Entropy-Driven Tension and Bending Elasticity in Condensed-Fluid Membranes

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Sensitive micropipet methods have been used to measure the relation between tension and the projected surface area in fluid membranes of vesicles over a 4-order-of-magnitude range in tension (10^{-3}–10 dyn/cm). In the low-tension regime (< 0.5 dyn/cm), the data confirm the prediction of equilibrium theory that the projected area should increase logarithmically with tension as shape fluctuations become progressively restricted. The slope of ln(tension) versus area dilation yields the elastic bending modulus of the membrane. In the high-tension regime, the projected area crosses over to vary linearly with tension due to direct expansion of area per molecule.

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In recent years, it has become apparent that thin condensed-fluid membranes behave as 2D generalizations of linear flexible polymers.[1] Random geometric conformations of the surface are used to define a configurational entropy which can be a dominant portion of the free energy in many situations. As for a polymer chain, a highly flexible surface is predicted to form compact "crumpled" shapes[2] at high temperatures (although there is some question about this prediction if conformations can only be achieved by "self-avoiding" displacements[3]). Likewise, lateral restriction of conformations (e.g., fixing the edges of the membrane to rigid walls) will reduce the configurational entropy and thus create an average lateral tension in the surface which must be supported by the boundaries or maintained by hydrostatic pressure on a curved portion of the surface. When the boundaries are displaced to extend the membrane, the tension is expected to increase and eventually reach a level sufficient to reduce the surface density by direct expansion of area per molecule. Hence, macroscopic observations of tension as a function of "apparent" (projected) surface area represent elastic compressibility relations for membranes in which the direct elastic area compliance is "renormalized" to some extent by thermal fluctuations. This behavior has been predicted by Helfrich and Servuss[4] with an equilibrium theory for bending elastic excitations of a planar membrane. Although tension in the model (and similar developments for quasispherical excitations of membrane vesicles[5]) has been invoked to improve empirical correlations of dynamic shape fluctuations,[6] there has been no direct verification of an entropy-driven tension in membrane surfaces or equilibrium aspects of the theory. Here, we report direct measurements of tension in single membranes as a function of lateral restriction over a 4-orders-of-magnitude range in tension that covers the crossover from an entropy-dominated surface compressibility to the direct elastic compliance. The results demonstrate elastic reversibility and agree with the functional prediction from equilibrium theory. Correlation with theory yields values of elastic bending (curvature) moduli which were determined for several types of lipid bilayer membranes.

In theoretical developments, two types of fluid membranes have received the most attention: "tethered" membranes,[2,3] which are compressible surface "nets," where direct interactions are dominated by confining pair potentials between surface "atoms" and dipole-dipole potentials are introduced to create bending rigidity; "continuous" membranes,[4,5] which are assumed to be nearly incompressible with large resistance to surface dilation, where direct interactions are represented by continuous energies for bending and expansion elasticity. Monte Carlo simulations of tethered-membrane capsules[2] have demonstrated that inflation of capsules required positive internal pressures as the shapes approached circular cross sections where conformations were tightly restricted. For infinitely flexible membranes (no bending rigidity), the results[2] implied that tension in the surface should increase in proportion to the projected-perimeter length raised to a power of about 3. By comparison, analyses of thermal undulations in continuous-membrane sheets[4] and vesicles[5] predict that the entropy-driven tension should increase exponentially with expansion of the projected surface area when fluctuations are strongly restricted. Here, the exponential rate or "stiffness" is proportional to the bending rigidity of the membrane.

Because molecularly thin membranes are extremely flexible, measurement of bending rigidity has not been simple or direct. The established method is to record a series of microscope images of a fluctuating vesicle over time, Fourier analyze the shapes to obtain the mean-square amplitudes $\langle u_q^2 \rangle$ of normal-mode displacements (relative to a fixed spheroidal shape) as a function of wave number q, and then correlate the results with the prediction from equipartition in the equilibrium theory.[6] To first approximation, the product $q^4 \langle u_q^2 \rangle$ should be constant and inversely proportional to the bending modulus k_c.[4–6] When the measured values for this product are not equal, then a tension parameter is introduced to improve the correlation. The tension reduces the amplitudes predicted for long-wavelength undulations relative to shorter-wavelength excitations. With the experiments reported here, the bending moduli are determined

directly from the ln(tension) versus projected area in the low-tension regime.

The experimental method[7] used here is very simple: A large membrane vesicle[8] is first slightly deflated from a spherical shape and then aspirated into a small-caliber suction pipet (Fig. 1). Because of the restricted permeability of the diacyl lipids used to form these membrane vesicles and the strong osmotic activity of the inside-outside solutions (which oppose displacement of water across the capsule surface), the volume encapsulated by the membrane remains constant throughout the aspiration process (to better than 1/1000). Consequently, the length of the vesicle projection inside the pipet provides a direct measure of the projected area of the vesicle. Likewise, diacyl-lipid surfactants have such low solubility in the aqueous environment that the membrane surface remains closed to exchange of amphiphiles with the aqueous environment. Thus, changes in length of the vesicle projection in the pipet represent expansions of the projected area due to reduction of surface undulations and direct dilation of area per molecule.

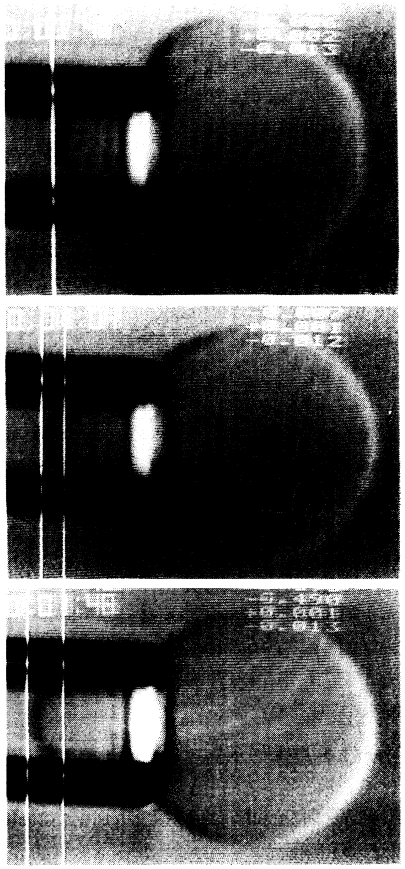

FIG. 1. Videomicrographs of vesicle pressurization (vesicle diameter ~ 20 μm, pipet caliber ~ 8 μm). (a) Lowest suction pressure, tension $\lesssim 4\times 10^{-4}$ dyn/cm. The vesicle contour fluctuates and deviates from a spherical shape. (b) Suction increased to produce ~ 0.01-dyn/cm tension. (c) Suction again increased, tension ~ 0.2 dyn/cm. Cursor lines mark the displacements produced by suction [e.g., about a 1% increase in projected area from (a) to (b) and similarly from (b) to (c)].

The membrane slides freely along the pipet wall but the vesicle is pressurized by the pipet suction so that the membrane forms an effective seal against the pipet wall (with a lubrication gap of few a nm). For fluid membranes, the (time-averaged) membrane tension $\bar{\tau}$ is uniform over the entire surface and easily calculated from the suction pressure ΔP when the segment of the vesicle exterior to the pipet is nearly spherical,[7] i.e.,

$$\bar{\tau} = \frac{\Delta P R_p}{2(1 - R_p/R_0)}, \tag{1}$$

where R_p and R_0 are the pipet and exterior segment radii, respectively. The experimental procedure is to initially determine the stable projection length in the tube at the lowest suction pressure (10^{-6} atm), which is the limit of experimental resolution. Then, the suction is increased to extend the projection length which expands the projected area of the capsule relative to the "zero-tension" state. Simple geometry shows that the increase in projection length ΔL represents the areal strain α (when normalized by the projected area at the zero-tension state) as approximated by

$$\alpha \simeq \tfrac{1}{2}[(R_p/R_0)^2 - (R_p/R_0)^3]\Delta L/R_p . \tag{2}$$

As shown in Fig. 1(a), the segment of the vesicle exterior to the pipet initially exhibits deviations from a spherical form; these undulate with time. The fluctuations are clearly visible when the suction pressure is within a few times the resolution level or equivalently for tensions up to $(2\text{-}3)\times 10^{-3}$ dyn/cm. When the suction is increased to produce tensions of 10^{-2} dyn/cm (or greater), the vesicle segment exterior to the pipet appears to be a smooth sphere at the level of optical detection as shown in Fig. 1(b). However, a significant area can still be drawn out from microscopic undulations as the tension is increased by another 2 orders of magnitude [note the change in projection length between Figs. 1(b) and 1(c)]. Figure 2(a) presents plots of ln(tension) versus area dilation from single vesicle tests for lipid bilayers with two different surface compressibilities.[9] Figure 2(b) shows the same data for tension versus area dilation plotted on a linear scale. The crossover from the exponential regime to the linear regime occurs at comparable levels of tension (~ 0.5 dyn/cm) because the ratio of the two slopes (linear to logarithmic regimes) is not very different for the two types of membranes.

In direct analogy to the statistical mechanics of linear flexible polymers, analyses of fluid-membrane fluctuations involve the assumption that fluctuations in microscopic surface density can be neglected and that conformations are determined by surface undulations just as axial vibrations of monomeric segments within polymers are neglected in the description of random walks of a po-

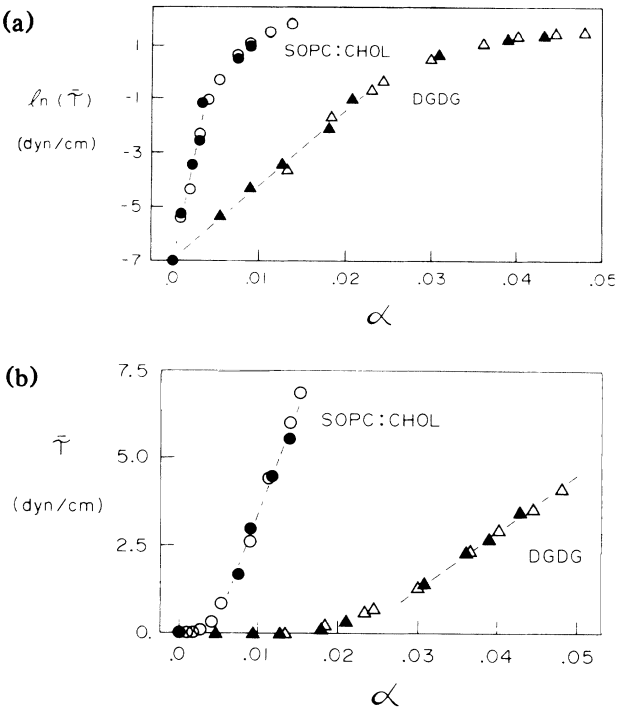

FIG. 2. Measurements of membrane tension and fractional area dilation for two vesicles with different lipid compositions. (a) A fluctuation-dominated regime appears at low tensions: a slope of $8\pi k_c/kT$. (b) Crossover to direct expansion at high tension: a slope of $\bar{K}$. (Open symbols, ascending pressure; solid symbols, descending pressure.)

lymer chain. For lipid bilayers, this is reasonable because the lifetime of molecular surface-density fluctuations is on the NMR time scale (10^{-6} sec or less), whereas long-wavelength undulations have decay times of fractions of a second.[10] Consequently, the area compressibility is approximated by the superposition of an area increase due to reduction of membrane undulations *plus* a direct expansion in area per molecule.[4] When the reference state is chosen as zero tension, both analyses of quasispherical vesicle excitations[5] and the plane-wave approximation[4] yield the same form for area dilation as a function of tension given by

$$\alpha \simeq (kT/8\pi k_c)\ln(1+c\bar{\tau}A)+\bar{\tau}/K_a\,, \qquad (3)$$

where k_c and K_a are the elastic moduli for bending and direct area expansion, respectively. The coefficient c for tension inside the logarithm is $1/\pi^2$ in the plane-wave approximation and $1/24\pi$ in the quasispherical approach. The lead term in Eq. (3) is derived from equipartition of energy among random normal modes of elastic excitation of the membrane where the Hamiltonian is taken as the surfaces integral of a continuous bending elastic energy density. In these analyses,[4,5] the tension is effectively a "chemical-potential" conjugate to the projected area of the surface and, thereby, is used to establish the projected area relative to a reference state.

In the low-tension regime [cf. Fig. 2(a)], the prediction of the equilibrium theory for "continuous" membranes correlates with the elastic response observed in these vesicle-pressurization experiments. It is important to emphasize that the measurements of tension versus projected area were taken from static pressures and stationary projection lengths inside the pipet, for both increasing and decreasing pressures. Thus, the work required to restrict membrane confirmations and to expand the area per molecule was reversible. As given by Eq. (3), the slope of the ln(tension)-versus-area-dilation plot is proportional to the elastic bending modulus, i.e., $8\pi k_c/kT$. After crossover to the high-tension regime, the slope of the tension versus area dilation approaches the direct elastic expansion modulus K_a. Table I cumulates measurements of elastic moduli for bilayers made with five different lipid compositions. These equilibrium measurements of elastic bending moduli are consistent with values obtained from Fourier analysis of dynamic fluctuations in vesicle shapes.[6] Inspection of the elastic moduli given in Table I clearly shows that bilayers with lower area compressibility are also less flexible. Predictions from simple mechanics indicate that the bending rigidity should scale as the area-compressibility modulus times the membrane thickness squared ($k_c \sim K_a h^2$).[10] Neglecting thickness variations, there is a consistent hierarchy (but not exact proportionality) among the lecithin (DAPC, DMPC, and SOPC) and lecithin:cholesterol (SOPC:CHOL) membranes. However, the sugar-lipid membrane (DGDG) is somewhat more flexible than implied by the area elastic modulus (also noted in a recent work by Mutz and Helfrich[6]). The obvious feature is that lipids with high degrees of unsaturation (e.g., DAPC and DGDG) produce very flexible membranes. The implication is that chain-packing irregularities and chain flexibility act to diminish the membrane rigidity relative to membranes made from more saturated lipids.

In addition to the initial entropy-dominated tension, microscopic fluctuations persist at all levels of tension which leads to a "renormalization" of the direct elastic compliance. The "apparent" surface-expansion modulus $\bar{K}$ is approximated by

$$\bar{K}/K_a \simeq (1+K_a kT/8\pi k_c \bar{\tau})^{-1}\,, \qquad (4)$$

TABLE I. Measurements of elastic moduli of bilayers.

Lipid[a]	k_c (10^{-12} dyn cm)	$\bar{K}$ (dyn/cm)
DAPC (18 °C)	0.44 ± 0.05	135 ± 20
DGDG (23 °C)	0.44 ± 0.03	160 ± 7
DMPC (29 °C)	0.56 ± 0.06	145 ± 10
SOPC (18 °C)	0.90 ± 0.06	190 ± 10
SOPC:CHOL (15 °C)	2.46 ± 0.39	640 ± 32

[a]Reference 9.

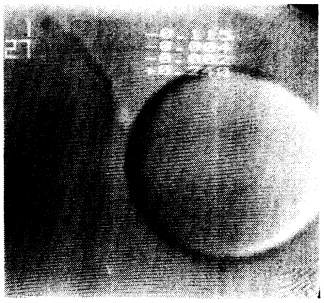

FIG. 3. Videomicrograph of a vesicle deflated to produce excess area.

which shows that the crossover from the fluctuation-dominated regime to direct expansion is determined by the ratio of elastic moduli, i.e., K_a/k_c. Based on the superposition of area dilations implicit in Eq. (4), the values deduced for the direct elastic expansion moduli of these lipid bilayers are about 10%–20% greater than slopes measured in the high-tension regime.

Even though these measurements support the predictions of equilibrium theory for a quasispherical excitations of vesicles,[5] we have found a significant deviation. When vesicles are deflated so that more than 3% excess area is available for large-scale undulations, then vesicle shapes are usually not stable as ellipsoids which is predicted by theory[5] (where the ellipsoidal shape implies a state of negative or compressive tension). Instead, vesicle surfaces become unstable and erupt to form an "archipelago" of satellite vesicles connected by microscopic umbilical tubes (Fig. 3). The vesicle surface remains topologically continuous and can be reinflated to a spherical form with full recovery of the appendaged surfaces. Unless supported or deformed by adjacent rigid structures, lipid bilayer vesicles prefer to stay closely spherical in shape and expel redundant area into either attached intravesicular or extravesicular bodies.

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[9]Five types of diacyl lipids were used to make bilayers, chosen to cover a wide range of surface compressibility: DAPC (diarachidonyl phosphatidyl-choline), DMPC (dimyristoyl phosphatidylcholine), SOPC (1-stearoyl-2-oleoyl phosphatidylocholine), SOPC:CHOL (a 1:1 mixture of SOPC and cholesterol), and DGDG (diagalactosyl diacylglycerol).

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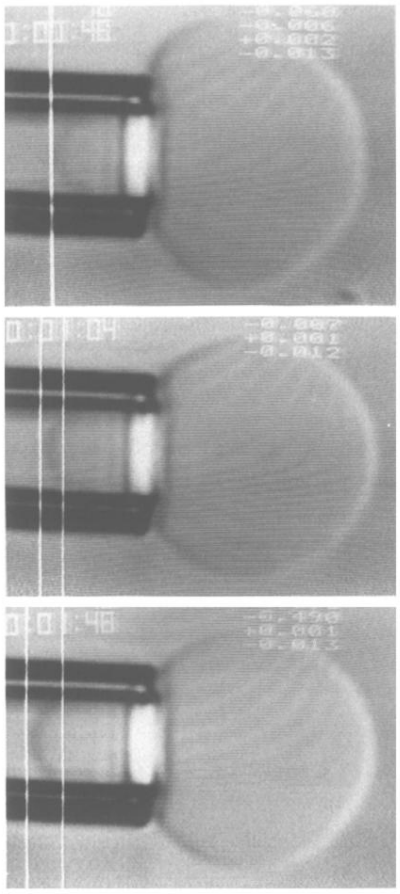

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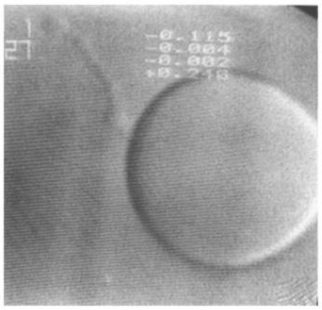

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