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Pulmonary Surfactant-Associated Protein A Enhances the Surface Activity of Lipid Extract Surfactant and Reverses Inhibition by Blood Proteins in Vitro†

Amanda M. Cockshutt,*,‡ Jeffrey Weitz,§ and Fred Possmayer‡,∥

Department of Biochemistry and Department of Obstetrics and Gynaecology, MRC Group in Fetal and Neonatal Health and Development, The University of Western Ontario, London, Ontario N6A 5A5, Canada, and Department of Medicine, McMaster University, Hamilton, Ontario L8N 3Z5, Canada

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ABSTRACT: Although a monolayer of dipalmitoylphosphatidylcholine, the major component of pulmonary surfactant, is thought to be responsible for the reduction of the surface tension at the air-liquid interface of the alveolus, the participation of unsaturated and anionic phospholipids and the three surfactant-associated proteins is suggested in the generation and maintenance of this surface-active monolayer. We have examined the effects of surfactant-associated protein A (SP-A) purified from bovine lavage material on the surface activity of lipid extract surfactant (LES), an organic extract of pulmonary surfactant containing all of the phospholipids and SP-B and SP-C, but lacking SP-A. Measurements of the surface tension during dynamic compression were made on a pulsating bubble surfactometer. Addition of SP-A to LES reduces the number of pulsations required to attain surface tensions near zero at minimum bubble radius. This increase in surface activity is dependent upon the presence of Ca^{2+} in the assay mixture. Maximal enhancement is observed at or below 1% of the lipid concentration (w/w). The addition of two blood proteins, fibrinogen and albumin, at physiological concentrations to LES causes severe inhibition of surface activity. Addition of SP-A in the presence of Ca^{2+} completely counteracts the inhibition by fibrinogen. The amount of SP-A required for full reversal of this inhibition was less than 0.5% of the lipid concentration. Complete reversal of inhibition by albumin was also observed, even though there was a ~5000-fold molar excess of inhibitor. Addition of lysophosphatidylcholine also inhibits LES; however, SP-A has no effect on this inhibition.

The type II epithelial cell produces and secretes pulmonary surfactant which serves to reduce the surface tension across the air-liquid interface of the alveolus, and hence facilitates breathing. This substance, composed of approximately 90% lipids and 10% protein, reduces surface tension during breathing by generating a monolayer at the interface enriched in the saturated phospholipid dipalmitoylphosphatidylcholine (DPPC)[1] (Goerke, 1974; King, 1984; Notter et al., 1984; Possmayer et al., 1985; Van Golde et al., 1988). DPPC constitutes only ~40% of the surfactant; the remainder is mainly unsaturated phosphatidylcholines, anionic phospholipids such as phosphatidylglycerol (PG) and phosphatidylinositol (PI), and the three surfactant-associated proteins SP-A, SP-B, and SP-C (Possmayer, 1988). It has recently been demonstrated both in vitro and in vivo that many of the surface tension reducing properties of surfactant lipids are potentiated by interactions with the small, hydrophobic proteins SP-B and SP-C which make up only ~1% of surfactant (Hawgood et al., 1987; Revak et al., 1988; Suzuki et al., 1986; Takahashi & Fujiwara, 1986; Yu et al., 1987, 1988).

SP-A is an abundant protein representing 5-10% of bovine surfactant (Yu et al., 1983). The structure elucidation of this highly conserved protein has revealed some very interesting features (Hawgood, 1989). The primary translation product is a ~28K protein that is posttranslationally modified to generate a glycoprotein with a molecular weight of ~36K that is acetylated, sulfated, and proline hydroxylated. The protein can be divided into 3 functional regions: the N-terminal 7 amino acids form a short tail containing a cysteine which participates in an interchain disulfide bridge, a collagen-like stretch consisting of 23 repeats of the Gly-X-Y triplet, and a glycosylated carboxy-terminal region which has considerable homology with several Ca^{2+}-dependent lectins. The oligomeric organization of SP-A has been elucidated (Voss et al., 1988) and appears to form an octadecameric structure very similar to the complement component C1q.

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* Address correspondence to this author at University Hospital, Room 8L-15, 339 Windermere Rd., London, Ontario N6A 5A5, Canada.
‡ Department of Biochemistry, The University of Western Ontario.
§ Department of Medicine, McMaster University.
∥ Department of Obstetrics and Gynaecology, The University of Western Ontario.

[1] Abbreviations: PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; LES, lipid extract surfactant; SP, surfactant-associated protein; lyso-PC, lysophosphatidylcholine; R_{min}, minimum bubble radius; R_{max}, maximum bubble radius; PG, phosphatidylglycerol; BSA, bovine serum albumin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; NRDS, neonatal respiratory distress syndrome; ARDS, adult respiratory distress syndrome.

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The physiological role of SP-A has remained somewhat elusive and is the subject of current research. The protein has the capacity to bind certain sugars (Haagsman et al., 1987), including mannose, in a Ca^{2+}-dependent manner. Other in vitro studies have demonstrated that this protein is capable of aggregating lipid vesicles, a process that is greatly enhanced by the inclusion of anionic phospholipids and Ca^{2+} (King, 1984; Wright & Clements, 1987). When added to type II epithelial cells in culture, SP-A stimulates the uptake of surfactant lipids from the medium and inhibits secretion of stored surfactant (Dobbs et al., 1987; Wright et al., 1987), suggesting an autocrine regulation of alveolar surfactant levels. More recently, immunological investigations, spurred by the structural similarity between complement component C1q and SP-A, have suggested an immune modulatory role for SP-A in the prevention of pulmonary infection (Tenner et al., 1989).

Previous investigations have demonstrated the deleterious effects of blood components on the activity of various surfactants both in vitro and in vivo (Fuchimukai et al., 1987; Holm et al., 1985a,b; Holm & Notter, 1987; Ikegami et al., 1984; Kobayashi et al., 1989; Seeger et al., 1985). Leakage of blood components into the alveolar space as a result of injury or increased vascular pressure has been implicated in the pathology of the neonatal and adult respiratory distress syndromes (NRDS and ARDS, respectively) (Burkhardt & Van Golde, 1989; Jobe & Ikegami, 1987; Robertson & Lachmann, 1988). The hyaline membranes associated with NRDS are composed largely of fibrin clots arising from the transudation of serum proteins. Examination of the interactions between surfactant components and blood components in vitro could lead to a better understanding of the basic mechanisms involved in RDS.

In this investigation, we have examined the effects of three blood components on lipid extract surfactant (LES). We have observed that SP-A can prevent inhibition by the proteins fibrinogen and albumin, even in the presence of a vast molar excess of inhibitor. We have also observed that the surface activity is compromised by lysophosphatidylcholine. This inhibition is unaffected by the addition of SP-A. We conclude that SP-A has a specific function in preventing protein inhibition in vitro.

Materials and Methods

Reagents. All chemicals were reagent grade or better. Bovine serum albumin, lysophosphatidylcholine, and fibrinogen were obtained from Sigma. Fibrinogen was also obtained from Kabivitrum, Helena Labs. Immobilized D-mannose was purchased from Pierce. The silver stain reagents were purchased as a kit from Bio-Rad.

Assays. Phospholipid concentrations were determined by using the phosphorus assay of Rouser (Rouser et al., 1960). Protein purity was assayed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using the method of Laemmli (1970), followed by staining with Coomassie R-250 or silver stain. Protein concentrations were determined by the method of Lowry (Lowry et al., 1951). Fibrinogen solution concentrations were also determined by using the Bio-Rad assay (Bradford, 1976) and the BCA assay (Smith et al., 1984) from Pierce. All protein concentration assays used bovine serum albumin as a standard. A large discrepancy was observed between the concentrations determined for fibrinogen by the three different protein assays. Fibrinogen concentrations reported in this paper refer to those obtained with the Bio-Rad method. Values obtained with the Bio-Rad method are ~70% of those obtained by Lowry; values obtained with the BCA assay are ~45% of the Lowry values.

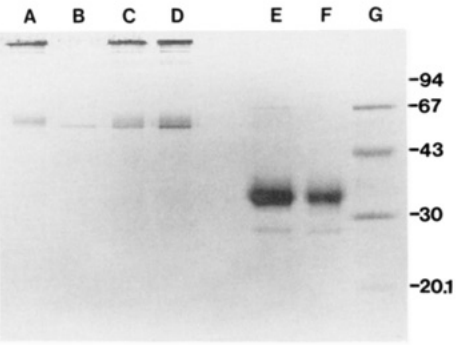

FIGURE 1: SDS–polyacrylamide gel of SP-A stained with Coomassie R-250. Lane A, SP-A nonreduced; lane B, BSA nonreduced; lanes C and D, SP-A and BSA nonreduced; lane E, natural surfactant reduced; lane F, SP-A reduced; lane G, molecular weight standard. Note the presence of albumin in natural surfactant. The "dimer" of SP-A observed under nonreducing conditions does not comigrate with albumin.

Surfactant Preparation. Pulmonary surfactant was prepared from bovine lavage material by a modification of the method described elsewhere (Yu et al., 1983; Weber & Possmayer, 1984). Briefly, lipid extract surfactant (LES) is obtained by organic extraction of lavage material. Neutral lipids are removed by acetone precipitation. The material is dried, made up in chloroform/methanol 9:1, and stored at −20 °C. This preparation contains all of the phospholipids of natural surfactant and the two small hydrophobic proteins SP-B and SP-C; however, it has been completely stripped of SP-A. Natural surfactant is prepared from lavage material by a series of washes and gradient centrifugations. The resulting material is lyophilized and stored at −20 °C.

Purification of SP-A. SP-A is purified from bovine natural surfactant by slight modifications to two previously published protocols (Haagsman et al., 1987; Ross et al., 1986). Briefly, an aqueous preparation of natural surfactant is delipidated by washing in butan-1-ol, ether/ethanol 3:1, and pure ether. The solvent is completely removed, and the precipitated protein is solubilized in 5 mM HEPES/0.1 mM Na_2EDTA, pH 7.4, and centrifuged at 30000*g* for 20 min to remove any insoluble material. The solubilized protein is then supplemented with $CaCl_2$ to a concentration of 1 mM and affinity-purified on a column of immobilized D-mannose. Bound protein is eluted with 2 mM EDTA in 5 mM HEPES, pH 7.4. A Coomassie-stained gel of the purified protein is shown in Figure 1. Note that under nonreducing conditions the band with an apparent molecular weight of 68K is distinct from albumin.

Fibrinogen Purification. Fibrinogen from Kabivitrum was purified as previously described (O'Brodovich et al., 1990). The fibrinogen solution containing Trasylol (American Diagnostica), a protease inhibitor, is passed over a lysine–Sepharose matrix (Pharmacia) to remove contaminating plasminogen. The preparation is then repeatedly precipitated in $(NH_4)_2SO_4$ (144 g/L final concentration) to concentrate the fibrinogen and remove factor XIII. The pellet is redissolved in 5 mM sodium citrate, pH 7.5, dialyzed against 0.1 M NaCl/50 mM Tris, pH 7.4, and stored at −70 °C.

Pulsating Bubble Surfactometer. Surface tension was measured during dynamic compression using a pulsating bubble surfactometer as described (Enhorning, 1977). Briefly, a bubble of ambient air drawn through a small tube is formed in a chamber containing a surfactant suspension. This bubble is pulsated at 37 °C at a rate of 20 cycles per minute between fixed radii (0.4–0.55 mm), resulting in a 50% change in surface area. The pressure across the bubble is measured by a pressure transducer, and the surface tension can be calculated from the

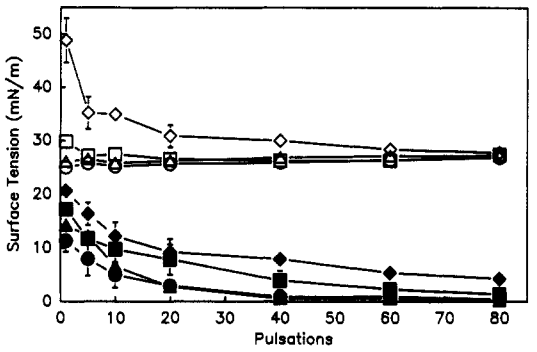

FIGURE 2: Effect of phospholipid concentration on the surface tension profile of LES. Surface tension (millinewtons per meter) is plotted vs number of pulsations for four different phospholipid concentrations of LES: (●, ○) 10 mg/mL; (▲, △) 5 mg/mL; (■, □) 2.5 mg/mL; (♦, ◊) 1 mg/mL. Closed symbols represent surface tension at minimum bubble radius; open symbols represent surface tension at maximum bubble radius. Values are the means ± SEM of three separate LES samples each assayed 3 times.

Table I: Effect of SP-A and Calcium on the Biophysical Activity of Lipid Extract Surfactant (LES)

sample	surface tension after 20 pulsations (mN/m)	
	R_{min}	R_{max}
5 mg/mL LES/1.5 mM $CaCl_2$	2.8 ± 1.5[a]	26.2 ± 0.4
+1 mg/mL SP-A	0.2 ± 0.3	24.7 ± 0.7
5 mg/mL LES/1.5 mM EDTA	10.1 ± 0.0	28.3 ± 1.2
+1 mg/mL SP-A	15.9 ± 2.0	37.9 ± 6.4
2.5 mg/mL LES/1.5 mM $CaCl_2$	7.8 ± 2.8	26.5 ± 0.3
+1 mg/mL SP-A	1.2 ± 0.4	26.2 ± 0.4
2.5 mg/mL LES/1.5 mM EDTA	14.8 ± 0.4	33.7 ± 0.9
+1 mg/mL SP-A	28.0 ± 3.7	46.3 ± 2.9

[a] Values are the means ± SEM of three separate LES samples each assayed 3 times.

Law of Laplace: $P = 2\gamma/r$. All surfactant samples assayed on the bubble machine were made up in buffers containing 0.9% NaCl/1.5 mM $CaCl_2$. Those sample assayed in the absence of Ca^{2+} had 1.5 mM EDTA added to the saline buffer in place of $CaCl_2$.

RESULTS

Lipid extract surfactant reduces the surface tension at maximum (R_{max}) and minimum (R_{min}) bubble radii in the pulsating bubble surfactometer. The number of cycles required to obtain near-zero surface tensions at R_{min} is inversely related to the phospholipid concentration (see Figure 2). Little change in the surface tension at R_{max} is observed except at very low surfactant concentrations when the initial surface tension is high. However, at the concentrations used in this study, usually 1–5 mg/mL surfactant phospholipid, the surface tension at R_{min} after 20 pulsations can be used as an indication of the rate at which surface tension decreases during dynamic compression. Table I shows the effect of addition of SP-A at a fixed concentration of 1 mg/mL to LES at 5 and 2.5 mg/mL. In the presence of Ca^{2+}, the addition of SP-A lowers the surface tension to near zero within 20 pulsations. In the absence of Ca^{2+}, a higher surface tension is observed, and the addition of SP-A actually inhibits the surface activity. When a concentration range of 1–10 mg/mL surfactant was assayed in this fashion, the most pronounced effect of SP-A was observed at the lower surfactant concentrations (data not shown).

The amount of SP-A required for maximal enhancement of surface activity was determined. Figure 3 shows a concentration curve for SP-A, where the SP-A concentration is expressed as a percentage of the lipid concentration, 2.5 mg/mL (w/w). Maximal enhancement of activity is observed by 0.5–1.0% SP-A, with no further enhancement observed as the value is raised to 10%. Similar results were observed when the phospholipid concentration was 5 mg/mL (data not shown). Bovine serum albumin (BSA) is a major contaminant of crude SP-A (Figure 1). Therefore, the effect of this protein over the same concentration was examined. BSA had only a small inhibitory effect on LES. This observation suggests that the stimulatory effect of SP-A cannot necessarily be duplicated by other proteins and may be specific.

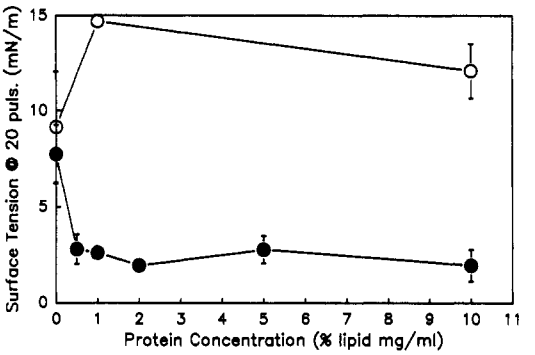

FIGURE 3: Concentration curve for the addition of SP-A to LES. Surface tension after 20 pulsations at minimum bubble radius (millinewtons per meter) is plotted vs protein concentration expressed as a percentage (w/w) of the lipid concentration of 2.5 mg/mL. (●) represents addition of SP-A; (○) represents addition of BSA as a control. Values are means ± SEM of three separate LES samples each assayed 3 times.

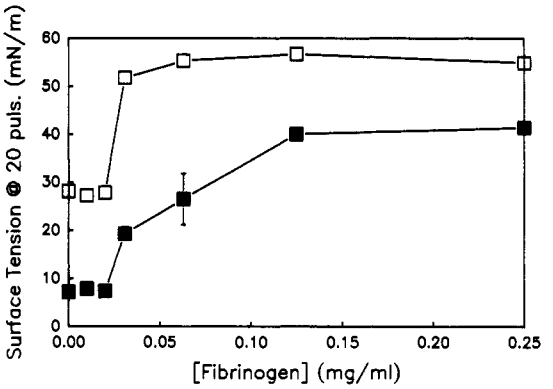

FIGURE 4: Effect of addition of fibrinogen to LES. Surface tension after 20 pulsations (millinewtons per meter) is plotted vs the concentration of fibrinogen added (milligrams per milliliter, as determined by the method of Bradford). The LES concentration is 2.5 mg/mL. The closed symbols represent values at minimum bubble radius, and the open symbols represent maximum bubble radius. Values are means ± SEM of three separate LES samples each assayed 3 times.

It has been previously demonstrated that the addition of various blood components to surfactant preparations impairs their ability to reduce surface tension. These studies have shown that fibrinogen, and in particular the fibrin monomer, is a potent inhibitor of surfactant (Seeger et al., 1985; Keough et al., 1987; Fuchimukai et al., 1987). Addition of fibrinogen to LES results in high surface tensions. Figure 4 shows the effect of addition of increasing concentrations of fibrinogen to LES at 2.5 mg/mL. Addition of >0.1 mg/mL fibrinogen results in surface tensions of >50 mN/m at R_{max} and ~40 mN/m at R_{min}, which are similar to the surface tensions of solutions containing 3 mg/mL fibrinogen in the absence of surfactant.

The effect of SP-A on the inhibition of LES by fibrinogen was determined. Inhibition of surface activity was abolished when SP-A was added prior to challenge with fibrinogen

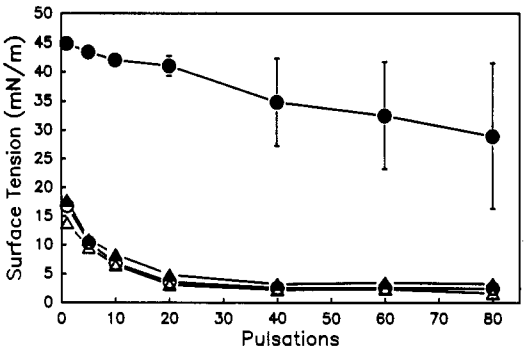

FIGURE 5: Effect of SP-A on the inhibition of LES by fibrinogen. Surface tension at minimum bubble radius (millinewtons per meter) is plotted vs number of pulsations. (○) represents LES; (●) LES + 3 mg/mL fibrinogen; (△) LES + 5% SP-A; (▲) LES + 5% SP-A + 3 mg/mL fibrinogen. LES is 5 mg/mL. Values are the means ± SEM of three separate LES samples each assayed 3 times.

Table II: Effect of SP-A on the Inhibition of LES by Albumin

sample	surface tension after 20 pulsations (mN/m) R_{min}	R_{max}
1 mg/mL LES/1.5 mM $CaCl_2$	14.9 ± 0.3[a]	33.7 ± 1.0
+10% SP-A (0.1 mg/mL)	7.6 ± 1.5	27.0 ± 0.7
+50 mg/mL BSA	40.1 ± 2.3	49.4 ± 0.5
+10% SP-A + 50 mg/mL BSA	6.5 ± 1.2	27.3 ± 0.6

[a] Values are the means ± SEM of three separate LES samples each assayed 3 times.

(Figure 5). In this experiment, LES was markedly inhibited by the addition of 3 mg/mL pure fibrinogen (open circles). However, when 5% SP-A was included in the LES preparation, the surface tension profiles in the presence and absence of fibrinogen were indistinguishable (compare closed and open triangles). In other experiments, it was observed that natural surfactant, which contains SP-A, behaved similarly to LES supplemented with SP-A (data not shown). This indicates that the effect of SP-A is not an artifact of the protein isolation procedure.

The inhibition of surfactants by albumin is much weaker than by fibrinogen (Seeger et al., 1985). When low concentrations of BSA (up to 5 mg/mL) were added to LES, no significant inhibition could be detected. When the concentration of albumin was increased to 50 mg/mL (and the surfactant phospholipid concentration was decreased to 1 mg/mL), a marked inhibition of surface activity, similar to that seen with fibrinogen, was observed (Table II). It should be noted that this approximates the physiological concentration of albumin in serum. The addition of SP-A at 10% of the lipid concentration (=0.1 mg/mL SP-A) completely prevented the inhibition.

The amount of SP-A required for the reversal of fibrinogen inhibition was determined by adding varying amounts of SP-A to fibrinogen-inhibited surfactant. The results, shown in Figure 6, demonstrate that only 0.5% by weight or less of the surfactant preparation need be SP-A for complete reversal of the fibrinogen inhibition.

The dependence of the reversal of fibrinogen inhibition on Ca^{2+} was determined by repeating the experiments after incubating the samples at 37 °C in the presence of 1.5 mM Na_2EDTA instead of $CaCl_2$. Table III shows that in the absence of Ca^{2+} SP-A has no effect on fibrinogen inhibition.

In order to obtain some indication of the manner in which SP-A reverses the inhibition by serum proteins, the effect of a potential nonprotein inhibitor was examined. Table IV shows that the addition of lysophosphatidylcholine (lyso-PC), which

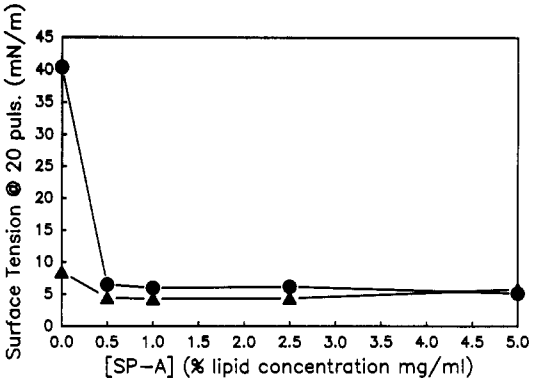

FIGURE 6: Concentration curve for the effect of SP-A on the inhibition of LES by fibrinogen. Surface tension at minimum bubble radius after 20 pulsations is plotted vs the concentration of SP-A added (expressed as a percentage of the lipid concentration). (▲) represents controls, with LES and buffer; (●) represents LES + 1 mg/mL fibrinogen. LES is 2 mg/mL. Values are means ± SEM of three separate LES samples each assayed 3 times.

Table III: Effect of Calcium on the Prevention of Fibrinogen Inhibition by SP-A

sample	surface tension after 20 pulsations (mN/m) R_{min}	R_{max}
2 mg/mL LES/1.5 mM EDTA	16.7 ± 0.2[a]	29.7 ± 1.0
+5% SP-A (0.1 mg/mL)	19.5 ± 0.3	52.6 ± 0.9
+1 mg/mL fibrinogen	40.6 ± 0.3	53.0 ± 0.1
+5% SP-A + 1 mg/mL fibrinogen	40.4 ± 0.7	53.3 ± 0.7

[a] Values are the means ± SEM of three surfactometer assays of one LES sample.

Table IV: Inhibition of LES by Lysophosphatidylcholine and the Effect of SP-A

sample	surface tension after 20 pulsations (mN/m) R_{min}	R_{max}
5 mg/mL LES/1.5 mM $CaCl_2$	4.5 ± 0.3[a]	26.6 ± 0.3
+5% SP-A (0.25 mg/mL)	3.8 ± 0.7	26.2 ± 0.7
+10% lyso-PC (0.5 mg/mL)	15.0 ± 2.8	36.7 ± 2.7
+5% SP-A + 10% lyso-PC	15.0 ± 4.0	35.2 ± 5.1

[a] Values are the means ± SEM of three separate LES samples each assayed 3 times.

is also present in serum, to LES at 10% of the surfactant phospholipid concentration (w/w) markedly retards the reduction of surface tension. The addition of SP-A at 5% of the lipid concentration has no effect on the inhibition by 10% lyso-PC (Table IV), an indication that the mechanism of inhibition by lyso-PC is distinct from that of fibrinogen and albumin. These results demonstrate that the addition of SP-A can counteract the inhibition of surface activity by blood proteins but not by lyso-PC.

Discussion

Edema has long been implicated in the etiology of both the neonatal and adult forms of respiratory distress syndrome (Burkhardt & van Golde, 1989; Robertson & Lachmann, 1988). Seeger et al. (1983, 1984, 1985a,b) clearly demonstrated the deleterious effects of different blood proteins on surface activity both in vitro and in situ. This study revealed that the development of edema in perfused lungs was correlated with alterations in pressure–volume characteristics and decreases in the surface activity of lavaged materials. Those perfusates that contained blood proteins, in particular fibrin and fibrinogen, caused greater inhibition of surface activity. The present investigation examines the role of SP-A in mi-

tigating surfactant inhibition by fibrinogen and albumin.

The data presented here confirm earlier observations on the enhancement of surfactant activity by the addition of SP-A to reconstituted and organic solvent-extracted surfactants (Hawgood et al., 1987; Chung et al., 1989). However, since the effect is most pronounced at low surfactant lipid concentrations, the significance of this role of SP-A in the lung is unclear. SP-A may serve to stabilize the surface tension during periods of reduced alveolar surfactant or during the establishment of the air–liquid interface upon initiation of breathing at birth. It was observed that the amount of SP-A required for maximal enhancement of activity is approximately 10–20% of the amount found in natural surfactant. This suggests that other functions of SP-A may account for the in vivo concentrations which are higher than required for optimal biophysical activity.

The mechanism by which SP-A enhances the surface activity of LES may be related to the formation of tubular myelin. It has been suggested that tubular myelin may be the source of the surface-active monolayer in vivo and under appropriate conditions in vitro (Goerke, 1983; Wright & Clements, 1987). Addition of SP-A to preparations containing the surfactant lipids DPPC and PG and SP-B in the presence of Ca^{2+} leads to the formation of tubular myelin as observed by electron microscopy (Suzuki et al., 1989). Conditions producing an increase in the content of tubular myelin correlate with more rapid adsorption and spreading of the lipids onto the monolayer (Benson et al., 1984). Thus, the action of SP-A in the present experiments may be to change the structure of LES from a predominantly lamellar structure to tubular myelin, thereby altering the spreading and monolayer-purifying abilities of the surfactant. SP-A may also accelerate the squeeze out of lipids other than DPPC.

Physiological serum concentrations of fibrinogen and albumin inhibited the biophysical activity of LES. During these investigations, a considerable variation in the extent of inhibition by different fibrinogen preparations was observed. Solutions of purified Kabivitrum fibrinogen are very potent. Crude preparations purchased from Sigma have only moderate inhibitory action. It was also noted that, with concentrated preparations (>10 mg/mL), a loss of inhibitory activity sometimes occurred which was correlated with the apperance of a white precipitate, presumably a fibrous clot, in the solution. These observations suggest that the fibrinogen present in the edema fluid in the lung may be a more potent inhibitor than the purified preparations.

Previous investigations with the Wilhelmy plate have revealed that, whereas a preformed surfactant film is barely affected by injection of albumin into the subphase, administration of albumin prior to or with surfactant injection results in a significant inhibition (Holm et al., 1988). These observations suggest that the inhibitory proteins compete with the surfactant lipids for the interface and thereby retard the adsorption and/or spreading of surfactant phospholipids. However, since the induction of edema in vivo alters pressure–volume parameters, it is clear that serum proteins can affect a preformed monolayer within the alveolus. The amounts of edema fluid introduced into the alveolar space in vivo can greatly exceed the alveolar subphase volume. Under these conditions, edema fluid would dilute the endogenous surfactant as well as inhibit its activity. In vitro studies conducted with the Wilhelmy plate and the pulsating bubble surfactometer use fixed hypophase volumes. Nevertheless, the reported measurements are congruous with the competitive model, since the initial high surface tensions observed with the inhibitors tend to fall during repeated pulsation, suggesting that the inhibitory protein may be gradually removed from the interface during dynamic compression.

Clearly, the nature of the inhibitory protein affects its potential to impair surfactant action. It is likely that the protein's structure (including the extent of denaturation in solution) and charge distribution influence its ability to occupy the interface such that one protein is a more effective inhibitor than another. In addition, some proteins may interact directly with surfactant lipids, preventing monolayer formation and purification. Our observations and those of others have determined that the polymerization or cleavage state of fibrinogen affects the inhibitory ability of this protein (O'Brodovich et al., 1990). Thus, the protein's conformation could have significant effects on its interaction with surfactant.

The precise mechanism by which SP-A prevents inhibition of surfactant by blood proteins is unknown. It is known that SP-A lowers the calcium level required for surface tension reduction by LES (Chung et al., 1989). SP-A has been shown to act synergistically with SP-B in surface tension reduction (Wright & Clements, 1987). SP-A does not enhance adsorption of mixtures containing only SP-C, a hydrophobic protein which is not required for the formation of tubular myelin (Pison et al., 1990). By the establishment of tubular myelin, SP-A could shift the equilibrium in favor of lipid monolayer formation. In the presence of SP-A, normal surface tensions are observed even with the first pulsation, indicating that compression of the bubble is not required for counteracting the inhibition. In the experiment in which SP-A prevented the inhibition by albumin, the concentration of inhibitor was 500 times (w/w) higher than that of SP-A. When the native molecular weight of SP-A (~650 000) is considered, this represents a 5000-fold molar excess of inhibitor over SP-A. In the fibrinogen experiments, a 200-fold molar excess of inhibitor over SP-A was estimated. Since SP-A effectively prevented inhibition in these instances, a stoichiometric model which relies on sequestration of the inhibitor by SP-A seems unlikely. It is possible, however, that SP-A may prevent undesirable associations between inhibitor proteins and surfactant lipids, behaving as a kind of surfactant chaperone.

To the authors' knowledge, inhibition of surfactant activity by high levels of lyso-PC has not been reported previously. Lyso-PC is present in the serum and could be produced in abnormal amounts by increased levels of alveolar phospholipase A_2 in certain types of ARDS (Hallman et al., 1982; von Wichert et al., 1981). Lyso-PC appears to inhibit LES by a different mechanism than the blood proteins. This inhibition is insensitive to the presence of SP-A. It is likely that the detergent-like properties of lyso-PC enable it to disrupt the monolayer spreading and purifying machinery of the preparation both in the presence and in the absence of SP-A.

In this investigation, the effects of addition of SP-A to LES on the ability of the surfactant to reduce surface tension in the presence and absence of inhibitory proteins were determined. SP-A enhances the surface activity of LES in a Ca^{2+}-dependent fashion. SP-A was also shown to have a protective function toward the inhibition of LES by fibrinogen and albumin. This process was also dependent on the presence Ca^{2+} and required only a small amount of SP-A. These results suggest that SP-A may play an important role in the prevention of surfactant inactivation by blood proteins which leak into the alveolus as a result of increased permeability or high pressures. Since it is known that disruption of surface activity in the lung results in high pressures across the alveolus, the ability of a surfactant to resist the initial effects of edema, the

inhibition by blood proteins, would enable it to more effectively maintain homeostasis and prevent the development of pulmonary disease. LES is currently being used in clinical trials for the treatment and prevention of NRDS (Jobe & Ikegami, 1987; Robertson & Lachman, 1988). Its use has been proposed for the treatment of ARDS as well. Consideration should be made for the possible inclusion of SP-A in this situation, where surfactant inhibition by blood proteins is well documented, for the prevention of surfactant inhibition by blood proteins.

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