Biochimica et Biophysica Acta 1798 (2010) 801–828

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Overcoming rapid inactivation of lung surfactant: Analogies between competitive adsorption and colloid stability

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ARTICLE INFO

Article history:
Received 22 September 2009
Received in revised form 13 December 2009
Accepted 15 December 2009
Available online 22 December 2009

Keywords:
Charge reversal
Chitosan
Inhibition
Depletion attraction
Albumin
Polyethylene glycol
Debye length

ABSTRACT

Lung surfactant (LS) is a mixture of lipids and proteins that line the alveolar air–liquid interface, lowering the interfacial tension to levels that make breathing possible. In acute respiratory distress syndrome (ARDS), inactivation of LS is believed to play an important role in the development and severity of the disease. This review examines the competitive adsorption of LS and surface-active contaminants, such as serum proteins, present in the alveolar fluids of ARDS patients, and how this competitive adsorption can cause normal amounts of otherwise normal LS to be ineffective in lowering the interfacial tension. LS and serum proteins compete for the air–water interface when both are present in solution either in the alveolar fluids or in a Langmuir trough. Equilibrium favors LS as it has the lower equilibrium surface pressure, but the smaller proteins are kinetically favored over multi-micron LS bilayer aggregates by faster diffusion. If albumin reaches the interface, it creates an energy barrier to subsequent LS adsorption that slows or prevents the adsorption of the necessary amounts of LS required to lower surface tension. This process can be understood in terms of classic colloid stability theory in which an energy barrier to diffusion stabilizes colloidal suspensions against aggregation. This analogy provides qualitative and quantitative predictions regarding the origin of surfactant inactivation. An important corollary is that any additive that promotes colloid coagulation, such as increased electrolyte concentration, multivalent ions, hydrophilic non-adsorbing polymers such as PEG, dextran, etc. added to LS, or polyelectrolytes such as chitosan, also promotes LS adsorption in the presence of serum proteins and helps reverse surfactant inactivation. The theory provides quantitative tools to determine the optimal concentration of these additives and suggests that multiple additives may have a synergistic effect. A variety of physical and chemical techniques including isotherms, fluorescence microscopy, electron microscopy and X-ray diffraction show that LS adsorption is enhanced by this mechanism without substantially altering the structure or properties of the LS monolayer.

Contents

1. Introduction: Why lung surfactant? . . . 802
2. Surfactant inactivation . . . 803
3. Equilibrium vs kinetic effects . . . 804
4. LS bilayer organization and interactions in solution . . . 805
5. Monolayer structure, organization and collapse . . . 807
6. Lung surfactant interfacial organization . . . 807
7. Kinetically hindered equilibrium and analogies to colloid stability . . . 811
8. Polymers — two distinct mechanisms of destabilizing colloids and enhancing adsorption . . . 815
9. Polymer depletion forces . . . 815
10. Optimizing polymer volume fraction and polymer molecular weight . . . 816
11. Cationic polyelectrolytes . . . 821
12. Conclusions . . . 823
Acknowledgements . . . 824
Appendix A. Supplementary data . . . 824
References . . . 824

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doi:10.1016/j.bbamem.2009.12.010

1. Introduction: Why lung surfactant?

The human lung bifurcates into numerous channels (bronchi and bronchioles) terminating in small spherical, liquid-coated chambers [1], called alveoli, in which gas exchange occurs. The surface area in adult lungs is ~70 m^2, about half the area of a tennis court [2–5]. This enormous surface area maximizes the exchange of oxygen and carbon dioxide between air and blood, but an air–water interface of this size could contribute a significant drain on metabolic energy as the interface expands and contracts against surface tension. Nature has minimized this energy drain by coating the lung air–water interface with a thin film of lipids and proteins, collectively called lung surfactant (LS).

LS is composed of 90% lipids (primarily dipalmitoylphosphatidylcholine, DPPC) and 10% of four lung surfactant specific proteins (SP-A, B, C, and D) [2–9]. Lung surfactant, like other surface-active substances, adsorbs spontaneously to an air–water interface because doing so lowers the energy of the interface [10]. Lung surfactant continues to adsorb until the normal air–water surface tension, γ, of ~70 mN/m (dyne/cm) decreases to ~30 mN/m at equilibrium [11,12]; this equilibrium surface tension is similar for native and most clinical replacement surfactants [13]. In normal lungs, after secretion of LS in the form of multilamellar bodies from alveolar type II cells [14–16] surfactant must unpack, move across the alveolar hypophase, adsorb to the air–water interface, and then transform from bilayer to monolayer and spread over the interface [4]. Similarly, aqueous mixtures of surfactant, introduced into the airway of a patient with lung disease, must travel to the periphery of the lung, adsorb and spread to cover the air–liquid interface, despite the presence of any other surface-active materials present in the alveoli. For both normal and exogenous surfactant, adsorption through the liquid subphase is the primary route of surfactant accumulation at the interface.

Why is this reduction in equilibrium surface tension so important to breathing? Surface tension causes the pressure in an air bubble (P_{in}) of radius, R, (a simple model for an alveolus with radius R) within a confining liquid (P_{out}) to increase according to Laplace's equation: P_{in}–$P_{out} = \Delta P \approx 2\gamma/R$. Breathing is initiated by motion of the diaphragm, which induces a negative pressure (vacuum) on the outsides of the alveoli (P_{out}). During breathing, since the alveolus is connected to the outside air (at ambient pressure, P_{am}), the increase in pressure in the alveolus due to surface tension, $\Delta P \approx 2\gamma/R$, must be such that an overall negative pressure ($P_{am} - P_{in} > 0$) remains between the air inside and outside the body so that air flows into the lungs. Hence, surface tension requires that the diaphragm generate a lower pressure (greater vacuum) than would be necessary in the absence of surface tension; the lower γ, the less force (Force = Pressure differential × surface area of lung) must be developed by the motion of the diaphragm, and the less work (Work = Force × Distance) is necessary for breathing. If the diaphragm cannot generate the necessary vacuum, air no longer flows into the lungs; if too much work is required to generate this vacuum, little energy is left for anything else. Simply put, *the evolution of air-breathing required the co-evolution of lung surfactant* [17].

A second consideration necessitating lung surfactant is that at any given time during breathing, different alveoli will be in different states of inflation. This means different values of R and different Laplace pressures, $\Delta P \approx 2\gamma/R$, with the less inflated, smaller alveoli having the larger Laplace pressures. Hence, the smaller alveoli tend to get even smaller and eventually collapse, and their high-pressure gas contents flow to the larger alveoli with their smaller Laplace pressures [18]. The corresponding liquid layer thickens in the less inflated alveoli, because the corresponding Laplace pressure inside the liquid in the deflated alveoli is less than in the liquid lining more inflated alveoli. The net result is that the smallest alveoli can collapse and fill with liquid and become difficult to re-inflate. While part of the lung collapses, other parts are over-extended.

Lung surfactant solves this second problem by further reducing the surface tension as the air–epithelial fluid interface in the alveolus shrinks during expiration. Surfactant molecules are effectively insoluble in the alveolar liquids, which traps the surfactant at the interface (at least over the time scales relevant to breathing). The area available per surfactant molecule at the interface decreases along with the decrease in the alveolar interfacial area. As the interfacial density of the surfactant increases, the surfactant molecules bump into each other more and more, which induces a force opposing the surface tension of the liquid. This "surface pressure", Π ($\Pi = \gamma_w - \gamma$; γ_w is the surface tension of a clean air–water interface, 72 mN/m, and γ the measured surface tension) exerted by the surfactant acts to expand the interfacial area in opposition to the surface tension of the liquid–air interface, which acts to decrease the interfacial area. These opposing forces cause the net interfacial tension to decrease during compression of the interface; a good lung surfactant can lower this dynamic interfacial tension to near zero. The minimum dynamic interfacial tension is limited ultimately by the strength and cohesion of the monolayer film. Eventually, the monolayer "collapses" and the film folds, buckles, deforms, cracks, etc. into either the subphase or the air [19–25] (See Fig. 7). After this monolayer collapse, enough lung surfactant must remain at the interface (or re-adsorb to the interface) to respread and cover the expanding alveolar interface during inspiration to restore the equilibrium surface tension and the low dynamic surface tension.

A good lung surfactant therefore provides both a low equilibrium surface tension and an even lower dynamic interfacial tension which minimizes the work of breathing, stabilizes alveoli against atelectasis during expiration, prevents excess liquid from accumulating in the lung, and insures uniform inflation on inspiration [2–6,8,9,18]. Every air-breathing animal with lungs has some form of lung surfactant, often very similar in composition to human lung surfactant [17,26–29]. This is why replacement surfactants for diseases associated with surfactant deficiency or inhibition are harvested from cows (Survanta), calves (Infasurf) and pigs (Curosurf), the most common large mammals raised for food in the US and Europe.

Although essential to breathing, lung surfactant [2,30–33] and its importance in the development of neonatal respiratory distress syndrome (NRDS; known as hyaline membrane disease at that time) [8,34] was only begun to be appreciated in the late 1950s. In NRDS, the lack of functional surfactant results in a progressive failure of the lungs, which is manifested clinically by atelectasis, decreased lung compliance (stiff lungs that require a greater pressure differential to inflate), decreased functional residual capacity (a measure of the amount of air left in the lungs after exhalation), systemic hypoxia (oxygen starvation), and lung edema (bleeding in the lungs) [2–4,8,30–32,34,35]. Only since the 1980s have infants with NRDS been treated with replacement surfactants, which has significantly reduced neonatal mortality [9,36]. Surfactant-deficient infants typically have less than 5 mg/kg of LS in their lungs, while typical healthy newborns have approximately 100 mg/kg. In 2002, RDS affected an estimated 24,000 newborns in the US [9]. Surfactant replacement is an expensive therapy; but it is cost-effective relative to neonatal intensive care [37].

The first clinically approved replacement lung surfactant, Exosurf, was a synthetic mixture of dipalmitoylphosphatidylcholine (DPPC, the major lipid component of native LS), hexadecanol, and the non-ionic surfactant, tyloxapol. Although efficacious, Exosurf does not contain the lung surfactant specific proteins SP-B and SP-C or any synthetic replacement peptide or protein [2]. Survanta, Curosurf and Infasurf, currently the three most-used clinical surfactants in the US, are organic solvent extracts from minced cow (Survanta) or pig (Curosurf) lungs, or extracted with organic solvents from the aqueous lavage of calf lung (Infasurf) [3,4]. The compositions of all four clinically approved surfactants vary widely in lipid composition; there still is no generally accepted lipid composition for a replacement