Critical shape transitions of monolayer lipid domains

(membranes/phase transitions/air–water interface/surface photochemistry)

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ABSTRACT Fluorescence microscopy can be used to visualize coexisting fluid phases in lipid monolayers composed of cholesterol and dipalmitoyl phosphatidylcholine under specified conditions of temperature, composition, and lateral pressure. At a critical composition of ≈ 30 mol % cholesterol, decreasing the average molecular area below $a_c \approx 50 \text{ \AA}^2$ per molecule forces the binary mixture through a critical point, where the monolayer becomes homogeneous. At molecular areas $\approx 10\%$ above this critical area, we observe shape transitions from liquid domains with circular shapes to domains with less symmetrical shapes. Shape transitions and critical shape fluctuations can also be triggered with light, due to photochemical effects on the monolayer. Shape fluctuations of lipid domains can thus be used to sense chemical events at the air–water interface.

Fluorescence microscopy has been used recently to show that binary lipid mixtures of dimyristoyl L- α -phosphatidylcholine ([Myr₂]PtdCho) and cholesterol can form immiscible fluid phases at the air–water interface (1). This result was anticipated by earlier spin-label studies of these lipids in bilayers (2). See Ipsen *et al.* (3) for leading references and theoretical models for the phase diagram of this binary mixture. The magnetic resonance and calorimetric data do not establish whether the coexisting liquids are in the same leaflet of a bilayer, or on different sides of the bilayer, or in different vesicle membranes. The fluorescence microscope results leave no doubt that two immiscible liquid phases can coexist in one monomolecular layer at the air–water interface (1). The present paper offers a preliminary physical chemical discussion of the properties of such coexisting fluid phases at the air–water interface.

The binary dipalmitoyl L- α -phosphatidylcholine ([Pam₂]PtdCho)/cholesterol mixture shows a mixing–demixing critical point. At a critical composition of ≈ 30 mol % cholesterol the two liquid phases, one rich in [Pam₂]PtdCho and one rich in cholesterol, merge into a single phase of uniform composition on increasing the lateral pressure. We expect lipid domains at the air–water interface to show a second type of critical point, which we term a *shape critical point* (4, 5). The mixing–demixing critical transitions and the shape transitions can be thought of as distinct transitions, at least under some conditions. The present paper develops this point. The present paper also describes a photochemical effect of light on lipid monolayers that can be used to induce shape transitions in individual lipid domains at the air–water interface.

BACKGROUND THEORY AND METHODS

Theory. Our earlier theoretical treatment of the shapes of solid lipid domains in a fluid lipid background was divided into two steps (4). We first assumed the solid and fluid phases to be incompressible, so that the area of the trough defines the relative proportion of solid and fluid in the monolayer. We then

included the long-range electrostatic intermolecular interactions to describe solid domain shapes and domain distributions that turn out to be a function of the fraction of the monolayer area that is solid (i.e., a function of monolayer compression). A related calculation has been made by Andelman *et al.* (6, 7); their calculation in addition treats a one-component monolayer system near its critical point in a Landau–Ginsberg approximation. The present approach is similar to our earlier calculation except that both coexisting phases in the monolayer are in the fluid state, and on compression of the monolayer both the compositions and densities of the two phases change. To simplify the discussion we consider a binary lipid mixture with the phase diagram illustrated in Fig. 1. [For a discussion of the phase diagram of cholesterol and [Pam₂]PtdCho, see Ipsen *et al.* (3).] This hypothetical mixture has a critical composition of $x_1 = x_2 = 0.5$ for the mole fractions of the two components. In a mixture of this composition, the relative proportions of the two phases remain constant as the temperature T is increased toward T_c , at which point the two phases merge to a homogeneous single phase. Following a familiar analysis (8), we let the chemical potential of component 1 in phase ν be μ_1^ν , where

$$\mu_1^\nu = \mu_1^0 + RT \ln x_1 + \alpha x_2^2 \quad [1]$$

and where μ_1^0 is the chemical potential of pure liquid 1. With this approximation for the two phases and two components one finds that the critical temperature is $T_c = \alpha/2R$. One can use the Gibbs–Duhem equation to show that

$$dT_c/d\pi = (1/2R)\Delta \quad [2a]$$

$$dT_c/d\ln A = (1/2R)(\Delta/A)(d\ln A/d\pi)^{-1}, \quad [2b]$$

where

$$\bar{A} - \bar{A}^{(0)} = x_1 x_2 \Delta \quad [3]$$

and $\bar{A}$ is the molar area of the mixture, and $\bar{A}^{(0)}$ is the molar area assuming ideal mixing. By this simple illustrative calculation we see how compressing or expanding a monomolecular film can raise or lower the critical temperature. In terms of this schematic model, the condensing effect of cholesterol on fluid phosphatidylcholine membranes corresponds to a negative Δ , and thus a decrease in T_c on compressing a monolayer. An order-of-magnitude estimate based on the molecular area data of Phillips (9) and the pressure–area data of Albrecht *et al.* (10) is that T_c changes by ≈ 10 K per $\text{\AA}^2$ change in molecular area!

Thus, if $T_c > T$, then as we increase the pressure, and lower the molar area $\bar{A}$, T_c approaches the bath temperature T from above. In a lipid mixture of critical composition, the compositions of the two phases approach one another. For $\bar{A}$ close

FIG. 1. Schematic phase diagram for a hypothetical binary lipid mixture with a miscibility gap and critical temperature T_c .

to $\bar{A}_c$ one expects critical point fluctuations associated with both variations in domain lipid composition as well as domain shape. Domain boundaries become diffuse and disappear. We refer to this critical point as the phase separation critical point.

It is convenient to define a second type of transition in monolayers with a critical point; this is the *shape transition*. We restrict this term to shape changes in lipid domains that have well-defined boundaries. For example, consider an isolated circular liquid domain surrounded by a second liquid. One can show that the equilibrium radius of this domain is given by the equation

$$R_{eq} = (e^3 \delta / 4) \exp(\lambda / \mu^2), \quad [4]$$

where λ is the line tension of the domain boundary, μ is the difference in dipole densities in the two fluids, and δ is the nearest neighbor dipole-dipole distance (4, 5). If for some reason the radius R of a circular domain becomes larger than R_{shape} , where

$$R_{shape} = e^{1/3} R_{eq}, \quad [5]$$

then the circular shape is unstable with respect to a transition to an elliptical shape (4, 5). Shape transitions are expected under nonequilibrium conditions. For example, if a circular lipid domain grows in size so that its radius R becomes larger than R_{eq} , and larger than R_{shape} , one predicts the onset of a shape transition. Under equilibrium conditions, shape transitions are not expected to be observed since $R_{eq} < R_{shape}$. However, if μ and the ratio λ/μ become small as T_c decreases, thermal fluctuations in equilibrium domain size can bring a domain into the region of critical shape fluctuations. To see this, consider two circular domains of equilibrium areas $a_o = \pi R_{eq}^2$, and consider a fluctuation so that the areas of these domains change to $a_o + \alpha$ and $a_o - \alpha$. The free energy of this fluctuation ΔF is

$$\Delta F = \left(\frac{\pi}{2} \right) \mu^2 R_{eq} \left(\frac{\alpha}{a_o} \right)^2 + \dots \quad [6]$$

The amplitude of this fluctuation reaches the critical radius R_{shape} when $(\alpha/a_o) = e^{2/3} - 1$, and thus the condition for critical shape fluctuations is

$$kT \sim \left(\frac{\pi}{2} \right) \mu^2 R_{eq} (e^{2/3} - 1)^2 + \dots \quad [7]$$

This condition is satisfied with plausible order-of-magnitude values of the parameters, $T = 300$ K, $R_{eq} \sim 10 \mu\text{m}$, and a

dipole density difference corresponding to 0.01 Debye per $100 \text{ \AA}^2$. Critical shape fluctuations should be distinguished from ordinary thermal fluctuations by their large amplitudes and low frequencies. We expect that as one approaches the region of critical shape fluctuations the microscopic appearance of the monolayer should be especially sensitive to chemical effects that change the dipole density difference, the line tension, and/or the average area per molecule.

Methods. The experimental set-up and methods used are similar to those described (1). The experiments employed binary mixtures of [Pam₂]PtdCho or [Myr₂]PtdCho and cholesterol (30 mol %), using 1-palmitoyl-2-[N-(7-nitro-2,1,3-benzoxadiazol-4-yl)aminocaproyl] phosphatidylcholine (NBD-PtdCho) as the fluorescent lipid probe (≈ 2 mol %), layered on distilled water. Experiments were carried out by compressing the monolayers until the gas-phase regions disappeared, so that only the two liquid phases were present. The monolayers were then further compressed slowly so as to lower the critical temperature. Shape transitions are then observed throughout the monolayer.

FIG. 2. Dependence of domain shapes on average molecular area in binary monolayer mixtures of [Pam₂]PtdCho (68 mol %) and cholesterol (30 mol %) at the air-water interface. The average molecular areas are $57 \text{ \AA}^2$ (A) and $51 \text{ \AA}^2$ (B). The fluorescent probe is NBD-PtdCho (2 mol %). Temperature is 21°C . Domains x, y, and z show transitions to elliptical (elongated) shapes, on which are superimposed shorter wavelength deviations from circular symmetry. The dark domains are thought to be the cholesterol-rich domains. (Bar = $100 \mu\text{m}$.) Illumination time is ≈ 5 sec for B. At the larger average area $57 \text{ \AA}^2$ per molecule in A, the shapes are not sensitive to light.

FIG. 3. Shape transitions of fluid lipid domains triggered by photochemical effects of light. (A–E) Photographs are of the same field and are separated in time by 7–8 sec. Light source is a 50-W microscope mercury lamp, followed by a Zeiss heat filter, a KG-1 heat filter, and a 430- to 500-nm band pass filter. The barrier filter blocks out radiation below 510 nm. (F) Photograph was taken after 2 min in the dark following the photograph in E. The photograph in F thus illustrates the reversibility of the shape change. The monolayer is composed of 68 mol % [Pam₂]PtdCho, 30 mol % cholesterol, and 2 mol % NBD-PtdCho. The average molecular area is 52 Å² per molecule. (Bar = 100 μm.)

Pressure-induced transitions were also reported in earlier work, but the published photographs do not distinguish clearly between phase separation critical points, where domain boundaries are diffuse and shape transition critical points (1). In the present work, domains undergoing shape transitions have sharp, well-defined domain boundaries. At higher monolayer pressures, domain boundaries do become diffuse, when the phase separation critical point is reached. In other words, the shape transition critical points and phase separation critical points can be distinguished experimentally.

RESULTS AND DISCUSSION

Fig. 2 illustrates the effect of increasing pressure π on a lipid monolayer composed of 30 mol % cholesterol, 68 mol % [Pam₂]PtdCho, and 2 mol % of NBD-PtdCho. The shape transitions seen here are, as expected, more complex than the simple circle-to-ellipse transition discussed previously. This is because the domains are not isolated electrostatically from one another and also because the ellipse is only one of a

number of shapes into which circular domains may distort. For a discussion of closely related shape transitions in ferrofluids, see Tsebers and Maiorov (11). It is clear from these results that increasing the pressure does bring the system through the critical region, as suggested by our simple model ($\Delta < 0$, $dT_c/d\pi < 0$). The negative value of Δ corresponds to the “condensing effect” of cholesterol (3).

We observed that when the monolayer is close to critical shape region, the radiation from the microscope lamp affects domain shapes. Thus, for the photographs in Fig. 2, domains were illuminated only for the time (≈ 5 sec) required to take a photograph. Specifically, the circular domains in Fig. 2A at an average area of 57 Å² per molecule are not noticeably affected by the illuminating radiation, whereas at lower areas per molecule, 51 Å² per molecule, the shapes in Fig. 2B are extremely sensitive to radiation. The photograph in Fig. 2B was taken in the shortest possible time to minimize this effect (5 sec = focus time plus exposure time).

Fig. 3 illustrates the effect of illumination on the shapes of the lipid domains at an average molecular area of 52 Å² per molecule, which is just at the onset of shape transitions. Fig.

3 A–E show progressive shape changes due to illumination. We attribute these changes to photochemical effects of the radiation that lead to changes in the dipole density difference μ , or the line tension λ , the sense of these changes being to increase μ and/or decrease λ . Another possibility is that a local pressure change (shock wave) is produced that acts to reduce a local T_c . The same shape changes can also be produced by a focused 488-nm argon ion laser beam, and the rate of shape change increases with increasing laser power. The photograph in Fig. 3F shows the reversal of the shape transition, when the monolayer is in the dark for 2 min. This reversal of the shape change may be due to the diffusion of photochemical products away from the region that was illuminated. If the photochemical products are dissolved in the monolayer they can diffuse out of a region 100 μm in diameter in about 2.5 min if they have diffusion coefficients typical of lipids in fluid monolayers ($10^{-7} \text{ cm}^2/\text{sec}$). Photochemical processes at the air–water interface have been described by Möbius (12). These photochemical effects at the air–water interface produce pressure changes (shock waves) that also have relaxation times of the order of a minute.

Throughout this present paper we have discussed shape transitions and shape fluctuations in terms of isolated, non-interacting lipid domains. It is evident from the microphotographs of the monolayer domains, as well as theoretical considerations (4, 6, 13–16), that under most experimental conditions, the lipid domains strongly repel one another. Thus, the shape of one domain can affect the shape of neighboring domains. The entire monolayer is a strongly interacting, highly cooperative system. It remains to determine the role critical shape fluctuations play in cooperative shape transitions in monolayers, such as the transition between hexagonal arrays and stripe arrays (6, 7, 13). Irrespective of this theoretical issue, it is clear that critical shape transitions can be used as sensitive indicators of chemical events at the air–water interface.

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