Enhanced Surfactant Adsorption via Polymer Depletion Forces: A Simple Model for Reversing Surfactant Inhibition in Acute Respiratory Distress Syndrome

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ABSTRACT Lung surfactant adsorption to an air-water interface is strongly inhibited by an energy barrier imposed by the competitive adsorption of albumin and other surface-active serum proteins that are present in the lung during acute respiratory distress syndrome. This reduction in surfactant adsorption results in an increased surface tension in the lung and an increase in the work of breathing. The reduction in surfactant adsorption is quantitatively described using a variation of the classical Smolukowski analysis of colloid stability. Albumin adsorbed to the interface induces an energy barrier to surfactant diffusion of order 5 k_BT, leading to a reduction in adsorption equivalent to reducing the surfactant concentration by a factor of 100. Adding hydrophilic, nonadsorbing polymers such as polyethylene glycol to the subphase provides a depletion attraction between the surfactant aggregates and the interface that eliminates the energy barrier. Surfactant adsorption increases exponentially with polymer concentration as predicted by the simple Asakura and Oosawa model of depletion attraction. Depletion forces can likely be used to overcome barriers to adsorption at a variety of liquid-vapor and solid-liquid interfaces.

INTRODUCTION

The surface tension control imposed by lung surfactant (LS), a unique mixture of lipids and proteins that lowers the interfacial tension in the lungs and facilitates normal breathing (1–3), is compromised during acute respiratory distress syndrome (ARDS) (4). Lung surfactant is a mixture of lipids (primarily dipalmitoylphosphatidylcholine) and four lung surfactant-specific proteins (SP-A, B, C, and D) that lines the interior of the lung alveoli and acts to lower the interfacial tension in the lungs, thereby insuring a negligible work of breathing and uniform lung inflation (1). The absence of lung surfactant due to prematurity leads to neonatal Respiratory Distress Syndrome (NRDS) (1,2,5,6). Treating NRDS with currently available replacement surfactants has significantly reduced neonatal mortality in developed countries (1,2,5,6). However, replacement surfactant treatment has been disappointing when used to treat lung diseases associated with lung injury and acute respiratory distress syndrome (7–9). In such cases, surfactant somehow loses the ability to reduce surface tension and is said to be ''inactivated'' (2,10–12). A common factor among ARDS patients is elevated levels of serum and inflammatory proteins in the bronchial and alveolar fluid (2,4,13–15) and reduced LS function (13). In vitro, LS mixed with serum proteins shows an ARDS-like depression of surfactant activity (2,16–18) and surfactant inactivation caused by serum leakage into alveoli is one reason why treatment of lung injuries with replacement surfactant are believed to be unsuccessful (16,19). Several nonionic and anionic polymers have recently been shown to enhance the ability of clinical surfactants to resist inactivation by serum and other substances both in vitro and in vivo (10,18,20–29). Lung function of animals with lung injury is markedly improved when polymers are added to clinical surfactants used for treatment of NRDS (10,12,24,30–32).

Here we show that one cause of surfactant inactivation is the formation of an interfacial film of albumin that reduces or even eliminates the normal adsorption of LS from solution to the interface in vitro, resulting in higher than normal surface tension (2,16–18,26,33). Albumin (and many other serum proteins found in the bronchial fluid of ARDS patients) is surface-active and has a surface pressure, Π ($\Pi = \gamma_w$-γ, γ_w is the surface tension of a clean water interface, 72 mN/m, and γ the measured surface tension) that is a logarithmic function of protein concentration up to a saturation concentration, which is $\sim$1 mg/ml for albumin (17,34,35). The surface pressure at the saturation concentration for albumin and other serum proteins is between 18 and 25 mN/m (surface tension of 47–54 mN/m).

This competitive adsorption of albumin to the alveolar air-liquid interface leads to an energy barrier to surfactant adsorption (18,26). If insufficient functional surfactant reaches the alveolar interface, the low surface tensions required for proper lung function are not reached and the work of breathing increases, along with the potential for further inflammation and injury, consistent with the development of ARDS. However, our experiments also show that a polymer-induced ''depletion attraction'' can overcome the repulsive energy barrier, thereby restoring normal surfactant adsorption, even in the presence of high albumin concentrations (16,18). The depletion attraction effectively pushes surfactant aggregates toward the interface due to the increased polymer entropy induced by the elimination of the ''excluded volumes'' of the surfactant aggregates and the interface (26,36) as the

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surfactant flocculates or is adsorbed to the interface. The depletion attraction is sufficiently strong that it can overcome the electrostatic and steric repulsion imposed by serum or albumin already adsorbed to the interface (26). One requirement for the depletion attraction to operate is that the polymer not adsorb significantly to the surfactant aggregates or to the air-water interface. Polyethylene glycol (PEG) is not particularly surface-active; a 1 wt % solution reduces the surface tension from 72 mN/m to ~64 mN/m.

METHODS

Survanta (Abbott Laboratories, Columbus, OH) was obtained from the San Francisco General Hospital nursery. Survanta is an organic extract of minced bovine lungs that contains 80–90 wt % phosphatidylcholine, of which ~70 wt % is saturated dipalmitoylphosphatidylcholine. There is <2 wt % of the lung surfactant specific proteins SP-B (<0.5 wt %) and SP-C (1.5 wt %). There are ~5 wt % negatively charged phospholipids including phosphatidylglycerol and phosphatidylserine, and ~10 wt % palmitic acid (37). Survanta and other clinical lung surfactants form multi-micron bilayer aggregates in buffered saline solution. Polyethylene glycol (PEG; 10 kDa), and bovine serum albumin were obtained from Sigma (St. Louis, MO). Isotherms were recorded at 25°C (no significant changes are seen from 23–37°C (38)) using a custom stainless steel ribbon trough (Nima, Coventry, England) equipped with a Wilhelmy plate (39,40). The trough had a surface area of 130 cm^2, a subphase volume of 150 mL, and a typical compression/expansion cycle took 8 min. Survanta was diluted in buffer (NaCl 150 mM, $CaCl_2$ 2 mM, and $NaHCO_3$ 0.2 mM, pH = 7) to a lipid concentration of 2 mg/ml and was deposited dropwise into the subphase of the Langmuir trough at the stated total surfactant concentrations to initiate each adsorption experiment. Albumin and PEG were dissolved in the same buffer at the stated concentrations for subsequent experiments.

A Nikon Optiphot optical microscope (Nikon, Tokyo, Japan) was positioned above the trough with a 50× extra-long working distance objective (Nikon) designed for fluorescent light. Full-length movies and individual frames were recorded directly to computer (Moviestar, Mountain View, CA). Contrast in the images was due to segregation of 1 mol % fluorescent lipid Texas Red-DHPE (Molecular Probes, Eugene, OR) between the fluid phase, which appears bright in images, and the condensed phase, which appears dark in the films containing Survanta. The albumin was not labeled and does not fluoresce.

RESULTS

Fig. 1 *a* is a typical expansion-compression cyclic isotherm for 800 μg of the clinical lung surfactant, Survanta, adsorbing to

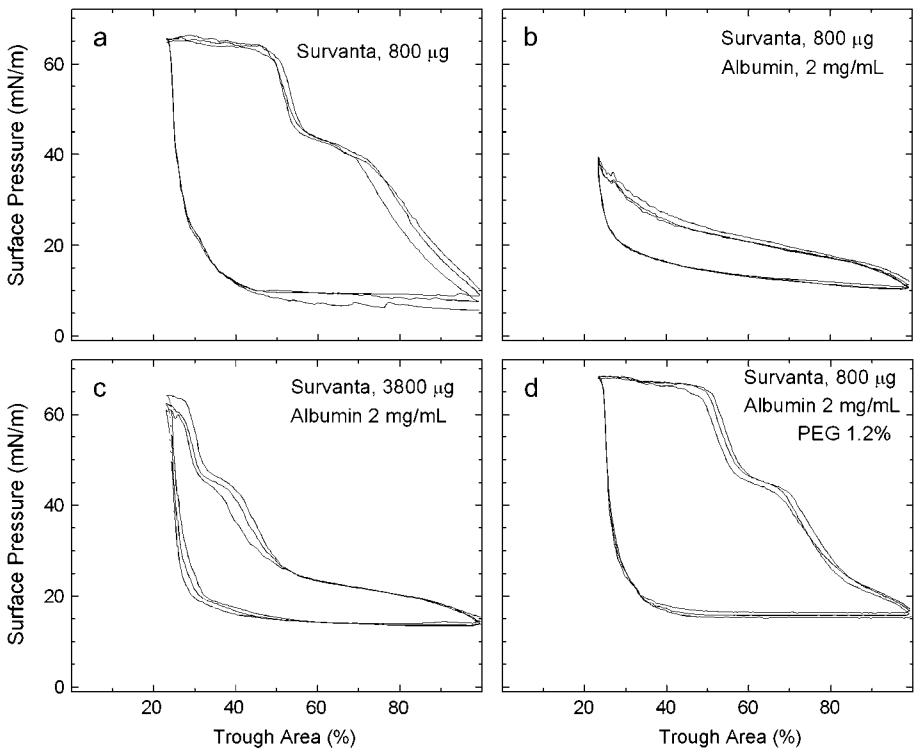

FIGURE 1 Cyclic isotherms of Survanta on various subphases. (*a*) 800 μg Survanta on a clean buffered saline subphase. On compression, the surface pressure increases until the isotherm has a characteristic shoulder at 40 mN/m. This corresponds to rearrangement of the unsaturated lipids and surfactant proteins SP-B and SP-C in the film (38). On further compression, the surface pressure rises abruptly to the collapse pressure of 65 mN/m. At this surface pressure, the film begins to ''collapse'' and forms cracks and folds as seen in Fig. 2 *b*. Film collapse determines the minimum surface tension possible for a given surfactant. On expansion, the surface pressure drops to 10 mN/m; lung surfactant isotherms exhibit significant hysteresis between the compression and expansion parts of the cycle. The cracks and folds in the monolayer begin to unfold and heal at these low surface pressures on expansion, which accounts for much of the hysteresis (17). (*b*) 800 μg Survanta on saline buffer containing 2 mg/mL albumin. The characteristic shoulder and collapse plateau on compression seen in panel *a* cannot be reached with albumin in the subphase, and the surface pressure does not rise to 40 mN/m. Albumin concentrations in ARDS alveolar fluid may reach 100 mg/ml, with an average concentration of 25 mg/ml (35); the concentrations used here are significantly lower than typically found in ARDS patients. The albumin prevents surfactant from reaching the interface and spreading as shown in Fig. 2, *c* and *d*. (*c*) Increasing the Survanta concentration to 3800 μg on saline buffer containing 2 mg/mL albumin does not restore the isotherm in panel *a*, and although the surface pressure does rise sufficiently that the shoulder at 40 mN/m is visible, the collapse plateau is not reached. Less surfactant adsorbs to the interface that for a clean interface, even though the total surfactant concentration has increased. (*d*) 800 μg Survanta on saline buffer containing 2 mg/mL albumin and 1.2 wt % polyethylene glycol (PEG) polymer of 10 K molecular weight. The characteristic shoulder and collapse plateau of panel *a* have been restored with little change in surface pressure showing that the PEG reverses the albumin inhibition. The only difference with the isotherm in panel *a* is that the minimum surface pressure is ~20 mN/m with PEG in the subphase (18). This suggests that the PEG also helps the collapsed monolayer to heal at higher surface pressures, along with increasing surfactant adsorption to the interface.

a clean, buffered-saline interface. The isotherm traces over itself on subsequent cycles, and on compression exhibits a characteristic shoulder at Π ~40 mN/m, and a collapse plateau at Π_{max} ~65 mN/m (38), indicative of good surfactant performance (2). The hysteresis between compression and expansion cycles is typical of Survanta and other clinical and natural lung surfactant isotherms (2,17,41). Reexpanding the interface after monolayer collapse leads to a rapid drop in surface pressure to ~10 mN/m, which is maintained until compression is resumed (2,38). In general, collapse structures do not readsorb to the interface until the surface pressure is rather low, resulting in the expansion and compression hysteresis (17). There is no significant change in the Survanta isotherm from 23 to 37°C (38), so most experiments were done at room temperature.

Fluorescence images of Survanta films (doped with 1 mol % Texas Red-DHPE; Molecular Probes) at the air-subphase interface show the mottled bright and dark textures typical of a phase-separated lipid/protein monolayer (Fig. 2 *a*; Π = 18 mN/m) and the cracks and folds (*arrows*) typical at monolayer collapse, which determines the maximum surface pressure, Π_{max} that can be achieved by a given film (Fig. 2 *b*; Π_{max} = 66 mN/m, surface tension of ~6 mN/m) (38,42,43).

In contrast, when 800 μg Survanta is deposited on a buffered subphase containing 2 mg/ml albumin (Fig. 1 *b*), the surface pressure does not increase above 40 mN/m even at the smallest trough area; the compression isotherm is not significantly different than that for albumin alone (see Fig. 3 *b*). The fluorescence images are featureless (Fig. 2 *c*) or show isolated, out-of-focus bright spots (Fig. 2 *d*) indicative

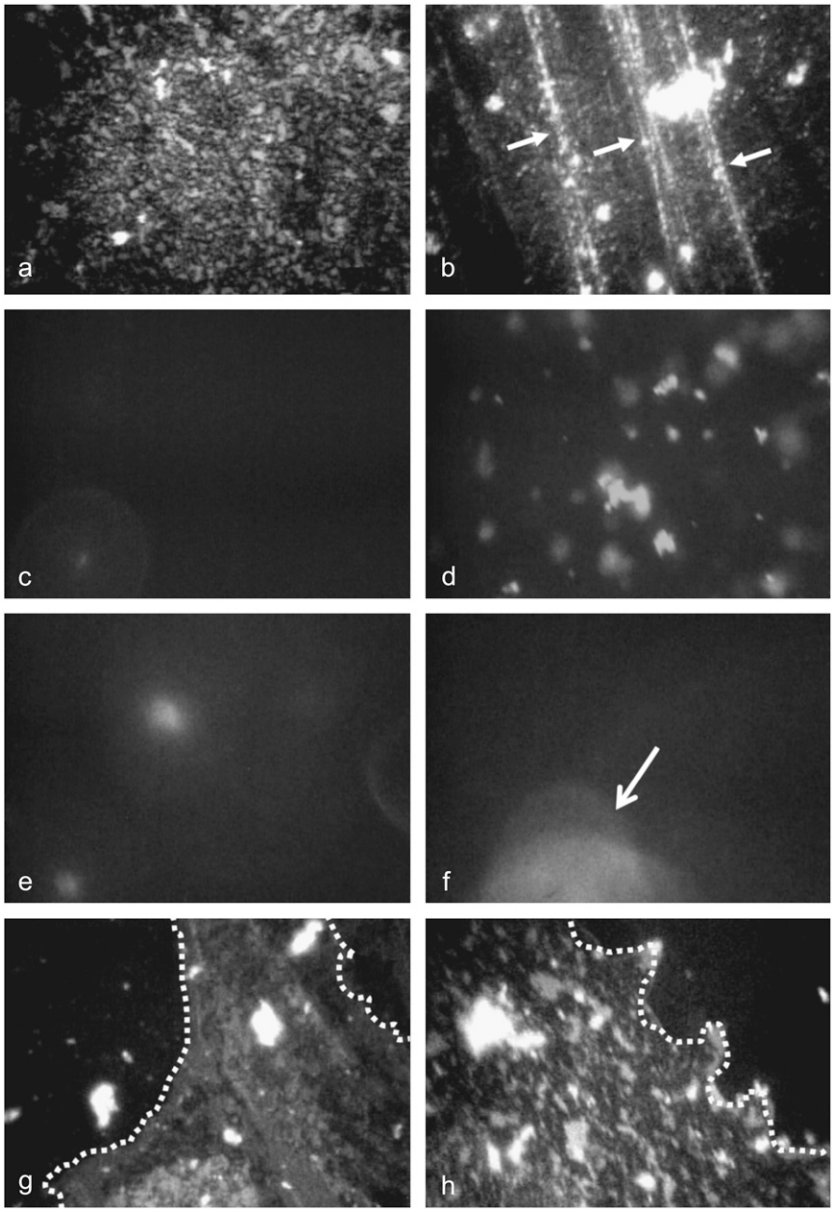

FIGURE 2 Fluorescence images of 800 μg Survanta spread at varying subphase compositions. Images are 180 μm by 250 μm. The left column is for each subphase composition at Π = 18 mN/m (*a*, *c*, *e*, *g*) and the right column is for each subphase at the maximum surface pressure reached during the cycle (66, 40, 31, and 38 mN/m, respectively for *b*, *d*, *f*, and *h*). (Row *1*) Survanta on a clean, buffered subphase. (*a*) Mottled texture typical of a phase-separated lipid/protein monolayer. The mottled texture is found at all surface pressures from 0 to collapse. (*b*) Arrows denote cracks where material is forced from the interface at the collapse plateau at 66 mN/m. (Row *2*) Survanta on buffer containing 2 mg/mL albumin. (*c*) At low surface pressure, no fluorescence is visible showing that the albumin prevents Survanta from adsorbing to the interface. (*d*) After several expansion and compression cycles (see Fig. 1 *b*), Survanta comes close to the interface, but does not spread due to the albumin film at the interface (compare to *e*–*h*). (Row *3*) (*e*) During the first cycle for Survanta spread on buffer containing 2 mg/mL albumin and 0.12 wt % PEG, small areas of the interface are starting to become covered with Survanta. (*f*) The Survanta monolayer begins to displace the albumin (*arrow*). (Row *4*) (*g*) By the third expansion-compression cycle for Survanta spread on buffer containing 2 mg/mL albumin and 0.12 wt % PEG, larger areas have a morphology similar to Survanta on a clean interface (Row *1*, *a* and *b*) in coexistence with areas similar to albumin (Row *2*, *c* and *d*). The dotted white lines denote the borders between the two regions. 0.12 wt % PEG is not sufficient to allow for sufficient Survanta adsorption to completely displace the albumin (See Fig. 3). For ~1 wt % PEG, the images are identical to Row 1 for all cycles (not shown).

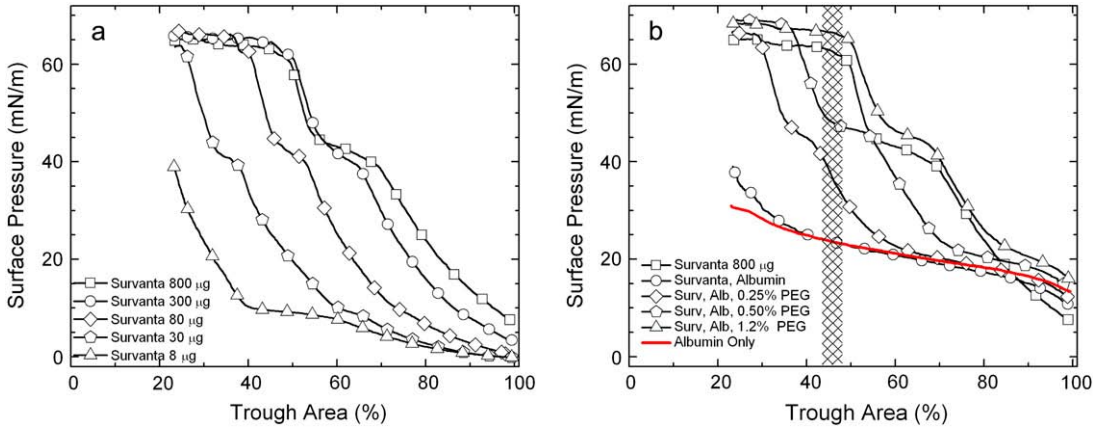

FIGURE 3 Fourth compression cycle isotherms of increasing concentrations of Survanta on a clean buffer subphase (*a*) and 800 μg Survanta on subphases containing 2 mg/ml albumin with increasing PEG concentrations (*b*). (*a*) Up-triangle, 8 μg Survanta; pentagram, 30 μg Survanta; diamond, 80 μg Survanta; circle, 300 μg Survanta; and square, 800 μg Survanta. At a given surface pressure, the isotherms are translated essentially unchanged from low trough area to high trough area with increasing Survanta concentration (note the characteristic shoulder at ~40 mN/m and the collapse plateau at ~65 mN/m). This shows that Survanta adsorption increases with increasing concentration as suggested by Eq. 1. The interface becomes saturated for concentrations >~300 μg; the 800 μg isotherm is not displaced significantly to higher trough areas. (*b*) square, Survanta on saline buffer subphase with no albumin; circle, Survanta-albumin; diamond, Survanta-albumin 0.25 wt % PEG; pentagram, Survanta-albumin 0.50 wt % PEG; and up-triangle, Survanta-albumin 1.2 wt % PEG. The red curve shows the surface pressure for the albumin subphase with no Survanta or PEG. Comparing to panel *a* shows that albumin in the subphase produces the same effect as decreasing the Survanta concentration from 800 μg to ~8 μg. Adding increasing amounts of PEG to the subphase shifts the isotherms to higher trough areas, the same effect as increasing the Survanta concentration in panel *a*. The shaded area denotes the trough area over which the surface pressure was averaged for each PEG concentration to obtain the relative surfactant adsorption plotted in Fig. 4.

of Survanta aggregates in the subphase kept from reaching the interface by the adsorbed albumin film. Comparing Fig. 1 *c* to Fig. 3 *a* shows that less surfactant adsorbs to the interface from 3.8 mg of Survanta on an albumin subphase than from 30 μg of Survanta deposited on a clean subphase. From the isotherm and the fluorescence images, this film still contains a significant albumin fraction.

However, adding 1.2 wt % 10 K molecular weight PEG (Fig. 1 *d*) to the albumin subphase restores the isotherm to that of the clean interface, showing that the polymer enhances surfactant adsorption (Fig. 1 *a*). The isotherm in Fig. 1 *d* is otherwise identical to that of the clean interface, confirming that the polymer does not alter the surfactant monolayer, only the adsorption. Fluorescence images show that as little as 0.12% PEG in the subphase (Fig. 2, *e* and *f*) causes Survanta to locally break through to the interface, perhaps at defects in the albumin monolayer (44). Survanta, which has a higher equilibrium spreading pressure than albumin (~40 mN/m vs. 22 mN/m (18)) partially replaces the albumin on the interface. Increasing the polymer concentration to ~1 wt % leads to interface images indistinguishable from Fig. 2 *a* (not shown).

These results can be explained and quantified using a variation of the classical Smolukowski analysis of colloid stability (26). Charged, surface-active serum proteins adsorbed at an interface induce an electrostatic and steric barrier to surfactant adsorption, similar in magnitude to the energy barriers responsible for colloidal stability against flocculation (45). Inactivation reversal by hydrophilic polymers results from adding the ''depletion attraction'' to the otherwise repulsive potential induced by the proteins at the interface. The depletion attraction lowers the energy barrier to the point that the surfactant aggregates are pushed toward the interface with sufficient force that the surfactant can displace the serum proteins.

The driving force for the change in surfactant interfacial concentration with time, $\frac{d\Gamma}{dt}$, is the gradient of a generalized chemical potential, which results in a diffusive flux multiplied by a potential energy barrier, as in the classic theory of colloid stability (26),

$$\frac{d\Gamma}{dt} = C_o\left(\frac{D_{eff}}{\pi t}\right)^{1/2}\exp[-(V_{max})/k_BT], \qquad (1)$$

where C_o is the bulk surfactant concentration, D_{eff} is the effective surfactant diffusivity, V_{max} is the maximum height of the potential energy barrier (located a distance l from the interface), k_B is Boltzmann's constant, and T is the temperature. For small V_{max}, surfactant adsorption becomes diffusion-limited and the interface saturates so adsorption no longer increases with bulk surfactant concentration. The height of the potential energy barrier results from several competing effects,

$$V_{max} \cong (E_1 - E_0) + \Pi\Delta A + E_{elect} + W, \qquad (2)$$

where $(E_1 - E_0)$ is the energy difference between surfactant molecules at the interface and in the bulk, which drives surfactant adsorption. This is opposed by the double-layer electrostatic potential, E_{elect}, which depends on the ionic strength of the subphase and the net charge distribution of the serum proteins at the interface (46). $\Pi\Delta A$ is a steric term,

which accounts for the energy necessary to clear an interfacial area of protein at a surface pressure of Π for new adsorption. The repulsive terms are opposed by the depletion attraction, as derived from the original Asakura and Oosawa model for a hard sphere of radius R next to a rigid interface: $W = W = -3(R/R_g)\phi_p k_B T\left[1 - (l/2R_g)\right]^2$ (36,47). This attraction is caused by moving a large sphere of radius R to within l of the interface, thereby freeing its excluded volume so as to increase the entropy of the polymer (47); ϕ_p is the volume fraction and R_g is the radius of gyration of the polymer. Albumin and the other serum proteins are of order 4–10 nm in diameter, while the surfactant particles are of the order of microns; hence, the depletion attraction is significantly greater for the surfactant particles. The depletion attraction is purely entropic, and is independent of the chemical composition of the surfactant, protein, and polymer as long as the polymer does not adsorb to the surfactant or the interface, which explains why PEG (10,20,24,48), dextran (21,30), and hyaluronan (18) are all effective at enhancing surfactant adsorption. Equations 1 and 2 predict an exponential dependence of surfactant adsorption on polymer concentration, but only a linear dependence on surfactant concentration in the presence of albumin.

Fig. 3 *a* shows the surface pressure as a function of trough area for increasing amounts of Survanta deposited on a clean saline subphase (38). The characteristic shape of the isotherms in Fig. 3 *a* are translated unchanged (note the shape of the collapse plateau and the shoulder at ~40 mN/m) from low to high trough area for a given surface pressure as the amount of Survanta added to the trough is increased from 8 μg up to 800 μg. This means that the total amount of surfactant at the interface has increased, as the relationship between surface pressure and area/molecule is fixed for a given surfactant composition and temperature (38). Hence, increasing surfactant adsorption is reflected in the isotherms as a translation from low to high trough area at a given surface pressure. Eventually, the adsorption saturates (note the small offset between 300 μg and 800 μg); at this point surfactant adsorption is diffusion-limited and further increases in surfactant concentration have little effect.

Fig. 3 *b* shows the effect of albumin on the isotherms is equivalent to decreasing the Survanta concentration (see Fig. 3 *a*). The red line in Fig. 3 *b* shows the compression isotherm of 2 mg/ml albumin with no Survanta or PEG, which differs little from that of 800 mg Survanta on the albumin subphase. This isotherm is most similar to that of the 8-μg Survanta isotherm in Fig. 3 *a*, which means that the effective surfactant adsorption is reduced by a factor of 100 or more by the albumin. A 100-fold reduction in adsorption at fixed bulk concentration, C_o, corresponds to a V_{max} of ~+5 k_BT in Eq. 1.

Increasing the PEG concentration in the subphase (Fig. 3 *b*) produces the same effect as increasing the total surfactant concentration on a clean interface (Fig. 3 *a*), indicating that the polymer increases surfactant adsorption. However, as in Fig. 3 *a*, the shapes of the isotherms are unchanged, just shifted to larger trough areas with increasing polymer concentration, confirming that the albumin and polymer do not affect the surfactant monolayer properties at the interface, just the total surfactant adsorption. This is consistent with the polymer inducing a depletion attraction between the interface and the surfactant aggregates.

Surfactant adsorption is restored to that of a clean interface for ~1 wt % PEG. This suggests that the magnitude of the depletion interaction is ~−5 k_BT to offset the repulsive potential. PEG of molecular weight 10 K has R_g ~ 4–5 nm, and 1 wt % has a volume fraction, ϕ_p ~ 0.2. R/R_g ~ 100 (corresponding to 1 μm diameter surfactant aggregates), hence the Asakura and Oosawa model gives $W = -60\,k_BT\left[1 - (l/2R_g)\right]^2$. This gives the correct magnitude for the depletion force for l ~1.4 R_g, suggesting that the maximum in the potential lies ~6 nm from the interface. This is roughly the dimension of the albumin molecules. It is not well established how the depletion force changes for nonspherical, rough, and deformable surfaces, as is the case for the surfactant aggregates. However, it is clear that the depletion interaction lowers the energy barrier to adsorption and diffusion-limited surfactant adsorption is restored.

To quantify the effect of polymer concentration on surfactant adsorption, Fig. 4 shows the relative rate of surfactant adsorption as a function of PEG concentration. The fluorescence images (Fig. 2 *c*) show that in the absence of polymer, no surfactant adsorbs to the interface. Hence, we define the relative adsorption (*RA*) as the difference between the sample surface pressure (Π) and the surface pressure of the albumin

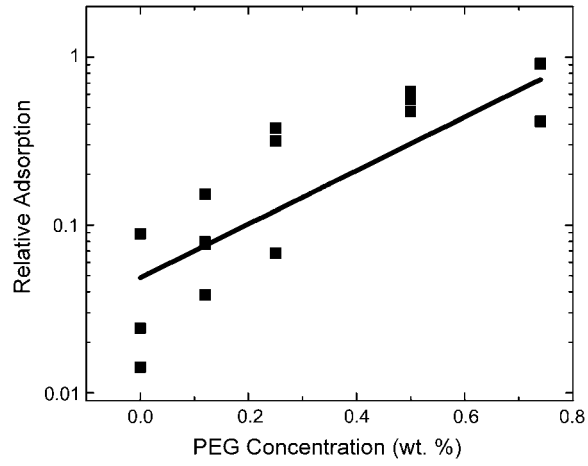

FIGURE 4 The relative adsorption (RA) is the difference between the sample surface pressure (Π) and the surface pressure of the albumin-only isotherm (Π_{Alb}, *red curve* in Fig. 3 *b*), divided by the difference between the surface pressure for the saturated isotherm (>1% PEG in Fig. 3 *b*) and Π_{Alb}, $RA = (\Pi - \Pi_{Alb})/(\Pi_{saturated} - \Pi_{Alb})|_{A_0}$. All surface pressures were evaluated by averaging over the same trough area denoted by the shaded area in Fig. 3 *b*. The solid line is a good fit to the data showing the exponential dependence of RA on the polymer concentration as predicted by Eqs. 1–3, consistent with the depletion attraction lowering the energy barrier to surfactant adsorption.

only isotherm (Π_{Alb}, *red curve* in Fig. 3 *b*), divided by the difference between the surface pressure for the saturated isotherm (>1% PEG in Fig. 3 *b*) and Π_{Alb}, $RA = (\Pi - \Pi_{Alb})/(\Pi_{saturated} - \Pi_{Alb})|_{A_o}$. All surface pressures were evaluated by averaging over the same trough area (A_o), denoted by the shaded area in Fig. 3 *b*. This region showed the maximum variation in adsorption. From Eqs. 1 and 2, the relative adsorption with albumin and PEG in the subphase compared to a clean interface should be an exponential function of PEG weight fraction, C_{PEG},

$$\ln\left(\frac{d\Gamma/dt_{albumin+PEG}}{d\Gamma/dt_{saturated}}\right) = \ln(RA) = \alpha + \beta\phi_p = \alpha + \beta' C_{PEG}, \quad (3)$$

in which β, β', and α are constants for a given Survanta and albumin concentration. While there is some scatter in the data, the exponential dependence of the surfactant adsorption on the PEG concentration is clear from the best-fit line. This shows that the depletion attraction lowers the steric and electrostatic energy barrier to adsorption. The relative adsorption increases by approximately a factor of 50 as the PEG concentration is increased from 0 to 0.8 wt %. Higher concentrations of PEG lead to minimal increases in adsorption, as the adsorption has been restored to the rate for a clean interface, which is likely diffusion-limited.

DISCUSSION

Inhibition of surfactant adsorption by surface-active contaminants (here serum proteins) are consistent with the physiological processes that accompany ARDS development and severity:

1. Surface-active proteins and lysolipids resulting from the combination of inflammation and increased alveolar epithelial permeability adsorb to the alveolar interface.
2. The protein layer creates an electrostatic and steric energy barrier that exponentially decreases surfactant adsorption.
3. Less surfactant adsorption means that low surface tensions cannot be reached during normal breathing.
4. Hydrophilic, nonadsorbing polymers can provide sufficient depletion attraction to overcome the energy barrier and reestablish normal surfactant adsorption.

The attractive depletion forces generated by the hydrophilic polymers balance the repulsive forces generated by steric and electrostatic interactions between the serum proteins and the anionic surfactant aggregates to restore diffusion-limited adsorption.

These results also suggest a role in the promotion of surfactant adsorption for the hyaluronan (HA) normally present in the alveolus. HA is a natural polysaccharide that is secreted by alveolar epithelial cells into the alveolar subphase fluid at concentrations of ~4000 μg/l (49); HA, like PEG, provides an attractive depletion attraction that can promote surfactant adsorption. The magnitude and range of the depletion attraction increases with molecular weight (26); however, there is likely both an optimal polymer concentration and molecular weight to enhance adsorption, as the diffusivity strongly decreases (D_{eff} in Eq. 1) with increasing polymer concentration and molecular weight. The HA in the lung epithelial fluid is ~220 kDa, which is dramatically less than that of the lung interstitium at ~3000 kDa (49). This suggests that the optimal molecular weight to promote surfactant adsorption may be different than that required to occupy the interstitial space between cells. During lung injury and disease, HA can be broken down by enzymatic action to produce smaller molecular weight fragments that may be inflammatory (49), but may also not provide sufficient depletion attraction to insure reliable surfactant adsorption, especially in the presence of serum proteins. Our results show that it is possible that the increased serum and inflammatory protein concentration and the modified hyaluronan molecular weight and concentration act in an unfortunate concert to reduce the rate of surfactant adsorption.

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