Tracking Giant Folds in a Monolayer

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The collapse dynamics of giant folds in a catanionic monolayer at the air—water interface are examined. A monolayer of dioctadecyldimethylammonium bromide (DODAB) and sodium dodecyl sulfate (SDS) in a 1:1 ratio is the system of study that previously was found to fold upon compression in a Langmuir trough. Carboxylate-coated polystyrene beads (1 μm diameter) are deposited and bound to the monolayer. Displacement of the beads is measured with epifluorescence microscopy and particle image velocimetry, yielding a measurement of the velocity of the monolayer around the fold. Reversibility is confirmed by measuring the amount of monolayer material entering and leaving the fold. Material near folds are found to have a maximum relative velocity on the order of 0.1 mm/s, and fold depths are found to be on the order of 1 mm. The folds exhibit regular unfolding behavior, which can be explained qualitatively by a simple mechanical model.

Introduction

Understanding interfacial dynamics has broad importance in many biological and engineering applications.1 In particular, a pulmonary surfactant is a critical interface between air and water in the lung, which lowers the surface tension to prevent lung collapse upon exhalation.2 Failure of the surfactant results in serious conditions characterized by respiration difficulty. Surfactant therapies3,4 have been developed to treat respiratory distress in premature infants with significant success, but little impact has been demonstrated with such therapies in adults.3 Understanding the dynamics of surfactants with respect to compression and expansion thus has significant practical value in that it may lead to ways to engineer improvements in these surfactant therapies.

Like all quasi-2D materials, surfactant monolayers must collapse when compressed. The collapse modes vary with the material and rate of compression6 and include buckling,7,8 budding,9 vesiculation, and folding.10 The folding process occurs in lung surfactant upon compression,10 which is likely critical in its function of preventing lung collapse modes. Stock solutions of SDS are dissolved in ethanol at the same potential energy barrier.11

Materials and Methods

Materials. Monolayers are formed from a 1:1 by number mixture of dioctadecyldimethylammonium bromide (DODAB, 97% purity) and sodium dodecyl sulfate (SDS, 99% (GC) purity), both purchased from Sigma-Aldrich. Stock solutions of DODAB are dissolved in chloroform to a 1.2 mM concentration. Stock solutions of SDS are dissolved in ethanol at the same potential energy barrier.11

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Like all quasi-2D materials, surfactant monolayers must collapse when compressed. The collapse modes vary with the material and rate of compression6 and include buckling,7,8 budding,9 vesiculation, and folding.10 The folding process occurs in lung surfactant upon compression,10 which is likely critical in its function of maintaining a low surface tension. The emergence of folds has received a recent theoretical treatment.8,11 Many thin membranes
concentration. Equimolar mixtures are prepared by adding equal volumes of the two stock solutions. An appropriate amount of chloroform is then added to bring the concentration of each surfactant to 0.12 mM, resulting in a final solvent concentration of 10% ethanol and 90% chloroform. A fluorescent probe, 2-(12-(7-nitrobenz-2-oxa-1,3-diazol-4-ylamino)dodecanoyl-1-hexadecanoyl-sn-glycero-3-phosphocholine (NBD C_{12}-HPC), is added to the mixture solution, resulting in a 0.5% probe concentration. To form monolayers, 60 μL of the mixture solution is spread across the surface of a water subphase. A time of 15 min is allowed to pass before any compression is done to ensure that the system has equilibrated.

To image carboxylate-modified polystyrene microspheres (1.0 μm diameter, Nile red fluorescence) from Invitrogen Molecular Probes are diluted by a factor of 1000 in 50% water, 50% isopropanol. This concentration was found to maximize the spreading of beads on the surface of the water subphase. Before deposition on the air–water interface, the diluted solution is ultrasonicated for 30 min to prevent bead aggregation. To achieve a useful surface concentration, 100 μL of the solution is deposited evenly across the surface. This concentration allows for a sufficient number of particles to be found in the field of view in order for the image analysis software to calculate velocities between frames for each window. Addition of the beads is not found to have a significant effect on the isotherm shape and did not affect the pressure at which folding occurs.

A temperature controller is used to increase the temperature of the subphase to 30 °C before the monolayer is deposited in order to maximize bead spreading. Once the bead concentration is seen to be roughly homogeneous, the temperature is reduced to room temperature (23 °C) and the SDS-DODAB monolayer is spread at the air–water interface. Bead imagery in a fluorescent SDS-DODAB monolayer shows that the beads track the fluorescent regions of the monolayer. We conclude that the beads adhere to the monolayer and remain attached for the timescale of the experiments performed.

**Methods.** The monolayer experiments are performed in a Langmuir trough constructed from poly(tetrafluoroethylene) (PTFE) and purchased from Nima Technologies (model 102M). The trough employs two PTFE barriers (7.0 cm in length) to compress and expand the surface to a minimum area of 15 cm² and a maximum area of 78 cm². The barriers are compressed at a rate of 10 cm² min⁻¹, corresponding to a linear velocity for each barrier of 0.12 mm s⁻¹, and do not pause between compression and expansion modes. Surface pressure measurements are made with a Wilhelmy plate system also manufactured by Nima. The trough is cleaned daily with ethanol, chloroform, and water. The Wilhelmy plate is allowed to soak in the subphase for 15 min until the force equilibrates. The water subphase is purified by a Millipore Milli-Q system such that the resistivity of the water reaches a resistivity of 18.2 MΩ cm at room temperature. An Olympus BX60MF5 microscope with a fluorescent cube (excitation filter, wide-band blue 450–480 nm; long-pass barrier filter, 515 nm) is used to view the air–water interface from above. A Cohu intensified CCD camera takes images and video of the monolayers, which are saved onto a hard drive via an NI frame grabber card and a custom LabVIEW program. An opaque box covers the system to minimize air currents and prevent ambient light from interfering with imaging.

Folding and unfolding events are captured at a video frame rate of 30 frames per second. The entire folding and unfolding process is recorded at approximately the same location in the monolayer, roughly in the center of the trough. To keep the same location in view, the stage upon which the trough sits is translated slightly during the course of the video. Folds often nucleate in groups, complicating the analysis. To isolate the behavior of a single fold, the only folding events analyzed were those with only one fold in the field of view. Care is taken to avoid disrupting the monolayer and subphase. Videos are analyzed with a custom-made MATLAB program that uses correlations to measure the movement

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**Figure 1.** Images of folding (a–c) and unfolding (d–f). The initial fold is shown to form in the image at the top left and progressively unfolds until the completely unfolded monolayer is shown in the bottom-right image. The bright spots are 1-μm-diameter fluorescent beads, which are also responsible for the fold’s fluorescence. The scale bar in the upper-right corner represents 300 μm.

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between small segments of the image. Images are rotated so that the motion of the monolayer is in the vertical direction. The fold is tracked throughout the video, and segments of the image are cropped so as to position the horizontally oriented fold in the center of each cropped image. Using the cropped images, particle image velocimetry (PIV) is used to track the motion of the beads and the monolayer above and below the fold. The PIV code is adapted from http://www.oceanwave.jp. Ybert et al. used a related method in which bright spots near a fold were tracked and the amount of material unfolded was estimated. Every 10th frame is analyzed with PIV because the change in position between successive frames does not rise significantly above the noise. The program uses a minimum quadratic difference (MQD) algorithm on 20 pixel square windows of the images to determine the movement of the window between successive images.

**Results.**

**Experimental Investigation of Folding.** Folding is observed at surface pressures of 47 mN m⁻¹, in agreement with limits proposed previously. Beads are at such a density that even small folds trap a significant number of beads and become immediately visible because of a line of fluorescence across the microscope’s field of view. As compression continues, small folds form in a jerklke fashion. Some of these folds continue to grow, swallowing other smaller folds and thus aggregating them. The image analysis program tracks these growing giant folds.

A region of interest in the tracked images is specified in which the PIV program is used to determine the mean motion. This region is chosen far enough away from the fold that during the unfolding process collapsed material moving away from the fold unfolds before passing through the region of interest. In this region, the velocity along a line of windows parallel to the fold was

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considered. Although there is some variation in the magnitude of the velocity, no section of the monolayer clearly prefers to fold or unfold. Therefore, averaging the velocity along the fold should reasonably represent the velocity of material above/below the fold. Similarly, the velocity of material along a line of windows perpendicular to the fold follows the linear elastic model described below, so the mean in this direction is an acceptable representation as well. The mean velocity for particles in the upper half of the image (above the fold) and the lower half of the image (below the fold) series is shown in Figure 2 for a typical data set. The sections labeled A–D in the Figure correspond to area compression with folding, expansion without unfolding, unfolding, and expansion after unfolding, respectively. The velocities are noisy in part because of the jerklke motion of the material associated with folding but also because of pixel-sized shifts due to imperfections in the image-tracking software. However, this noise is significantly suppressed by subtracting the velocities above the fold and below the fold as shown in Figure 3. This plot of relative velocity between the top and bottom of the fold shows a characteristic, uncorrelated jerklke motion of folding as observed by Gopal et al. The peak velocity of unfolding averaged approximately 130 μm s⁻¹. The mean velocity during folding was 48.4 μm s⁻¹. Because the velocities between successive frames can be determined, summing them gives the displacement of material within the image. The displacement of material above and below a characteristic fold is shown in Figure 4. Combining the two measures of material above and below the fold yields a measure of the total displacement into and emanating from the fold. The total displacement is expected to depend on the rate of compression and any extra compression that occurs after the fold nucleates.

Figure 4 shows the total displacement found by tracking the beads with the PIV program. It does not represent the total amount of material displaced, however, because the highly compressed monolayer behaves as a linear elastic material and is capable of stretching. To understand the stages of the folding process better, we consider two simple models of the system. In the first, we consider only the kinematics of the expansion of the monolayer outside of the fold. In the second, we allow for the effect of the unfolding process itself on the dynamics of the monolayer.

**Linear Strain Model.** Images of a fully compressed monolayer look at a small segment of the overall monolayer. When barrier expansion begins at a constant velocity, the material in the
field of view responds by expanding. If we assume that the material in the trough moves at a rate proportional to the distance from the center of the trough (as in a linear elastic material), then the observed velocity of the material should follow: \( v(x,t) = 2v_b L(t) \) where \( v_b \) is the velocity of each boundary, and \( L(t) \) is the distance between the barriers as a function of time, and \( x \) is the distance from the center axis. If the barriers move at a constant velocity, then \( L(t) = L_0 + 2v_b t \) where \( L_0 \) is the distance between the barriers in the most compressed state at \( t = 0 \). If we take the center of the images to be the origin and \( t = 0 \) to be the time of initial expansion, then the amount of material displaced outside of an upper and lower boundary at \( x = x_1 \) and \( x = -x_1 \) in the image is

\[
\int_{x_1}^{t_1} (v(x_1, t) - v(-x_1, t)) \, dt = 2v_1 \log \left( \frac{2t_2 v_b + L_0}{2t_1 v_b + L_0} \right)
\]

The velocity of the material in regions above and below the fold is measured. An effective \( v_1 \) value is found by choosing the midpoint of the top region and a corresponding midpoint of the bottom region. This allows eq 1 to be compared to plots of the experimentally determined displacement. The difference between the expected displacement due to expansion and the displacement measured elucidates the amount of material that folds and unfolds.

Evidence of the linear expansion of the monolayer occurs both in the initial stages of expansion and in the tail of the expansion curve, after the unfolding process is completed. Comparing this tail shape to what is expected from eq 1 shows that the measured displacement curve closely follows the expected expansion curve, supporting the assumption that the monolayer acts as a linear elastic material. Because the monolayer behaves this way after unfolding (as well as just before), it is assumed that it behaves as such during the unfolding process. Therefore, subtracting the expected expansion from eq 1 gives a more precise measure of the amount of material inside the fold at its most folded. Effectively, this subtracts out the linear expansion of the monolayer. Figure 5 shows a comparison of eq 1 with a typical displacement curve, and Figure 6 shows the result after subtracting the expected displacement from the measured displacement.

Performing this analysis on a series of five folds seems to indicate that folding is reversible or nearly so. The total displacement into the folds studied averaged 915 \( \mu \)m, and the total unfolded displacement averaged 905 \( \mu \)m, for a 1% loss of material. This small difference may be explained by error in the method of calculating displacements. For some windows in the image, there is little visible material that causes the MQD method to produce an erroneous result. In addition, these folds oftentimes do not simply unfold upon expansion but break apart into segments that move with the monolayer-like particles. It is possible that small segments move outside the image region, causing a measured loss of material.

**Model of the Unfolding Process.** To better understand the unfolding itself, we describe the monolayer as a continuum linear elastic object mechanically coupled to the fold. This is consistent with our observation of three distinct periods of unfolding: (a) the initial phase of expansion, (b) the unfolding, and (c) the final expansion. During unfolding, tension applied to the monolayer at a barrier distant from the fold disrupts the folded state at a critical value. We treat this unfolding event in a manner analogous to the fracture of a solid. Beyond this critical tension, the amount of monolayer trapped in the fold continuously decreases until the fold disappears. We describe the monolayer by a single elastic modulus \( \mu \) and define a displacement field \( u(x) \) everywhere outside the fold and in the tangent plane of the monolayer. We use a simplified model that assumes an infinitely long fold to avoid the consideration of end effects. Therefore, we consider the energy per unit length \( E_{\text{el}} \) of the fold in terms of the surface tension \( \tau \), or force per length, on the monolayer. Assuming the fold to lie perpendicular to the \( \hat{x} \) direction and assuming translational symmetry along the fold, we may set \( u(x) = x \tau(x) \) and write the elastic energy of the monolayer having extent \( L \) in the direction normal to the fold as

\[
E_{\text{el}} = \frac{1}{2} \mu \int_0^L \left( \frac{du}{ds} \right)^2 ds
\]

For Figure 6, net unfolded displacement of monolayer material calculated by subtracting the elastic expansion component from the measured displacement curve. The maximum unfolded displacement is shown by the top red line, and the zero value, by the bottom green line. The solid black line represents a fit to the discussed unfolding model. Note how the data have two regions with roughly the same slope, indicating that these regions are expanding like an elastic material. The shape of this curve is seen in all observed unfolding events and seems to be characteristic of unfolding in this material.
We choose our coordinate system so that the unfolding process moves material in the \( \hat{x} \) direction because of an external tension applied to the right edge of the material, \( s = L \), by the moving barrier. During the unfolding process, we assume that both viscous and inertial stresses are negligible so that the monolayer remains in local stress balance:

\[
\frac{d^2u}{dt^2} = 0
\]  
(3)

For a surface tension \( \tau \) at the moving barrier, one boundary condition for eq 3 is that \( du/ds = \tau/\mu \) at \( s = L \). We also insist that the monolayer is pinned at the edge of the fold so that \( u(0) = 0 \). We find that \( u(s) = sx/\mu \), or, more usefully, for a total extension of the monolayer due to the displacement of the barrier by \( x \), we note that the displacement field and the applied tension are given by

\[
u = u(s) = \frac{sx}{\mu} \quad \tau(x) = \frac{\mu x}{L}
\]  
(4)

It is clear from the data that the unfolding process involves a threshold or critical tension for unfolding. By examining Figure 5, we see that the barrier moves \( x^* \approx 200 \mu m \) before there is a discontinuous change in the rate of change of the net displacement versus time, which we attribute to the initiation of unfolding. Using this result and a measure of the surface pressure during the unfolding event (\( \Pi \approx 35 \text{ mN m}^{-1} \)), we find from eq 5 that the elasticity of the monolayer at this surface pressure is \( \mu = \tau^* L/\lambda^* \approx 5000 \text{ mN m}^{-1} \). To analyze the unfolding dynamics, we must introduce a model for the work done to remove a length of fold \( \delta \), where \( \delta \) is measured along the fold and into the aqueous subphase. We make the following assumptions: (i) Once the critical tension \( \tau^* \) is reached, the structure of the fold at its boundary with the monolayer is modified, allowing the monolayer trapped in the fold to flow out. (ii) After this threshold has been reached, the work done to decrease the fold by \( \delta \) is constant: 

\[
\delta w = \varepsilon \delta \tau
\]  
(6)

We attribute the force scale \( \varepsilon \) to an attractive interaction between the surfactants on two sheets making up the fold. To account for the observation that the monolayer can be stretched a finite distance before unfolding, it must be that \( \varepsilon < \tau^* \). Moreover, we assume, at least initially, that the attractive interaction determining \( \varepsilon \) is independent of position along the depth of the fold so that the work required to pull out a length of the fold is independent of the amount of the fold already pulled out.

Using this minimal model, we see that the combination of force balance at the monolayer/fold boundary and eq 6 requires that

\[
\varepsilon = \frac{\mu x}{L}
\]  
(7)

The fold acts to pin the force in the two-phase (i.e., fold and monolayer) system in a manner analogous to the way in which a two-phase liquid–gas system fixes its pressure during volume change along an isotherm. We fix the velocity of the barrier to be \( v \) so that \( v = L + \dot{x} \). Using this and the time derivative of eq 7, it is easy to show that during the continuous unfolding regime

\[
\dot{L} = \frac{v}{1+z}
\]  
(8)

\[
\dot{x} = \frac{v^2}{1+z}
\]  
(9)

where \( z = \varepsilon/\mu \). If the intrafold binding is strong so that \( z \gg 1 \), then the displacement of the barrier results in a large extensional velocity of the monolayer and a small unfolding rate that adds new material to it (i.e., \( \dot{x} \gg \dot{L} \)). If the monolayer is highly incompressible elastically and the binding energy of the fold is sufficiently small (\( z \ll 1 \)), then the extension of the barrier rapidly pulls out the fold. For all cases, however, we note that this simple model predicts that the rate of unfolding is constant in time once the critical tension has been reached. Figure 5, however, suggests that the unfolding rate accelerates upon the onset of unfolding.

To account for this acceleration, we consider a somewhat more complex model of the work of unfolding. Consider a cross section of the fold in a plane perpendicular to the symmetry direction along the fold (\( \hat{y} \)). Near the fold/monolayer boundary, we expect a sufficient separation between the two monolayers making up the fold to decrease the local binding energy per unit length at this point significantly. Similarly, the finite bending modulus of the monolayer suggests that near the bottom of this cross section the two monolayer sheets are also poorly connected. The greatest binding energy must come from the central section of the fold away from the deformed regions at the monolayer fold boundary and the curved bottom of the fold. The work done per unit length of unfolding must depend on the total amount of the remaining fold. As the fold becomes smaller, a larger fraction of it is taken up by the deformed boundary region of the curved region. Thus, the work of unfolding is a monotonically increasing function of the length \( F \) of the fold. The minimal extension of our constant \( \varepsilon \) model is then

\[
\varepsilon(F) = \frac{\tau + \alpha F}{L + f}
\]  
(10)

where \( \alpha \) is a positive constant. We now write the force balance equation between the fold and the monolayer of initial length \( L \) that is obtained during the unfolding process as

\[
\varepsilon - \alpha f = \frac{\mu x}{L + f}
\]  
(11)

where we have assumed that a length \( df \) of fold that is removed increases the length of the monolayer by \( df \). Here, \( f(t) \) is the length of the fold released at time \( t \) so that the remaining fold has length \( F = f(t) \) and \( \varepsilon = \varepsilon + \alpha F \). The constant velocity condition is now \( v = f + \dot{x} \). Using this and the time derivative of eq 11, we find a first-order differential equation satisfied by \( g = L + f \):

\[
\frac{d}{dt} \left( \frac{\alpha (z - 1) g}{\mu} \right) = \frac{v \dot{L}}{g}
\]  
(12)

Upon integrating, we find that

\[
g(t) = L + f(t) = \frac{\mu}{\alpha} \left( z - 1 \right) + \sqrt{(z - 1)^2 + \frac{2\alpha}{\mu} \left( vt + \dot{L} \right)}
\]  
(13)

with the constant length \( \dot{L} = \alpha L^2/2 \mu - (z - 1)L \) set by the initial condition that \( f(0) = 0 \).

The most interesting result occurs for the case where the typical fold binding energy is comparable to the elastic constant of the monolayer, \( z \approx 1 \). In the case of the equality of these energy scales, we find that the fold rapidly unfolds initially but that this rate slows continuously as the process continues. The amount of the fold that has vanished at time \( t \) after the initiation of unfolding is given by

\[
f(t) = -L + \sqrt{\frac{2\alpha vt}{\alpha} + L^2}
\]  
(14)
This dependence of the unfolding on time is in qualitative agreement with the data shown in Figure 6.

Conclusions

This study sheds light on the processes of folding and unfolding in a catanionic monolayer, which may relate to the processes in alveoli of the lung. We confirm the general picture of folding in which small, individual folding events aggregate to form a large fold. Direct measurement of the unfolding process reveals a number of interesting features. An unfolding event usually occurs in one cascading process, unlike folding. Tracking the material around the fold suggests that little if any material is lost during the folding and unfolding process. A method of accurately gauging the quantity of material entering and leaving the fold has been implemented in these measurements through the use of micrometer-sized fluorescent beads and a PIV program. The plot of the corrected displacement of material leaving the fold seems to have a characteristic shape that is seen in all unfolding events. A simple mechanical model of the unfolding process qualitatively captures the time dependence of the displacement of material. Although the model describes our experimental system, the alveolar lining exists on a much thinner viscous layer, possibly perturbing the folding dynamics of our system. Future work is needed to verify the correspondence of the two systems.

Velocities of material near the fold have been measured and were found to be on the order of tens of micrometers per second for a single barrier speed. Previous work measured velocities of material in the monolayer\(^1\) but not the relative velocity of material near a collapsing fold. In addition, folding here occurs on slower timescales than previously reported, which allows the dynamics of folding to be investigated. These dynamics may be important in the proper functioning of lung surfactant and should be considered in surfactant analysis. The model essentially assumes an elastic material coupled to the fold and a binding energy in the fold that depends on the length of the fold. This model captures the qualitative behavior of the unfolding, but more quantitative measurements of the monolayer/fold properties are needed. In particular, the binding energy and elastic modulus of the fold and monolayer, respectively, are free parameters in the model. Future work will focus on measuring these characteristics of the monolayer.

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