Experimental and Theoretical Study of Toroidal Vesicles

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We report the observation of toroidal and higher genus vesicles of diacetylenic phospholipids, a class of polymerizable amphiphiles. When unpolymerized, the vesicles exhibit different toroidal shapes in quantitative agreement with recent theoretical predictions. When partially polymerized, only a specific family of shapes has been observed: the Clifford torus or the branch of nonaxisymmetric shapes obtained by its conformal transformations. Assuming that partially polymerized vesicles are permeable on short time scale, we give a physical explanation of our findings. We also report the results of a variational calculation which approximates the nonaxisymmetric shape problem for finite spontaneous curvature.

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When dispersed in an aqueous solution, phospholipid molecules form giant unilamellar vesicles of typical size $R > 1$ μm. A conceptual framework for the study of their shapes was set by Canham [1], Helfrich, and others, who formulated the shape problem as the minimization of a curvature Hamiltonian under the constraints of constant area and volume. Following the work of Deuling and Helfrich [2] on the morphology of red blood cells, recent work has focused on vesicles of spherical topology (genus zero). In these studies, theoretical phase diagrams for shapes calculations have been confronted with experimental observations [3].

In this Letter we report the first observations of *fluid vesicles of nonzero genus* made from a polymerizable phospholipid and we contrast them with partially polymerized systems [4]. The shapes observed are in quantitative agreement with recent theoretical predictions [5–7] where the constraints select the minimum energy shapes. Partially polymerizing the membrane, however, has the rather surprising effect of selecting one generic class of toroidal shapes. In that case, we have only observed [4] the so-called Clifford torus or its conformal transforms, a branch of the Dupin cyclides [4,7,8]. Considering that partially polymerized vesicles are more permeable than unpolymerized ones in the early stages of their formation, we give a heuristic explanation of our findings, showing that partially polymerized vesicles select the shape with absolute minimum bending energy available for a toroidal topology [4,9]. Proceeding further, we include a stability analysis of the Clifford torus where it is shown that a positive spontaneous curvature favors nonaxisymmetric solutions akin to the Dupin cyclides. Finally, we derive a variational ansatz to approximate these nonaxisymmetric shapes at constant area and volume and show that the absolute energy minimum is obtained for axisymmetric shapes.

The polymerizable phospholipids used in our experiments $DC_{23}PC$ [1,2bis(10,12-tricosadinoyl)-*sn-glycero*-3-phosphocholine] were purchased from Avanti Polar Lipids as a crystalline powder. A small amount (~10 mg) of the slightly hydrated powder was spread on a glass plate (Petri dish) to obtain a thin lipid film. Water was added, at $T = 50\,^{\circ}C$ (melting temperature, $T_m = 43\,^{\circ}C$ [10]), to allow the film to swell. After several hours, some μl of the solution containing the swollen *unpolymerized* lipid were introduced by capillarity in a microchamber (typical thickness about 100 μm) which was then sealed and placed in a temperature-controlled oven on a microscope stage [4]. For the observations an inverted phase contrast microscope (Nikon) was used. Membranes are seen in the focal plane when they are parallel to the optical axis of the microscope.

Though long tubular fluid vesicles predominated, we also observed circular toroidal vesicles with reduced volume $v \equiv 6\sqrt{\pi}V/S^{3/2} \leq v_{\text{Clifford}}$ and nonaxisymmetric tori with $v > v_{\text{Clifford}}$ (see Fig. 1). These shapes can be obtained by minimizing the curvature energy under the

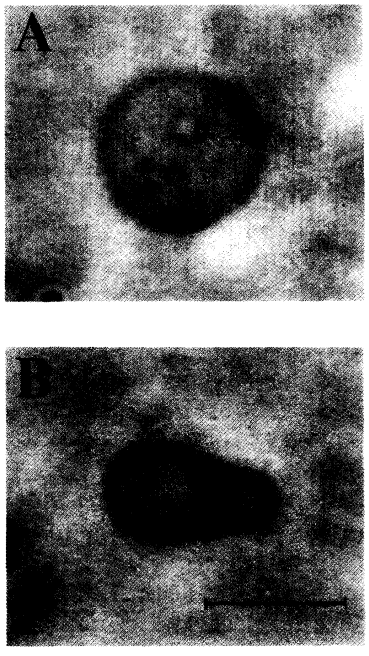

FIG. 1. Nonaxisymmetric *unpolymerized* fluid toroidal vesicle; (a) top view, (b) side view. The bar indicates 10 μm. The reduced volume $v \approx 0.77$.

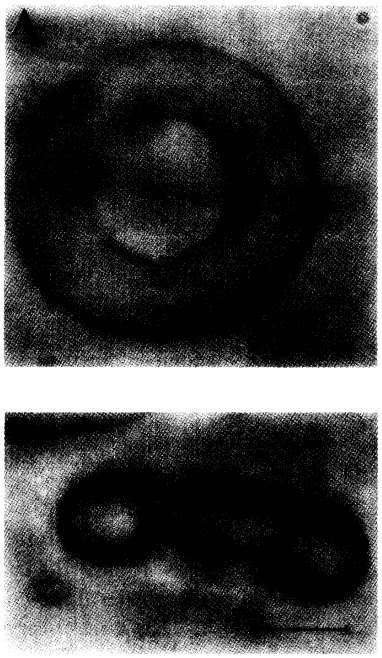

FIG. 2. Two views of a toroidal *unpolymerized* fluid vesicle obtained from the film swelling method at $T=50\,^{\circ}\mathrm{C}$; (a) top view, (b) side view. The bar indicates 10 μm. The reduced volume $v \approx 0.5$.

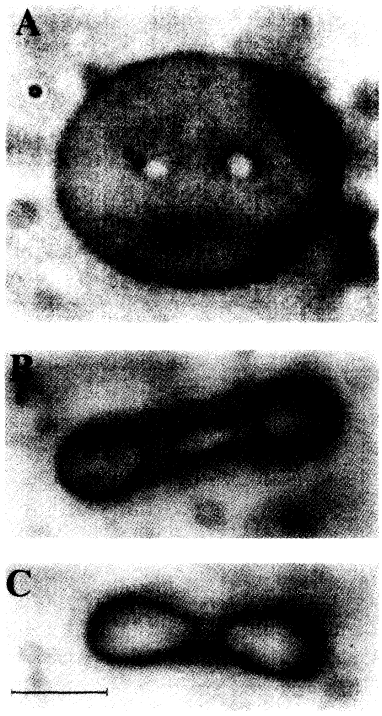

FIG. 3. An *unpolymerized* fluid two-holed torus. (a) Top view; (b) side view—cut through the two holes; and (c) side view—cut perpendicular to the line joining the two holes. The bar indicates 10 μm.

constraints of constant volume V and area S [6]. However, it is interesting to notice that the toroidal vesicle of Fig. 2 is apparently only a local minimum of the energy, since for this value of v minimization the bending energy yields sickle-shaped tori [6]. We did not observe the formation of these toroidal vesicles, but we did see tubular vesicles forming ring-shaped structures. Although, the final step of fusion of the ends of these tubes has not been observed, they could present a preliminary stage in the formation of fluid toroidal vesicles. Vesicles of genus 2 were also observed [Fig. (3)]. There are as yet no shape calculations for vesicles of this genus to compare our observations with, but Willmore surfaces of genus 2 are reported in the mathematical literature [9].

Partially polymerized toroidal vesicles were described previously [4]. The major observational difference with the tori reported here is the apparent selection of the toroidal shape which is close to either a Clifford circular toroid with reduced volume $v=v_C$ [5], or, less often, to its conformal transforms, nonaxisymmetric tori known as the Dupin cyclides [6,7]. As argued below this observational difference can be attributed to the different mechanisms of formation of these vesicles.

When unpolymerized, vesicles are obtained by the gentle swelling of a lamellar phase with many defects. As the lamellas peel off, they may form vesicles whose topology is related to the topology of the defects in the lamellar phase, but in any case the vesicles thus obtained are fluid and their area and volume is constant.

Partially polymerized vesicles are obtained by polymerization of the ordered phase of the diacetylenic molecules at $T < T_m$. This phase is achieved by cooling down an unpolymerized vesicular solution below T_m. As the molecules order in an $L_{\beta'}$ phase, the membrane of the vesicle warps around to form long soda-straw-like structures [11]. These double-membrane long needles are obtained after *squeezing out* the water inside the vesicles. During that process the membrane is thus *pierced* at least at one place. In this solid phase the membrane is first partially polymerized (the polymerization percentage $<40\%$ is below percolation) and the solution is heated to a temperature $T > T_m$ where the needles *inflate* to reform flaccid fluctuating vesicles. This inflation stage, during which the *vesicular volume is unconstrained,* lasts a few minutes and ends when the free monomers have reverted to the fluid state, allowing the closing of the membrane pores. The reduced volume v of the vesicle is then fixed.

The following is a plausible explanation of these experimental findings. Since the volume constraint is relaxed in the early stage of partially polymerized vesicle formation, the system can settle at the absolute minimum of the bending energy

$$H = \tfrac{1}{2} k_c \int\int (c_1 + c_2 - c_0)^2 dS\,, \qquad (1)$$

where $\frac{1}{2}(c_1+c_2)$ is the mean curvature and c_0 is the spontaneous curvature. Being only interested in shapes of toroidal topology, we have dropped out the constant Gaussian term. For $c_0=0$, the absolute minimum of (1) is the so-called Clifford torus or due to the conformal invariance of (1) [6,7] its conformal equivalents, the Dupin cyclides [12].

Since partially polymerized membranes when heated back to the fluid state increase their volume to reach equilibrium, the first equilibrium shape accessible to the system corresponds to the absolute minimum of (1) with the smallest reduced volume v: This shape is the Clifford torus, see Fig. 4(a). However, if the kinetics which governs the plugging of the pores is sufficiently slow, thermally induced conformal fluctuations [7] can drive the hole off center, so that the final (fixed volume) is locked on a Dupin cyclide with a small eccentricity.

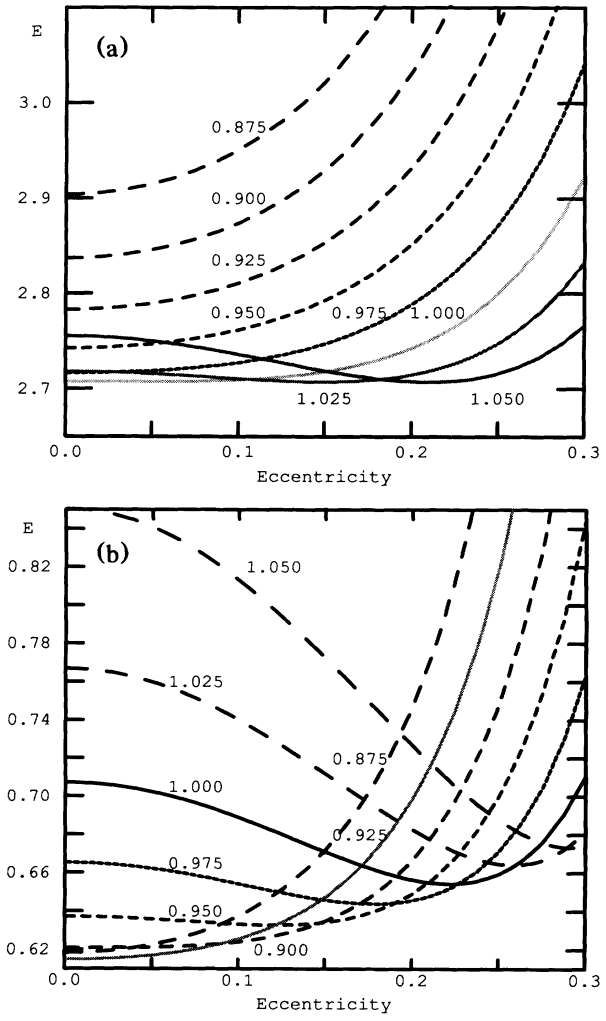

FIG. 4. Energy $E=H/2\pi^2k_c$ vs eccentricity for the variational ansatz at (a) $c_0=0$ and (b) $c_0=1$. The various curves correspond to different reduced volumes (v/v_C) for a given area $S=2\sqrt{2}\pi^2$. (a) Notice that the minimal energy is the same for all $v\geq v_C$. This degeneracy is suppressed when $c_0\neq 0$, see (b). (b) Notice that the curve corresponding to $v=v_C$ has a nonaxisymmetric minima, but that the absolute minima (for free volume) is still axisymmetric.

An effect, previously neglected, is the possible induction of a mean spontaneous curvature $c_0\neq 0$ by partial polymerization [4]. In the following, we show that a nonzero spontaneous curvature does not modify the conclusions of the preceding discussion and may also induce a symmetry-breaking transition to a nonaxisymmetric torus. For $c_0\neq 0$, Ou-Yang Zhong-can [5] has shown that the symmetric Clifford torus is still a stationary solution of the shape problem, but by studying its stability to shape deformations, we shall see below that for $c_0>0$ it is unstable with respect to a shift of the hole [6].

Let us consider azimuthal deformations of the toroidal shape $\eta=\eta_0[1+a_m\cos(m\phi)]$ with η parametrizing the toroidal surface $\mathbf{R}$ as in Ref. [4] ($\sinh\eta_0=1$ for the Clifford torus): $\mathbf{R}=[\sinh\eta\cos\phi,\ \sinh\eta\sin\phi,\ \sin\theta]/(\cosh\eta-\cos\theta)$. The $m=0$ term corresponds to a breathing mode, but the $m=1$ component moves the hole off center. For a permeable membrane, i.e., the relevant limit in the inflation stage, the second variation of the appropriate free energy reads as [13]

$$\left.\frac{\delta^{(2)}(H+pV)}{2\pi^2k_c}\right|_S = -\tfrac{3}{2}\eta_0^2a_m^2m^2+\eta_0^2a_m^2(1-2c_0\sqrt{2}) + \tfrac{1}{2}\eta_0^2a_m^2m^4. \qquad (2)$$

When $c_0=0$, the $m=1$ harmonic is a Goldstone mode of the curvature Hamiltonian (1), since the bending energy is a conformal invariant. However, for $c_0>0$, this $m=1$ mode makes the Clifford torus unstable with respect to a displacement of the hole [6].

Since the prime effect of the unstable mode is to move the hole off center, it is natural to look for stationary shapes which break the azimuthal symmetry. We shall approach this problem by a variational ansatz for shapes with a circular meridian cross section due to Kléman [8]. Coupling this ansatz, which includes arbitrary breathing and eccentric deformations, to a global rescaling [14] one can look for the minimal energy shapes at given volume V and surface S [15]. The results for $c_0=0$ and 1 are presented in Fig. 4, where the elastic energy is drawn as a function of the hole eccentricity for various values of the reduced volume v. For a given reduced volume

$$v>v_{\rm crit}(c_0)=v_C(1-c_0\sqrt{2}/18)+O(c_0^2),$$

the minimum energy is nonaxisymmetric. Thus, as expected from the stability analysis, for $c_0>0$ the Clifford torus (with $v=v_C$) is not an equilibrium shape. If, however, the volume constraint is relaxed, the vesicle shape and volume are given by the smallest energy minimum in Fig. 4, and this corresponds to an axisymmetric torus with $v_{\rm crit}(c_0)<v_C$ (when $c_0>0$).

In conclusion, the toroidal shape resulting from the

inflation stage of partially polymerized membranes will be an axisymmetric shape close to the Clifford torus or a toroidal shape with small eccentricity, due either to conformal fluctuations or (if $c_0 > 0$) to a decentralization of the hole at fixed volume (or fixed osmotic pressure). The previous discussion provides a possible explanation of the difference between the observed fluid and partially polymerized toroidal vesicles. As an experimental test of these ideas, it might be interesting to punch a toroidal fluid vesicle (for example, with antibiotics, some of which are known to form pores in the membrane) and look for an evolution to the Clifford torus and conformal fluctuations (if $c_0 = 0$).

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Note added.— We have recently generalized the observations reported here to vesicles of higher genus and more common phospholipids (e.g., egg lecithin) [16].

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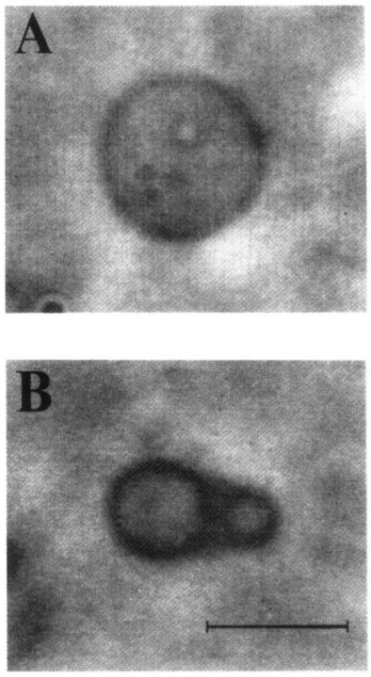
[13] The second variation includes the work done by the osmotic pressure, so H is replaced by $H + pdV$; see M. A. Peterson, Phys. Rev. Lett. **61**, 1325 (1988). A similar calculation, but at constant volume, leads to the same variation for the free energy H [15].

[14] In the notations of Kléman [8], our variational ansatz consists in choosing r, the amplitude of the breathing deformation, a, the scale, and c/a, the eccentricity, to minimize the energy for a given surface and volume.

[15] B. Fourcade (to be published).

[16] X. Michalet and D. Bensimon (unpublished).



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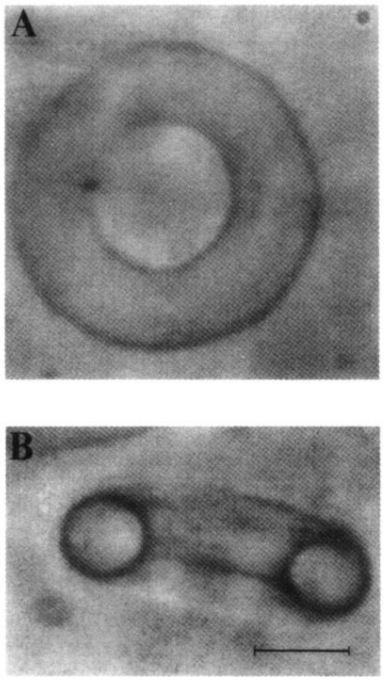

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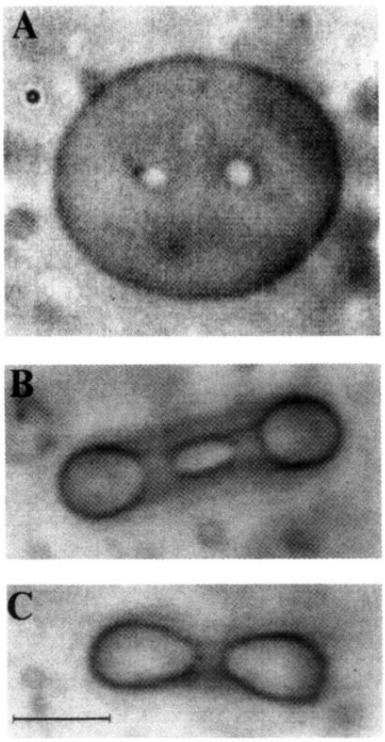

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