Competitive Adsorption of Lung Surfactant and Serum Proteins at the Air-Liquid Interface: A Grazing Incidence X-Ray Diffraction Study

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ABSTRACT

The competitive adsorption of lung surfactant (LS) and albumin at the air-liquid interface and the ability of polyethylene glycol (PEG) to mediate LS adsorption are analyzed using pressure-area isotherms and grazing incidence x-ray diffraction (GIXD). The addition of albumin drastically reduces the amount of LS on the interface and slightly increases the LS lattice spacing. The addition of PEG restores the characteristic LS peaks, yielding a slightly more compact lattice. The scattering results are consistent with recent work which proposed that albumin creates a physical barrier which eliminates LS adsorption and that PEG enhances LS adsorption but does not significantly change LS surface ordering.

INTRODUCTION

Lung surfactant (LS) is a unique mixture of lipids and proteins that lines the alveolar air-liquid interface and lowers the surface tension in the lungs, thereby insuring negligible work of breathing and uniform lung inflation [1]. The surface tension control imposed by LS is compromised during Acute Respiratory Distress Syndrome (ARDS) which afflicts 140,000 annually with a 40% mortality rate in the US [2]. The complex pathogenesis of ARDS includes increased permeability of the alveolar-capillary barrier yielding an influx of blood serum proteins into the bronchial and alveolar fluid [3]. In vitro, LS mixed with serum proteins shows an ARDS-like decrease in performance; surfactant inactivation caused by serum protein leakage into the alveoli is one reason why treatment of ARDS with replacement LS is unsuccessful [4].

One possible cause of surfactant inactivation is the competitive adsorption of surface-active serum proteins (such as albumin) that reduces or even eliminates the normal adsorption of LS to the interface [4]. Albumin is surface-active and has a surface pressure, $\Pi$, ($\Pi = \gamma - \gamma_w$; $\gamma_w$ is the surface tension of a clean air-water interface, 72 mN/m, and $\gamma$ 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 [4]. The saturation surface pressure for albumin is $\Pi \sim 18$ mN/m, much lower than the $\Pi \sim 70$ ($\gamma$ near zero) required for proper respiration [4]. This competitive adsorption of albumin to the alveolar air-liquid interface leads to a steric and electrostatic energy barrier to LS adsorption which can lower the rate of LS transport to the interface [5]. Several hydrophilic polymers, such as PEG have recently been shown to enhance the ability of replacement LS to resist serum inactivation both in vitro and in vivo [6, 7]. For PEG, the inactivation reversal effect that restores the normal rate of LS adsorption can be
explained via the “depletion attraction” between the air-liquid interface and LS aggregates in the bulk [5, 7]. The depletion attraction acts like an attractive force that pushes the LS toward the interface offsetting the steric and electrostatic repulsion induced by the interfacial albumin.

The present study utilizes GIXD to investigate the surface ordering of LS at the air-liquid interface. While multicomponent lipid monolayers with a composition similar to LS have been investigated with GIXD [8], only very limited data on complex LS mixtures exists [9]. Additionally, the present study gives the first quantitative information on the effect of albumin and PEG on the LS surface ordering.

EXPERIMENT

Survanta (Abbott Laboratories, Columbus, Ohio) was a generous gift of the Santa Barbara Cottage Hospital nursery. Survanta is an organic extract of minced bovine lungs that contains 80-90% wt. phosphatidylcholine, of which, ~70% wt. is saturated dipalmitoylphosphatidylcholine (DPPC), ~10% wt. palmitic acid (PA) and < 2% wt. LS specific proteins [10]. 10 kDa PEG and bovine serum albumin were obtained from Sigma (St. Louis, MO) and used as received. Isotherms were recorded at 20°C in a Langmuir trough equipped with a Wilhelmy plate for surface pressure measurements and a continuous stainless steel ribbon barrier to change the trough area (Nima, Coventry, England). The trough had a surface area of 130 cm² and a typical compression/expansion cycle took 8 min (~0.42 cm²/sec). The isotherms were similar at speeds as low as 36 min/cycle, which corresponds to the barrier speed in the GIXD experiments. Albumin and PEG were dissolved in the subphase buffer (NaCl 150 mM, CaCl₂ 2 mM, NaHCO₃ 0.2 mM and pH = 7) at the stated concentrations for all experiments. Survanta was diluted in the same buffer to a lipid concentration of 2 mg/mL and was deposited dropwise onto the subphase of the Langmuir trough at the stated total surfactant amounts to initiate each adsorption experiment.

All GIXD experiments were carried out at the BW1 (undulator) beam line at HASYLAB (Hamburg, Germany) using a dedicated liquid surface diffractometer with an incident x-ray wavelength of $\lambda \sim 1.3$ Å [11]. A thermostated Langmuir trough (surface area of 410 cm²) was mounted on the diffractometer and all experiments were carried out at 20°C. For consistency with the isotherms, the amount of Survanta spread was scaled by the GIXD trough surface area. For phospholipid monolayers at the air–water interface, diffraction is observed only from the lateral order of the alkyl chains; the lipid headgroups do not contribute to the diffraction [11]. The GIXD intensity resulting from a powder of 2-D crystallites may be represented by its projection onto the $q_{xy}$ axis to yield Bragg peaks or onto the $q_z$ axis to yield Bragg rods, where $q_{xy}$ and $q_z$ are the horizontal and vertical components of the scattering vector $q$, respectively. The Bragg peaks ($q_{xy}$) allow for the determination of the 2-D lattice spacing of the alkyl chains ($d = 2\pi / q_{xy}$). The intensity distribution along a Bragg rod can be analyzed to infer the direction and magnitude of the molecular tilt in the crystalline part of the monolayer. Due to lack of vertical crystalline repeat, there is no restriction on the scattering vector component $q_z$ along the direction normal to the crystal: Bragg scattering from a 2-D crystal extends as a continuous Bragg rod through reciprocal space [11]. The dimensions of the footprint of the incoming x-ray beam were ~2 x 50 mm². As a precaution against beam damage, the trough was translated by 0.025 mm horizontally across the x-ray beam in the direction along the barrier compression at every step of the $q_{xy}$ scan.
RESULTS AND DISCUSSION

Figure 1a shows a typical compression-expansion cyclic isotherm for 180 µg of the clinical LS, Survanta, adsorbing to a clean, saline buffered interface. The isotherm traces over itself on subsequent cycles and on compression exhibits a characteristic shoulder at $\Pi \approx 42$ mN/m and collapse plateau, $\Pi_{\text{max}} \approx 68$ mN/m, which determines the minimum surface tension possible for a given surfactant [7]. The hysteresis between compression and expansion cycles is typical of Survanta [1] and is due to the partial readsoption of the collapse structures into the monolayer [4]. On a clean interface, reexpanding the interface after monolayer collapse leads to a rapid drop in surface pressure until compression is resumed. There is no significant change in the Survanta isotherms from 20 – 37°C [9].

![Figure 1: Cyclic isotherms of Survanta on buffered subphases of varying composition.](image)

(a) 180 µg Survanta on a clean buffered saline subphase. (b) Black curve: 180 µg Survanta deposited onto a saline buffer containing 2 mg/mL albumin. Red curve: The isotherm for the albumin subphase, with no Survanta. (c) 180 µg Survanta on saline buffer containing 5% wt. 10 kDa PEG. (d) 180 µg Survanta on saline buffer containing 2 mg/mL albumin and 5% wt. 10 kDa PEG.

However, when the same amount of Survanta (180 µg) is deposited onto a subphase containing 2 mg/mL albumin (Figure 1b, black curve), the surface pressure does not increase above 35 mN/m even at the smallest trough area. Both the compression and expansion isotherms are not different than that of albumin alone (Figure 1b, red curve). Survanta inactivation under these conditions results from an inability of the LS to adsorb to the interface. Figure 1c shows that Survanta deposited onto a subphase containing 5% wt. 10 kDa PEG still exhibits a characteristic shoulder at $\Pi \approx 42$ mN/m and a collapse plateau at $\Pi_{\text{max}} \approx 68$ mN/m similar to that of Figure 1a. PEG addition results in increased Survanta adsorption; less compression is required to reach the collapse plateau. Additionally, the minimum surface pressure during expansion increases
compared to Survanta (Figure 1a), suggesting that PEG facilitates readsorption of the collapse structures resulting in less hysteresis. Survanta deposited onto a subphase containing 2 mg/mL albumin and 5% wt. 10 kDa PEG (Figure 1d) exhibits an isotherm which resembles Survanta on a clean interface after two expansion-compression cycles, showing that the PEG reverses the inactivation. The compression cycle in Figure 1d displays the characteristic shoulder at Π ~42 mN/m and collapse plateau at Π_{max} ~68 mN/m, similar to that of the clean interface (Figure 1a). This supports the hypothesis that PEG does not significantly alter the surface ordering of the LS but rather enhances the adsorption of LS to the interface to displace the albumin [7].

Figure 2 shows the Bragg peaks from GIXD scans of Survanta on varying subphases at Π = 20 mN/m. For Survanta on a saline buffered subphase (Figure 2a), two peaks are observed with the integrated intensity of the \( q_{xy} = 1.44 \ Å^{-1} \) peak roughly twice that of the \( q_{xy} = 1.48 \ Å^{-1} \) peak indicating a distorted hexagonal lattice. The higher intensity from the four coincident (1,1) reflections leads to the assignment of the (1,1) reflection to \( q_{xy} = 1.44 \ Å^{-1} \) and the (0,2) reflection to \( q_{xy} = 1.48 \ Å^{-1} \). A distorted hexagonal lattice with similar peak positions occurs in simple lipid models of LS containing DPPC/PA 3:1 (w/w) at similar conditions [8]. The Bragg rod (profile not shown) exhibits a local maximum above the horizon \( q_z = 0 \ Å^{-1} \) at \( q_z = 0.41 \ Å^{-1} \), indicating that the molecules are tilted relative to the normal.

![Figure 2 Bragg peaks from GIXD scans of Survanta on varying subphases](image)

Figure 2 Bragg peaks from GIXD scans of 600 µg Survanta at the air-liquid interface subphases of varying composition at Π = 20 mN/m (a,b,d) or Π = 25 mN/m (c). The points indicate instrument data, the black curve is the overall fit and the blue curves are fits of the individual peaks. (a) A saline buffer subphase. (b) A saline buffer subphase containing 2 mg/mL albumin. (c) A saline buffer subphase containing 5% wt. PEG. (d) A saline buffer subphase containing 2 mg/mL albumin and 5% wt. PEG.
A saline buffer subphase containing 2 mg/mL albumin yields an equilibrium surface pressure of $\Pi \sim 18$ mN/m, indicating albumin adsorption at the air-liquid interface. GIXD scans show no signal for $q_{xy} = 0.04$-2.50 Å$^{-1}$ indicating a lack of crystalline order from the albumin on the interface. When Survanta is spread on the aforementioned albumin subphase, the Survanta-albumin (SA) film shows great heterogeneity at $\Pi = 20$ mN/m. Some regions show no signal indicating a lack of Survanta in the footprint others while others yield the Bragg peaks shown in Figure 2b. Here, two peaks are present with the intensity of the peak at $q_{xy} = 1.39$ Å$^{-1}$ roughly twice that of the $q_{xy} = 1.47$ Å$^{-1}$ peak indicating a distorted hexagonal lattice. These results are consistent with fluorescence images which show coexistence of extended regions of albumin and Survanta on the air-liquid interface which can be larger than the 1 mm. While the isotherm maximum surface pressure (35 mN/m) indicates that the albumin dominates the interface, the distorted hexagonal lattice demonstrates that some Survanta is present. Compared to Survanta at $\Pi = 20$ mN/m, the SA peaks are shifted left, indicating a disruption of the lattice packing. The Bragg rod for the SA system exhibits a local maximum higher above the horizon ($q_z = 0.57$ Å$^{-1}$) than Survanta, indicating that the molecules are more tilted in the SA system.

GIXD scans of a subphase containing 5% wt. PEG yielded no signal from $q_{xy} = 0.04$-2.50 Å$^{-1}$. Figure 2c shows Bragg peaks of Survanta spread on the aforementioned PEG subphase at $\Pi = 25$ mN/m, the minimum surface pressure obtained for this system. Two peaks are observed with the integrated intensity of the $q_{xy} = 1.44$ Å$^{-1}$ peak roughly twice that of the $q_{xy} = 1.48$ Å$^{-1}$ peak indicating a distorted hexagonal lattice. The Bragg rod for the Survanta-PEG system exhibits a local maximum above the horizon at $q_z = 0.41$ Å$^{-1}$. Overall, the Bragg peaks and Bragg rods are equivalent to Survanta on a clean subphase, indicating that PEG does not influence the Survanta surface ordering.

A saline buffer subphase containing 2 mg/mL albumin and 5% wt. PEG also yields an equilibrium surface pressure of $\Pi \sim 18$ mN/m, indicating adsorption to the air-liquid interface. GIXD scans show no signal from $q_{xy} = 0.04$-2.50 Å$^{-1}$ indicating a lack of crystalline order from PEG or albumin on the interface. Figure 2d shows Bragg peaks of Survanta spread on the aforementioned albumin-PEG subphase; the Survanta-albumin-PEG (SAP) system exhibits the characteristic Survanta diffraction peaks at $\Pi = 20$ mN/m. The SAP system shows two peaks with the intensity of the peak at $q_{xy} = 1.48$ Å$^{-1}$ roughly twice that of the $q_{xy} = 1.49$ Å$^{-1}$ peak indicating a distorted hexagonal lattice. Compared to Survanta, the SAP peaks are shifted right, indicating a more compact lattice. The Bragg rod for the SAP system exhibits a local maximum closer to the horizon than Survanta ($q_z = 0.09$ Å$^{-1}$) indicating less tilted molecules at the interface.

For Survanta, the calculated $d$-spacings, $d_{11} = 4.38$ Å and $d_{02} = 4.24$ Å, yield a distorted hexagonal lattice with $a_H = 4.95$ Å and $\alpha = 118^\circ$. The addition of albumin increases the size of the distorted hexagonal lattice at 20 mN/m to $a_H = 5.04$ Å and $\alpha = 116^\circ$ while the addition of PEG yields a distorted hexagonal lattice with $a_H = 4.94$ Å and $\alpha = 118^\circ$, similar to Survanta. The SAP system yields a slightly more compact lattice with $a_H = 4.88$ Å and $\alpha = 119^\circ$.

**CONCLUSIONS**

Isotherms and GIXD measurements were used to investigate the surface ordering of LS and the impact of albumin and PEG addition. Albumin addition drastically changes the LS isotherm to an albumin-like isotherm showing that albumin substantially reduces the amount of LS reaching the air-liquid interface. PEG addition enhances LS adsorption to the interface resulting in a restoration of the LS isotherm from albumin inactivation. GIXD measurements
show that LS packs in a distorted hexagonal lattice at $\Pi = 20$ mN/m on all of the subphases investigated. They also confirm that albumin addition drastically reduces the amount of LS on the interface; scans also revealed increased lattice spacing compared to LS, indicating that albumin disrupts the LS packing. Addition of PEG alone does not change the LS packing while PEG addition restores the characteristic LS peaks in the Survanta/albumin/PEG system, yielding a slightly more compact lattice. The isotherm and GIXD results are consistent with a recently proposed competitive adsorption model: (1) albumin substantially reduces LS absorption by creating a physical barrier on the air-liquid interface and (2) PEG addition enhances LS adsorption to the air-liquid interface, restoring the LS isotherms while only minimally changing the LS surface ordering.

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