Stripe Phases in Lipid Monolayers near a Miscibility Critical Point

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(Received 8 September 1998)

Some mixtures of lipids in monolayers at the air-water interface exhibit immiscible liquid phases. Theory predicts an exponential scaling for the width of liquid domains that depends on a competition between line tension and electrostatic dipolar repulsion. It is shown that the scaling law is valid for the stripe phase in a binary mixture of lipids, dihydrocholesterol, and dimyristoylphosphatidylcholine. This stripe phase appears only near a miscibility critical point. The agreement of observed widths with theoretical scaling provides strong evidence for equilibrium in such mixtures, an essential condition for using data from monolayers to model the liquid regions of bilayers and biological membranes. [S0031-9007(99)08450-1]

PACS numbers: 87.19.–j, 64.60.Fr, 68.35.Rh

Liquid regions of biological membranes are formed by lipid bilayers and membrane proteins. These two-dimensional liquids are typically composed of hundreds of different lipids and show little in-plane order. The structural and functional roles of the different lipids have been a resistant problem in membrane biology. One approach is to study the thermodynamic phases of simple lipid mixtures in monolayers, and to extrapolate such studies to more complex mixtures in bilayers and biological membranes. Understanding miscibility critical points and conditions for thermodynamic equilibrium are key elements in this approach. To this end we have studied mixtures of a sterol, dihydrocholesterol, and phospholipids in monolayers at the air-water interface.

As observed by epifluorescence microscopy, several mixtures of cholesterol and phospholipids form immiscible liquid domains in monolayers at the air-water interface [1–4]. The domain sizes and shapes are used to determine phase diagrams of the lipid mixtures. For example, stripe domains indicate proximity to a miscibility critical point [4,5]. The present work was undertaken to show that the stripe width w reflects a state of thermodynamic equilibrium in these mixtures.

In lipid monolayers, the equilibrium sizes and shapes of *liquid* domains are determined by a competition between line tension λ and electrostatic dipolar repulsion. Theory predicts the equilibrium radii of circular domains and widths of stripes [6]. We measure stripe widths of immiscible liquid domains formed by a binary mixture of dimyristoylphosphatidylcholine (DMPC) and dihydrocholesterol (Dchol) near the miscibility critical point. In this mixture it is known that circular domains are not at equilibrium with respect to domain size [7,8]. Here we show that, on the contrary, the stripe widths do reflect equilibrium near the critical point. In demonstrating this result, we relate our results to earlier measurements of the domain deformability [9], surface potentials [10], and critical exponents [11].

Background.—Figure 1(a) is a schematic phase diagram for a monolayer of two lipid species, both assumed to be liquids. Within the two-phase region, a mixture of lipids at monolayer surface pressure $\Pi_{\text{low}} < \Pi_C$ separates into "black" and "white" immiscible liquid domains. In our work the black phase is Dchol rich and the white phase is DMPC rich. There is extensive work showing that these phases are liquid (e.g., [9]). Theoretically, the two-phase region is divided into stripe (S), hexagonal (H), and inverse hexagonal (H') phases bounded by coexistence regions as in Fig. 1(b) [6,12,13]. In an "equivalent dipole" model, a lipid molecule in a black or white domain has an average electrostatic dipole moment density m_B or m_W

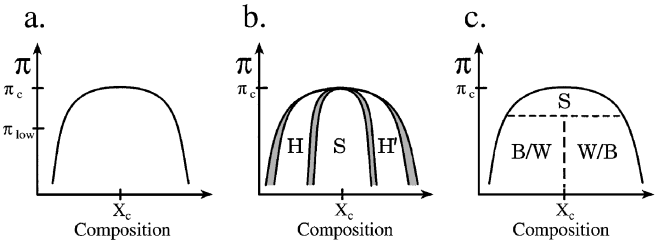

FIG. 1. Binary mixture with miscibility critical point. (a) Schematic phase diagram for a binary lipid mixture: Two liquid phases coexist up to the critical monolayer surface pressure Π_C at critical composition X_C. Within the two-phase region, a monolayer at $\Pi_{\text{low}} < \Pi_C$ separates into liquid domains designated white and black. (b) Schematic *theoretical* phase diagram of a cholesterol-phospholipid binary mixture: Below the critical pressure and within the two-phase region three superstructure morphologies arise from a competition between long-range dipolar forces and line tension. There are two phases of hexagonally packed circular domains (H and H') and a stripe phase (S) [12]. The boundary between the stripe phase and the hexagonal phases is adapted from [13]. Coexistence regions of adjacent phases are in grey. The equilibrium widths of the stripes and the radii of circles depend on the proximity to the critical point. (c) Schematic *experimental* phase diagram of cholesterol-phospholipid binary mixture: Stripes are observed only within a few dyn/cm of the critical point [5]. At lower surface pressures, domains are primarily black circles on a white background (B/W) or vice versa (W/B). Fingering, in which stripes emanate from circular domains, is observed in the transition between circular domains and stripes.

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oriented perpendicular to the air-water interface [6]. We assume that the difference in dipole densities, $m = m_B - m_W$, approaches zero at the critical pressure Π_C with the critical exponent β [9],

$$m = m_0\left(1 - \frac{\Pi}{\Pi_C}\right)^{\beta}. \quad (1)$$

The difference in dipole density at zero pressure m_0 is measured at pressures just above the gas phase (0–0.5 dyn/cm). The dipole density difference is proportional to the composition difference for ideal mixtures. Phase diagrams of a number of binary mixtures of phospholipids and cholesterol have yielded the critical exponent $\beta \approx 0.25$ [11]. This exponent is predicted to be 0.125 in two dimensions or 0.32 in three dimensions [14]. In the following, Eq. (1) is assumed to be valid from pressures near zero up to the critical pressure [11].

The dipole density difference and the line tension play against each other to determine the size of a domain. We define the van der Waals line tension λ as the dipole-independent component of the free energy that is proportional to the perimeter of a domain. The line tension λ can be related to the critical exponent μ, where λ_0 is the line tension at zero pressure [9],

$$\lambda = \lambda_0\left(1 - \frac{\Pi}{\Pi_C}\right)^{\mu}. \quad (2)$$

Experimentally, $\mu \approx 1$ for a DMPC and cholesterol mixture for a wide range of pressures [9]. Theoretically, it is predicted to be 1 (two dimensions) or 1.26 (three dimensions) [14].

A large dipole density difference $|m|$ favors elongated domains whereas a large tension λ favors compact domains. The energy per unit area E reflects this competition. For black stripes of width w, area fraction ϕ, and cutoff distance Δ, between molecular dipoles [6],

$$E = 2\left(\frac{\phi}{w}\right)\left\{\lambda - m^2 \ln\left[\frac{ew}{\Delta}\,\frac{\sin \pi\phi}{\pi\phi}\right]\right\}. \quad (3)$$

The energy is minimized to yield an equilibrium stripe width w [6] where,

$$w = \Delta\left(\frac{\pi\phi}{\sin \pi\phi}\right)\exp\left(\frac{\lambda}{m^2}\right). \quad (4)$$

Near a critical point, the areas of black and white domains are equal so that $\phi = \frac{1}{2}$ [6],

$$w = \Delta\,\frac{\pi}{2}\,\exp\left[\frac{\lambda_0}{m_0^2}\left(1 - \frac{\Pi}{\Pi_C}\right)^{\mu-2\beta}\right]. \quad (5)$$

Since $\mu > 2\beta$, $\lambda/m^2 \to 0$ as $\Pi \to \Pi_C$. This is the reason that, as the critical pressure is approached from a lower pressure, stripes become thinner as in Fig. 1(b). Experimentally, the stripe width w is measured as Π is varied.

Experiments.—In the experiments, mixtures are made from DMPC (66.9 mol%), Dchol (32.9%), and a small amount (0.2%) of fluorescent dye TR-DMPE (Texas red dimyristoylphosphatidylethanolamine). Dchol is used instead of cholesterol to minimize photo-oxidation of the monolayer. Domains rich in Dchol exclude dye and appear black. For this lipid mixture, there are equal areas of black domains on white backgrounds and white domains on black backgrounds so that $\phi = \frac{1}{2}$. Lipids were deposited from 1 mg/ml chloroform solutions on the air-water interface of a 9×3 cm^2 Teflon trough. DMPC (Avanti Polar Lipids, Alabaster, AL), Dchol (Sigma, St. Louis, MO), and TR-DMPE (Molecular Probes, Eugene, OR) were used without further purification. A minimal amount of TR-DMPE was used to reduce photo-oxidation as much as possible. Oxidation was insignificant for a monolayer in the dark. For a constantly illuminated monolayer of 65:35 DMPC:Dchol, photo-oxidation causes the critical pressure to increase on the order of 0.016 dyn/cm · min. Experiments were repeated 3 times and each completed within 15 min. Only the most uniform monolayers were analyzed. Stripe widths were inversely proportional to radii of k-space rings produced by Fourier transforms of 0.074 mm^2 fluorescence micrographs [15]. Errors in pressure and width were ± 0.01 dyn/cm and ± 1 μm.

Although stripes are theoretically stable for all pressures well below Π_C [Fig. 1(b)], experimentally they only appear close to the critical pressure as in Fig. 1(c) [4,5]. At lower surface pressures, domains are primarily black circles on a white background or vice versa. The radii of circular domains were not uniform and did not equilibrate on our experimental time scales of minutes.

Monolayers were compressed above Π_C to a homogeneous liquid phase. The trough barrier allowed leakage so the pressure slowly decreased $\sim$0.05 dyn/cm · min as measured with a Wilhelmy plate. As the pressure dropped below Π_C, stripe phases appeared and stripes became thicker. Approximately 0.15 dyn/cm below Π_C, domains became circular, because equilibration of domain areas is slow far from the critical point [7].

Close to the critical point, stripe domains pinch apart and rejoin frequently, freely exchanging material. At lower surface pressures below the critical point, domain areas cease to fluctuate on the time scale of experiments, although domain shapes and perimeters still undulate [16]. With further decrease of pressure, domains with a constant area evolve from long, thin stripes to form short, thick shapes. At pressure Π, which defines a stripe width $w(\Pi)$ as in Eq. (5), domains with areas smaller than $\pi[w(\Pi)/2]^2$ are circular, whereas larger domains are elongated. Determination of a width for a field of domains is limited at low pressures (large widths) by the formation of circular domains and at high pressures (small widths) by the microscope resolution.

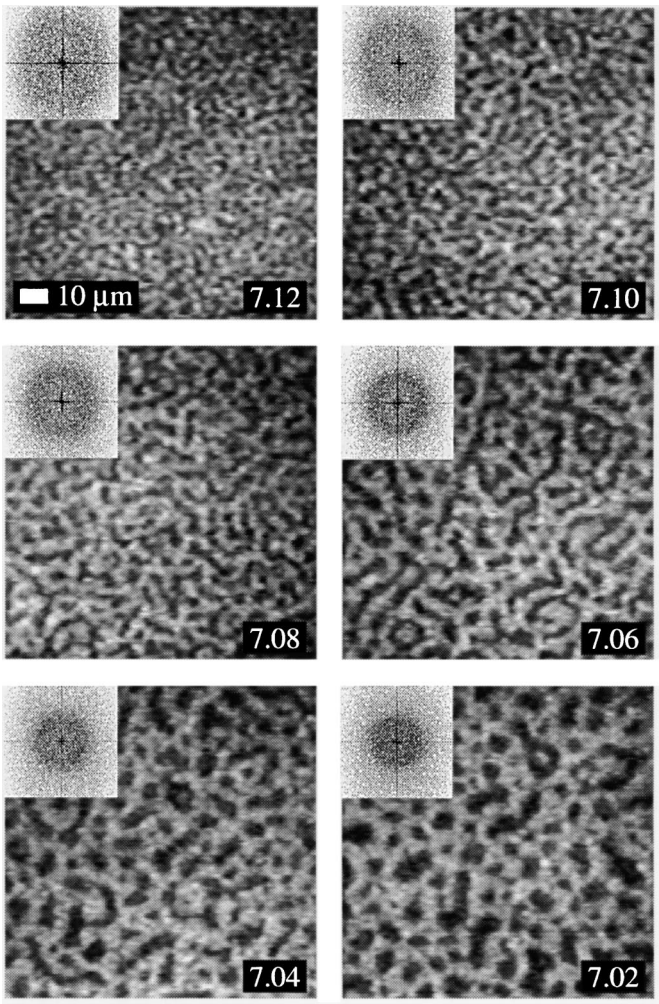

FIG. 2. Experimental determination of stripe widths: Micrographs of stripe phase domains at different surface pressures (inset values in dyn/cm). At high pressure ($\Pi = 7.12$ dyn/cm), near the critical point, stripes are thin. As pressure decreases, stripe widths increase. At $\Pi = 7.02$ dyn/cm many of the stripes become circular domains. Domains with larger areas are still elongated. Micrographs 4 times as large were used to create the inset Fourier transforms. Stripe widths are inversely proportional to the radii of the rings in the Fourier transforms as measured from the center to the peak intensity of the ring.

Results.—Fluorescence micrographs of the stripe phase are shown in Fig. 2. Starting above the critical pressure, the pressure was slowly decreased and labyrinthine stripes became visible. As the pressure fell, the stripe width increased until stripes were supplanted by circular domains. Values of the stripe width were found from 2D Fourier transforms of the micrographs (inset, Fig. 2). Figure 3 plots the stripe width versus the monolayer surface pressure for the experiment shown in Fig. 2. The experiment was repeated 2 more times. As in previous work, we have taken the dipole cutoff distance Δ to be 10 Å [9]. We use $\mu = 1.0$ and $\beta = 0.25$, based on experimental values of these critical exponents for a number of bi-

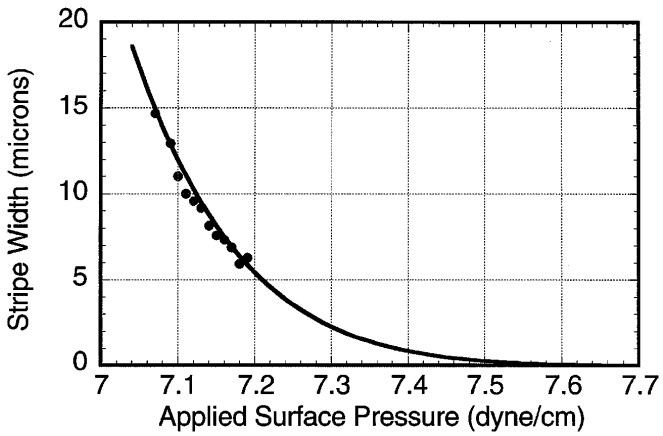

FIG. 3. Stripe width as a function of monolayer pressure Π: As monolayer surface pressure decreases, the widths of stripe domains increase. The data from the experiment in Fig. 2 (dots) are fit (line) to Eq. (6) and yield $\lambda_0/m_0^2 = 32.2$, $\Pi_C = 7.69$ dyn/cm. Other data sets are similar and do not show the dip at 7.1 dyn/cm.

nary cholesterol-phospholipid mixtures [11]. (The Dchol-DMPC phase diagram itself has a somewhat peculiar shape [10], but we have nonetheless used $\beta = 0.25$ in our calculations.) The data in Fig. 3 are fit to the equation

$$w = \frac{\pi}{2}\,(10^{-3}\ \mu\mathrm{m})\exp\left[\frac{\lambda_0}{m_0^2}\left(1 - \frac{\Pi}{\Pi_C}\right)^{0.5}\right]. \quad (6)$$

The fits to three data sets yield an average value of λ_0/m_0^2 of 29.7 ± 4.9. Fits of the data to Eq. (6) using $\mu - 2\beta$ in the range 0.3 to 0.7 give substantially the same value of λ_0/m_0^2 to within a factor of 2.

It is reassuring that the value of λ_0/m_0^2 obtained in the present work is close to a previous result for a mixture of DMPC and cholesterol, where it was reported that $\lambda_0/m_0^2 \approx 32$ to within an error of about a factor of 2 [9]. The tension term λ_0 was found by measuring the rate at which an elongated domain recovers its circular shape [9]. In this measurement line tension and dipolar forces compete with hydrodynamic drag. The dipolar term m_0^2 was found from measurements of the surface potential [10], Brownian motion [10], and electrophoretic mobility [17]. The close agreement in the values of λ_0/m_0^2 is probably fortuitous. We regard any agreement to within a factor of 2 to 3 to be satisfactory. The standard deviation of our measurements is 4.9. A larger source of uncertainty in λ_0/m_0^2 is the narrow useful pressure range in which the stripe phase is observed. That is, the stretch exponential of Eq. (6) is fit over only 12 values of the stripe width w that vary by less than an order of magnitude. If domains with very large areas could be prepared, then as the pressure is raised, the stripe phase should be seen over a larger pressure range. At the present time there is no adequate theory for the equilibrium distribution of the individual stripe areas near the critical point. The theory that leads

to Eq. (5) assumes that stripes are of an infinite length and therefore an infinite area. Near the critical point the aspect ratio of the stripes is large enough for this theory to apply.

The dependence of the stripe width on pressure in monolayers can be contrasted with the effect of magnetic fields on liquid ferromagnetic domains [18,19]. In the latter case, increasing field strength perpendicular to a thin film increases the magnetic dipole density, and presumably not the dipole-independent part of the line tension. With increasing field, magnetic domains form stripes, because the magnetic equivalent of λ/m^2 decreases because m^2 increases. In the monolayer case, both λ and m^2 decrease with increasing pressure, but λ decreases more rapidly than m^2.

Our evidence for the state of equilibrium of the stripe phase entails the following points. (a) The stripe widths are a reversible function of the monolayer pressure near the critical point. (b) Near the critical point, stripes freely exchange material with one another. (c) The observed widths conform to the theoretical scaling law in Eq. (5). (d) The magnitude of the observed widths agrees with numerical values of the several parameters in Eq. (5) obtained from independent measurements using different techniques.

In epifluorescence microscopy experiments, the critical pressure is usually quoted as the pressure at which it is no longer possible to distinguish white and black domains at the critical composition ($\sim$7.2 dyn/cm in Fig. 3). However, stripes which are thinner than the camera's resolution may persist to higher pressures, especially if λ_0/m_0^2 is small. Fitting Eq. (5) to the stripe widths is a useful way to extrapolate monolayer properties to the true critical point and to measure the critical pressure.

Phase diagrams of lipid mixtures offer one of the few methods available for the study of intermolecular interactions between lipid molecules. The appearance of the stripe phase by epifluorescence microscopy provides a useful diagnostic tool for finding critical points in the phase diagrams of complicated mixtures such as those found in biological membranes [4]. This is because the stripe phase is observed over only a small range of surface pressures and lipid compositions near the critical point. As shown in the present work, the appearance of a stripe phase conforming to the scaling of Eq. (5) provides evidence that these mixtures have reached chemical composition equilibrium.

We thank Jay Groves for helpful discussions. This work was supported by the NSF.

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