Coexistence of Buckled and Flat Monolayers

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(Received 20 February 1998)

The minimum surface tension and respreadability of a surfactant monolayer is limited by a two to three dimensional instability called collapse. Liquid-condensed or solid phase monolayers collapse via fracture followed by loss of material. Liquid-expanded phase monolayers collapse by solubilization into the subphase. Monolayers that retain a continuous liquid-expanded phase network surrounding islands of liquid-condensed or solid phase collapse at low surface tensions via a localized, large amplitude buckling. The buckled regions coexist with the flat monolayer, remain attached to the interface, and reversibly reincorporate into the monolayer upon expansion. [S0031-9007(98)06943-9]

PACS numbers: 68.10.Cr, 68.35.Rh, 87.15.–v, 87.64.Dz

Many amphiphilic molecules that are nearly insoluble in water can form a monolayer at an air-water interface. The area available to the monolayer, A, can be decreased by imposing an external surface pressure π, which lowers the normal air-water surface tension γ_0 to γ:

$$\pi = \gamma_0 - \gamma . \quad (1)$$

Measurements of π versus A at constant temperature (Fig. 1) are known as isotherms [1,2]. At large areas per molecule, monolayers are "gaseous"; the hydrophobic portions of the molecule make significant contact with the water surface but little contact with each other. As the monolayer is compressed into the liquid-expanded (LE) phase, the hydrophobic parts of the molecules come into contact with each other and lift from the water surface but remain largely disordered and fluid. Further compression leads to a first order transition to the "liquid-condensed" (LC) phase, marked by a plateau in the isotherm corresponding to LE and LC coexistence (Fig. 1). In the LC phase, the molecules exhibit long range order, are less compressible, and less fluid than in the LE phase. Impurities, such as the fluorescent dye added to visualize the monolayers (Figs. 2 and 3), preferentially locate in the more disordered LE phase and are expelled from the better ordered LC phase. A kink in the isotherm at higher π marks the transformation to a better ordered "solid" (S) phase, in which the area per molecule corresponds to the two dimensional packing of three dimensional crystals of the amphiphile. Above a critical temperature that depends on the subphase, many phospholipids exhibit only the LE phase; the better ordered LC and S phases are absent [1,2].

Eventually, the molecular area (or π) reaches a limiting value beyond which the monolayer cannot be compressed further. At these limiting conditions, a flat monolayer is similar mechanically to a plate under compression; as the pressure is increased past the limiting value, the plate can (i) fracture and break, (ii) buckle at constant area, or (iii) lose material (and hence interfacial area) depending on the elastic and solubility properties of the monolayer [3–7]. The surface pressure at collapse, π_c, determines the minimum surface tension for a given monolayer; the collapse mechanism determines the reversibility, i.e., what fraction of the monolayer remains at the interface and how well the monolayer respreads to cover the interface as π is decreased.

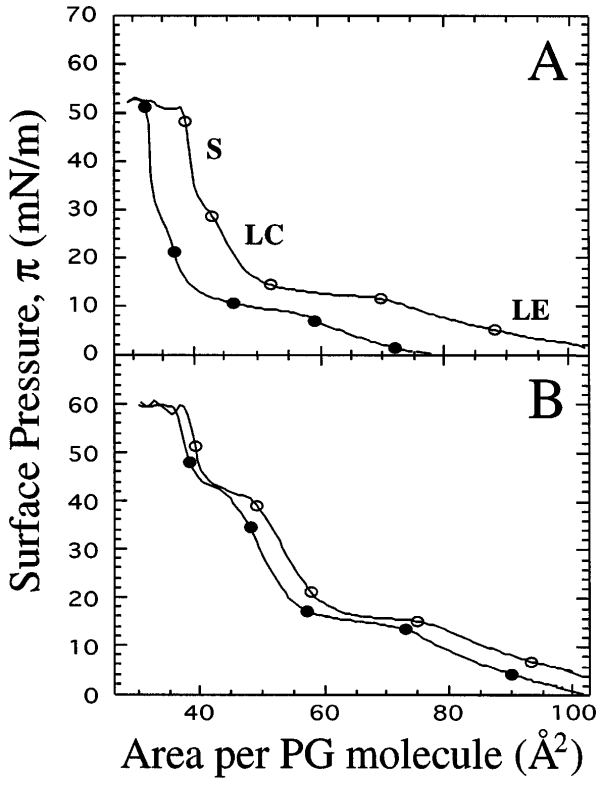

FIG. 1. Cycling isotherms (compression speed = 0.08 Å^2 per molecule per second with the first and second compressions indicated by ⊖ and ●, respectively) of (A) a DPPG monolayer at 37 °C (with the locations of the LE, LC, and S phases indicated), showing an area offset upon recompression due to irreversible monolayer collapse and (B) a DPPG/10 wt % SP-$B_{1\text{-}78}$ monolayer at 37 °C, showing a more reversible collapse than without the peptide.

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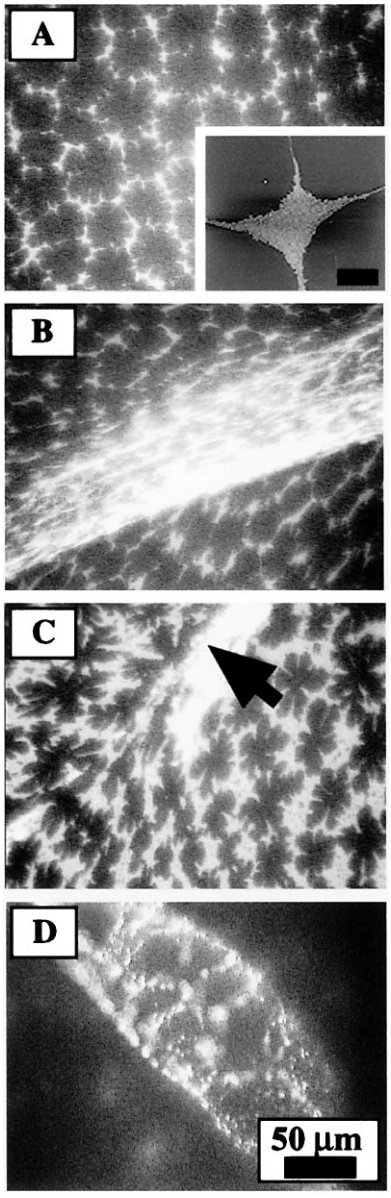

FIG. 2. (A) FM image of a monolayer of DPPG/10 wt% SP-$B_{1\text{-}25}$/1 mol% NBD-PG at 37 °C immediately prior to collapse ($\pi = 55$ mN/m), showing a bright, fluid phase network segregating the condensed phase domains. The inset is an AFM image of an interstitial region; the height difference between the protein-rich network phase and the condensed phase domains is 5 nm. (B) FM image of a folded region in a collapsed monolayer ($\pi \approx 60$ mN/m) of DPPG/10 wt% SP-$B_{1\text{-}25}$/1 mol% NBD-PG. The folds extend several microns into the subphase and retain the monolayer morphology. (C) FM image of the expansion of a collapsed DPPG/10 wt% SP-$B_{1\text{-}25}$/1 mol% NBD-PG monolayer showing unfolding of the monolayer (arrow). (D) FM image of a fold in a collapsed monolayer of DPPG/1 mol% NBD-PG on buffered saline subphase (2 mM Ca^{2+}, 150 mN NaCl, 0.2 mM $NaHCO_3$, *p*H 6.9).

In general, fluid LE monolayers, such as those formed by phospholipids above their critical temperatures, collapse at relatively low π_c via the ejection of material to the subphase [6,8]. More ordered and rigid LC or S phase monolayers collapse at higher π_c, usually by fracturing, followed by loss of portions of the monolayer in the subphase or formation of multilayered crystalline aggregates at the air side of interface [3–7]. Collapse via fracture or solubilization is irreversible; the collapse phase material does not reincorporate into the monolayer as π is decreased [5,8–10].

Here we show, using a combination of isotherms, optical fluorescence microscopy, and atomic force microscopy (AFM) that monolayers with a continuous LE phase separating islands of LC or S phase at π_c can undergo a large amplitude buckling into the aqueous subphase. The folded regions coexist with the flat monolayer—further compression changes the fraction of material in the folds relative to the flat monolayer at constant π_c, indicative of a first order phase transition. The morphology of a continuous network of fluid LE phase separating LC phase domains alters the elasticity of the monolayer, which allows the monolayer to bend rather than break. The folds remain attached to the monolayer at the interface and are reincorporated into the monolayer upon expansion with little loss of material. While dipalmitoylphosphatidylglycerol (DPPG) monolayers on a saline subphase containing calcium buckle on collapse, the buckling transition can be more readily induced in a variety of single component and mixed phospholipid monolayers by the addition of a protein found in lung surfactant (LS). LS is a complex mixture of lipids and proteins that forms a monolayer at the alveolar surface of the lungs in mammals and humans and acts to reduce the work of breathing [11]. LS consists primarily of dipalmitoylphosphatidylcholine (DPPC), with smaller fractions of anionic lipids such as saturated and unsaturated phosphatidylglycerols (PG) and fatty acids [12,13], along with an amphipathic, polycationic protein called SP-B [14]. Our results show that the SP-B protein inhibits lipid ordering while simultaneously preventing solubilization of the LE fraction of the monolayer. Monolayers with SP-B can achieve low tensions and respread easily on expansion due to this novel buckling collapse mechanism.

The full native sequence of SP-B, SP-$B_{1\text{-}78}$, a shorter peptide based on the amino terminus of SP-B, SP-$B_{1\text{-}25}$, and a fluorescein-labeled version of the peptide, F-SP-$B_{1\text{-}25}$ were synthesized as described previously [5]. The proteins or peptides were mixed with the appropriate amounts of zwitterionic DPPC, saturated anionic DPPG, unsaturated anionic palymitoyloleoylphosphatidylglycerol (POPG), and anionic palmitic acid (PA) (Avanti, 99%) in 3:1 chloroform:methanol (Fisher spectranalyzed). The fluorescent probe 1-palmitoyl, 6-(N-7-nitrobenz-2-oxa-1,3-diazol-4-yl-)-PG (NBD-PG, molecular probes) was added at lipid mole ratios of 0.5% to 1%. Solutions were spread onto pure water (Milli-Q, Millipore) or buffered (0.2 mM $NaHCO_3$, *p*H 6.9), 150 mM NaCl, 2 mM $CaCl_2$ subphases in a temperature-controlled

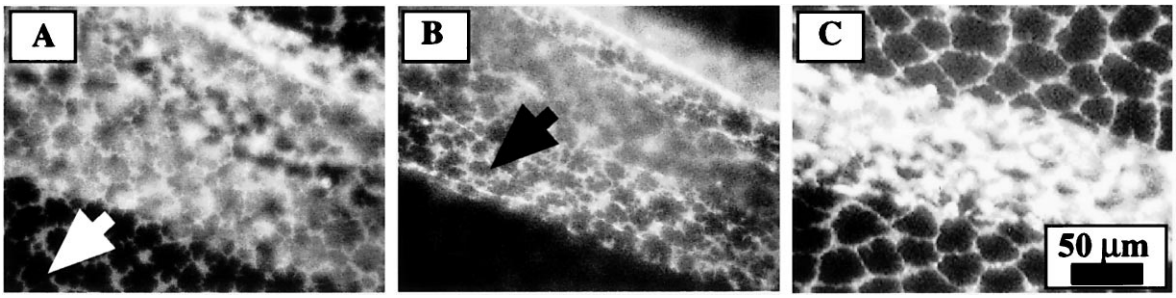

FIG. 3. (A),(B) FM images of monolayers of 3:1 mol:mol DPPG:POPG/1 mol % NBD-PG containing 10 wt % SP-B_{1-78} at 37 °C. (A) An image in the focal plane of the monolayer showing the bright LE phase network between dark LC phase domains (arrow) at collapse pressure of $\pi = 60$ mN/m, coexisting with a folded region. (B) The focal plane within a folded region underneath the monolayer; the domain morphology in the folded regions is the same as the monolayer. (C) FM image of the collapse behavior of a model lung surfactant monolayer containing 67:22:8:3 wt:wt DPPC, POPG, PA, and synthetic SP-B_{1-78}; similar LE network, LC islands, and folded regions are observed. The collapse pressure was about 65 mN/m.

microfluorescence film balance [15]. Selected monolayers were transferred to mica substrates for AFM imaging with a Nanoscope III FM (Digital Instruments) [16].

Isotherms of DPPG at 37 °C on pure water showed a phase progression of G → LE (or fluid) → LC → S. The monolayer consists entirely of the S phase prior to collapse at $\pi_c \approx 52$ mN/m [Fig. 1(A)] via fracture followed by loss of material to the subphase. After expansion and subsequent recompression, the isotherm was offset to lower molecular areas [Fig. 1(A)], with an irreversible loss of material. Adding 10 wt % SP-B_{1-25} or SP-B_{1-78} (approximately the physiological ratio) led to the formation of a fluid, LE phase network that remained at π in excess of 55 mN/m [Fig. 2(A)] [5,8]. Similar fluid networks were observed with fluorescein labeled F-SP-B_{1-25}, showing that the protein partitioned into the LE phase. A new plateau appeared in isotherms of DPPG/10% SP-B_{1-25} or SP-B_{1-78} monolayers near the collapse pressure of the pure peptides (approximately 40 mN/m) [8]; however, this plateau was reproducible upon cyclic compression [Fig. 1(B)], indicating that both SP-B and DPPG remained in the monolayer. AFM images of DPPG/SP-B_{1-25} films transferred to mica substrates at a π of 50 mN/m [16] showed that the SP-B_{1-25}-rich network formed a 5 nm high rim around the condensed phase domains [inset of Fig. 2(A)]. This peptide-rich network remained associated with the monolayer up to collapse, which occurred at an elevated $\pi_c \approx 60$ mN/m [Fig. 1(B)].

Upon collapse, the monolayer no longer fractured, but buckled, forming protrusions that extended several microns into the subphase [Fig. 2(B)]. The buckled regions occurred at random, and coexisted with the undeformed, flat monolayer; further compression changed the fraction of the monolayer in the folds relative to the flat regions at a constant π_c. These folds are distinctly different than previous observations of a second order "buckling transition" in which uniform, nanometer-amplitude undulations were observed experimentally [17,18] or predicted theoretically [19,20], but may be related to a first order flat to buckled transition predicted by Guitter *et al.* [21,22] and Milner *et al.* [20]. The network of LE phase surrounding islands of LC or S phase appeared unchanged within the folds; in monolayers with 10 wt % F-SP-B_{1-25} the fluid phase appeared bright in the images, indicating that the network remained protein rich. Upon expansion of the collapsed monolayers, the buckled regions reincorporated reversibly into the monolayer, with the film pulling apart in directions perpendicular to the fold lines and the folds "unzipping" back into the monolayer [Fig. 2(C)]. The offset in cycling isotherms was greatly reduced in the presence of SP-B [Fig. 1(B)], indicating a significantly smaller loss of material.

The folding transition was not just a feature of this particular lipid and protein mixture; folding could also be induced in a pure DPPG monolayer on a saline subphase containing from 2–5 mM calcium at 23 °C [Fig. 2(D)]. The calcium also induced a LE phase network that persists in the folded regions [Fig. 2(D)]. As discussed above, on pure water, the fluid network is not stable at collapse in pure DPPG films [Fig. 1(A)], and folds do not occur.

Folding transitions were also observed in mixed saturated and unsaturated lipid monolayers containing SP-B peptide. At temperatures in excess of 20 °C, pure POPG monolayers are in a homogeneous LE phase at all π and collapse at approximately 50 mN/m via solubilization to the subphase. Adding increasing amounts of POPG to DPPG monolayers results in an increase in the amount of fluid phase present at a given π [8]. SP-B preferentially partitioned into the fluid phase in mixed DPPG:POPG monolayers, as in the pure DPPG films. The bright fluid phase network remained up to the collapse pressure of approximately 60 mN/m [Fig. 3(A), arrow]. Collapse occurred via a coexistence between buckled and flat monolayers [Figs. 3(A) and 3(B)] in which both LC and LE domains folded into the subphase. The folds had the same average morphology and composition as the monolayer, and all components rapidly reincorporated into the monolayer upon expansion [23]. SP-B was again critical to the existence of a fluid phase network prior to

collapse; in the absence of the peptide a POPG-rich bright fluid phase was removed from 3:1 mol:mol DPPG:POPG monolayers at low π (approximately 50 mN/m, the collapse pressure of pure POPG) via solubilization to the subphase (not shown). Similar folds also occurred in a model synthetic lung surfactant monolayer [24] composed of 67% DPPC, 22% POPG, 8% PA, and 3% SP-B by weight [Fig. 3(C)]. The DPPC and PA were primarily localized in the condensed domains and the SP-B and POPG in the fluid phase domains; hence, folding appears to be independent of the lipids forming the condensed phase. The essential feature of buckling is the formation of a fluid, LE phase network that separates the LC or S phase domains at surface pressures up to π_c.

The mechanism of monolayer collapse depends primarily on the elasticity and cohesiveness of the monolayer. For ordered LC or S phase monolayers, the semicrystalline monolayer is apparently too brittle to bend, and collapse occurs by fracture. For the disordered and fluid LE monolayers, collapse occurs by solubilization of material into the subphase, either by molecular solubility or the formation of liquid crystalline aggregates such as vesicles or liposomes. However, monolayers that retain a continuous LE network separating islands of LC or S phase at π_c collapse via a reversible buckling in which the monolayer is flexible enough to bend but retains enough cohesion to prevent loss of material to the subphase. The folds have the same composition as the flat monolayer and reversibly reincorporate into the monolayer on expansion. The folds are inherently asymmetric in that folding always occurs into the aqueous subphase. The folding process allows collapse to occur at elevated surface pressures (low surface tensions) while making it possible for the protein and unsaturated lipid components to remain associated with the monolayer, facilitating rapid respreading.

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