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Observation of toroidal vesicles

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In a recent article by Ou-Yang Zhong-can [Phys. Rev. A **41**, 4517 (1990)], rings with generating circles of radii in the ratio 1$\sqrt{2}$ have been found as stable equilibrium shapes of vesicle membranes, for some negative values of their spontaneous curvature. Here we report experimental observations of this topology in partially polymerized phospholipid membranes. Evaluating the radii of the generating circles of several toroidal vesicles we found agreement with the predicted value.

Above the chain-melting transition temperature (T_m) phospholipids in excess water form spontaneously different aggregates, among which are giant unilamellar vesicles. The spherical vesicles are simple models for biological cells, such as the red blood cell. Their equilibrium shapes are determined by a minimization of the shape energy[1,2]

$$F=\frac{1}{2}k_c\oint(c_1+c_2-c_0)^2dA+\Delta p\int dV+\lambda\oint dA \ , \tag{1}$$

where dA and dV are, respectively, the surface area and the volume elements, k_c is the bending rigidity, c_1 and c_2 are the two principal curvatures, and c_0 is the spontaneous curvature. The first term of Eq. (1) is the curvature elastic energy of the vesicle membrane. The spontaneous curvature c_0 describes the effect of an asymmetry of the membrane bilayer. The second and third terms take account of the constraints of constant volume and area, where Δp, the pressure difference across the membrane, and λ, the mechanical lateral tension, serve as Lagrange multipliers.

From the equation for the mechanical equilibrium of vesicle membranes,[3] obtained from Eq. (1), Ou-Yang Zhong-can has found the shape of vesicles of a further genus (besides those with spherical topology) which he called anchor rings.[4] His analytical solution predicts the existence of toroidal vesicles, whose generating circles have radii in ratio $1/\sqrt{2}$, as shown in Fig. 1. By investigating their stability he has shown that these rings are stable for negative values of the spontaneous curvature c_0, satisfying

$$c_0r_0<-(\pi\sqrt{2})^{1/2}(\tfrac{3}{2}+\sqrt{2}/4)\approx-3.9 \ , \tag{2}$$

where $A=4\pi r_0^2$ is the ring-vesicle area. Up to now a vesicle with this topology has not been observed in experiments.

We now report the first observation of this topology in partially polymerized diacetylenic phospholipid membranes. The polymerizable phospholipid used in our experiments was 1,2-bis(10,12-tricosadiynoyl)-*sn*-glycero-3-phosphocholine. It was purchased as a crystalline powder from Avanti Polar Lipids (Alabama) and used without further purification. The purity was checked prior to use by silica gel thin-layer chromatography (TLC) in a chloroform-methanol-water (65:25:4) solution. Only a single spot was visible when developed in iodine vapor. In view of the sensitivity of the procedure, impurity concentrations larger than 1% can be excluded. Lipids were kept below $-20\,^{\circ}\mathrm{C}$ under nitrogen.

A small amount (<0.1 mg) of the powder was spread on a glass cover slide. A quartz cover slip was put on top with a paraffin film as spacer. This ≈ 100-μm-high microchamber was filled by capillarity with bidistilled wa-

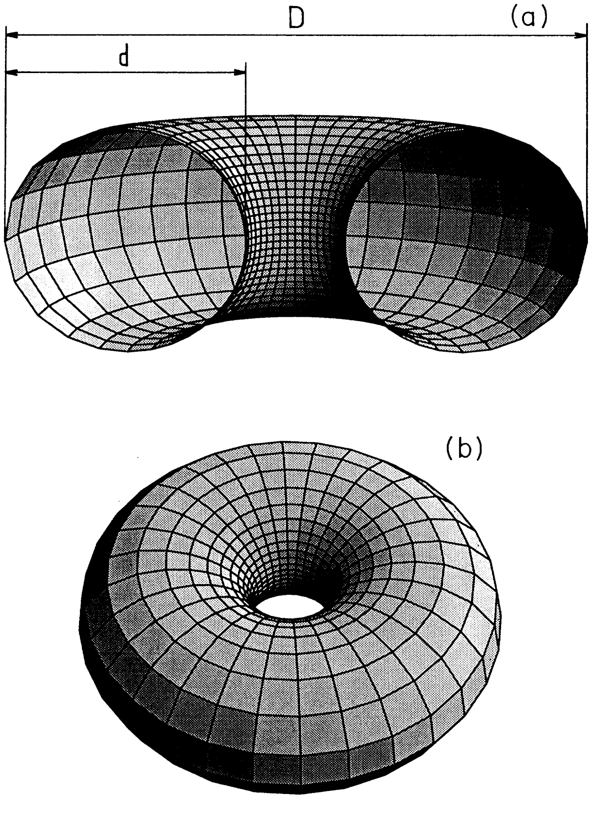

FIG. 1. Two views of a ring vesicle with generating radii in the ratio $1/\sqrt{2}$. D represents the outer diameter and d the width of the ring.

ter, and was sealed with paraffin to prevent evaporation. The sample was then placed in a small temperature-controlled oven on a microscope stage. For the observation of the sample an inverted phase contrast microscope (Nikon) was used, equipped with a charge coupled device (CCD) videocamera (Sony). Membranes are seen in the focal plane where they are parallel to the optical axis of the microscope.

Above T_m [$\approx$43°C (Ref. 5)], the unpolymerized lipids swelled spontaneously, forming giant unilamellar vesicles and other dilute structures. After several hours of swelling at $T = 50\,^\circ\mathrm{C}$ we started to investigate the samples for ring vesicles. However, no unpolymerized vesicle with the topology of a ring was detected, even after several days.

The polymerizable lipids, containing a diacetylene group halfway down in both of their hydrocarbon chains, can be polymerized into three-dimensional sheets by uv irradiation. The lipids polymerize only if they are in the solid gel state, i.e., at $T < T_m$. It is known that upon cooling slowly below T_m unpolymerized vesicles convert into long cylindrical tubules formed by winding sheets of bilayers or break up into "shards" if cooled rapidly,[6] whereas partially polymerized vesicles exhibit a striking wrinkling transition, well below T_m, from a rather smooth fluctuating vesicle to a shape where the mem-

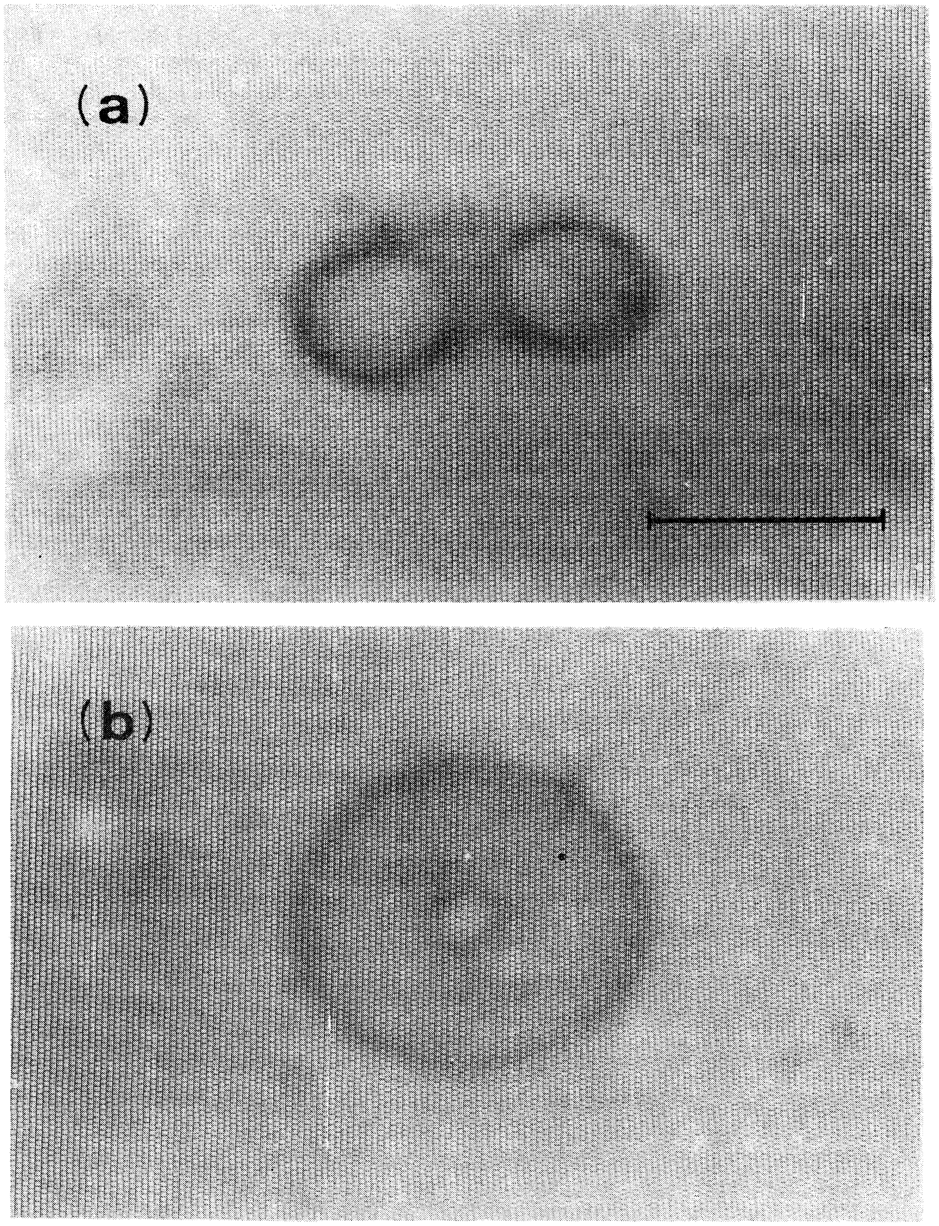

FIG. 2. Ring vesicle of partially polymerized phospholipid membrane at $T = 50\,^\circ\mathrm{C}$, (a) side view, (b) top view. The bar indicates 10 μm.

brane is highly convoluted and rigid.[7]

The membranes were partially polymerized in the solid gel state, i.e., as tubular structures, by uv radiation (254 nm) at $T=25\,^\circ\mathrm{C}$ for about 1–10 min. Reheating above T_m causes a reversal to a shape in which fluctuations are not significantly different from those observed with the unpolymerized ones. Investigating these partially polymerized samples we were aware of several giant ring vesicles (with diameters $>4\ \mu\mathrm{m}$). As the vesicle rotates slowly one has a view of the different projections of the vesicle in the focal plane. A top (side) view, where the larger (smaller) generating circle is parallel to the focal plane, is shown in Fig. 2. The side view clearly exhibits the toroidal form of the vesicle and rules out other interpretations. Ring vesicles were stable over days, without changing their mean characteristic shape. They displayed thermal undulations with amplitudes similar to other membrane structures in the samples. We evaluated the radii of the two generating circles of more than 20 ring vesicles to verify the predicted ratio of $1/\sqrt{2}$. The results are shown in Fig. 3. The outer diameters (D) range from 5 to 18 μm. The ratios of the outer diameter (D) to the width (d) of each ring are aligned on a straight line. The mean ratio of 0.41 ± 0.02 is in good agreement with the theoretical prediction, i.e., $\sqrt{2}-1$ (the appropriate value for the ratio of the diameters). Toroidal vesicles with distinctly different ratios have not been observed. In some rare cases where the ring vesicles were not axisymmetric, the mean width of the ring was used.

The number of toroidal vesicles is limited for several reasons. As outlined in Ref. 4, the necessary condition for a stable equilibrium is a negative spontaneous curvature (2). This condition fixes both the lateral tension λ and the pressure difference across the vesicle Δp [see Ref. 4, Eq. (14)], while in the case of spherical vesicles one of them can be chosen freely. Obviously this condition restricts the number of observable ring vesicles, besides the always small number of giant unilamellar vesicles.

The occurrence of spontaneous curvature due to partial polymerization was proposed recently[7] as a possible explanation of the wrinkling transition observed in partially polymerized vesicles. It seems reasonable to assume that partial polymerization of diacetylenes—done in the solid gel state, when the membrane forms a tubular structure of diameter $\approx 1\ \mu\mathrm{m}$—results in polymer patches (or networks) which may induce a spontaneous local curvature with a radius $|c_0^{-1}|\approx 0.5\ \mu\mathrm{m}$. Inserting that value in the stability condition, Eq. (2) implies that ring vesicles with a diameter greater than $\approx 4\ \mu\mathrm{m}$ should be stable, as they are indeed observed to be. Moreover, using that value for c_0, one can estimate the lateral tension λ [Eq. (14) of Ref. 4] for a ring vesicle of outer diameter $D=8\ \mu\mathrm{m}$ to be $1.6\times10^{-5}\ \mathrm{mN\,m^{-1}}$, where we assumed a realistic bending rigidity k_c of 1×10^{-19} J.[8,9] This lateral tension λ is small enough to allow for out-of-plane fluctuations with large amplitudes, in agreement with the microscopic observation.

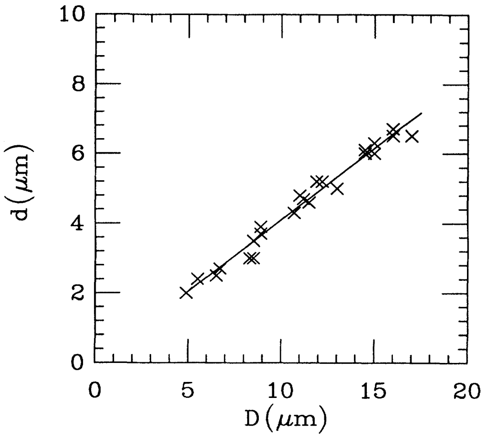

FIG. 3. Results for 24 individual ring vesicles. The solid line represents the theoretical prediction. For further details see Fig. 1.

The observation of toroidal vesicles shows that the spontaneous curvature c_0 is a relevant parameter in a description of the state of partially polymerized membranes. Experiments with partially polymerized vesicles are still at their beginnings but these first results will hopefully stimulate further work, particularly as these heterogeneous membranes are interesting and relevant models for biological cells.

Note added. After submission of this Brief Report, we received a copy of unpublished work by U. Seifert, where he described different families of toroidal vesicles that he obtained from numerical calculation of the minimum of the vesicle shape energy (for $c_0=0$). Our experimental observation is in agreement with one type, the circular toroids.

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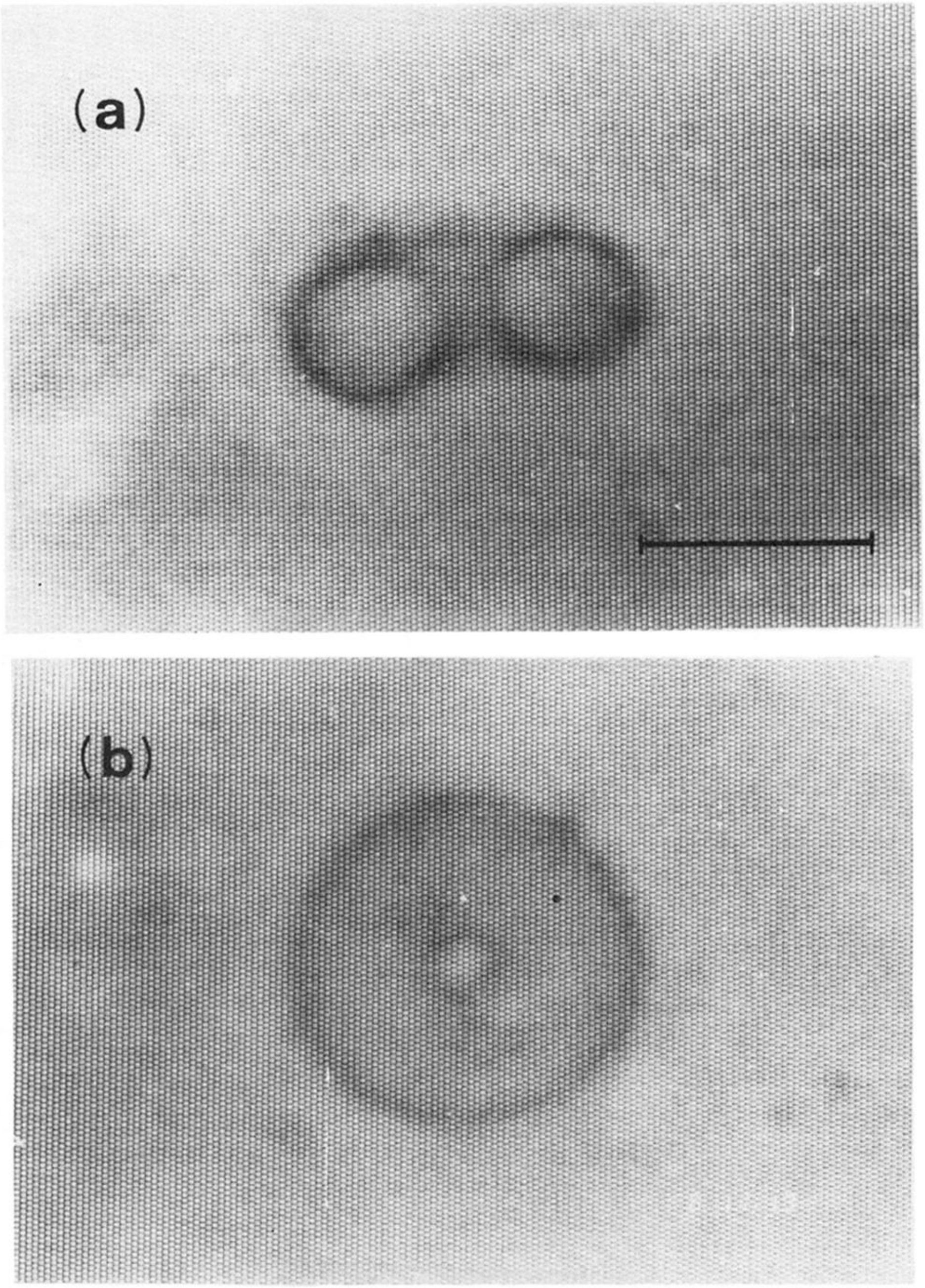

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