How does pulmonary surfactant reduce surface tension to very low values?

Background: Although initially proposed by von Neergaard in 1929 (14), direct evidence for surface-active material at the air-fluid interface of the lung was first reported by Richard Pattle and John Clements in the 1950s (5, 10).

Pattle deduced that microbubbles formed from lung washings could reduce surface tension (γ) to near 0 mN/m. Using his modified Langmuir-Wilhelmy balance, Clements demonstrated that pulmonary extracts generated surface films that could be compressed to γs below 10 mN/m. These were (and remain) extraordinarily low values. Von Neergaard’s studies went virtually unnoticed, but Avery and Mead employed a Clements-type balance to demonstrate that infants succumbing to the respiratory distress syndrome lacked a lung substance present in infants dying of other causes (2). Not surprisingly, this discovery focused attention on the clinical significance of surfactant, resulting in the enormous scientific interest that surfactant research still enjoys today (7, 8, 12). However, as emphasized by the article of Yan et al. (16) in this issue of the Journal of Applied Physiology, despite being recognized for over 80 years and investigated for over 50, the mechanism by which pulmonary surfactant reduces γ at the air-liquid interface to <1 mN/m is still not understood.

The classical model for surfactant function: Pattle proposed that surfactant was a lipoprotein, and it was soon discovered that surfactant contained phospholipids (PL), particularly phosphatidylcholine (PC), and that dipalmitoylphosphatidylcholine (DPPC) was the major constituent (12). DPPC bilayers (e.g., liposomes) at atmospheric pressure undergo a gel to liquid-crystalline phase transition at 41°C. Below this temperature, (Tc), hydrated DPPC molecules are essentially motionless, although they may rotate occasionally. Above Tc, the fatty acyl chains become mobile (i.e., melt) and individual DPPC molecules can diffuse within the bilayer leaflet. Lateral compression of DPPC molecules at the air-water interface (i.e., monolayers) on a Langmuir-Wilhelmy balance leads to analogous but not identical phase transitions. Such monolayers can be compressed from the very dilute gaseous phase (>120 Å²/molecule) to the interacting liquid-expanded (LE) phase (~100 Å²/molecule) and then to the tightly packed tilted-condensed (TC) phase (~40 Å²/molecule). DPPC isotherms (γ vs. surface area at constant temperature) exhibit LE/TC phase coexistence plateaus. DPPC monolayers can be readily compressed to γs = 0 mN/m at either room temperature or 37°C and can remain at these low surface tensions for considerable periods of time. In contrast, 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) monolayers cannot normally be compressed to low γs at these temperatures, presumably because they are far above their bilayer Tc of ~6.5°C. (Note that Tc cannot be defined for a monolayer unless lateral compression is specified. This is because PL isotherms vary with temperature due to thermal agitation. See Refs. 11 and 12 for additional details.)

The classical model for surfactant function was formulated to explain how pulmonary surfactant, which normally contains only 30–40% DPPC/total PL, can achieve γs near zero during compression. According to the classical model, as this term is used by Hall’s group (11, 16), the alveolar surface is covered by a TC monolayer of almost pure DPPC. The mechanistic explanation for the classical model, known as the squeeze-out hypothesis, suggests that lateral compression of mixed PL monolayers results in the loss of the least stable PL components, thereby generating enrichment in the most stable component, DPPC (3, 6, 15). Extensive surface area reductions or multiple compressions would, at least theoretically, result in sufficient DPPC enrichment to allow reduction of γ to near zero.

The classical model and the accompanying squeeze-out hypothesis have dominated thinking in the surfactant field for over 30 years. Yan et al.'s studies (16) attempted to determine whether the classical model can apply to surfactant films in vitro and at the alveolar air-fluid interface. It should be mentioned that the squeeze-out hypothesis, as stated above, has been updated to include the surfactant-associated proteins and surfactant reservoirs. Surfactant proteins somehow connect the surface monolayer to underlying surfactant layers, which can respread when the films are expanded. Theoretically, this would allow unstable fluid PLs to leave the monolayer during exhalation (compression) and replenish the film through respreading during inspiration (expansion). Surfactant reservoirs can be formed during surfactant adsorption (film formation) as well as through compression (12). Such reservoirs have been demonstrated functionally (by γ studies), by autoradiography, and by atomic force microscopy (1, 13, 17).

Supercompressed monolayers: When monolayers of calf lung surfactant extract (CLESE) or POPC are compressed on a Langmuir trough γ is reduced to ~24 mN/m, where a collapse plateau ensues. This γ corresponds to equilibrium (γe) and to the equilibrium spreading pressure (πe = 46 mN/m). This latter value can be obtained (experimentally) by placing flakes or powders of dry or partially hydrated PLs on water above Tc. The surface pressure, π, is equal to γ0 (clean surface) minus the observed γ (thus at 37°C, πe = 70 – 24 = 46 mN/m). Because γ is normally derived as an equilibrium process, DPPC monolayers below γe (24 mN/m) are unstable by definition. However, such films are metastable and can remain so, kinetically, far longer than is required to stabilize our alveoli.

Hall’s group recently discovered unexpected behavior when spread monolayers of PCs such as POPC at 37°C are compressed rapidly with a captive bubble surfactometer. These films do not collapse at πe, but attain γs near 70 (γ = 0 mN/m). (Such rates have not yet been achieved with the Langmuir trough.) They propose that these films are compressed so quickly that they do not have time to collapse near πe, but instead they form an amorphous, noncrystalline phase perhaps similar to a glass. Once formed, these films remain stable when cycled below γc. The observation that monolayers of so-called fluid PL can support πs near 70 (γ = 0 mN/m) was astonishing.

As indicated by Yan et al. (16) there are only two apparent ways in which PL monolayers of surfactant PLs can attain the low γs (high πs) required to stabilize the alveoli (i.e., DPPC enrichment or supercompression). They therefore investigated the melting behavior of monolayers of supercompressed DPPC, POPC, and CLSE at different πs. This involved isobars...
(i.e., measuring surface area while heating the monolayers at constant $\pi$). They observed that heating DPPC monolayers resulted in an initial large increase in surface area, followed by a slow and then a rapid decline in surface area as the monolayers collapsed under the force of lateral compression. They defined the temperatures at which surface area is maximum as the “melting temperature.” This allows comparison of these values with those obtained with the other monolayer films and also with physiological measurements of pulmonary mechanics as the point at which collapse begins. They observed that POPC and CLSE monolayers do not show an initial large increase in surface area. The lack of an increase in this area (which with DPPC can be attributed to thermal expansion and LE formation within the crystalline TC monolayers) with CLSE monolayers shows these monolayers are not composed of DPPC-enriched TC. Furthermore, supercompressed CLSE melts at $\sim 10^\circ C$ lower than DPPC. Thus there is no indication of high DPPC content, as hypothesized by the classical model.

The failure to detect TC films on the air-water interface at low $\gamma$ is supported by other investigations. Fluorescence-probe studies demonstrated that PL from CLSE can be overcompressed to $\gamma = 2 \text{ mN/m} (\pi = 68 \text{ mN/m})$, whereas over 60% of the surface monolayer remains in the LE state (11). This implies that LE phase can be compressed to high $\pi$s where it is no longer fluid. Radioactive experiments failed to demonstrate DPPC enrichment during monolayer formation (18). It must, however, be stressed that overcompressed CLSE films also differ from overcompressed POPC films. Consequently, the actual nature of surfactant films at low $\gamma$s remains unknown. In addition to the above, the surface area reductions that occur during breathing or sighing are probably slower, in some animals at least, than those required to generate supercompressed films.

In conclusion, as with most good research, the findings of Yan et al. (16) raise a number of questions, including the following. 1) Do surfactant reservoirs contribute to film stability at low $\gamma$? 2) Does the presence of surfactant protein B and/or surfactant protein C stabilize monolayer LE phase, permitting such films to withstand high $\pi$s? 3) Do DPPC-rich domains have a crucial role in film stability? 4) Why do mammalian surfactants have such a wide range of DPPC concentration (i.e., 10–50%; Ref. 9)? 5) What is the role of cholesterol in surfactant biophysical function (4)?

These and other questions highlight our lack of understanding of the manner in which surfactant stabilizes our alveoli at low lung volumes in healthy lungs and how this stability is disrupted by pulmonary diseases. We submit that the nature of the surfactant monolayer and how it regulates $\gamma$ defines a unique, critical property of the lungs that should be of fundamental interest to all pulmonary clinicians and lung biologists.

We hope that the paper by Yan et al. (16) and this invited editorial will serve to stimulate further investigations on these important issues.

REFERENCES


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