3$) or 11.97 ± 2.49%, respectively. Incubation with SP-A significantly ($P < 0.001$) increased the liposomes recovered in the pellet. Inclusion of 5 mM EGTA or 100 μg/ml anti-SP-A polyclonal antibody in the incubation mixture reduced the percent of radioactivity recovered in the pellet; 4.0% or 3.9% (mean of two experiments), respectively, when that of control was 9.5%. When boiled SP-A was used instead of native SP-A, only 1.9% (mean of two experiments) of radiolabeled liposomes was recovered in the pellet fraction. When SP-D (10 μg/ml) was used instead of SP-A, only 2.45% (mean of two experiments) of liposome was associated with type II cell plasma membrane. Each fraction obtained was assayed for alkaline phosphatase activity (Fig. 1C). Approx. 70% of alkaline phosphatase activity was associated with the pellet fraction, indicating that most of the plasma membrane was recovered in the pellet. These data indicate that SP-A increases the association of phospholipid liposomes with type II cell plasma membrane, and suggest that SP-A mediates the transfer or the binding of lipid to plasma membrane.

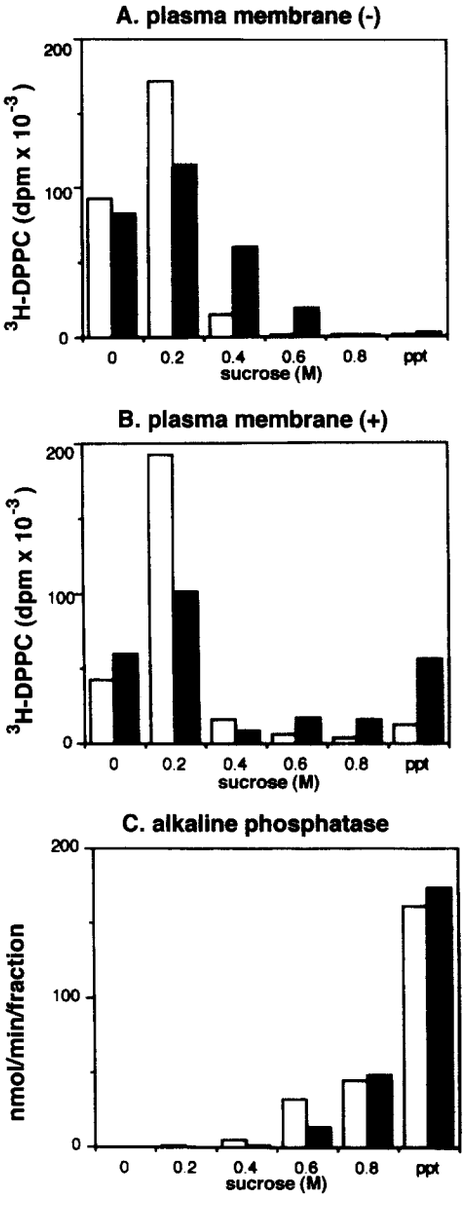

Fig. 1. Effect of SP-A on interaction of phospholipid liposomes with plasma membrane isolated from type II cells. Phospholipid liposomes (40 nmol as dipalmitoylphosphatidylcholine, DPPC) containing 60% DPPC, 20% egg phosphatidylcholine, 15% phosphatidylglycerol and 5% cholesterol, and a trace amount of [^{3}H]DPPC were incubated without (panel A) or with (panel B) plasma membrane (40 μg protein) isolated from type II cells in the absence (□) or the presence (■) of 10 μg/ml SP-A at 37°C for 60 min. The incubation mixture was then layered on a top of a gradient consisting of 2 ml each of 0.2, 0.4, 0.6 M sucrose and 4 ml of 0.8 M sucrose and centrifuged to separate the liposomes associated with plasma membrane from unbound liposomes. After centrifugation, 2 ml each from the top was collected and the final 2 ml was collected with the pellet. The radioactivity in each fraction was then counted. Each fraction obtained in the experiment shown in panel B was assayed for alkaline phosphatase activity (Panel C) as described in Section 2. The data shown are representative for three experiments.

To examine whether SP-A mediates the transfer or the binding of lipid to plasma membrane, plasma membranes isolated from type II cells were incubated with liposomes

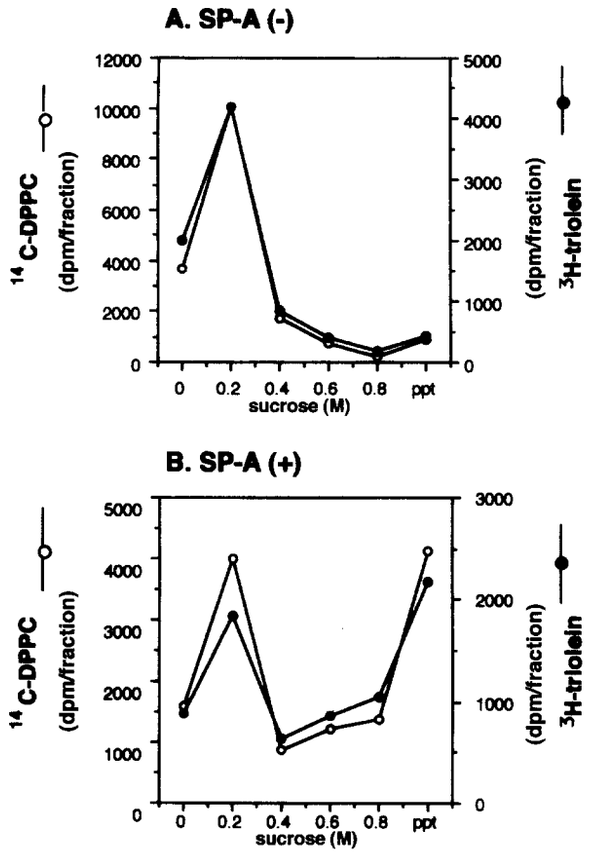

Fig. 2. SP-A mediates the binding of phospholipid liposomes but not the transfer of lipids to plasma membrane isolated from type II cells. The plasma membrane (40 μg protein) isolated from type II cells was incubated liposomes (40 nmol as dipalmitoylphosphatidylcholine, DPPC) containing 60% DPPC, 20% egg phosphatidylcholine, 15% phosphatidylglycerol and 5% cholesterol, and a trace amount of [^{14}C]DPPC (○) and [^{3}H]triolein (●) in the absence (panel A) or the presence (panel B) of 10 μg/ml SP-A at 37°C for 60 min. The reaction mixture was then immediately processed for sucrose density gradient centrifugation and the radioactivity in each fraction collected was counted as described in Section 2. The data shown are representative for three experiments.

containing [^{14}C]DPPC and [^{3}H]triolein, non-exchangeable marker, in the absence or the presence of SP-A, and processed for sucrose density gradient centrifugation. The radioactivities of ^{14}C and ^{3}H in the fractions obtained were shown in Fig. 2. The profiles of ^{14}C and ^{3}H in the sucrose density gradients were almost identical in both the absence and the presence of SP-A. The data clearly indicate that SP-A mediates the binding of liposomes to plasma membrane but not the transfer of DPPC.

We examined the liposome association with type II cell plasma membrane in various concentrations of SP-A (Fig. 3). The percent of radioactivities associated with the plasma membrane increased in a manner dependent upon SP-A concentration. Only two to three percents of radioactivity was recovered in the pellet fraction without plasma membrane in the presence of 20 and 40 μg/ml SP-A. Next, we compared the liposomes containing 1-palmitoyl-2-linoleoyl-phosphatidylcholine (PLPC) to those containing

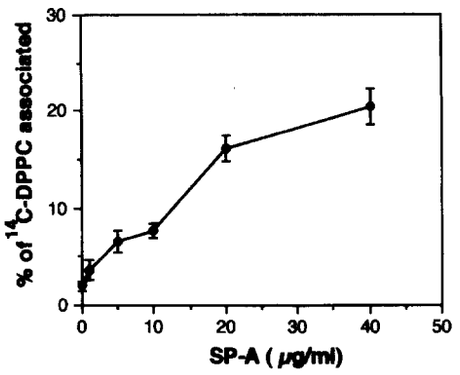

Fig. 3. The association of phospholipid liposomes with type II cell plasma membrane is dependent upon SP-A concentration. The plasma membrane (40 μg protein) isolated from type II cells was incubated with liposomes (40 nmol as dipalmitoylphosphatidylcholine, DPPC) containing 60% DPPC, 20% egg phosphatidylcholine, 15% phosphatidylglycerol and 5% cholesterol, and a trace amount of [^{14}C]DPPC with various concentrations of SP-A at 37°C for 60 min. The reaction mixture was then immediately processed for sucrose density gradient centrifugation and the radioactivity in each fraction collected was counted. The data represent percents of radiolabeled liposomes associated with the plasma membrane fraction in total radioactivities as described in Section 2. The values are means ± S.D., $n = 3$.

DPPC on the association with type II cell plasma membrane. When DPPC liposomes were incubated with type II cell plasma membrane in the presence of 10 μg/ml SP-A, 6.67% of liposomes was associated with the membrane (Table 1). In contrast, only 3.70% of PLPC lipsomes was associated with the membrane ($P < 0.005$ compared to DPPC liposomes). When the background (without SP-A) was subtracted, SP-A increased the association of DPPC

Table 1
The SP-A-mediated association of dipalmitoylphosphatidylcholine (DPPC)-containing liposomes with type II cell plasma membrane is significantly greater than that of 1-palmityol-2-linoleoylphospatidylcholine (PLPC)-containing liposomes

Phosphatidylcholine consisiting of liposomes	SP-A	% of [^{14}C]phosphatidylcholine associated [a]
DPPC	−	2.15 ± 0.42
DPPC	+	6.67 ± 1.32 [b,c]
PLPC	−	1.51 ± 0.57
PLPC	+	3.70 ± 0.75 [d]

[a] Phospholipid liposomes (40 nmol as DPPC or PLPC) containing 60% DPPC or PLPC, 20% egg phosphatidylcholine, 15% phosphatidylglycerol and 5% cholesterol, and a trace amount of [^{14}C]DPPC or [^{14}C]PLPC were incubated with plasma membrane (40 μg protein) isolated from type II cells in the absence or the presence of 10 μg/ml SP-A at 37°C for 60 min. The incubation mixture was then processed for sucrose density gradient centrifugation to separate the liposomes associated with plasma membrane from unbound liposomes as described under Section 2. The data represent percents of radiolabeled liposomes associated with the membrane fraction in total radioactivities. Values are means ± S.D., $n = 5$.
[b] $P < 0.005$ when compared to PLPC, SP-A(+).
[c] $P < 0.001$ when compared to DPPC, SP-A(−).
[d] $P < 0.002$ when compared to PLPC, SP-A(−).

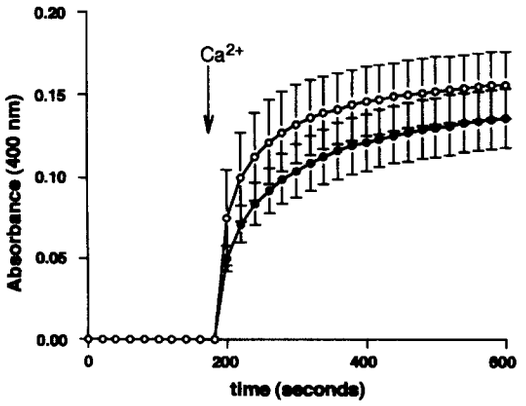

Fig. 4. The SP-A-induced aggregation of phosphlipid liposomes consisting of dipalmitoylphosphatidylcholine (DPPC) and 1-palmitoyl-2-linoleoyl-phosphatidylcholine (PLPC). SP-A (20 μg/ml) in 20 mM Tris buffer (pH 7.4) containing 0.15 M NaCl were preincubated for 3 min with 100 μg/ml unilamellar liposomes consisting of 60% DPPC (○) or PLPC (●), 20% egg phosphatidylcholine, 15% phosphatidylglycerol and 5% cholesterol. After equilibration, turbidity was measured at 400 nm at room temperature. Following the initial absorbance readings, $CaCl_2$ was added to a final concentration of 5 mM at a time of 30 s and turbidity was further measured until a time of 10 min. The values are means ± S.D., $n = 4$.

liposomes with the type II cell plasma membrane by 2-fold more than that of PLPC liposomes (4.52% vs. 2.19%). Since SP-A causes aggregation of phospholipid vesicles [2], we examined whether this difference is simply because SP-A induces aggregation of liposomes containing DPPC by a 2-fold greater extent than those containing PLPC. The turbidity induced by SP-A was measured using liposomes containing DPPC or PLPC. SP-A was able to induce both types of phospholipid vesicles; the final turbidity of DPPC or PLPC liposomes was 0.156 ± 0.02 A_{400} unit (mean ± S.D., $n = 4$) or 0.136 ± 0.018 A_{400} unit, respectively (Fig. 4). Only 15% difference in the final turbidity was found between these species of PC in the SP-A-mediated vesicle aggregation.

Next, we performed the vesicle association experiments with the plasma membranes isolated from liver and type II cells. 7.8% of the vesicle radioactivity was recovered in the membrane fraction isolated from type II cells, while only 3.7% was associated with the membrane isolated from liver ($P < 0.005$) in the presence of SP-A (Table 2). This indicates that the plasma membrane from type II cells possesses the higher activity on liposome association induced by SP-A than that from liver.

Table 2
The SP-A-mediated association of liposomes with the plasma membrane derived from type II cell is significantly greater than that derived from liver

Source of plasma membrane	SP-A	% of [^{14}C]phosphatidylcholine associated [a]
Type II cell	−	1.88 ± 0.16
Type II cell	+	7.81 ± 0.48 [b,c]
Liver	−	0.93 ± 0.17
Liver	+	3.73 ± 1.01 [d]

[a] Phospholipid liposomes (40 nmol as dipalmitoylphosphatidylcholine, DPPC) containing 60% DPPC, 20% egg phosphatidylcholine, 15% phosphatidylglycerol and 5% cholesterol, and a trace amount of [^{14}C]DPPC were incubated with plasma membrane (40 μg protein) isolated from type II cells or liver in the absence or the presence of 10 μg/ml SP-A at 37°C for 60 min. The incubation mixture was then processed for sucrose density gradient centrifugation to separate the liposomes associated with plasma membrane from unbound liposomes as described in Section 2. The data represent percents of radiolabeled liposomes associated with the membrane fraction in total radioactivities. Values are means ± S.D., $n = 5$.
[b] $P < 0.005$ when compared to liver plasma membrane, SP-A(+).
[c] $P < 0.001$ when compared to type II cell plasma membrane, SP-A(−).
[d] $P < 0.002$ when compared to liver plasma membrane, SP-A(−).

4. Discussion

Several studies have shown that surfactant lipids are taken up by alveolar type II cells and cleared from the alveoli [7–12]. This process contributes to the recycling and reutilization of surfactant phospholipids. The hydrophobic surfactant proteins as well as SP-A enhances the uptake of lipids by type II cells [13]. Synthetic SP-B peptides and the native protein also augment lipid uptake by fibroblasts [25]. In contrast, SP-A failed to enhance lipid uptake by fibroblasts [15], indicating that the mechanism of uptake process by this protein is different from that by the hydrophobic protein; the latter appears to be due to the nonspecific liposome fusion. Wright et al. [15] described that the metabolic inhibitors decreased the stimulatory effect of SP-A by 30–50% on liposome uptake by type II cells. This indicates that the uptake process is in part dependent on cellular process. However, the details of the uptake process of lipids into type II cells have not been well understood. In this study we demonstrated that SP-A preferentially enhances the binding of phospholipid vesicles containing DPPC to the plasma membrane derived from type II cells. This may contribute, in part, to the process of the SP-A-mediated lipid uptake by type II cells.

We used density gradient centrifugation with several layers of sucrose. Since alkaline phosphatase is located on the plasma membrane of alveolar type II cells [24], we collected the pellet fraction as the plasma membrane in which most of alkaline phosphatase activity is localized. There raised a possibility that the liposomes aggregated by SP-A may be simply cosedimented with the pellet fraction. However, the control experiments without plasma membrane showed that the sedimentation profile shifted slightly to the right in the presence of SP-A, but that the liposomes cosedimented with the pellet fraction without plasma membrane was negligible when compared to the experiments with plasma membrane. Thus, the radioactive liposomes cosedimented with the pellet fraction is considered to be those associated with the plasma membrane.

When the isolated plasma membrane was applied to the sucrose density gradient centrifugation after the incubation

with radiolabeled liposomes, approx. 66% of the membrane was recovered in the pellet fraction in the absence of SP-A; this was estimated by the distribution of the alkaline phosphatase activity (see Fig. 1C). Some of the plasma membranes were cosedimented with the gradients with lower densities. However, the addition of SP-A in the incubation mixture resulted in only 6% of increase in the alkaline phosphatase activity in the pellet fraction, indicating that SP-A does not appear to increase the aggregation of the plasma membrane significantly when estimated by sucrose density gradients. On the other hand, SP-A increased the binding of liposomes to type II cell membranes more than 4-fold. Thus, there appears to be little possibility that the enhanced association of liposomes with the type II cell membrane could be simply due to the SP-A-mediated aggregation of the plasma membrane and subsequent trapping of the radiolabeled liposomes.

Our previous study [16] has shown that the SP-A-mediated uptake of phosphatidylcholine by type II cells is preferential for DPPC and suggested that SP-A facilitates the incorporation of DPPC into lamellar bodies. Our present study is on this line. Lumb et al. [26] demonstrated that a protein derived from canine lung lavage, but not SP-A, catalyzed the transfer of phospholipids. This protein transferred saturated and unsaturated phosphatidylcholine and phosphatidylinositol but failed to transfer phosphatidylglycerol. The present study confirmed their result of SP-A possessing no activity of lipid transfer. The phospholipid transfer protein may play a role in the recycling of phospholipid in concert with surfactant proteins.

This study indicates that the SP-A-mediated association of phospholipid vesicles is preferential for those containing DPPC; this is approx. 2-fold higher than that of those containing PLPC. The aggregation activity of DPPC liposomes induced by SP-A appeared to be only by 15% higher than that of PLPC liposomes; the 2-fold difference in the membrane association between DPPC- and PLPC-containing liposomes does not appear to be explained only by the vesicle aggregation activity. In the absence of SP-A the percent of DPPC liposomes associated with type II cell plasma membrane is higher than that of PLPC liposomes. The uptake experiments by Wright et al. [15] with type II cells, alveolar macrophages and fibroblasts showed that in the absence of SP-A the amount of DPPC taken up by type II cells was increased approximately by two-fold greater than those by alveolar macrophages and fibroblasts. Suwabe et al. [27] reported that DPPC inhibited surfactant secretion by type II cells. This inhibition was cell-type specific. Unsaturated phosphatidylcholine such as PLPC failed to inhibit secretion. This suggests that DPPC may selectively fuse with the plasma membrane of type II cells. The present result that DPPC liposomes preferentially associate with type II cell plasma membrane in the absence of SP-A may support this assumption.

We also found that the plasma membrane derived from alveolar type II cells possessed higher activity as a acceptor for the binding of DPPC liposomes than that derived from liver in the presence of SP-A. The phospholipid to protein ratio of the plasma membranes isolated from type II cells and liver were 0.775 ± 0.083 nmol phospholipid/μg protein (mean $\pm$ S.D., $n = 3$) and 0.630 ± 0.014 nmol/μg, respectively. Although the content of phospholipids in type II cell membrane appears to be slightly higher than that in liver membrane, the amount of radiolabeled liposomes associated with type II cell membranes was more than 2-fold greater than those associated with liver membrane. Thus, it is unlikely that the 2-fold difference in liposome binding between the membranes from type II cells and liver may be caused by approx. 20% difference of the phospholipid contents between these membranes. Alveolar type II cells express a high-affinity receptor for SP-A [5,6]. Chemical modification studies demonstrate that cell surface binding activity of SP-A is directly related to its capacity to inhibit surfactant lipid secretion by type II cells [28]. Additional studies are needed to clarify whether lipid uptake by type II cells and the lipid association with type II cell plasma membrane utilize the same receptor that regulates surfactant secretion, although the membrane topology may differ from that of intact cells after the plasma membrane is isolated. SP-A also increased the amount of liposomes associated with liver plasma membrane, albeit to a lesser extent, as well as type II cell plasma membrane. In the study described by Wright et al. [15], SP-A at 15 μg/ml also increased the association of DPPC with two lines of fibroblasts, albeit total amounts increased by SP-A is clearly lower than those with type II cells. The mechanism by which SP-A increases the association of liposomes with the membranes, which are not derived from type II cells, is unclear.

In conclusion, SP-A increased the binding of liposomes containing DPPC to the plasma membrane isolated from type II cells. The SP-A-mediated association of liposomes containing DPPC with type II cell plasma membrane was greater than liposomes containing PLPC or the plasma membrane derived from liver.

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