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Hydrophobic lung surfactant proteins B and C remain associated with surface film during dynamic cyclic area changes

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Abstract

The biophysical activity of lung surfactant depends, to a large extent, on the presence of the hydrophobic surfactant proteins B (SP-B) and C (SP-C). The role of these proteins in lipid adsorption and lipid squeeze-out under dynamic conditions simulating breathing is not yet clear. Therefore, the aim of this study was to investigate the interaction of spread hydrophobic surfactant proteins with phospholipids in a captive-bubble surfactometer during rapid cyclic area changes (6 cycles/min). We found that SP-B and SP-C facilitated the rapid transport of lipids into the air–water interface in a concentration-dependent manner (threshold concentration $\geq 0.05{:}0.5$ mol% SP-B/SP-C). Successive rapid cyclic area changes did not affect the concentration-dependent lipid adsorption process, suggesting that SP-B and SP-C remained associated with the surface film.

Keywords: Pulmonary surfactant; Surfactant associated protein B (SP-B); Surfactant associated protein C (SP-C); Spread film; Surface activity; Captive-bubble surfactometer

1. Introduction

The biophysical activity of lung surfactant depends, to a large extent, on the presence of the hydrophobic surfactant proteins B (SP-B) and C (SP-C) (reviewed in [1–3]). In particular, SP-B and SP-C enhance formation of surfactant film at the air–water interface and thereby promote rapid lowering of surface tension upon film compression.

Recent findings obtained in in vitro model systems suggest that SP-B and SP-C, together with lipid components, are squeezed out from the surface film upon area compression [4–6] and are stored in a multilayered phase closely associated with the residual film at the air–water interface [7,8]. Upon subsequent area expansion, they respread into the surface film [4,9].

These findings have provided important insights into the movement of SP-B and SP-C in relation to the air–water interface during compression and expansion of a complex surfactant film. However, the fate of SP-B and SP-C during compression and expansion at rates similar to breathing rates is still unclear.

Abbreviations: ata, absolute atmospheric pressure; DPPC, 1,2-dipalmitoyl-*sn*-3-phosphocholine; γ, surface tension; γ_{max}, maximum surface tension; γ_{min}, minimum surface tension; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; *p*, system pressure inside the glass cuvette; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-(phospho-*rac*-(1-glycerol)); SP-, surfactant-associated protein; SUV, small unilamellar vesicle

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We report the results of a study designed to investigate the interaction of SP-B and SP-C with phospholipids in a captive-bubble surfactometer during rapid cyclic area changes (6 cycles/min).

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-(phospho-*rac*-(1-glycerol)) (POPG) (Avanti Polar-Lipids, Alabaster, AL), *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), EDTA, calcium-, potassium- and sodium chloride (Sigma-Aldrich, Vienna, Austria), chloroform ($CHCl_3$) and methanol (MeOH) (HPLC-grade, Burdick and Jackson, Muskegon, MI), isopropylalcohol and triethylamine (Fluka Chemie, Buchs, Switzerland) were used as purchased. Agarose (SeaKem ME agarose, FMC BioProducts, Rockland, ME) and silicone rubber gaskets (Cal-Neva Supply, Oakland, CA) were extracted as described [10]. Water used for chemical analyses was filter purified (Modulab ModuPure, US Filter Corporation, Lowell, MA), and water used for surface activity measurements was quartz distilled (Muldestor, Wagner and Munz, Munich, Germany).

2.2. Reconstitution of hydrophobic surfactant protein–phospholipid and phospholipid mixtures and preparation of small unilamellar vesicles for subphase injection

Hydrophobic surfactant protein–phospholipid mixtures used for spreading of film were reconstituted as follows. The concentration of both DPPC (10.8 mg/ml) and POPG (9.7 mg/ml) stock solutions dissolved in $CHCl_3$/MeOH (1:1; v/v) was determined according to [11]. The concentration of both SP-B (0.088 mg/ml) and SP-C (0.087 mg/ml), isolated from pig lung lavage [12] and stored in $CHCl_3$/MeOH (1:1; v/v) at −20°C, was determined by fluorescamine assay [13] and quantitative amino acid analysis. In the SP-B stock solution, phospholipid content was unmeasurable; in the SP-C stock solution, it was 0.22 nmol phospholipid/μg SP-C. Aliquots from DPPC and POPG stock solutions were mixed (DPPC/POPG, 80:20 mol%). 9-μl aliquots from this lipid mixture were transferred (801, Hamilton, Bonaduz, Switzerland) into pointed glass vials (100 μl; Roth, Karlsruhe, Germany) and dried under N_2 gas at 37°C. Then, aliquots from SP-B and SP-C stock solutions were mixed (SP-B/SP-C, 10:90 mol%). Aliquots of 173.5, 86.8, 43.4, 17.4 or 8.7 μl were withdrawn from the mixed protein solution (825, Hamilton) and added to the dried lipids. In controls, 173.5 μl solvent without SP-B/SP-C was added. The final molar compositions of SP-B/SP-C/phospholipid (DPPC/POPG, 80:20 mol%) in sample solutions prepared for spreading of film were as follows: 0.20:2.00:97.80 mol%; 0.10:1.00:98.90 mol%; 0.05:0.50:99.45 mol%; 0.02:0.20:99.78 mol%; and 0.01:0.10:99.89 mol%. Control samples only contained phospholipid. The fixed molar ratio of 1:10 for SP-B/SP-C was chosen to represent the ratios we observed in a variety of surfactant preparations [14]. After gentle mixing on a vortexer, SP-B/SP-C/phospholipid or phospholipid mixtures were dried again under N_2 gas at 37°C and stored at −80°C.

Small unilamellar vesicles (SUVs) for subphase injection were prepared for each experiment by sonication of freshly prepared multilamellar vesicles (DPPC/POPG, 80:20 mol%) as described in [15]. A 30-μl aliquot was slowly injected (805, needle gauge 22S, Hamilton) into the stirred subphase (final subphase concentration 1.0 mg DPPC/ml). SUVs rather than large unilamellar vesicles were chosen because their higher curvature favors interaction with the surface film [16] and imitates the high curvatures found in membranes of tubular myelin [17]. To measure the size of SUVs generated by sonication of multilamellar vesicles, negative staining was used. An aliquot was withdrawn from a sample used for subphase injection and stored at −80°C until morphological analysis. The thawed vesicle suspension was adsorbed on formvar–carbon-coated nickel grid for 2 min and negatively stained with 2% uranyl acetate in distilled water. The grid was viewed and photographed in a Philips CM10 at 80 kV, and the diameters of 169 vesicles were measured. To exclude the possibility that phospholipids were degraded by sonication, the temperature was measured in the suspension of SUVs before and after 2 min of sonication.

2.3. Surface activity measurements and definition and calculation of film parameters used to characterize the surface activity of spread film

The surface activity of hydrophobic surfactant protein–phospholipid and phospholipid films was measured in a captive-bubble surfactometer according to [15]. Briefly, a bubble (0.5 cm^2) was formed in subphase buffer (140 mM NaCl, 10 mM HEPES, 0.5 mM EDTA, 2.5 mM $CaCl_2$, pH 6.9) by air injection (29 μl). Dried SP-B/SP-C/phospholipid samples were dissolved in 25 μl $CHCl_3$/MeOH (1:1; v/v). The wall of the glass vial was rinsed carefully. The sample solution was mixed by repeated vortexing and aspiration (805, Hamilton). A 0.05 μl aliquot from the sample solution was spread along the bubble's air–water interface. The subphase was stirred at 100 rpm for 60 min, and thereafter the sample chamber was perfused with subphase buffer for 30 min (at 37°C). The amount of material spread was standardized to 0.15 μg DPPC plus relative amounts of POPG, SP-B, and SP-C, which is 11% more DPPC than the interface can hold at a surface tension (γ) of 25 mN/m. Thus, film was formed at conditions of surface excess.

Minimum surface tension was defined as surface tension of film reached during dynamic compression before onset of film collapse, and film collapse was defined as a decrease in bubble diameter at constant bubble height. Maximum surface tension was defined as highest surface tension of film reached during dynamic expansion. Overcompression of film was defined as percentage of bubble area compressed beyond that measured at the onset of film collapse (collapse point). An exact description of the calculation of surface tension and area, film compressibility, and film stability is given elsewhere [15].

2.4. Experimental protocol

First, a film was spread along the bubble's air–water interface as described above. Then the subphase was stirred (100 rpm) for 60 min. After perfusion of the sample chamber, SUVs (without hydrophobic surfactant proteins) were injected into the subphase, and stirring continued for another 15 min. Next, the bubble area was increased by sudden lowering of system pressure inside the glass cuvette (p) to 0.5 ata for 10 s to study adsorption. Then the bubble was cycled five times (6 cycles/min) between 2.8 and 0.5 ata to measure the surface activity of film during dynamic cyclic area changes. Then the system pressure was kept constant at the end of the fifth compression over a period of 5 min (2.8 ata) to determine film stability after cyclic area changes. Finally, the bubble was cycled another five times (6 cycles/min) between 2.8 and 0.5 ata, and the surface activity of film was measured.

2.5. Statistical analysis

Experiments were performed in random order. Data are reported as mean ± S.E. For statistical analysis, the program SPSS version 6.1 was used. To determine the critical concentration of hydrophobic proteins required for biophysical activity, we analyzed the data with the Kruskal–Wallis statistic. When we found a significant difference, we performed a one-way analysis of variance (post hoc Bonferroni test) to identify differences. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Film formation

The surface tension reached after 1 s by spread film with proteins after a sudden increase in bubble area was significantly lower when the concentration of proteins used was $\geq 0.05{:}0.5$ mol% SP-B/SP-C (Fig. 1). For concentrations of $< 0.05{:}0.5$ mol% SP-B/SP-C, the surface tension was as high as that in the control (phospholipid without SP-B/SP-C). Similarly, surface tension was significantly lower after 10 s when the concentration of proteins was $\geq 0.05{:}0.5$ mol% SP-B/SP-C (Fig. 1) and as high as that in the control for concentrations of $< 0.05{:}0.5$ mol% SP-B/SP-C.

3.2. Minimum and maximum surface tension

The minimum surface tension reached on the first compression by spread film with proteins during rapid cyclic area changes was equally low for the various protein concentrations tested and as low as that in

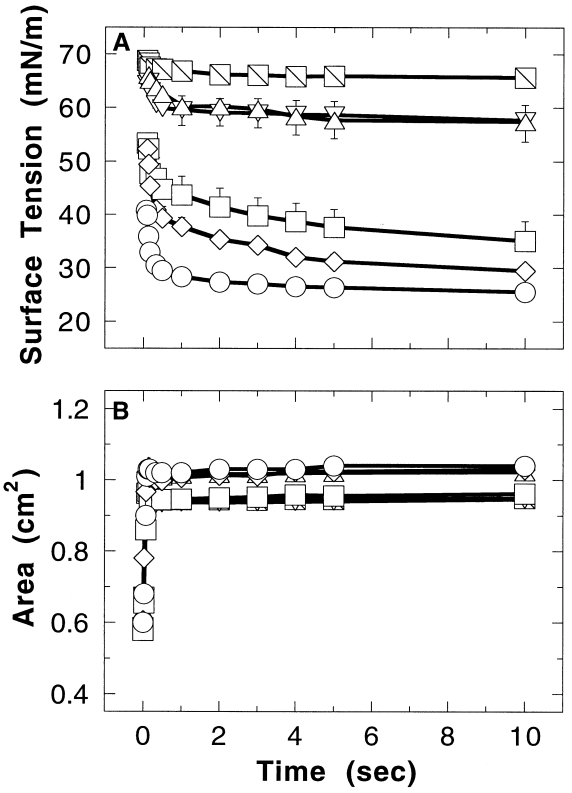

Fig. 1. Adsorption kinetics for spread hydrophobic lung surfactant protein–phospholipid and phospholipid films. (A) Bubble surface tension and (B) area response of films to the first rapid decrease in cuvette pressure are plotted vs. time. SP-B/SP-C/phospholipid (DPPC/POPG, 80:20 mol%) ratios: ○, 0.20:2.00:97.80 mol%; ◇, 0.10:1.00:98.90 mol%; □, 0.05:0.50:99.45 mol%; △, 0.02:0.20:99.78 mol%; ▽, 0.01:0.10:99.89 mol%; open square with diagonal line, 0:0:100 mol% (control). Data are expressed as mean ± S.E. for three to ten experiments. S.E. bars are partly obscured by symbols. For clarity, data obtained for surface tension within ≤0.07 s (first and second frames after sudden bubble expansion) are not shown. The surface tension after 1 and 10 s for spread films with proteins was significantly lower for concentrations ≥0.05:0.5 mol% SP-B/SP-C.

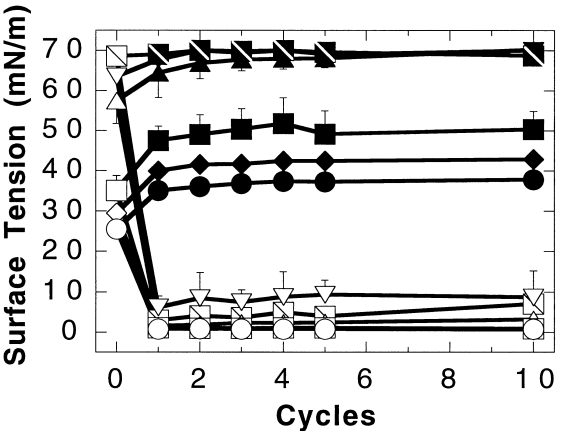

Fig. 2. Minimum surface tension (open symbols) and maximum surface tension (closed symbols) of spread hydrophobic lung surfactant protein–phospholipid and phospholipid film during cyclic area changes. Symbols are the same as those in Fig. 1. Data are expressed as mean ± S.E. for three to ten experiments. S.E. bars are partly obscured by symbols. The minimum surface tension on the first rapid compression and thereafter was equally low for the various protein concentrations tested except for 0.01:0.1 mol% SP-B/SP-C, when it was significantly higher for compressions two to five. The maximum surface tensions were always significantly higher for concentrations <0.05:0.5 mol% SP-B/SP-C.

the control (Fig. 2). Upon repeated compressions during rapid cyclic area changes, minimum surface tension remained as low as that in the control when the concentration of proteins used was ≥0.02:0.2 mol% SP-B/SP-C. However, for 0.01:0.1 mol% SP-B/SP-C, minimum surface tension was significantly higher for compressions two to five (Fig. 2). The maximum surface tension reached on expansion by spread film with proteins during rapid cyclic area changes was always significantly higher, and as high as that in the control, when the concentration of proteins used was <0.05:0.5 mol% SP-B/SP-C (Fig. 2).

3.3. Film compressibility at surface tension of 15 mN/m

On the first compression, the compressibility of spread film with proteins during rapid cyclic area changes was significantly higher for 0.01:0.1 mol% SP-B/SP-C and for the control (Table 1) than for the higher protein concentrations. However, on the third and fifth compression of rapid cyclic area changes, no difference was found for the various concentrations of proteins.

3.4. Film stability

The stability of spread film with proteins at similar decreases in bubble area during constant system pressure was equally high for all concentrations of proteins tested (Fig. 3) and as high as that for the control.

Table 1
Surface activity of spread hydrophobic lung surfactant protein–phospholipid and phospholipid films at 37°C

Ratio of film components[a] (mol %)	Film compressibility at 15 mN/m (m/mN)		
	1st Compression	3rd Compression	5th Compression
0.20:2.00:97.80	0.008 ± 0.001 (7/7)[b]	0.008 ± 0.001 (7/7)	0.008 ± 0.001 (7)
0.10:1.00:98.90	0.012 ± 0.001 (3/3)[b]	0.009 ± 0.001 (3/3)	0.008 ± 0.001 (3/3)
0.05:0.50:99.45	0.014 ± 0.002 (3/3)[b]	0.011 ± 0.002 (3/3)	0.011 ± 0.002 (3/3)
0.02:0.20:99.78	0.016 ± 0.001 (3/3)[b]	0.013 ± 0.002 (3/3)	0.012 ± 0.002 (3/3)
0.01:0.10:99.89	0.020 ± 0.002 (5/6)	0.017 ± 0.001 (5/6)	0.013 ± 0.002 (4/6)
0:0:100	0.017 ± 0.002 (10/10)	0.013 ± 0.002 (10/10)	0.013 ± 0.002 (10/10)
	Overcompression (%)		
	1st Compression	3rd Compression	5th Compression
0.20:2.00:97.80	16 ± 2 (47)[c]	12 ± 2 (44)[c]	13 ± 2 (44)[c]
0.10:1.00:98.90	5 ± 1 (46)	2 ± 1 (40)	3 ± 1 (41)
0.05:0.50:99.45	3 ± 2 (48)	2 ± 1 (46)	2 ± 2 (46)
0.02:0.20:99.78	0 (54)	0 (55)	0 (57)
0.01:0.10:99.89	0 (61)	0 (61)	0 (63)
0:0:100	0 (58)	0 (58)	0 (59)

Film compressibility = $(1/A)\times(dA/d\gamma)$ at $\gamma = 15$ mN/m; numbers in parentheses, number of experiments analyzed/number of experiments performed. Overcompression, expressed as percentage of total area reduction (for definition, see Section 2); numbers in parentheses, mean percent total area reduction. Data are mean ± S.E.
[a]SP-B/SP-C/phospholipid (DPPC/POPG, 80:20 mol%).
[b]Significantly different from concentrations ≤ 0.01:0.1 mol% SP-B/SP-C ($P < 0.05$).
[c]Significantly different from concentrations ≤ 0.1:1.0 mol% SP-B/SP-C ($P < 0.05$).

3.5. Overcompression

Observed overcompression of spread film during rapid cyclic area changes was significantly lower for concentrations ≤ 0.1:1.0 mol% SP-B/SP-C than for the highest protein concentrations in all compressions (Table 1).

3.6. Dynamic surface tension–area compression–expansion isotherms

Representative surface tension–area compression–expansion isotherms for spread film with and without hydrophobic surfactant proteins recorded during rapid cyclic area changes are shown in Fig. 4. When the concentration of proteins used for spreading was ≥ 0.05:0.5 mol% SP-B/SP-C, the formation of film during cycling was fast and, as a result, maximum surface tension remained < 50 mN/m. In contrast, when the concentration of proteins was < 0.05:0.5 mol% SP-B/SP-C, the formation of film was slow and, as a result, maximum surface tension increased to > 50 mN/m. Similarly, when the concentration of proteins was ≥ 0.05:0.5 mol% SP-B/SP-C, hysteresis and overcompression during cycling could be observed. However, when the concentration of proteins was < 0.05:0.5 mol% SP-B/SP-C, hysteresis disappeared and overcompression could no longer be observed. Close superimposition of the first (expansion only), second, and third cycles was found for concentrations of proteins ≥ 0.05:0.5 mol% SP-B/SP-C and for concentrations of proteins ≤ 0.01:0.1 mol% SP-B/SP-C. Moreover, at these protein concentrations no shift of the compression–expansion isotherms toward lower areas was seen. For 0.02:0.2 mol% SP-B/SP-C cycles were not superimposable. Instead, they showed a shift toward lower areas.

3.7. Quality assurance and quality control of data obtained

The surface tension of bubbles in buffer before spreading of hydrophobic surfactant protein–phospholipid film was 70.1 ± 0.6 mN/m at 1.0 ata and 70.2 ± 0.5 mN/m at 2.8 ata (area reduction, 51%; $n = 34$ experiments). The ratio of side-to-side to

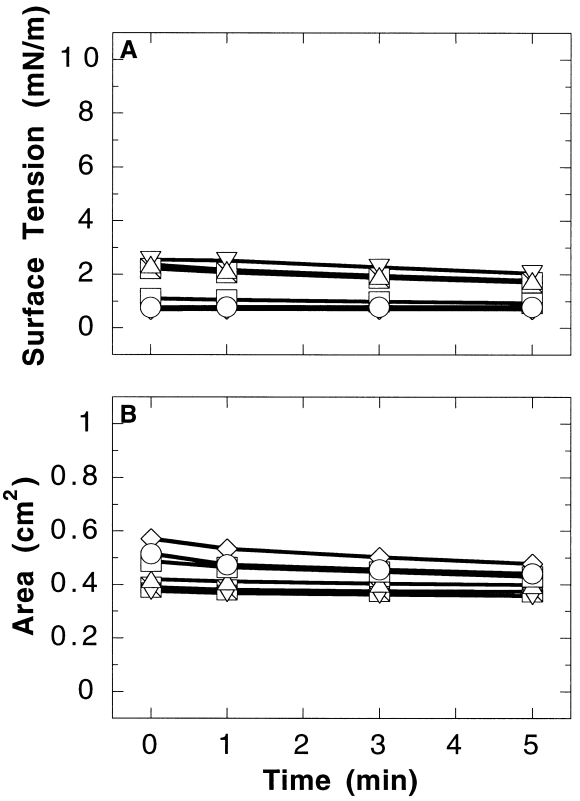

Fig. 3. Desorption kinetics for hydrophobic lung surfactant protein–phospholipid and phospholipid film. (A) Bubble surface tension and (B) area response of spread film to constant cuvette pressure are plotted vs. time. Symbols are the same as those in Fig. 1. Data are expressed as mean ± S.E. for three to eight experiments. S.E. bars are partly obscured by symbols. The stability of spread film with proteins was equally high for the various concentrations of proteins tested.

front-to-back diameters of bubbles from spread film experiments was 1.00 ± 0.02 ($n = 258$ frames). The average diameter of SUVs used for subphase injection was 47 ± 1 nm ($n = 169$ measurements). The temperature measured in the suspension of SUVs used for subphase injection was 49.3 ± 0.2°C before sonication and 47.0 ± 0.3°C after 2 min of sonication ($n = 15$ experiments).

4. Discussion

The aim of this study was to investigate the interaction of SP-B and SP-C with phospholipids during rapid cyclic area changes. The results show that the hydrophobic surfactant proteins SP-B and SP-C catalyze adsorption and spreading of phospholipids at the air–water interface in a concentration-dependent manner. Successive rapid cyclic area changes did not affect the concentration-dependent lipid adsorption process, suggesting that SP-B and SP-C remained associated with the surface film.

SP-B and SP-C enhance the rapid transport of phospholipids into the air–water interface and contribute to the formation of a highly surface-active film. The threshold concentration of SP-B and SP-C found in this study to result in high surface activity of the spread film was 0.05:0.5 mol% SP-B/SP-C. At or above this concentration, new material was rapidly transported into the air–water interface during adsorption (Fig. 1). Moreover, the newly formed film reached a minimum surface tension of < 5 mN/m on the first compression (Fig. 2) and showed progressive refinement with enrichment of DPPC (Table 1), kept the maximum surface tension at < 50 mN/m during rapid cyclic area changes (Figs. 2 and 4), and desorbed slowly upon static compression (Fig. 3). These findings are in accordance with the characteristic features defined for a good surfactant [18]. In contrast, at a concentration of < 0.05:0.5 mol% SP-B/SP-C, we found that the surface activity was clearly lower, resembling more closely that of film without proteins. The results obtained in this in vitro model system are similar to the findings of other groups under a variety of experimental conditions [19–27] and confirm the important role of SP-B and SP-C in interactions with phospholipids. The results further show that it is possible to control the composition of film and to study their behavior during dynamic conditions, thus providing the opportunity to gain new insights into structure–function relationships of individual surfactant components.

Binding, lipid mixing, and rearrangement of lipid packing are thought to be prerequisites for fast formation of a surface film at the air–water interface. It was previously shown that SP-B and SP-C, when spread in combination with lipids at the air–water interface of a surface balance, are responsible for binding of lipid vesicles to the monolayer [12,28]. It was furthermore reported that SP-B enhances lipid mixing between vesicles, particularly at concentrations around 0.2 mol% [29–31] and that SP-C alters lipid packing at concentrations above 0.5 mol% [32].

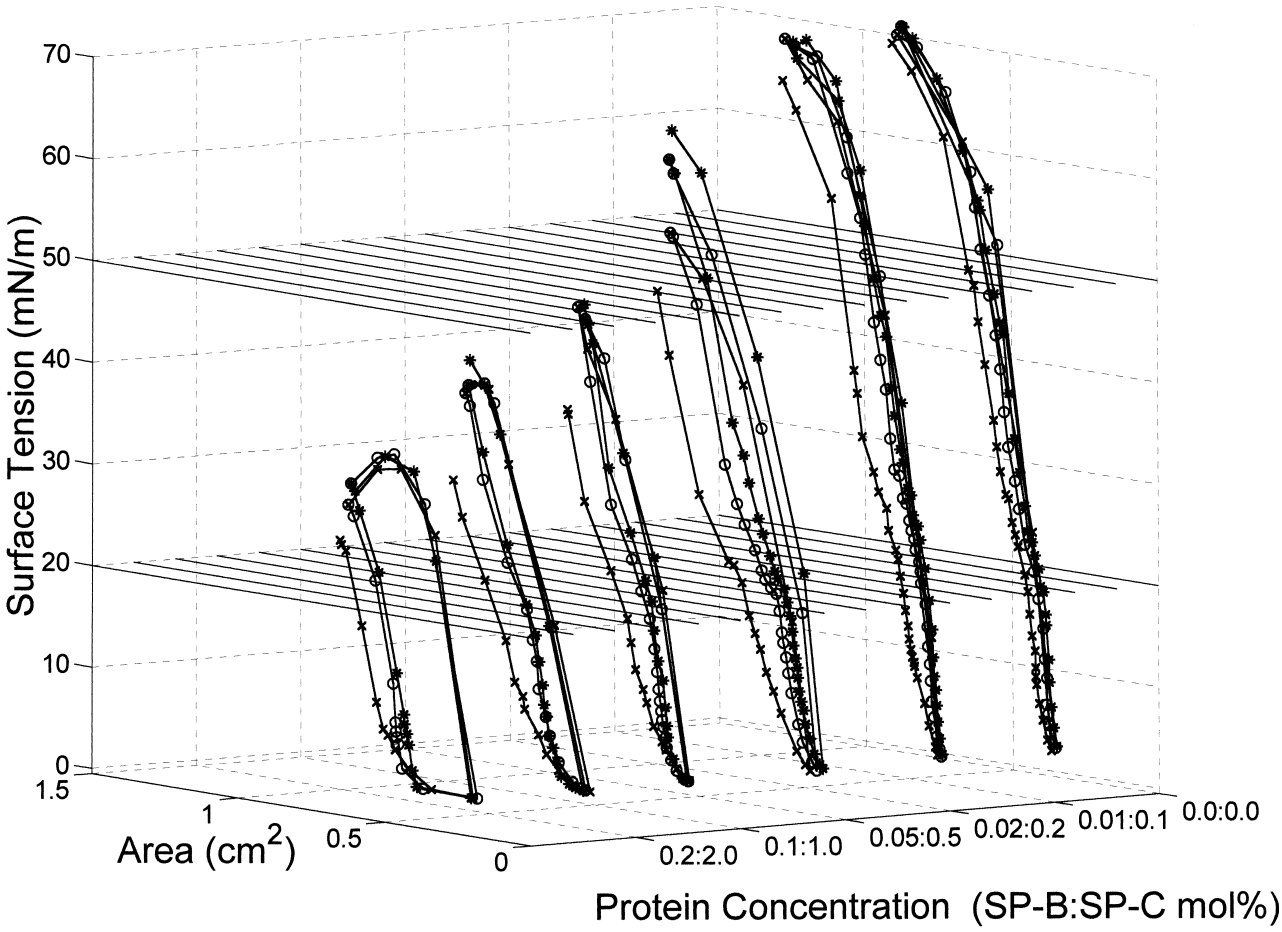

Fig. 4. Surface tension–area compression–expansion isotherms for hydrophobic lung surfactant protein–phospholipid and phospholipid film during cyclic area changes (6 cycles/min). A representative experiment is shown for each concentration tested. ×, ○ and ∗, first, second, and third compression–expansion cycles, respectively. For clarity, no other compression–expansion cycles are shown. Between γ_{max} and γ_{min} (bubble compression), data points are shown for every 1/30 s. Note that the maximum surface tension is >50 mN/m for concentrations $<0.05{:}0.5$ mol% SP-B/SP-C.

From the conditions used in our experiments, we do not have direct evidence that such processes took place at or near the air–water interface of the bubble. However, our findings that the surface tension of the bubble dropped quickly during the first bubble expansion at concentrations $\geq 0.05{:}0.5$ mol% SP-B/SP-C (Fig. 1) and that the maximum surface tension remained <50 mN/m during rapid cyclic area changes above the threshold concentration of 0.05:0.5 mol% SP-B/SP-C (Figs. 3 and 4) provide indirect evidence that interactions between the hydrophobic proteins and the phospholipids similar to those described above could have been present in these experiments. Whether this is more an effect of SP-B or SP-C is difficult to say. Data obtained in a detailed in vitro study suggested that surface activity of mixed phospholipid film was higher with SP-B than with SP-C [33]. Preliminary data obtained in a different system suggested that activity was somewhat higher for SP-C [34]. In contrast, in vivo experiments performed to determine the effect of both proteins on the lung mechanics of premature rabbits found no differences between the two proteins [35]. Thus, further studies are clearly needed before this question can be answered definitively. We are currently studying SP-B and SP-C separately to further elucidate their roles in protein–phospholipid interaction during dynamic cyclic area changes.

Studies recently performed in surface balances at low compression rates have demonstrated directly that SP-B and SP-C are reversibly squeezed out from the surface film during compression [4,9]. Furthermore, results obtained by electron microscopy, surface tension measurements, and fluorescence light microscopy strongly suggest that during this reversible process material is stored in a multilayered phase closely associated with the residual, surface-active film at the air–water interface [7,8]. Both SP-B and SP-C, as well as SP-A [36], are thought to contribute to the formation of such a multilayered phase. Although we have analyzed the surface tension–area compression isotherms as precisely as possible with respect to kinks between 35 and 5 mN/m during compression of film, no clear proof could be obtained for a sudden squeeze-out of SP-B– and SP-C–lipid complexes. It was shown in a very elegant study that fluorescently labeled SP-C can remain in the film down to a γ of 5 mN/m [32]. Whether this indicates that no squeeze-out of SP-B– and SP-C–lipid complexes was present in our experiments, or that squeeze-out was present and we were unable to detect it, is unclear to us. Perhaps the high compression speed used in our experiments stabilized the film and thereby prevented a sudden exit of film components not necessarily present at $\gamma < 35$ mN/m. Alternatively, differences in the experimental protocol and/or technical limitations of the captive-bubble surfactometer used could explain this difference. At this point we can say that in the presence of protein concentrations $\geq 0.05{:}0.5$ mol% SP-B/SP-C, spread film reached a minimum surface tension of < 5 mN/m upon rapid compression within a few video frames (with one frame being equal to 1/30 s) (Fig. 4). Thus, for this series of experiments, a detailed reconstruction of the compression isotherm in the most interesting region between 35 and 5 mN/m, a must for a solid interpretation of squeeze-out phenomena of SP-B– and SP-C–lipid complexes, could not be achieved.

Another interesting finding is that at concentrations $\geq 0.05{:}0.5$ mol% SP-B/SP-C, film collapse (Table 1) and hysteresis (Fig. 4) were present in a concentration-dependent manner during rapid cyclic area changes. These observations are consistent with the view that SP-B and SP-C promote insertion [12] and repeated respreading of lipids from a collapse phase [4,37,38]. It has been shown [32] that SP-C induces packing rearrangements in lipid arrays and increases membrane fluidity; it was suggested that such structural perturbations facilitate the transport of lipids into the air–water interface. Both the repeated film collapse and the hysteresis observed in our study indicate that these structural rearrangements are present during rapid cyclic area changes. Furthermore, they suggest that SP-B and SP-C remain closely associated with the surface film during such rapid cyclic area changes.

It is not easy to extrapolate the findings obtained in our in vitro model system to an in vivo situation, particularly in a pathological situation. However, the following conservative interpretation seems legitimate. In lavage fluid obtained from patients with acute respiratory distress syndrome, the concentration of SP-B measured in the pelleted surfactant material was significantly lower than that in the lavage fluid of normal subjects [39]. When the surface activity of the pelleted material was determined, minimum and maximum surface tension was elevated in patients with acute respiratory distress syndrome and, to a lesser degree, in at-risk patients. Interestingly, the results obtained in our in vitro model system showed that for a threshold concentration of $< 0.05{:}0.5$ mol% SP-B/SP-C, the surface activity of spread film during rapid cyclic area changes (6 cycles/min) was low when compared to adsorbed film of natural surfactant [10,20,23]. In particular, maximum surface tension was clearly > 50 mN/m (Figs. 3 and 4). Thus, it is possible that in in vivo conditions of reduced concentrations of SP-B inhibitory plasma proteins, as in in vitro conditions [33], can successfully compete against pulmonary surfactant components for the air–water interface and damage the surface properties of the surfactant film. Therefore, we would like to emphasize that maximum surface tension achieved during enlargement of the surface area under dynamic conditions is an important parameter for defining the quality of a surfactant preparation.

In conclusion, we have shown that spread SP-B and SP-C catalyze the rapid transport of lipids into the air–water interface and that the interaction of SP-B and SP-C with phospholipids is concentration dependent. Our results suggest that SP-B and SP-C remain associated with the surface film during rapid cyclic area changes.

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