POLYMER DEPLETION FORCES AND SURFACANT ADSORPTION

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Introduction

Lung surfactant is a mixture of lipids, primarily dipalmitoylphosphatidylcholine, and several specific proteins, that lines the interior of the lung alveoli. Lung surfactant lowers the interfacial tension in the lungs, thereby insuring a negligible work of breathing and uniform lung inflation. The absence of lung surfactant in premature infants leads to neonatal Respiratory Distress Syndrome, which can be treated by delivering replacement surfactants, often derived from animals, to the lungs. Such surfactants often provide immediate relief from symptoms and improved oxygenation and gas exchange. However, in some infants and adults, the presence of blood serum proteins or other surface-active species not normally present in the alveolar space may inhibit lung surfactant performance and may play a major role in the development of Acute Respiratory Distress Syndrome (ARDS) [1]. ARDS is often fatal and there is no known treatment. We have found that adding ionic or non-ionic polymers to mixtures of organic extracts of surfactants enhance surface activity and reduce inactivation in vitro and in vivo [2]. The origin of this inhibition reversal is likely the enhanced adsorption of surfactant due to the polymer induced depletion force [3,4]. In this work, a theoretical description of the depletion force on surfactant adsorption is presented and predictions are made to suggest how to optimize surfactant/polymer mixtures for treatments of ARDS.

Origin of Inhibition by Soluble, Surface-Active Substances

Inhibition is known to be strongly dependent on both the species and concentration of inhibitor [1]. In Figure 1, surface pressures of fibrinogen, albumin and IgG, three proteins commonly implicated in surfactant inhibition, are given as a function of concentration up to a certain bulk concentration at which the surfactant concentration is adjusted by the addition of inhibitor. Inhibitor concentrations .1 mg/ml and greater exert surface pressures of about 20 mN/m, while IgG requires 100 times that concentration to reach the same level. Thus, albumin and fibrinogen should not inhibit below .1 mg/ml and IgG should have little effect until 1mg/ml. This prediction agrees well with experiments [5] establishing the relative inhibitory capacity of serum proteins.

Figure 1. Many common inhibitors of surfactant exhibit a concentration dependent surface pressure, similar to simple soaps and detergents. The surface pressure, $\Pi_c(c)$, depends on the log of the soluble species concentration, up to a concentration at which the surface is saturated and the surface pressure no longer increases.

Inhibition likely occurs when a surface active species not normally present in the alveoli—such as serum proteins, competes successfully for the air-water interface with lung surfactant [5]. Most lung surfactant inhibitors are water soluble and rapidly adsorb to the air-water interface and produce moderate (20 – 30 mN/m) equilibrium surface pressures, $\Pi_c(c)$ (Fig.1). However, as the interfacial area is reduced during exhalation, inhibitor molecules at the interface easily exchange with the subphase, and the surface pressure stays roughly constant at $\Pi_a$ until most of the inhibitor leaves the interface. Only then does the surface pressure start to rise due to the remaining surfactant at the interface.

The lipids and hydrophobic proteins in lung surfactant are essentially insoluble as molecular species in the subphase – lung surfactants exist at the air-water interface as a monolayer or multilayer film, as bilayer aggregates in the subphase, or as some intermediate structure (tubular myelin surfactant is one such structure [6]) that occurs during conversion from subphase to interface [7]. The energy difference between surfactant in the subphase and at the interface determines the equilibrium spreading pressure, $\Pi$, of the surfactant. As the surfactant film is compressed, the surface pressure increases to levels above $\Pi_a$ until the film collapses, generally by the generation of folds, cracks, etc. in the film [8-10]. The maximum surface pressure is set by the collapse pressure. Proper lung function requires that the surface tension in the alveoli drop to near zero as the alveolar surface area decreases. Mixed surfactant-inhibitor films require greater compression to reach the maximum surface pressure, and breathing becomes more difficult [11].

Experimental

The unifying features of the polymers that reverse inhibition are: (1) they do not specifically adsorb to surfactant aggregates; (2) they are small compared to surfactant bilayer aggregates in solution (nm vs microns); (3) inhibition reversal occurs for all surfactant and polymer mixtures studied so far. This suggests that a generic interaction, the so-called “depletion interaction” (Fig. 2), leads to inhibition reversal, rather than a specific interaction between particular surfactants and polymers. A mixture of two different sizes of non-interacting “hard spheres” maximizes its entropy by maximizing the volume accessible per “sphere” [3,4]. Here, the small spheres are the polymers with radius of gyration, $R_g$ (typically nm), and the large spheres are surfactant aggregates of radius $R$ (typically microns). As the large sphere moves toward another large sphere or the wall, the volume excluded from the centers of the small spheres (hatched regions) overlap causing the volume accessible to the small spheres to increase. This increases the entropy of the mixture (decreases the free energy) by an amount proportional to the size of the excluded volume overlap region, multiplied by the osmotic pressure of the small spheres. Moving a single large sphere to a rigid surface decreases the mixture’s free energy by the depletion interaction energy, $E_D^\text{free}$, per unit time, is proportional to the average concentration of surfactant in the solution, $c_a$, the diffusion constant for the surfactant, $D$, across a boundary layer whose thickness grows with time, $t$ [12]:

$$
\frac{d\Pi}{dt} = c_a \left( \frac{D}{\sigma} \right)^{1/2} \exp \left[ -\left( E_1 + \Pi \Delta A + E_{\text{elect}} - E_m - E_a \right) / k_B T \right].
$$

The effective surfactant concentration is adjusted by the probability of crossing an “energy barrier” whose height is given by $E_{\text{elect}} - E_a$ $k_B T$. The energy difference between surfactant in bilayers and in the monolayer, which leads to adsorption onto a clean interface with zero surface pressure, $\Pi \Delta A + E_{\text{elect}}$ to the barrier. The rate of spreading of anionic lipids from solution is well described by the rate equation above and changes with $E_{\text{elect}}$ [13]. To stabilize colloidal particles against aggregation, the energy barrier height need only be $\sim 15 k_B T$ [14]. For the relatively fast cycles of expansion and compression under normal breathing, the energy barrier does not have to be that high to effectively inhibit surfactant adsorption.
The origin of depletion forces pushing surfactant to the interface. Small spheres (polymers with radius \( R_g \)) cannot occupy the excluded volume within \( R_g \) of the surface of the interface or the large spheres of radius \( R \) (surfactant aggregates). (Bottom) As the large spheres move to the interface or toward each other, their excluded volumes overlap, increasing the volume available to the small spheres, thereby reducing the energy of the system and forcing the spheres toward each other and towards the interface. This increases the rate of adsorption and can overcome the energy barrier caused by serum as described in the equations.

However, if polymer is present in the subphase, \( E_o \) reduces the energy barrier. The depletion interaction effectively “pushes” the surfactant to the interface (Fig. 2). For the volume fractions (\( \phi = 1-10\% \)) and aspect ratios (\( R/R_g \approx 10–300 \)) that reduce inhibition, \( E_o \approx 10-100 \) kJ/mol. For a polymer in a good solvent, \( R_g \) scales as \( M^{0.6\pm0.3} \), in which \( M \) is the polymer molecular weight. Hence, the depletion potential: \( E_o \propto N (R+R_g)^{12-13}/R_g^{11} \). If both large and small spheres have the same charge the electrostatic repulsion between the large and small particles increase the effective radius of both large and small particles by roughly \( \kappa^{-1} \), the Debye length [15], and \( E_o = 4\pi N \rho (R+R_g)^{12-13}/k_BT V \). In physiological saline, \( \kappa^{-1} \approx 1 \) nm. Lung surfactant contains a substantial fraction of anionic lipids, so this suggests that an anionic polymer might provide a larger depletion interaction for a given concentration. This is why we find that inhibition reversal occurs for \( 125 \) wt/vol 1250 kDa anionic hyaluronic acid, and it takes 5 wt/vol 10 kDa non-ionic polyethylene glycol to provide the same inhibition reversal [12]. In a recent paper, Yu et al. [16] measured the rate of adsorption of Bovine Lung Extract Surfactant (BLES) in a pendant drop apparatus as a function of PEG molecular weight, \( M \). They found that the rate of adsorption increased with \( M \) from 3000 – 35,000 kDa at 10 mg/ml mass concentration, consistent with the theoretical predictions. The osmotic pressure of the polymer-surfactant solution, which should be minimized to prevent infiltration of liquid into the lung, is proportional to \( N/\kappa^2 \), so high molecular weight polymers should have a distinct advantage in treatments of ARDS and other lung injuries.

Conclusions

The range of the depletion force is \( 2R_g \), which shows that using higher molecular weight polymers will increase the range as well as the magnitude of the depletion interaction. The range of electrostatic interactions are roughly \( \kappa^{-1} \), the Debye length [15], which is proportional to the ionic strength of the subphase. It is not known what the range of the steric interactions with albumin or other serum proteins might be – the albumin molecule has traditionally been considered a prolate ellipsoid with length of \( \sim 14 \) nm and a diameter of \( \sim 4 \) nm [17]. Hence, we expect that we will need polymers with a radius of gyration of at least about \( 4 \) nm. 4 kDa PEG has \( R_g = 2.7 \) nm, and 6 kDa PEG has \( R_g = 3.3 \) nm [18], so even relatively small molecular weight polymers should have a sufficient range of depletion forces. 1 kDa PEG has \( R_g = 1.3 \) nm, which may be too small for depletion forces to enhance adsorption. However, physiological conditions suggest that the maximum molecular weight possible be used to minimize osmotic effects in the lungs. Polymers might be added to all surfactant treatments to enhance adsorption to anticipate the possibility of reabsorbed interfaces.

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