Metastability of a Supercompressed Fluid Monolayer

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ABSTRACT   Previous studies showed that monomolecular films of extracted calf surfactant collapse at the equilibrium spreading pressure during quasi-static compressions but become metastable at much higher surface pressures when compressed faster than a threshold rate. To determine the mechanism by which the films become metastable, we studied single-component films of 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC). Initial experiments confirmed similar metastability of POPC if compressed above a threshold rate. Measurements at different surface pressures then showed that rates of collapse, although initially increasing above the equilibrium spreading pressure, reached a sharply defined maximum and then slowed considerably. When heated, rapidly compressed films recovered their ability to collapse with no discontinuous change in area, arguing that the metastability does not reflect transition of the POPC film to a new phase. These observations indicate that in several respects, the supercompression of POPC monolayers resembles the supercooling of three-dimensional liquids toward a glass transition.

INTRODUCTION

Insoluble monolayers at an air-water interface have long provided simple two-dimensional systems for studying phase transitions (Gaines, 1966; McConnell, 1991; Knobler and Desai, 1992). At appropriate surface areas and surface pressures[1], phospholipid monolayers, for instance, form gas, liquid-expanded (LE), and condensed phases analogous to structures in three-dimensional materials (Gaines, 1966; Kaganer et al., 1999). An additional transition, however, known as collapse has no three-dimensional analog. The coexistence of a two-dimensional film with its three-dimensional bulk phase determines a maximal equilibrium surface pressure (π_e) that limits access to higher surface pressures (Gaines, 1966; Smith and Berg, 1980; De Keyser and Joos, 1984; Vollhardt and Retter, 1991). Monolayers spread at an air-water interface and compressed to smaller areas reach surface pressures above π_e only in nonequilibrium states, achieved when the rate of compression exceeds the rate at which the films can relax to equilibrium by collapse. For substances that are liquids in the bulk phase, collapse at π_e has been considered "essentially instantaneous" (Smith and Berg, 1980). For hydrated phospholipids, which form bulk liquid-crystals, the disordered LE phase collapses at rates that are finite but also rapid (Goerke and

Gonzales, 1981; Hawco et al., 1981). Only condensed phases have previously sustained surface pressures well above π_e in static films for more than transient intervals.

In the lungs, films at the air-water interface of the alveolar air spaces undergo compression during exhalation by the decreasing alveolar surface area, but avoid collapse. Pulmonary mechanics (Horie and Hildebrandt, 1971) and the contact angles of fluorocarbon droplets deposited in the peripheral alveoli (Schürch et al., 1976) both indicate that surface pressures reach nonequilibrium values well above π_e, and that those pressures are sustained in static lungs for at least tens of minutes. The classical model of pulmonary surfactant concludes from those observations that the functional film in the lung must occur in the condensed phase (Clements, 1977; Bangham et al., 1979). Dipalmitoyl phosphatidylcholine (DPPC), which constitutes 30–50% of pulmonary surfactant (Yu et al., 1983; Kahn et al., 1995; Postle et al., 2001), is the only component that at physiological temperatures can form the condensed phase below π_e. The model therefore contends that the film in situ has a composition that differs from the complete mixture of freshly secreted constituents, and that it must be sufficiently enriched in DPPC to form the condensed phase (Clements, 1977; Bangham et al., 1979).

Compressions in the lungs, however, occur at rates considerably faster than the slow speeds commonly used on Langmuir troughs to characterize films under quasi-equilibrium conditions. Recent experiments using captive bubbles, which allow variation of compression rates over a much wider range than standard surface balances, suggest that the speed of compression is a critical determinant of a film's ability to avoid collapse. Films of extracted surfactant from calf lungs (calf lung surfactant extract, CLSE) collapse at π_e during standard slow compressions, but reach much higher surface pressures when compressed faster than a threshold rate (Crane and Hall, 2001). Static films show minimal collapse for prolonged periods not only

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[1]Surface pressure, defined as the extent to which a film reduces the surface tension of a clean interface (Gaines, 1966), denotes the two-dimensional equivalent of pressure in three dimensions, or the force exerted by an interfacial film on its one-dimensional confining boundaries, with units of force/length.

at the highest surface pressures immediately after the rapid compression, but also after expansion to lower surface pressures. When expanded to π_e and then recompressed at the same slow rates that initially caused collapse, the films again reach higher surface pressure. The films become kinetically trapped by the rapid compression in a transformed structure that is metastable well above π_e.

Our previous studies considered the mechanism for transformation suggested by the classical model. Dimyristoyl phosphatidylcholine (DMPC) at 37°C demonstrated the same difference in behavior between fast and slow compressions (Crane and Hall, 2001). These results with a single-component film indicate that transformation does not result from a change in composition. They also show that transformation can be studied with systems that are much simpler than the multicomponent mixture of pulmonary surfactant. Here we have used a major constituent of pulmonary surfactant, 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC), that at ambient laboratory conditions is even farther than DMPC above the temperature at which formation of the condensed phase below π_e becomes impossible. We first confirm that the same phenomena that characterized transformation for CLSE also occur for POPC. We then determine properties of the highly compressed films and of the transition itself to test specific models drawn from three-dimensional materials of how transformation might occur.

MATERIALS AND METHODS

Materials

DPPC and POPC (purity >99%) were obtained from Avanti Polar Lipids (Alabaster, AL) and used without further characterization or purification. Chloroform and methanol, used as spreading solvent, were obtained from Honeywell, Burdick & Jackson (Muskegon, MI). Water was distilled and then filtered through Macropure, Ultrapure DI, and Organic Free Cartridges from Barnstead/Thermolyne (Dubuque, IA). N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid (HEPES) (GibcoBRL brand, Life Technologies, Grand Island, NY), CaCl$_2$·2H$_2$0 (J. T. Baker, Inc., Phillipsburg, NJ), and NaCl (Mallinckrodt Specialty Chemicals, Paris, KY) were purchased commercially and used without further analysis. Buffer used to suspend the bubble was filtered through 0.45 μm micropore filters (Millipore, Bedford, MA) to remove particulate contaminants.

Methods

These studies used a ''surface balance'' (Kwok et al., 1996) consisting of a captive bubble floating in an aqueous subphase below an agarose dome (Putz et al., 1994; Crane et al., 1999). The captive bubble offers several advantages for the studies conducted here. In addition to better control of atmospheric humidity and temperature, the bubble provides a much wider range of compression rates. The continuous interface removes the film from contaminants in the ambient environment (Klopfer and Vanderlick, 1996) and also eliminates the possibility of escape by the film along or around any artificial confining barriers (Goerke and Gonzales, 1981). The films are spread in organic solvent at the air-water interface, and the spreading solvent is then removed by exhaustive exchange of the subphase while the film is held at a constant surface pressure of ~40 mN/m. The films are then compressed and expanded by varying the hydrostatic pressure applied to the subphase. Measurements of the bubble's height and diameter by CCD camera allow calculation of surface pressure and surface area (Malcolm and Elliott, 1980; Schoel et al., 1994). Surface pressure-surface area (π-A) isotherms for films compressed on the captive bubble closely reproduce curves obtained on Langmuir troughs (Heikkila et al., 1970; Albrecht et al., 1978) for DPPC (Crane et al., 1999) and palmitic acid (data not shown).

Our experiments used small volumes (0.08 μl) of phospholipid solutions in chloroform:methanol (1:1, v/v) spread on air bubbles (100–120 μl) floating in 150 mM NaCl, 1.5 mM CaCl$_2$, 10 mM HEPES, pH 7.0 (HSC). CCD cameras recorded the profiles of the bubble in the vertical axis to ensure axisymmetric shape and in the horizontal axis to measure the height (h) and diameter (d) from images captured via framegrabber (National Instruments, Austin, TX) to computer. Programs constructed in LabVIEW (National Instruments) calculated surface pressure, surface area, and volume from h and d (Schoel et al., 1994), and manipulated the bubble via a motion controller (MicroLYNX4, Intelligent Motion Systems, Marlborough, CT) to regulate a stepper-motor–driven syringe pump (MBP 2000, Advanced Liquid Handling, Milwaukee, WI) to infuse and withdraw buffer from the chamber (Crane et al., 1999). During initial rapid compressions of predetermined duration, data were obtained from each frame at the 30 Hz rate of the CCD camera. During subsequent manipulations, which often used simple feedback to maintain experimental variables such as surface pressure at constant values, the program recorded measurements averaged over several frames. Temperature was monitored with a thermistor probe (YSI, Yellow Springs, OH) and manipulated by a temperature-controller (Cole-Palmer, Vernon Hills, IL) and heating pads (Minco, Minneapolis, MN) applied along the sides of the cuvette.

Isothermal compressions through a range of surface pressures used infusions from the syringe pump at constant rates. Although volume changed linearly, area varied at a rate that depended on the nonlinear relationship between area and volume. This relationship depends on the shape of the bubble, which in turn varies with surface pressure. The rate of area-compression therefore was determined in part by the performance of the film. Bubbles with monolayers that collapse at π_e maintained a constant surface pressure and a relatively similar shape. For the same change in volume, their rate of area-compression was faster than with films that avoided collapse and reached high surface pressures, at which the bubbles became dramatically flattened with surface area relatively well preserved. Rates of area-compression, reported here as fractional rates, or rates of strain, $(dA/A)/dt = d(\ln A)/dt$, were obtained during the initial change in area where collapse of fluid films was least, so that with different films, the same rate of volume-compression generated the most reproducible change in area.

RESULTS

Experiments with POPC demonstrated the same essential features that previously defined transformation for films of CLSE. When compressed on bubbles at 0.03 min^{-1} (1.2 Å^2/ (molecule·min); see Methods) at 26°C, comparable to rates commonly used to produce quasi-equilibrium conditions on Langmuir troughs, π-A isotherms for POPC displayed a plateau at 47 mN/m of nearly constant surface pressure (Fig. 1). This value is just above π_e, established as 46.8 ± 0.2 mN/m by allowing surface pressure to equilibrate at constant area after compression to the onset of the plateau. Microscopic studies on Langmuir troughs with similar systems demonstrate the appearance and growth of three-dimensional structures (Schief et al., 2003), confirming that the plateau results from collapse. At rates three orders of magnitude faster, however, surface pressure increased to 68 mN/m (Fig. 1). Subsequent manipulation of the films to
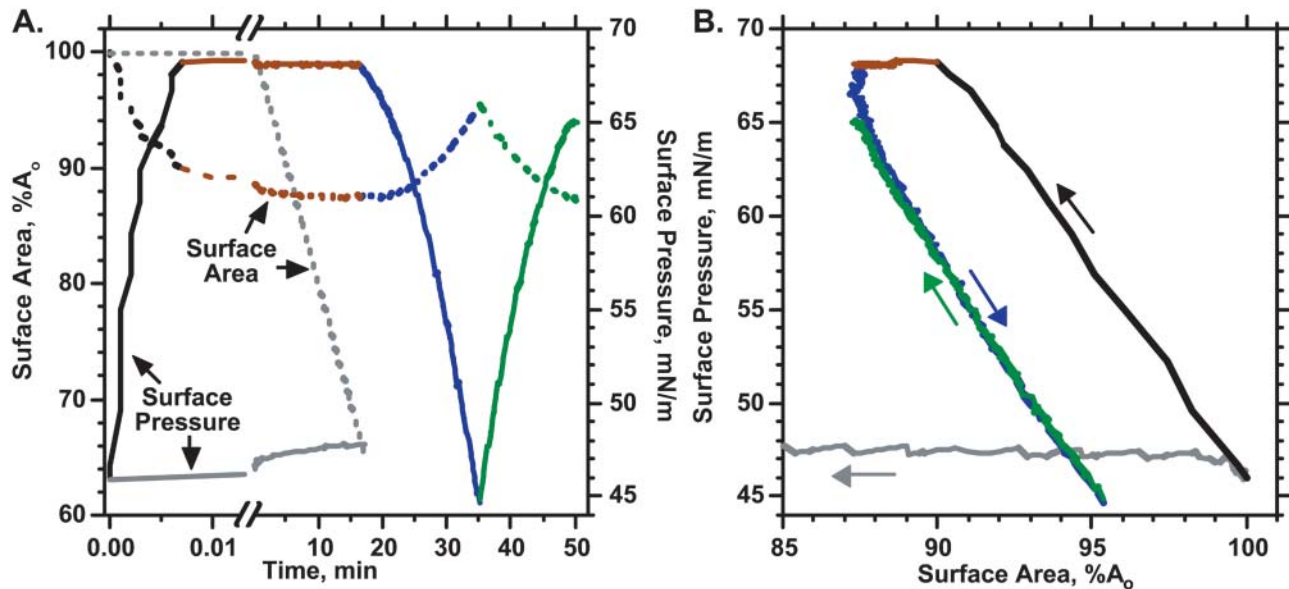
FIGURE 1 Transformation of POPC monolayers by rapid compression. Films spread to initial surface pressures <42 mN/m were compressed at 26°C and 0.005 min^{-1} to 46 mN/m, just below π_e. The bubble was then manipulated by infusion of buffer at a constant rate of either 1.4% initial volume/min (*gray lines*; initial area compression of 0.01 min^{-1}) for 17 min, or 109% initial volume/s (*black lines*; area compression of 23.1 min^{-1}) until surface pressure reached 68 mN/m. Surface pressure of the rapidly compressed film was held constant for 15 min (*red lines*), after which it was expanded to lower surface pressure at 2.5% initial volume/min (*blue lines*), and then recompressed at the same rate (*green lines*). (*A*) Variation of surface pressure (*solid lines*, right axis) and area (*dashed lines*, left axis) with time. Surface area is expressed relative to the initial area at 46 mN/m (A_o). Split timescale shows that the more rapid compression lasted ~0.4 seconds. (*B*) The same measurements plotted as surface pressure versus surface area.

maintain constant surface pressure required as little as a 2.7% further reduction in surface area over a period of two hours (6.1 ± 2.4%, mean ± SD, for 11 experiments; Fig. 1 *A*). (For comparison, films of DPPC in the condensed phase at 37°C fell by 6.1 ± 2.9% during isobaric compression at 66 mN/m over the same two-hour period.) The rapidly compressed films resisted collapse not only at the very high surface pressures achieved at the end of compression, but also at lower pressures after slow expansion (Fig. 1). Although the films recovered their original behavior when expanded to sufficiently low surface pressures (data not shown), if returned only to their initial conditions just below π_e, slow recompression produced no evidence of collapse (Fig. 1 *B*). The initial fast compression to high surface pressure therefore transformed the film from a structure that collapsed readily at π_e to a kinetically trapped metastable form that sustained much higher surface pressures for prolonged periods.

The dependence on the rate of compression demonstrated previously for CLSE (Crane and Hall, 2001) also occurred for POPC (Fig. 2). Increasing the rate of compression at 26°C from 0.03 to 0.12 min^{-1} produced little difference in the behavior of the film. Surface pressure increased only to 49.5 ± 0.2 mN/m, and fell promptly as soon as compression stopped (data not shown). With a slightly faster rate of 0.19 min^{-1}, however, surface pressure reached 66.7 ± 1.5 mN/m. Even faster compression simply reduced the change in area required to achieve these high surface pressures (Fig. 2).
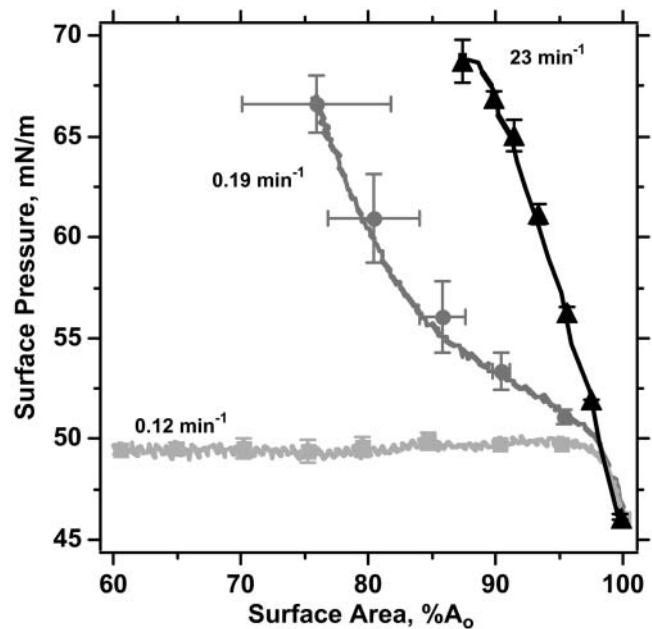

FIGURE 2 Compression at different rates. Monolayers of POPC spread to surface pressures <42 mN/m were first compressed at 26°C and 0.03 min^{-1} to 46 mN/m, and then to higher surface pressures at the indicated rates of area-compression expressed as $d(\ln A)/dt$ (see Methods). Area is normalized relative to the initial area at 46 mN/m (A_o). Curves give data from representative experiments. Symbols give mean ± SD at selected points averaged at common volumes for both area and surface pressures in three experiments.
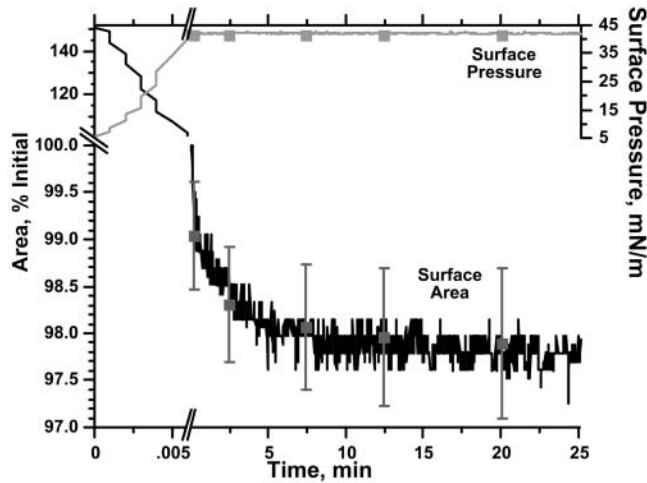
FIGURE 3 Relaxation after a rapid change in surface pressure below π_e. Films of POPC spread to 5 ± 4 mN/m were compressed at 74 ± 6 min^{-1} to 41 ± 1 mN/m and then held at constant surface pressures. Area is expressed relative to the initial value when the film first reached its isobaric surface pressure. The split time and area scales distinguish the initial rapid compression from the subsequent isobaric relaxation. The curve shown is representative of four experiments. Symbols indicate mean $\pm$ SD at selected times.

Rather than changing continuously over a broad range of compression speeds, the behavior of the monolayers changed

at a discrete rate representing a threshold for reaching high surface pressures.

A threshold rate of compression has specific implications concerning the behavior of the film. For any film above π_e, surface pressure changes according to the relative rates of relaxation, either by rearrangement within the interface or by collapse, and compression. Rearrangements in POPC were limited. After pulsed changes of 30 mN/m at surface pressures below π_e, where collapse cannot occur, area fell during isobaric compression by only $2.1 \pm 0.8\%$ (Fig. 3). The contribution of rearrangements to relaxation above π_e was therefore also assumed to be small. To reach high surface pressures, the rate of compression must then exceed the rate of collapse. If the thermodynamic drive alone determined the rate of collapse, then the transition would proceed faster at surface pressures progressively further above π_e (Rapp and Gruler, 1990). Compressions at different rates would produce π-A isotherms with an initial increase in surface pressures to the point at which compression and collapse occur at the same rate, followed by an isobaric plateau. A threshold rate, at which a relatively small change in the speed of compression abruptly increases the final surface pressure, implies that collapse, rather than increasing inexorably at surface pressures farther above π_e, reaches a limiting value.
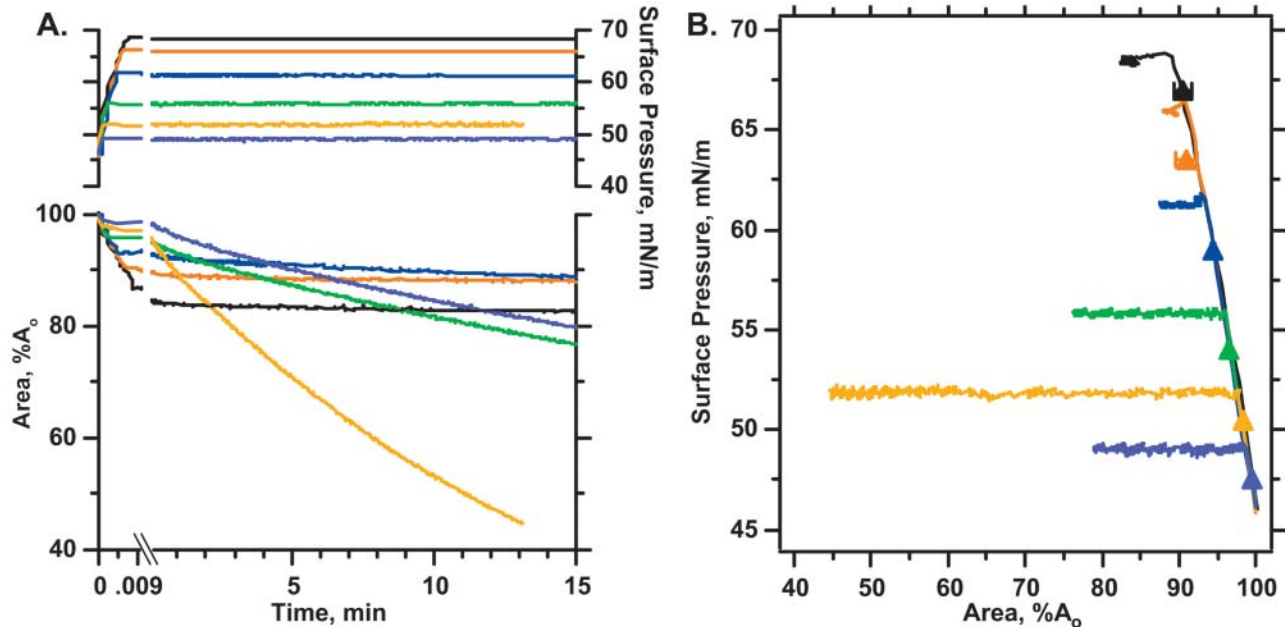

FIGURE 4 Rapid initial compression followed immediately by isobaric compression. Monolayers of POPC spread to <42 mN/m were first compressed at 26°C and <0.005 min^{-1} to 46 mN/m and then at 23 ± 5 min^{-1} to different target surface pressures. Isobaric compression then maintained the final surface pressure achieved at the end of the rapid compression. Experiments lasted at least 15 min or until hydrostatic pressure exceeded the limits of the apparatus (3.5 atm). (A) Variation of surface area (lower left axis) and surface pressure (upper right axis) with time. Area is normalized to the initial value at 46 mN/m (A_o). (B) Surface pressure-surface area isotherms. Curves of the same color in Fig. 4, A and B, indicate presentations of the same data. For purposes of clarity, results are presented for individual experiments. Symbols in Fig. 4 B give mean area $\pm$ SD at specific surface pressures during the rapid compression for at least five experiments in each case. Error bars that are not evident are less than the size of the symbols. Error of the measurements during the isobaric compressions is given in Fig. 5.
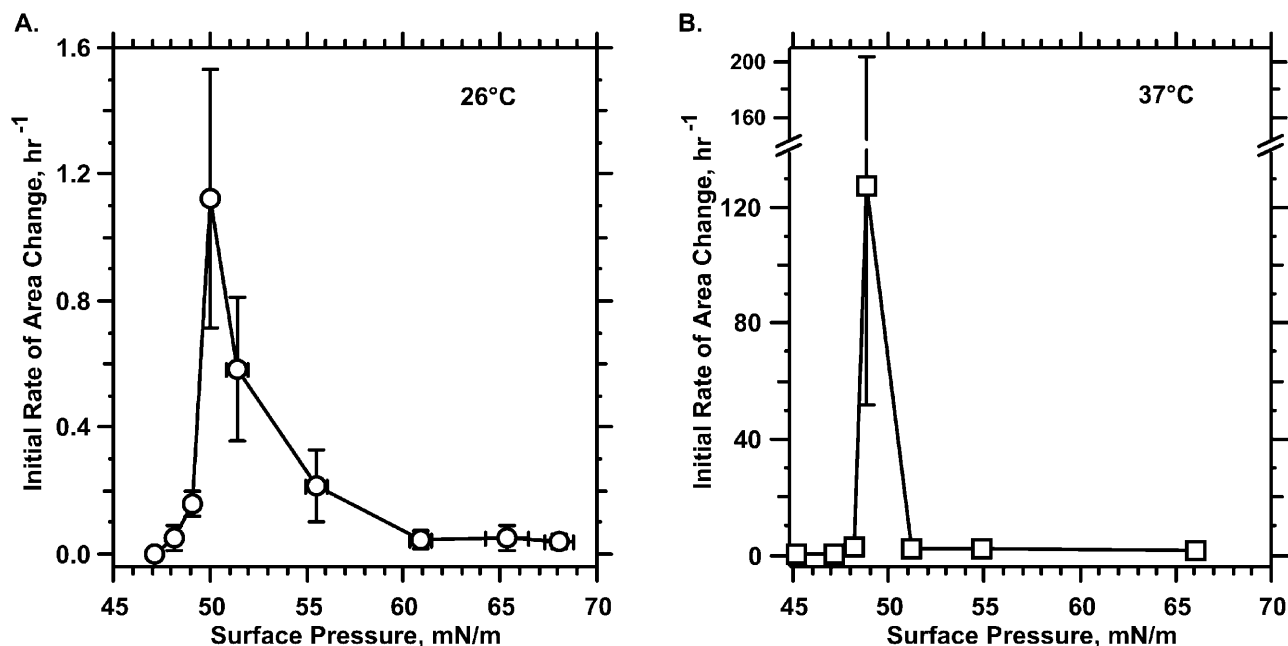
FIGURE 5   Rate of initial collapse at different surface pressures. Rates of collapse were obtained from the slope of the least squares linear fit of ln A versus time between 20 s and 1 min during isobaric compression after the initial pulsed compression. Symbols give mean $\pm$ SD of the collapse rate and of the measured surface pressure for each target surface pressure for at least five experiments. (*A*) 26°C; (*B*) 37°C.

To determine how the rate of collapse varied at different surface pressures, we compressed the films at 23 $\pm$ 5 min^{-1} and 26°C along a common isotherm to different target surface pressures (Fig. 4). Isobaric compression then maintained the final surface pressure achieved at the end of the pulsed compression (Fig. 4). Relaxations within the interface were ignored, and the decrease in area was accepted as equal to the area occupied by constituents leaving the monolayer. The rate of isobaric compression was then used to measure the rate of collapse.
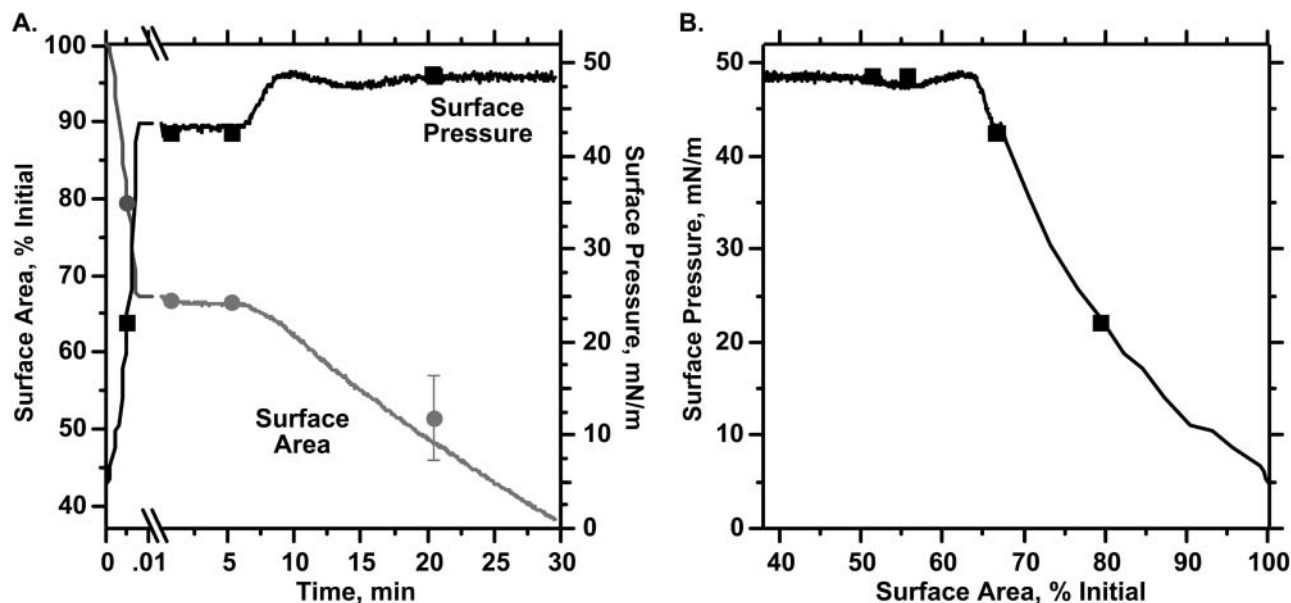

FIGURE 6   Collapse of films after rapid compression below π_{e}. POPC monolayers were compressed at 54 $\pm$ 6 min^{-1} from 5.2 $\pm$ 0.1 mN/m to 42.5 $\pm$ 3.1 mN/m. The films were then held at constant surface pressure for roughly 6 min, comparable to the standard manipulation of films compressed to high surface pressures (Fig. 1), before undergoing further compression at 0.024 $\pm$ 0.002 min^{-1}. Area is expressed relative to the initial area of the spread film before compression. Curves are representative of four experiments. Symbols indicate mean $\pm$ SD at selected points, with error bars less than the size of the symbols in some instances. (*A*) Variation of surface pressure and surface area with time. (*B*) The same data expressed as surface pressure versus surface area.
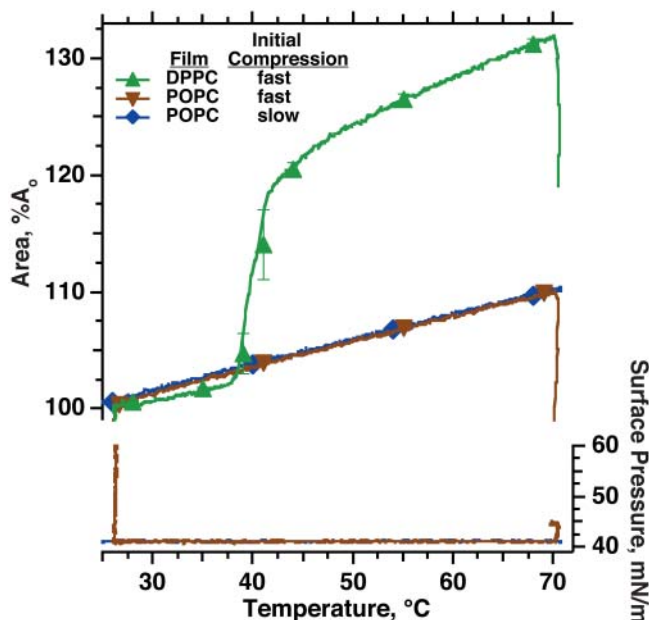
FIGURE 7 Heating isobars for DPPC and POPC. Films containing each compound were spread initially at ~23°C to <40 mN/m, heated to 26°C, and then subjected either to only a "slow" compression (<0.008 min^{-1}) to 46 mN/m, or, after reaching that surface pressure, to a "fast" compression (19 min^{-1} for DPPC and 30 min^{-1} for POPC) that reached 68 mN/m. Both rapidly and slowly compressed films were then expanded slowly (0.005 min^{-1}) to 41 mN/m, and then heated isobarically at 1.0°C/min to 70°C. The film's ability to sustain surface pressures above 50 mN/m was tested by slow compression before and after heating. Surface area (left axis) was expressed relative to the initial value (A_o) at 26°C and 41 mN/m. The isobars for rapidly compressed DPPC in these studies are comparable to curves for films compressed only at standard slow rates (Crane et al., 1999). Each curve is representative of three experiments. Symbols at selected temperatures indicate mean ± SD to provide the magnitude of variation. Error bars that are not evident are less than the size of the symbols.

The rate of collapse decreased with time (Fig. 4 A). At all surface pressures, the isobaric compression required to maintain the target surface pressure for most experiments during the 15-min measurements became progressively slower. In a few experiments at 50 mN/m, where the rate of collapse was fastest and the results most variable, collapse first slowed and then accelerated. For all other experiments, in which the extent of collapse during the 15-min observation ranged from 1% to 60% of the interface, collapse slowed continuously.

The rate of collapse varied dramatically at different surface pressures (Fig. 5 A). Immediately above π_e, the initial rate of collapse, measured during the first minute after the rapid compression, increased steeply. At 50 mN/m, however, collapse reached a maximal rate and then declined. From its peak value of 1.12 ± 0.41 h^{-1}, collapse slowed to 0.037 ± 0.022 h^{-1} at 68 mN/m (Fig. 5 A). The limiting rate of collapse predicted by the existence of a threshold compression rate therefore occurred at a sharply defined maximum.

Because of the physiological relevance of these studies for pulmonary surfactant, we also measured the dependence of collapse on surface pressure at 37°C (Fig. 5 B). At the higher temperature, collapse became detectable at surface pressures 1 mN/m lower than at 26°C. The surface pressure of the maximum rate of collapse was shifted by a similar amount to 49 mN/m. The maximum rate of 127 ± 76 h^{-1} was two orders of magnitude greater, approaching the limits of our ability to maintain isobaric conditions. The peak was also considerably sharper at the higher temperature. Increasing surface pressure above π_e, however, produced the same initial increase in collapse to a maximum and subsequent decrease to much lower rates with further deviation from equilibrium conditions. The basic behavior observed at 26°C therefore also occurred at the physiological temperature.

The metastable films could result either from the rapid, pulsed compression or from the high surface pressures achieved. To distinguish between these possibilities, films were compressed rapidly through the same difference in surface pressures but to values below π_e (Fig. 6). During subsequent slow continuous compression, isotherms reached only 48 mN/m before forming a collapse plateau. These results indicate that the rate of compression could not itself produce the metastability, and suggest that the surface pressure reached determined the film's behavior.

Previous studies have demonstrated metastable behavior above π_e only for films in the condensed phase. To determine if the rapidly compressed films of POPC had reached conditions, normally inaccessible because of collapse, at which they formed the condensed phase, we conducted melting experiments. A phase transition produced by isothermally increasing surface pressure can often be reversed during isobaric heating. When held at constant surface pressure, the condensed phase of DPPC, for instance, melts to the LE phase with an abrupt expansion over a narrow range of temperatures (Crane et al., 1999; and Fig. 7). At the high surface pressures at which the POPC films become metastable, the slow loss of area caused by collapse would complicate efforts to detect expansion of a film at a first-order phase transition. The persistence of the transformed state at lower surface pressures, however, where the films remained resistant to collapse during recompression, allowed the measurements to be performed below π_e where collapse does not occur.

During heating, transformed films returned substantially to their original behavior. After rapid compression (30 min^{-1}) at 26°C to >68 mN/m, the films of POPC were slowly (0.005 min^{-1}) expanded to 41 mN/m (Fig. 7). Slow (0.005 min^{-1}) recompression first confirmed that the films remained resistant to collapse above π_e. After returning to 41 mN/m, the films were heated isobarically at 1°C/min from 26°C to 70°C. During slow recompressions (0.005 min^{-1}) at 70°C, the films reached a maximum surface pressure of 44.8 ± 0.3 mN/m, only slightly higher than values of 42.1 ± 0.1 mN/m for films that were heated to the same temperature

without first undergoing the rapid compression. In control experiments, rapidly compressed films of POPC were expanded to 41 mN/m and then incubated at 26°C for an hour, longer than the total interval required for heating the experimental films. The ability to reach high surface pressures during slow compressions persisted (data not shown), confirming that the altered behavior of the heated films resulted from the change in temperature rather than the incubation at the lower surface pressure.

For the heated film, the substantial reversion of the transformed film to the original behavior occurred without any discontinuous change in area or in the slope of the heating isobar. In contrast to condensed DPPC, POPC melted with only a linear increase in area over the entire range of temperatures (Fig. 7). The heating isobar for the transformed monolayers remained virtually superimposable on the curve for films that had experienced no rapid compression (Fig. 7). Return to the initial state without evidence for a phase transition, either first or second order, argues that transformation represents some process other than formation of a condensed phase.

DISCUSSION

Previous studies showed that films of CLSE become transformed if compressed above a threshold rate. Films that collapse at π_e during slow compressions become metastable at much higher surface pressures. That metastability and resistance to collapse persist during subsequent expansion to π_e. The same transformation occurs for LE films containing the disaturated phosphatidylcholine DMPC. Our results here confirm that the phenomenon extends at least to POPC, a phosphatidylcholine with unsaturated acyl groups that represents the third most prevalent constituent of calf surfactant (Kahn et al., 1995).

Our prior studies left unresolved what particular aspect of the rapid compression is essential for transformation. Of the several factors considered previously for CLSE, experiments excluded only the possibility that transformation resulted from a change in composition, which is impossible for single-component films containing only DMPC. Our results left uncertain whether the critical factor in producing the metastable films was the speed of the pulsed compression, the surface pressure achieved, or the degree of collapse that occurred at the end of the compressions when the films reached very high surface pressures. Our findings here resolve that issue. Films compressed at a common rate faster than the threshold level necessary to achieve high surface pressures continue to collapse rapidly if they stop short of high surface pressures. Compressions that are more controlled than in our original experiments, and that are halted before reaching the very high surface pressures and low surface tensions at which any film becomes unstable, show that transformation requires no final collapse. Our

results indicate that the extent of metastability is determined by the surface pressures to which the films are compressed.

The most obvious mechanism by which transformation might occur is a first order phase transition, either to a condensed phase or to some other tightly packed equilibrium structure such as the equivalent in monolayers of the subgel phase observed in bilayers (Katsaras et al., 1995). Such a transition would occur at a surface pressure above π_e, which would be inaccessible during slow compressions because of collapse and achieved only when the films are compressed fast enough to reach the appropriate conditions. A first order transition would involve a change to a structure with a lower molecular area. Because of the restrictions of the Gibbs phase rule for one-component systems, the change in area would occur discontinuously at a single surface pressure during isothermal compression or at a single temperature during isobaric heating. No discontinuous change occurs in either the compression isotherm or the heating isobar.

The absence of these features might in each case result from slow kinetics. The very rapid compression required to reach the high surface pressures at which transformation occurs could obscure any isobaric plateau in the compression isotherm. The slow kinetics with which the films relax to their initial state could extend the change in area over a broad range of temperatures and hide the expected isothermal expansion. During heating, however, over an interval in which the transformed film returns substantially to its original behavior, the change in area for the transformed and LE films is the same. The metastable film melts with no increase in area beyond the thermal expansion of the LE phase. This result indicates that any change in molecular area or thermal expansion coefficient is undetectably small, and effectively excludes the possibility that transformation results from a standard phase transition.

The supercompressed POPC films behave in a number of respects like three-dimensional liquids supercooled toward a glass transition. If supercooled to a sufficient extent, liquids form amorphous solids, or glasses, in which they retain their disordered structure but acquire the immeasurably high viscosity that defines a solid (Debenedetti, 1996; Ediger et al., 1996). We lack direct information to confirm the essential structural and rheological elements of the model. Our indirect evidence, however, fits well. The melting isobars for POPC suggest that like supercooled liquids, the markedly altered behavior of the supercompressed films occurs without a major structural change. Concerning the film's ability to flow, the rate of collapse provides a useful indication. Phospholipid monolayers collapse by flow of a continuous lamella into a liquid-crystalline stacked structure (Schief et al., 2003). The rate of collapse at any particular extent of supercompression above π_e then provides an indication of the viscosity of flow into the third dimension (Rapp and Gruler, 1990). Immediately above π_e, the rate of collapse indicates that the film flows readily. The marked slowing of collapse at surface pressures well above π_e suggests a greatly

increased viscosity. Like the supercooled liquids, super-compression changes the LE films from a state that readily flows to one with a much higher viscosity, while apparently retaining their original disordered structure.

In addition to the characteristics of the films themselves, several features of the transition by which transformation occurs during supercompression resemble the supercooling of liquids. Transformation of the monolayers involves a kinetic transition to a metastable state, analogous to the kinetic formation of metastable liquids. Both systems must avoid a standard phase transition—crystallization for the supercooled liquids, and collapse for the supercompressed monolayers—to achieve the metastable state. Bypassing the transition requires that conditions change faster than a threshold rate determined by the maximum rate of the transition. For three-dimensional liquids at temperatures progressively farther below the freezing point, the increasing thermodynamic drive becomes dominated by the rising viscosity, producing a rate of crystallization that first increases but then passes through a maximum and slows (Uhlmann, 1972). The rate at which the fluid monolayers collapse shows a similar profile above π_e. Transformation to the metastable state then requires that liquids cool fast enough to reach the low temperatures at which crystallization slows, and that compression of fluid monolayers outruns collapse to reach high surface pressures.

The apparent correspondence of the effects achieved by supercooling and supercompression fits with the paradigm of jamming (Liu and Nagel, 1998). According to that theory, granular matter has the general characteristic that it can be trapped, whether driven by applied forces or a reduction in temperature, in rigid but disordered structures. Under appropriate conditions, systems as diverse as sand piles, colloids, and supercooled liquids form structures that resist flow when sheared below a yield stress (Liu and Nagel, 1998). A jammed state in two dimensions, however, is unexpected. Analog (Quickenden and Tan, 1974) and digital (Mason, 1976) simulations indicate that two-dimensional arrays of hard disks or spheres have a high tendency to crystallize, making it difficult to form amorphous solids (Speedy, 1994). We speculate that the thickness of the phospholipid monolayers, although small relative to dimensions within the interfacial plane, may be sufficient to avoid the constraints of a strictly two-dimensional system.

A second characteristic that is unusual at least for a supercooled state is the persistence of the altered behavior when returned to the thermodynamic conditions at which they originally flowed. Liquids supercooled beyond the glass transition commonly crystallize during heating well before reaching the freezing point. The elongated shape of the phospholipid molecules and the extensive intermolecular contacts in the highly compressed state may contribute to the slow response to the altered conditions.

Irrespective of the variation of collapse with surface pressure, its temporal course at any single surface pressure is also unusual. The rate of most phase transitions accelerates with time. Once a nucleus of the new phase exceeds a critical size, movement of constituents across the interfacial boundary commonly increases any one dimension at a constant rate (Christian, 1965). Consequently at constant thermodynamic conditions, the total amount of the new phase, whether two- or three-dimensional, grows at an increasing rate. This characteristic behavior describes the collapsed phase formed from monolayers that in the bulk phase are liquids (Smith and Berg, 1980; Vollhardt and Retter, 1991). Films of POPC, however, collapse at a rate that slows.

Two processes that could contribute to this behavior are rearrangements within the monolayer and friction. We have ignored rearrangements because of their limited effect on films below π_e. Their contribution to the post-compression change in area could be greater at higher surface pressures. Rearrangements presumably begin with the initial perturbation, and at lower surface pressures, where viscosity is lower, the more rapid rearrangements might be largely completed before compression ends. If the rearrangements were delayed at higher surface pressures until after the compression, they would produce a change in area that would slow with time. Rearrangements, however, seem unlikely to explain effects of the magnitude observed here, with changes that reach 60% of initial area. The difference from the standard accelerating rates that have been observed previously with materials which form bulk liquids seems more likely to reflect the different mechanisms of collapse. Rather than growing by movement of constituents across an interface, liquid-crystalline collapse occurs by flow of continuous lamella into a bilayer that slides along the monolayer (de Mul and Mann, 1994; Friedenberg et al., 1994; Fang et al., 1997; Schief et al., 2003). Friction between the bilayer and monolayer, which should increase with the extent of collapse, may explain the progressively slowing rate.

The behavior of the films after reaching high surface pressures is particularly significant for pulmonary physiology. The widely accepted classical view of surfactant function explains the metastability of surfactant films in situ in terms of a film in the condensed phase. DPPC is the only constituent of pulmonary surfactant that can form the condensed phase in single-component films at physiological temperatures. The model therefore contends that, whether by selective adsorption of DPPC (Schürch et al., 1995) or selective exclusion above π_e of other constituents (Watkins, 1968; Clements, 1977; Bangham et al., 1979), the functional film is sufficiently enriched in DPPC to form the condensed phase (Hildebran et al., 1979). This theory is based primarily on the assumption that LE films must be unstable above π_e, and that only films in the condensed phase can demonstrate the metastability of films in the lungs. Our current results with POPC, a major constituent of pulmonary surfactant, contradict that fundamental assumption.

Although our results do not disprove the classical model, they do suggest an alternative mechanism by which films in

the lungs might become metastable. Monolayers of extracted CLSE and POPC demonstrate the same essential features of transformation. The films of calf surfactant collapse at π_e when compressed slowly, but sustain surface pressures well above π_e after compression faster than a threshold rate that lies within the physiological range (Crane and Hall, 2001). The similar behavior of calf surfactant and POPC suggest that they become metastable by similar mechanisms. The transformation would result not from a change in composition, which is impossible for single-component films containing only POPC, but from the attainment of surface pressures at which fluid monolayers become jammed into solid but amorphous structures.

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