Published in final edited form as:

Langmuir. 2015 December 8; 31(48): 13063–13067. doi:10.1021/acs.langmuir.5b03094.

The Equilibrium Spreading Tension of Pulmonary Surfactant

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Abstract

Monomolecular films at an air/water interface coexist at the equilibrium spreading tension (γ_e) with the bulk phase from which they form. For individual phospholipids, γ_e is single-valued, and separates conditions at which hydrated vesicles adsorb from tensions at which overcompressed monolayers collapse. With pulmonary surfactant, isotherms show that monolayers compressed on the surface of bubbles coexist with the three-dimensional collapsed phase over a range of surface tensions. γ_e therefore represents a range rather than a single value of surface tension. Between the upper and lower ends of this range, rates of collapse for spread and adsorbed films decrease substantially. Changes during adsorption across this narrow region of coexistence between the two- and three-dimensional structures at least partially explain how alveolar films of pulmonary surfactant become resistant to collapse.

Graphical Abstract

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Author Contributions

The manuscript was generated through contributions of both authors. Both authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Supporting Information

An additional experimental section provides a more detailed description of the experimental methods. Graphs show the compression isotherms that indicate the onset of collapse for the full set of experiments, and the reversible isotherm for CLSE during compression and expansion. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.5b03094.

INTRODUCTION

The equilibrium spreading tension (γ_e) is defined as the surface tension at which a two-dimensional monolayer at an air/water interface coexists with its three-dimensional bulk phase.¹ As required for a true equilibrium, γ_e is independent of the pathway by which the state is achieved. Hydrated phospholipids, for instance, form a bulk phase that is a smectic liquid crystal. Whether dispersed in aqueous media as multilamellar vesicles or collapsed from compressed monolayers,² phospholipids form stacks of bilayers. Overcompressed monolayers collapse to the same γ_e achieved by multilamellar vesicles that equilibrate to form adsorbed films.³

For individual phospholipids that form fluid structures, γ_e is single-valued. Collapse of compressed monolayers begins abruptly at a discontinuity in the compression-isotherm.³ Further compression converts components from the monolayer to the collapsed phase with minimal effect on surface tension. Adsorbing vesicles reach the same final surface tension.^{3,4} The two- and three-dimensional structures coexist at a unique surface tension just below 25 mN/m.

Compression-isotherms suggest that monolayers of pulmonary surfactant behave differently.² Pulmonary surfactant is the mixture of lipids and proteins that forms a thin film on the liquid that lines the alveolar air sacks.⁵ When compressed by the decreasing surface area during exhalation, the alveolar film is remarkably resistant to collapse at very low surface tensions.^{6,7} The structure of this metastable film and the process by which it forms have remained uncertain.⁷ As with individual fluid phospholipids, the compression-isotherm in vitro for surfactant monolayers features a prolonged plateau during which changes in surface area produce limited decreases in surface tension.² Microscopic studies confirm that the plateau corresponds to the collapse of the monolayer to form three-dimensional structures.² Unlike films of the individual phospholipids, however, the compression-isotherm enters the collapse-plateau gradually, with a progressive rather than discontinuous change in slope.² Unless this difference represents a kinetic phenomenon, the gradual change would indicate that the two- and three-dimensional phases coexist over a range of surface tensions. This coexistence raises the possibility of compositional and structural changes that might have functional consequences. The studies here, with films of extracted calf surfactant formed on the surface of bubbles, determined the range of surface tensions over which the collapsed phase and the monolayer coexist at equilibrium. Measurements then determined whether traversing the region of coexistence changes the functional characteristics of the films.

EXPERIMENTAL SECTION

Our studies used pulmonary surfactant from calves, purified by extraction into chloroform (calf lung surfactant extract, CLSE).⁸ To provide the complete set of neutral and phospholipids (N&PL), gel permeation column chromatography removed the surfactant proteins from CLSE.⁹ 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC), obtained from Avanti Polar Lipids (Alabaster, AL), was used without further characterization or purification.

Our experiments manipulated films on the surface of small bubbles floating in buffered electrolyte below an agarose dome (Figure 1). Measurements of the height and maximum diameter provided the surface tension and area.^{10,11} Films were formed either by depositing solutions in chloroform–methanol at the air/water interface, or by injecting concentrated aliquots of dispersed material below the surface. Adjustments in the volume of the subphase manipulated the size of the bubble, and allowed feedback to hold surface tension constant during isobaric experiments.¹² The Supporting Information provides a detailed description of these measurements.

RESULTS AND DISCUSSION

Fluid films of POPC, when compressed on the surface of bubbles, produced expected results. POPC forms monolayers in the disordered liquid-expanded phase that persists at ambient temperatures during compression to the onset of collapse.¹³ The isotherm demonstrated the discontinuous change in slope that characterizes the beginning of collapse for single-component fluid monolayers (Figure 2). The surface tension of the discontinuity at 23.8 ± 0.5 mN/m approximated the γ_e measured previously for fluid phosphatidylcholines.^{3,4}

At constant temperature, the Gibbs phase rule limits two-phase coexistence for films containing a single component to a unique surface tension.¹⁴ Nucleation of a collapsed phase can delay its initial formation,¹⁵ but thereafter, the equilibrated two- and three-dimensional structures should coexist at a single surface tension. With pulmonary surfactant, the presence of multiple constituents eliminates that constraint. Coexistence of the monolayer and bulk phase is allowed over a range of surface tensions. The complete set of surfactant lipids, however, deviated minimally from the behavior of POPC. The slope of the compression-isotherms for the neutral and phospholipids (N&PL), obtained from calf surfactant by removing the proteins, changed abruptly, if not discontinuously, at 24.9 ± 0.3 mN/m (Figure 2). The presence of the multiple constituents produced only a limited alteration in the onset of collapse.

The additional presence of the surfactant proteins produced a more substantial change. Extracted calf surfactant (calf lung surfactant extract, CLSE) contains the hydrophobic surfactant proteins, SP-B and SP-C, as well as the complete set of lipids in N&PL. For CLSE, the isotherms showed a more gradual flattening at the beginning of the collapse-plateau (Figure 2). In microscopic studies,² deviation of the compression-isotherm from its linear decrease in surface tension provides the earliest indication of collapse, directly preceding the appearance of three-dimensional structures. The change in slope occurred at 27.4 ± 1.0 mN/m for CLSE, significantly before reaching the collapse-plateau for N&PL and POPC. The more gradual change in slope suggested that the collapsed structures coexisted with the monolayer over a range of surface tensions. The isotherms for CLSE were reversible, following the same trace during compression and expansion (Supporting Information), consistent with equilibrium-behavior. Previous studies have produced divergent results concerning whether the hydrophobic surfactant proteins produce slower¹⁶ or faster⁹ collapse of monomolecular films. The results here provide no kinetic information,

but indicate that the proteins induce formation of the three-dimensional collapsed phase earlier during compression.

The hydrophobic surfactant proteins in CLSE allowed comparison of the surface tension reached at the end of adsorption with the range of tensions over which the spread monolayers collapse. The proteins greatly accelerate the adsorption of the lipids, which by themselves adsorb slowly and incompletely.¹⁷ Adsorption reduced surface tension to 23.6 ± 0.3 mN/m, well below the onset of collapse.

The surface tension at end-adsorption provided the lower boundary of the coexistence region. With progressive increases in surface concentration, produced either by added constituents or decreasing area, conversion of the monolayer to the bulk phase continues until the three-dimensional structure exists in excess. At the experimentally convenient mM concentrations, CLSE is several orders of magnitude above the critical micelle concentration for phospholipid,¹⁸ and present in excess. The surface tensions at the onset of collapse and the completion of adsorption therefore establish the limits of coexistence between the two- and three-dimensional structures. The surface tensions of this region represent the γ_c for pulmonary surfactant.

Over this region of coexistence, the behavior of the films changed. At ~ 27 mN/m, the compressibility of the monolayers, whether formed by spreading or adsorption, indicated formation of the collapsed phase (Figure 3, squares and triangles, respectively). These experiments obtained adsorbed monolayers by replacing the subphase and interrupting adsorption before surface tension reached the value at the onset of collapse. At the lower surface tensions achieved at the end of adsorption, the isotherms were again parallel for the different films, whether formed by spreading or adsorption, and whether adsorption was interrupted or proceeded to completion (Figure 3). Rather than extending the collapse-plateau, compression produced a progressively steeper slope to reach low surface tensions (Figure 3). During transition through the narrow region of coexistence, whether by compression or adsorption, the compressibility fell substantially, indicating a greatly reduced tendency to collapse.

Isobaric compressions, at constant surface tension, measure the kinetics of collapse directly. If a surface tension corresponds to a unique surface concentration, then the change in the area of a film held at constant surface tension indicates the gain or loss of interfacial constituents.¹⁹ Following a pulsed compression, monolayers formed by interrupted adsorption or spreading at high surface tensions, above the region of coexistence, collapsed quickly (Figure 4). Films that adsorbed to completion collapsed at rates that were slower by 2 orders of magnitude (Figure 4). The isobaric experiments confirm that transition through the coexistence of two- and three-dimensional structures induced the resistance to collapse of the alveolar films.

These experiments were performed at the ambient temperature of 22.5 °C. Pulmonary surfactant functions instead at physiological temperatures. The compression-isotherm at 37 °C included the gradual change in slope that extended over a range of surface tensions (Figure 5). Adsorption reached a final surface tension well below the onset of collapse

indicated by the change in slope (Figure 5). Between surface tensions before the onset of collapse and at the end of adsorption, isobaric measurements following a pulsed compression showed that collapse slowed significantly (Figure 6). The coexistence of two- and three-dimensional structures over a range of surface tensions and the stabilization of films during adsorption through this region occurred at 37° as well as 22.5 °C.

Two previously considered possibilities might explain how the functional transformation occurs during transit across γ_e . The first is the formation of a multilayer. Greater thickness might reduce the rate at which a film collapses. Whether the proteins promote adherence of collapsed structures or vesicles, the multilayered bulk phase formed in the region of coexistence would collapse more slowly.

The alternative possibility involves a compositional change. The classical model of pulmonary surfactant has long proposed that the alveolar film which resists collapse must have the highly ordered structure of the tilted-condensed (TC) phase.^{7,20} Dipalmitoylphosphatidylcholine (DPPC), which commonly represents ~35–40% of the surfactant phospholipids,²¹ forms the TC phase at physiological temperatures. Formation of a TC film from pulmonary surfactant, however, would require a substantial enrichment in DPPC. Microscopic studies indicate that the initial adsorbed monolayer, formed by fusion of surfactant vesicles with the interface,^{22,23} contains the full complement of surfactant constituents. In adsorbed and spread monolayers, the area of the coexisting two-dimensional phases is equivalent,²⁴ strongly suggesting equal compositions. In contrast, the TC phase that forms from monolayers of the surfactant phospholipids contains essentially pure DPPC.²⁵ A process that would produce the enrichment of DPPC necessary to form a TC film has remained elusive.

The γ_e that extends over a range of surface tensions offers a possible explanation. Systems with a single-valued γ_e allow only collapse or adsorption at any specific surface tension. For γ_e that extends over a range, adsorption continues at conditions that cause monolayers to collapse. Exchange of different constituents between the monolayer and the adjacent material, based on partitioning rather than differential kinetics of adsorption or collapse and promoted by the proteins, provides a possible mechanism for the compositional change necessary to form a TC film.

OUTLOOK

Regardless of the mechanism, the change in behavior across the range of γ_e provides insight into the formation of the functional surfactant film. Two kinds of structures can replicate the resistance to collapse of the alveolar film. Formation of each structure has seemed likely to require a nonphysiological process. The enrichment of DPPC necessary to form a TC film could occur by selective exclusion of other constituents.^{26,27} (The alternative, selective adsorption of DPPC,²⁴ is incompat-adsorbed monolayers).²⁴ In addition to the crystalline TC monolayers, fluid films become persistently metastable if supercompressed to low surface tensions.¹² Both the super-compressed fluid and crystalline structures would require initial compression, whether to exclude constituents or reach low surface tensions, before the adsorbed monolayers resist collapse. Physiological results suggest instead that the adsorbed

film is functional from the beginning of the first exhalation.^{28,29} Our results, that the surfactant film develops resistance to collapse during the final stage of adsorption across the range of γ_e , fit with the physiological observations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

These studies were supported by the National Institutes of Health (HL60914).

Dr. Edmund Egan of ONY, Inc. provided the extracted calf surfactant. Dr. Shankar Rananavare contributed helpful discussions. Hamed Khojini and Drs. Wenfei Yan and Samares Biswas generated unpublished results that led to these studies.

ABBREVIATIONS

A_{init}	area at the beginning of the compression
A_{27}	area when surface tension is 27 mN/m
γ_e	equilibrium spreading tension
CLSE	calf lung surfactant extract
DPPC	dipalmitoylphosphatidylcholine
N&PL	neutral and phospholipids
POPC	1-palmitoyl-2-oleoylphosphatidylcholine
SP-B	surfactant protein B
SP-C	surfactant protein C
TC	tilted-condensed

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Figure 1.

A bubble used to manipulate interfacial films, viewed along its horizontal axis. The $17\ \mu\text{L}$ bubble floats in buffered electrolyte below a ceiling formed by an agarose dome.

Calculations that find the solution to the Young–Laplace equation which best fits the interfacial profile,³⁰ or that use only the height and diameter,^{10,11} provide the area and surface tension, which in this case was $4.2\ \text{mN/m}$.

Figure 2.

Onset of collapse. Initial films were formed by depositing small aliquots of solutions in chloroform:methanol (1:1, v:v) on the surface of bubbles. Compressing the bubble at $0.2 \mu\text{L}/\text{min}$ changed area at an average fractional rate of $0.198 \pm 0.006 \text{ h}^{-1}$. Area is expressed relative to the value at 27 mN/m (A_{27}). The continuous traces give data from representative experiments to preserve features that can be lost during averaging. Supporting Information provides the full set of isotherms. Symbols give mean $\pm$ SD for $n = 4$ experiments. The gray line superimposed on the isotherm for CLSE demonstrates the beginning of curvature, which indicates the onset of collapse. The dashed horizontal line gives the surface tension achieved by CLSE at end-adsorption. For clarity of presentation, data for POPC and N&PL, plotted against the upper axis, are displaced horizontally relative to the lower axis for CLSE.

Figure 3.

Compression-isotherms for adsorbed films and spread monolayers of CLSE. The subphase contained 1 mM phospholipid for films adsorbed to completion, or 0.25 mM phospholipid for films formed by interrupted adsorption. Traces give data from representative experiments, with symbols indicating mean $\pm$ SD for $n = 4$ at 22.5 ± 0.4 °C. Area is expressed as the fraction of the value for the initial film (A_{init}), before beginning compression at an average fractional rate of $2.44 \pm 0.88 \text{ h}^{-1}$. The horizontal dashed line indicates the final surface tension reached during adsorption.

Figure 4.

Compression-isobars for CLSE at 22.5 °C. Films were formed by spreading, by interrupted adsorption to reach an initial surface tension of 32.5 ± 0.1 mN/m, or by allowing adsorption to reach completion. A pulsed compression then lowered surface tension to 22.4 mN/m, which was held constant using feedback (upper left axis, representative trace). Curves give the mean area (left lower axis) relative to the initial value after the pulsed compression, $\pm$ SD ($n = 4$) at selected times.

Figure 5.

Compression-isotherms at 37 °C. The turbidity of the subphase limited the concentration of phospholipid to 0.5 mM phospholipid. Continuous curves give results for representative experiments during area-compression at an average fractional rate of $3.72 \pm 0.34 \text{ h}^{-1}$. Symbols give the mean $\pm$ SD for $n = 4$ experiments. The horizontal dashed line indicates the surface tension reached at the end of adsorption.

Figure 6. Compression-isobars at 37 °C. Conditions for this figure and Figure 4 were equivalent except for the temperature and the surface tension, which was held here at 20.2 mN/m.