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Interaction of Lipid Vesicles with Monomolecular Layers Containing Lung Surfactant Proteins SP-B or SP-C†

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ABSTRACT: Pulmonary surfactant contains two families of hydrophobic proteins, SP-B and SP-C. Both proteins are thought to promote the formation of the phospholipid monolayer at the air–fluid interface of the lung. The Wilhelmy plate method was used to study the involvement of SP-B and SP-C in the formation of phospholipid monolayers. The proteins were either present in the phospholipid vesicles which were injected into the subphase or included in a preformed phospholipid monolayer. In agreement with earlier investigators, we found that SP-B and SP-C, present in phospholipid vesicles, were able to induce the formation of a monolayer, as became apparent by an increase in surface pressure. However, when the proteins were present in a preformed phospholipid monolayer (20 mN/m) at similar lipid to protein ratios, the rate of surface pressure increase after injection of pure phospholipid vesicles into the subphase at similar vesicle concentrations was 10 times higher. The process of phospholipid insertion from phospholipid vesicles into the protein-containing monolayers was dependent on (1) the presence of (divalent) cations, (2) the phospholipid concentration in the subphase, (3) the size of the phospholipid vesicles, (4) the protein concentration in the preformed monolayer, and (5) the initial surface pressure at which the monolayers were formed. Both in vesicles and in preformed monolayers, SP-C was less active than SP-B in promoting the formation of a phospholipid monolayer. The use of preformed monolayers containing controlled protein concentrations may allow more detailed studies on the mechanism by which the proteins enhance phospholipid monolayer formation from vesicles.

Pulmonary surfactant is a complex lipid–protein mixture that lowers surface tension at the air–water interface in the lung. Lipids comprise the majority (approximately 90%) of this surface-active material, and their composition has been studied in detail (Van Golde et al., 1988). The most abundant phospholipid components are dipalmitoylphosphatidylcholine (DPPC),[1] unsaturated phosphatidylcholine (PC) species, and phosphatidylglycerol (PG). There is general agreement that DPPC is the principal surface-active component of pulmonary surfactant. It is this compound that is responsible for decreasing the surface tension at the alveolar surfaces to low values at end expiration (Clements, 1977).

At least three families of proteins, SP-A, SP-B, and SP-C, are thought to be unique constituents of pulmonary surfactant (Possmayer, 1988). SP-A is a glycoprotein of M_r 26 000–38 000 under reducing conditions (Possmayer, 1988; Hawgood, 1989). SP-A has been shown to play a role in the formation of tubular myelin in the presence of calcium ions (Benson et al., 1984; Suzuki et al., 1989) and the hydrophobic protein SP-B (Suzuki et al., 1989). SP-A may also be important in the regulation of surfactant homeostasis (Wright et al., 1987; Rice et al., 1987; Dobbs et al., 1987; Kuroki et al., 1988) and in alveolar defense (Tenner et al., 1989; Van Iwaarden et al., 1990). SP-B and SP-C are very hydropobic proteins that copurify with the lipids during extraction of surfactant with organic solvents. SP-B has a molecular weight of 18 000 under nonreducing conditions and a molecular weight of 5000–8000 under reducing conditions (Possmayer, 1988; Hawgood, 1989). It has been shown that the presence of SP-B in phospholipid vesicles enhances the adsorption of phospholipids to an air–water interface (Hawgood et al., 1987). SP-C has a molecular weight of 5000–8000 under both reducing and nonreducing conditions (Possmayer, 1988; Hawgood, 1989) and has two palmitoyl groups covalently linked to the polypeptide chain

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[1] Abbreviations: SP-A, SP-B, and SP-C, surfactant proteins A, B, and C, respectively; PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; PG, phosphatidylglycerol; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid; EGTA, ethylene glycol bis-(β-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid; MLV, multilamellar vesicle(s); SUV, small unilamellar vesicle(s); LUV, large unilamellar vesicle(s).

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(Curstedt et al., 1990). The presence of SP-C in phospholipid vesicles also led to an enhanced adsorption of phospholipids to an air–water interface (Takahashi & Fujiwara, 1986; Warr et al., 1987; Arjomaa & Hallman, 1988).

The mechanism by which SP-B and SP-C enhance the phospholipid adsorption to the air–water interface is not known. It is also not known whether these proteins become part of the phospholipid monolayer after adsorption of protein-containing phospholipid vesicles to the air–water interface.

In the present study, we used a model system in which we investigated the rate of surface pressure increase after injection of phospholipid vesicles underneath a preformed monolayer containing either SP-B or SP-C. The results of this new approach show that (1) SP-B and SP-C can form very stable monolayers in the absence or presence of phospholipids and (2) SP-B and SP-C, present in a preformed monolayer, are capable to induce phospholipid insertion from pure phospholipid vesicles into the monolayer.

Materials and Methods

Materials. Dipalmitoylphosphatidylcholine (DPPC) was purchased from Roth (Karlsruhe, Germany). Phosphatidylglycerol (PG; sodium salt, prepared from egg yolk phosphatidylcholine) was obtained from Sigma Chemical Co. (St. Louis, MO).

Isolation of SP-B and SP-C. SP-B and SP-C were isolated from porcine lung lavage. Porcine lungs were obtained from the slaughterhouse and lavaged 3–5 times with a solution of 154 mM NaCl. Pulmonary surfactant was prepared from the bronchoalveolar lavage by the method of Hawgood et al. (1985). Lung surfactant was extracted with 1-butanol (Haagsman et al., 1987). Butanol was dried by rotary evaporation, and the residue was dissolved in chloroform/methanol/0.1 M HCl (1:1:0.05, v/v). Insoluble material was removed by centrifugation. SP-B and SP-C were separated from lipids and purified to homogeneity by Sephadex LH-60 chromatography (column size 2.5 cm × 80 cm; Pharmacia, Uppsala, Sweden). The column was eluted with the same solvent at a flow rate of 15 mL/h. Fractions of 5 mL were collected and were analyzed by SDS–PAGE and silver staining (silver stain, from Bio-Rad Laboratories, Richmond, CA). Fractions containing SP-B or SP-C were pooled. The bulk of solvent was removed by rotary evaporation, and the remaining water phase was lyophilized. SP-B and SP-C were stored in chloroform/methanol (1:1, v/v) at −20 °C. Protein was determined by the fluorescamine procedure using bovine serum albumin as a standard (Böhlen et al., 1973).

Protein Electrophoresis. Protein electrophoresis was performed by one-dimensional Tricine/SDS–PAGE as described by Schägger and von Jagow (1987). Gels were stained with silver stain.

Multilamellar Vesicles (MLV). Lipids (DPPC:PG ratio 7:3, w/w) with or without SP-B or SP-C dissolved in chloroform/methanol (1:1, v/v) were dried under a stream of nitrogen at 37 °C. The lipid films were hydrated in 25 mM Hepes (pH 7.0) supplemented with 0–150 mM NaCl (2 μmol of lipid/mL). The suspensions were vortexed for 5 min.

Small Unilamellar Vesicles (SUV). Small unilamellar vesicles were prepared from MLV by sonication with a Branson B12 sonifier equipped with a 0.5-in. flat-top disrupter tip for 1 min at 50 W.

Large Unilamellar Vesicles (LUV). Large unilamellar vesicles were prepared from MLV by extrusion through two 0.4-μm Unipore polycarbonate membranes (Mayer et al., 1986). All vesicles were prepared freshly each day at 45 °C and kept at 37 °C.

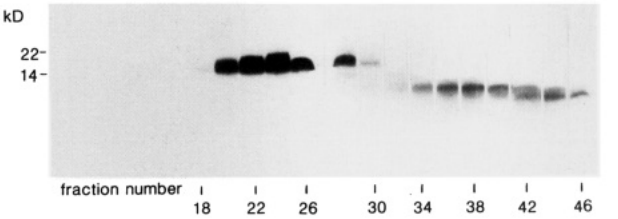

FIGURE 1: Separation of SP-B and SP-C. Aliquots from sequential fractions eluted from a Sephadex LH-60 column were analyzed by Tricine/SDS–PAGE under nonreducing conditions. The gels were stained with silver stain. Fractions containing only SP-B (M_r 18 000; fractions 20–28) or SP-C (M_r 8000 and/or M_r 5000; fractions 34–46) were pooled.

Monolayer Studies. For constant-area monolayer experiments, a 5.5-mL Teflon trough (r = 1.25 cm) was used. Surface pressure was determined at 37 °C by the Wilhelmy plate method, using a Cahn 2000 electrobalance (Demel, 1982). As a subphase, a buffer was used containing 25 mM Hepes (pH 7.0). The subphase was stirred continuously with a magnetic bar. Lipids (DPPC:PG ratio 7:3, w/w) and/or SP-B or SP-C dissolved in chloroform/methanol (3:1, v/v) were spread at the air–water interface with a glass capillary. Vesicles and salt solutions were injected into the subphase through an injection hole.

The results are given as a representative of at least three separate experiments. The percentage of variability between individual experiments was less than 10%.

Results

Protein Purification. SP-B and SP-C were purified from porcine bronchoalveolar lavage. After butanol extraction of pulmonary surfactant, the proteins were separated from lipids and purified to homogeneity by Sephadex LH-60 chromatography. Fractions were analyzed by Tricine/SDS–PAGE and silver staining (Figure 1). Fractions containing only SP-B (M_r 18 000; fractions 20–28) or SP-C (M_r 8000 and/or M_r 5000; fractions 34–46) were pooled. The elution of the phospholipid peak started with fraction 54.

Biophysical Studies with SP-B or SP-C Present in Phospholipid Vesicles. Multilamellar vesicles of DPPC/PG and SP-B (10:1, w/w) injected into a subphase of 25 mM Hepes (pH 7.0) did not adsorb to the air–water interface. The maximal pressure increase was induced by injection into the subphase of NaCl (maximal adsorption rate at 150 mM NaCl) or different divalent cations (Ca^{2+}, Mg^{2+}, Mn^{2+}, Sr^{2+}, Ba^{2+}; maximal adsorption rate at 3 mM) or by lowering the pH of the subphase from pH 7.0 to pH 4.0, leading to a final surface pressure of 51 ± 1 mN/m. Figure 2 shows that the addition of divalent cations (3 mM $CaCl_2$) was more effective in inducing adsorption of the MLV of DPPC/PG and SP-B to the air–water interface than the addition of monovalent cations (150 mM NaCl). Although the initial adsorption rate was the same in both cases, the maximum surface pressure was reached much faster if 3 mM $CaCl_2$ was added to the subphase (60 min) instead of 150 mM NaCl (240 min). The presence of 150 mM NaCl did not further enhance the $CaCl_2$-mediated adsorption rate. Comparable adsorption curves were recorded with SUV of DPPC/PG and SP-B (10:1, w/w). Similar results were obtained with either MLV or SUV of DPPC/PG and SP-C (5:1, w/w), although the adsorption rates were lower than in comparable experiments with SP-B-containing vesicles (data not shown).

In the experiments with SP-B or SP-C incorporated in the phospholipid vesicles, it is not clear whether the proteins will end up in the monolayer at the air–water interface and at what

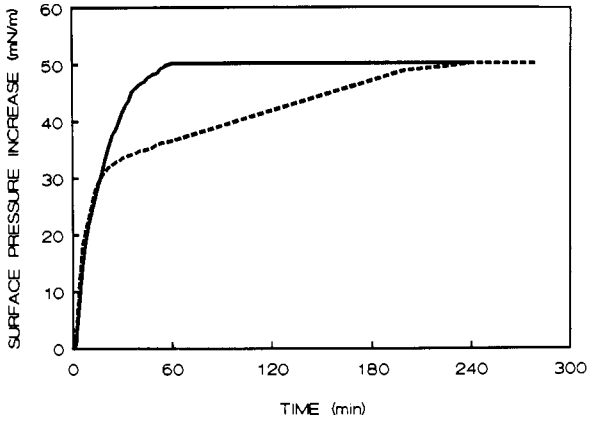

FIGURE 2: Adsorption of MLV-containing SP-B to the air–water interface. MLV of DPPC/PG and SP-B (10:1, w/w) were injected into the subphase (20 nmol of lipid/mL). At zero time, divalent cations [(—) $CaCl_2$, final concentration in the subphase 3 mM] or monovalent cations [(---) NaCl, final concentration in the subphase 150 mM] were added.

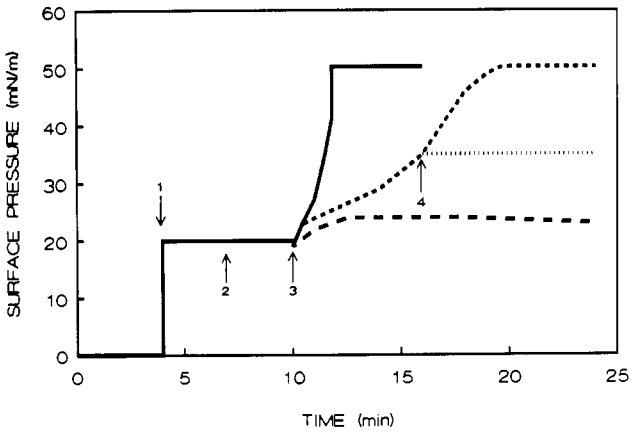

FIGURE 3: Experimental design of the experiments. (1) Spreading of the monolayer to 20 mN/m on a subphase containing 25 mM Hepes (pH 7.0). (2) Vesicle injection (SUV of DPPC/PG) into the subphase. (3) Addition of $CaCl_2$ to a final concentration of 3 mM. The surface pressure was measured with time. (--) Preformed monolayer containing DPPC/PG without hydrophobic protein. (—) Preformed monolayer containing DPPC/PG and SP-B 10:1 (w/w) and a vesicle concentration in the subphase of 20 nmol of lipid/mL. (---) Preformed monolayer containing DPPC/PG and SP-C 5:1 (w/w) and a vesicle concentration in the subphase of 20 nmol of lipid/mL. (4) Addition of EGTA to a final concentration of 5 mM (vertical dashes).

concentration. In order to study the role of SP-B and SP-C in the vesicle–monolayer interaction in more detail, the proteins were incorporated in the monolayer before phospholipid vesicles were added.

Biophysical Studies with SP-B or SP-C in Preformed Phospholipid Monolayers. The hydrophobic surfactant proteins, SP-B and SP-C, formed extremely stable monolayers. The maximum pressures of preformed pure SP-B and SP-C monolayers were 36 and 37 mN/m, respectively. The monolayers were stable with time.

All experiments with SP-B or SP-C in preformed monolayers were done in the same controlled way, as illustrated in Figure 3: (1) formation of the DPPC/PG monolayer containing either SP-B or SP-C (20 mN/m) on a subphase of 25 mM Hepes (pH 7.0); (2) injection of the DPPC/PG vesicles into the subphase; (3) injection of $CaCl_2$ to a final concentration of 3 mM 2 min after vesicle injection; (4) registration of the surface pressure increase in time until the maximum pressure was reached. The initial surface pressure decrease of 0.25 mN/m can be explained by the condensing effect of

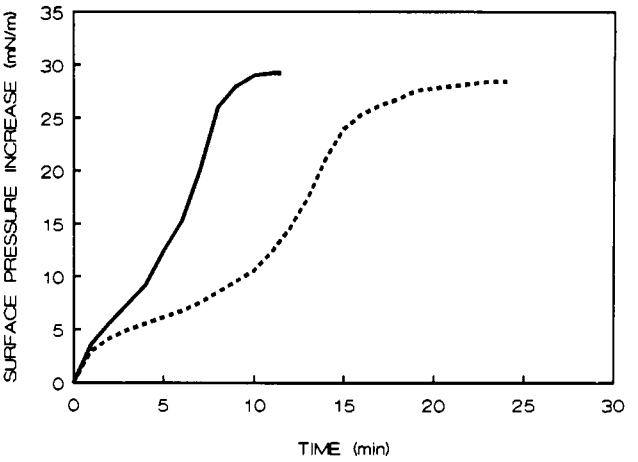

FIGURE 4: Effect of $CaCl_2$ and NaCl on the insertion of phospholipids from SUV into a preformed monolayer of DPPC/PG and SP-B. SUV of DPPC/PG were injected into the subphase (10 nmol of lipid/mL) underneath a preformed monolayer of DPPC/PG and SP-B (10:1, w/w; 20 mN/m). At zero time, divalent cations [(—) $CaCl_2$, final concentration in the subphase 3 mM] or monovalent cations [(---) NaCl, final concentration in the subphase 150 mM] were added.

Ca^{2+} ions on the PG in the monolayer. With SP-B [lipid to protein ratio 10:1 (w/w) and vesicle concentration in the subphase 20 nmol of lipid/mL], the maximum pressure (51 ± 1 mN/m) was reached within 2 min, showing an almost linear increase of the surface pressure with time. With SP-C [lipid to protein ratio 5:1 (w/w) and vesicle concentration in the subphase 20 nmol of lipid/mL], the surface pressure increase with time had a typical sigmoidal shape (Figure 3).

The insertion of phospholipids from DPPC/PG vesicles into a preformed monolayer containing SP-B or SP-C could be inhibited or terminated by injecting 5 mM EGTA into the subphase before or after (Figure 3) $CaCl_2$ injection into the subphase. Phospholipid insertion into the monolayer after $CaCl_2$ injection was only very limited when the phospholipid vesicles were injected underneath pure phospholipid monolayers (Figure 3).

The maximal insertion rate of phospholipids from DPPC/PG vesicles (SUV or LUV) into preformed monolayers of DPPC/PG and SP-B (10:1, w/w) was obtained with a final $CaCl_2$ concentration in the subphase of 3 mM. The time required to reach the maximum surface pressure (20 nmol of lipid/mL) was 2 min. When other divalent cations (Mg^{2+}, Mn^{2+}, Sr^{2+}, or Ba^{2+}, 3 mM) were injected into the subphase, the maximum surface pressure was also reached after 2 min. NaCl, which gave a maximal insertion rate at 150 mM, was less effective than divalent cations in inducing the SP-B-dependent insertion of phospholipids into the monolayer (Figure 4). The $CaCl_2$-mediated insertion rate was not further enhanced by the presence of 150 mM NaCl. Similar results were obtained with preformed monolayers of DPPC/PG and SP-C (5:1 w/w), although the insertion rates were lower than in comparable experiments with SP-B in the preformed monolayer. The insertion rate of phospholipids from LUV of DPPC/PG was about 10 times lower than the insertion rate of phospholipids from SUV of DPPC/PG. No significant change in surface pressure was observed when MLV of DPPC/PG were used. When the proteins were present both in the preformed phospholipid monolayers and in the phospholipid vesicles, high rates of surface pressure increase could also be obtained with MLV (data not shown).

Figure 5 shows that the time to reach the maximum surface pressure was dependent on the vesicle concentration in the subphase. Although the amount of SP-B in the preformed

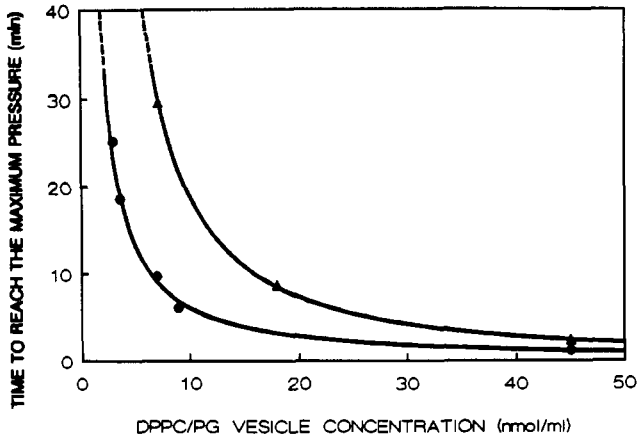

FIGURE 5: Effect of vesicle concentration on phospholipid insertion into a preformed monolayer. Time required to reach the maximum pressure was measured as a function of the vesicle concentration (SUV of DPPC/PG) injected into the subphase. (●) Monolayers containing DPPC/PG and SP-B (10:1, w/w; 20 mN/m). (▲) Monolayers containing DPPC/PG and SP-C (5:1, w/w; 20 mN/m).

monolayers was the same [(DPPC/PG):SP-B ratio 10:1, w/w], the maximum pressure was reached much faster when the vesicle concentration in the subphase was increased. Below a vesicle (SUV) concentration in the subphase of 3 nmol of lipid/mL, the insertion of phospholipids into the monolayer was limited. Comparable adsorption curves were obtained when SP-C instead of SP-B was present in the preformed monolayers, but SP-C was less efficient than SP-B in inducing phospholipid insertion from DPPC/PG vesicles into the preformed monolayer. Maximal insertion rates into monolayers containing (DPPC/PG):SP-B (10:1, w/w) and (DPPC/PG):SP-C (5:1, w/w) were obtained at vesicle (SUV) concentrations of 20 and 30 nmol of lipid/mL, respectively (Figure 5).

Figure 6 shows that the time to reach the maximum pressure was highly dependent on the SP-B or SP-C concentrations in the preformed monolayers. At higher protein concentrations, the maximum pressure was reached much faster than at low protein concentrations. At lipid to protein ratios higher than 30:1 (w/w) in the preformed monolayers, the insertion of phospholipids (20 nmol of lipid/mL) into the monolayers was limited. The surface pressure increase with time obtained with SP-C present in the monolayer had a more sigmoidal shape than with SP-B present in the monolayer (Figure 6). Again, it was shown that SP-C was less efficient than SP-B in inducing phospholipid insertion from DPPC/PG vesicles into the preformed monolayer (Figure 6).

Generally, the insertion rates into monomolecular layers decrease at higher initial pressures. However, the pressure profile as demonstrated in Figure 6A indicates that the rate of pressure increase is accelerated at higher pressures. Therefore, we determined the insertion rates of phospholipids into SP-B-containing monolayers [(DPPC/PG):SP-B ratio of 10:1, w/w] at different initial surface pressures. The insertion rates were higher at high initial surface pressures than at low initial surface pressures (Figure 7). Below an initial surface pressure of 15 mN/m, the insertion of phospholipids (5 nmol of lipid/mL) into the monolayer was limited.

Discussion

Davies et al. (1986) have described that the surface properties of surfactant suspensions isolated from bovine lung lavage were dependent on the presence of cations and that divalent cations were far more effective in inducing surface activity than monovalent cations. Studies in which SP-B or

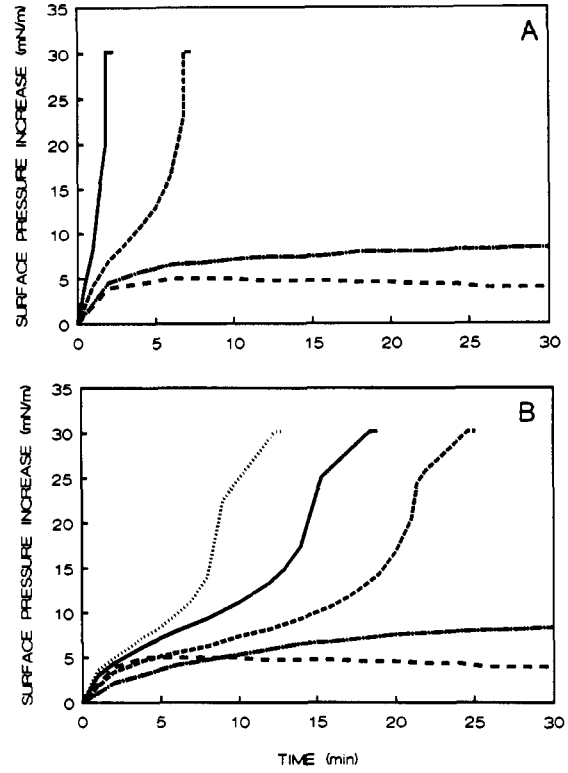

FIGURE 6: Effect of monolayer protein concentration on phospholipid insertion into a preformed monolayer. Time required to reach the maximum pressure was measured as a function of the protein concentration in the preformed monolayers. (A) Monolayers containing DPPC/PG and SP-B (20 mN/m). (B) Monolayers containing DPPC/PG and SP-C (20 mN/m). Lipid to protein ratio in the preformed monolayer: (···) 5:1; (—) 10:1; (---) 25:1; (-·-) 50:1. (--) Monolayer containing only DPPC/PG. The vesicle concentration in the subphase was 20 nmol of lipid/mL.

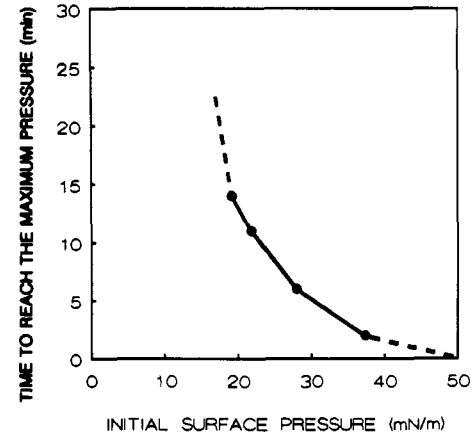

FIGURE 7: Effect of initial surface pressure on phospholipid insertion into a preformed monolayer. Time required to reach the maximum pressure was measured as function of the initial surface pressures in preformed monolayers containing DPPC/PG and SP-B (10:1, w/w). The vesicle concentration in the subphase was 5 nmol of lipid/mL.

SP-C were present in phospholipid vesicles showed that the adsorption of phospholipids to an air–water interface was enhanced by these hydrophobic proteins in a concentration-dependent way (Takahashi & Fujiwara, 1986; Whitsett et al., 1986; Hawgood et al., 1987; Warr et al., 1987; Arjomaa & Hallman, 1988; Shiffer et al., 1988). In the present study, we showed that this function of both SP-B and SP-C was not only dependent on the SP-B or SP-C concentration in the vesicles but also on the presence of cations. The presence of divalent cations (3 mM $CaCl_2$, $MgCl_2$, $MnCl_2$, $SrCl_2$, or

$BaCl_2$) in the subphase was far more effective in inducing surface activity than the presence of monovalent cations (150 mM NaCl). These results indicate that not only the interactions of SP-A with phospholipids are influenced by (divalent) cations (King & MacBeth, 1981; Hawgood et al., 1985; Efrati et al., 1987; Haagsman et al., 1990) but also the interactions between the hydrophobic surfactant proteins (SP-B and SP-C) and phospholipids. In earlier studies, Kobayashi and Robertson (1983) showed that the adsorption rate of extracted surfactant lipids to an air–water interface was similar to that of natural surfactant if $CaCl_2$ (4.5 mM) was present in the subphase. In retrospect, this Ca^{2+} dependency is in agreement with our results, because now it is known that fractions of extracted surfactant lipids contain the surfactant proteins SP-B and SP-C.

The rate of phospholipid adsorption to the air–water interface was independent of the type of vesicles injected into the subphase if the proteins were present in the vesicles. Adsorption of MLV to the air–water interface was as effective as adsorption of SUV of DPPC/PG and SP-B (maximum pressure 51 ± 1 mN/m). The maximum pressure was also reached when protein-containing phospholipid vesicles (MLV or SUV) were injected underneath preformed phospholipid monolayers with or without protein.

Interestingly, when SUV of DPPC/PG were injected underneath preformed phospholipid monolayers containing SP-B or SP-C (20 mN/m), phospholipids were also inserted into the monolayer (maximum pressure 51 ± 1 mN/m). Actually, at similar lipid to protein ratios and similar vesicle concentrations, the insertion rate of phospholipids from pure phospholipid vesicles (SUV) into protein-containing monolayers (20 mN/m) was 10 times higher than the adsorption rate of protein-containing phospholipid vesicles (SUV) (cf. Figures 2 and 3). Phospholipid insertion into the monolayer after $CaCl_2$ injection was only very limited when SUV of DPPC/PG were injected underneath a pure phospholipid monolayer. This indicates that the proteins can enhance monolayer formation if present in (1) the phospholipid vesicles, (2) the (pre)formed monolayer, or (3) both the phospholipid vesicles and the (pre)formed monolayer. In the second case, the insertion of phospholipids into the monolayer was dependent on the type of vesicles injected into the subphase. Phospholipids from SUV or LUV of DPPC/PG were inserted into preformed monolayers containing SP-B or SP-C. Phospholipids from SUV were inserted 10 times faster than phospholipids from LUV. This may be due to the difference in radius of curvature between SUV and LUV. A smaller radius of curvature may favor the interaction between the vesicles and the monolayer. It has been described that SUV are more effective in protein-induced fusion events than LUV (Gad et al., 1982, 1985). LUV and SUV of DPPC/PG at the concentrations used had no measurable surface activities themselves. Phospholipids from MLV of DPPC/PG were not inserted into SP-B- or SP-C-containing monolayers, not even with 100-fold lipid concentrations in the subphase. Probably, the phospholipids in MLV of DPPC/PG are so tightly packed that vesicle–monolayer interaction is inhibited. The observation that inclusion of SP-B and SP-C in MLV does enhance the adsorption of phospholipids to the air–water interface can be explained by the interaction of SP-B or SP-C with the individual membranes of MLV.

Both the adsorption of the phospholipids at the air–water interface enhanced by SP-B or SP-C present in the vesicles and the insertion of phospholipids into a monolayer enhanced by SP-B or SP-C present in the monolayer were dependent on the presence of cations. In both cases, divalent cations were far more effective than monovalent cations. This indicates that the functions of both SP-B and SP-C are highly influenced by electrolytes. Experiments were done in the absence of NaCl in the subphase because in this way the system could be "triggered" with 3 mM $CaCl_2$ after injection of vesicles into the subphase. In case 150 mM NaCl would be present in the subphase, the protein-enhanced insertion of the phospholipids into the monolayer would start immediately after vesicle injection into the subphase.

The SP-B- or SP-C-enhanced insertion of phospholipids into a preformed monolayer was also dependent on the vesicle concentration in the subphase and on the protein concentration in the preformed monolayers. Higher subphase concentrations of vesicles or higher protein concentrations in the preformed monolayers most likely enhanced the probability of vesicle–monolayer interaction. With SP-B (lipid to protein ratio 10:1, w/w, and vesicle concentration 20 nmol of lipid/mL), the surface pressure increase with time had an almost linear tracing, whereas with SP-B [lipid to protein ratio >10:1 (w/w) or vesicle concentration ≤20 nmol of lipid/mL] or with SP-C [lipid to protein ratio ≥5:1 (w/w) and/or vesicle concentration ≤30 nmol of lipid/mL] the surface pressure increase with time had a typical sigmoidal shape. Kobayashi and Robertson (1983) also described linear and S-shaped adsorption tracings with time for natural surfactant and lipids extracted from natural surfactant (containing SP-B and SP-C) depending on the lipid concentrations in the subphase. The differences in curves (Figure 3) may be related to the fact that SP-C present in the preformed monolayer was less effective in enhancing the insertion of phospholipids into a preformed monolayer than was SP-B. This is in agreement with our results and those of Hawgood et al. (1987) and Warr et al. (1987) that SP-C present in phospholipid vesicles is less effective in enhancing phospholipid adsorption to the air–water interface than SP-B.

When the phospholipid/SP-B monolayers were spread at different initial surface pressures, it appeared that the insertion rates were much higher at high initial surface pressures than at low initial surface pressures. This indicates that the proteins, even at high surface pressures, are able to induce phospholipid insertion into an existing monolayer.

Our results strongly support the general idea that the hydrophobic surfactant proteins play an important role in the surface properties of lung surfactant. When present in phospholipid vesicles (MLV or SUV), the proteins enhance the adsorption of phospholipids to the air–water interface; when present in preformed monolayers, the proteins induce the insertion of phospholipids from phospholipid vesicles (SUV or LUV) into the monolayer. High rates of surface pressure increase were obtained with MLV if the proteins were present in both the preformed phospholipid monolayers and the phospholipid vesicles.

Our results suggest that protein–protein interactions between protein in the monolayer and protein in the phospholipid vesicles are not required in the adsorption and/or insertion process. Divalent cations may be involved in the binding of the phospholipid vesicles to the monolayer by (1) formation of cation–PG bridges, (2) phospholipid–protein interactions, (3) conformational changes of the protein, or (4) dehydration of the polar head-group region of the phospholipids involved.

The model system presented in this paper opens perspectives for further studies on the mechanism(s) by which the hydrophobic surfactant proteins enhance the formation of phospholipid monolayers. Furthermore, this model system, which is based on preformed monolayers under which vesicles are

injected, may be a good reflection of the physiological situation in which lamellar bodies are secreted underneath an existing surfactant monolayer.

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