Wrinkling Transition in Partially Polymerized Vesicles

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Upon cooling partially polymerized vesicles are observed to undergo a spontaneous transition to a wrinkled rigid structure. The transition is reversible: The vesicles unwrinkle upon heating. A model is presented suggesting that this transition could be the membrane equivalent of a glass transition.

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Partially polymerized phospholipid vesicles have been proposed as models of the cell membrane and as a way to stabilize lyposomes for drug delivery [1,2]. Much theoretical work has been devoted to the statistical mechanics of homogeneous fluid or fully polymerized membranes [3], but not to the interesting and biologically more relevant subject of heterogeneous (e.g., partially polymerized) membranes. Here we report that upon cooling, partially polymerized vesicles may undergo a spontaneous transition to a wrinkled and rigid structure often accompanied by a tearing of the membrane and the subsequent expulsion of the vesicular content. The transition is reversible: The vesicles unwrinkle upon heating. Based on a model for a heterogeneous membrane, we suggest that this transition could be the membrane equivalent of the spin-glass transition observed in magnetic systems. Our observations may open a new avenue for the development of drug delivery vectors.

Polymerizable phospholipids are a class of phospholipids with the ability to covalently crosslink upon catalysis by a chemical or photochemical process [2]. Among the many different lipids and polymerization mechanisms to have been investigated, the most extensively studied class is that of the diacetylenic phosphatidylcholine lipids which upon UV irradiation polymerize into two-dimensional sheets [1,2]. The polymerizable phospholipid used here [1,2-bis(10,12-tricosadiynoyl)-*sn*-glycero-3-phosphocholine] belongs to that class. It was purchased as a powder from Avanti Polar Lipids and stored at −30 °C. Its purity was checked prior to use by silica-gel thin layer chromatography (TLC) in a chloroform-methanol-water mixture (65:25:4). Small amounts (3–30 mg) of the powder were transferred to a Hellma quartz cuvette, dissolved in chloroform, and evaporated overnight in a vacuum chamber protected from light. The lipids were then rehydrated with ultrapure water to a concentration of 1–10 mg/ml at 50 °C for 1–3 h. They swelled spontaneously to form a rather heterogeneous population of large (> 1 μm) unilamellar and multilamellar vesicles. That solution was then cooled below the chain melting temperature ($T_m = 43\,°\mathrm{C}$) and polymerized by UV irradiation (at 254 nm) either during cooling or afterwards (polymerization is only possible below T_m). Samples of the solution were taken for analysis by TLC (after evaporation of the water content and dispersion in chloroform). Polymerized solutions exhibit two spots: one at the origin of deposition exhibiting the characteristic red color of the polymer and another at the same height as the unpolymerized sample. The relative amount of polymerized molecules can be estimated by densitometry analysis of the TLC spots. Alternatively it can also be estimated by differential scanning calorimetry (DSC) on a Perkin-Elmer DSC2 instrument: The endothermic peak at $T_m = 43\,°\mathrm{C}$ decreases as a function of irradiation time as more and more molecules are frozen-in by the polymerization. Samples from the solution were taken for observation by phase-contrast microscopy. They were introduced by capillarity in a cell (of thickness 100 μm) made of a paraffin film sandwiched between two microscope cover slides. The cell was sealed with paraffin and placed in a small temperature-controlled oven on the microscope stage, where the morphological changes of the vesicles could be observed as a function of temperature.

Unpolymerized samples of the solution, if cooled slowly ($\approx 1\,°\mathrm{C/min}$) below T_m, show the formation of long (10–200 μm) cylindrical tubules of diameter ≈ 1 μm [4]. If cooled rapidly ($\approx 10\,°\mathrm{C/min}$) below T_m, the vesicles break up into "shards," i.e., small tubular sections [4]. These structures have been the subject of intense research in the last five years. The tubules are helical ribbons consisting of a pair of phospholipid bilayers wrapped around cylindrically like a paper soda straw [4]. The lipid molecules in the gel state (i.e., below T_m) have a smectic-*C* order, their acyl chains being tilted at $\approx 28°$ from the bilayer normal [5]. A recent model [6] shows that such a microscopic ordering of chiral molecules may favor tubular structures over spherical ones in spite of the elastic energy associated with the small cylindrical curvature.

Partially polymerized vesicles can be obtained by UV irradiation of tubules, followed by reheating above T_m to reform vesicles. Tubules which are more than 40% polymerized do not revert to vesicular form (presumably the polymerized network has percolated through). Alternatively partially polymerized vesicles can be obtained by UV irradiation of unpolymerized ones while cooling through T_m. The vesicles observed at $T < T_m$ have rather large fluctuations (not significantly different from those observed at $T > T_m$), in spite of the fact that the acyl chains have frozen, as indicated by the presence of a peak near T_m in the DSC thermogram. Probably the existence of a large number of microcrystalline smectic-C domains nucleated around polymerized patches allows for a large number of 2D dislocations, thus weakening the membrane rigidity. As these vesicles are further cooled down to 18–22 °C, they exhibit a striking wrinkling transition from a state where the membrane is "floppy," fluctuating and rather smooth, to a state where the membrane is rigid and highly convoluted (Fig. 1). Wrinkling usually initiates at a single site on the bilayer and proceeds by an invasion or percolation process throughout the membrane. In phase-contrast microscopy, wrinkling is first identified by the nucleation of a darker (i.e., denser) spot on the membrane. As that spot grows, the fluctuations of the vesicle are reduced and the tension in the membrane increases. Vesicles which are far from spherical become spherical as their excess area wrinkles. Very often the tension in the bilayer is large enough to tear it. The content of the vesicle is then expelled out as the membrane continues to crumple into a rigid convoluted structure (Fig. 2). The whole process usually takes a few seconds and is faster the less polymerized the membrane. That transition does not yield a detectable peak in the DSC thermogram. It is reversible, though hysteretic. Unwrinkling seems to occur below T_m, but on time scales of hours. Above T_m, unwrinkling occurs within minutes. In contrast to wrinkling, which seems to be an energetic transition proceeding like a shock wave from a single nucleation center, unwrinkling seems to be driven by entropy. It begins at various places on the membrane, some areas fluctuating while still possessing some wrinkled rigid parts, which randomly unfold and reverse to a flop-

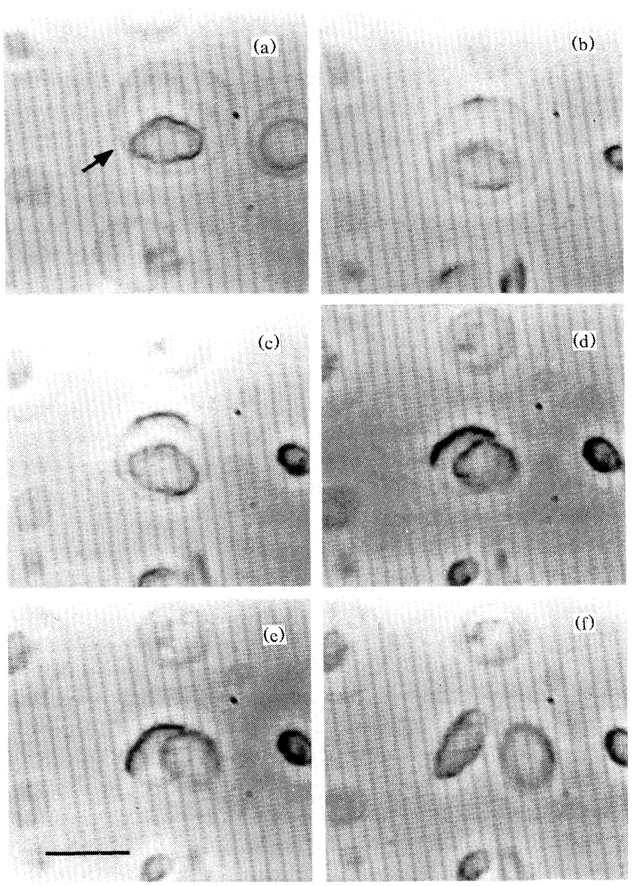

FIG. 1. The wrinkling transition observed at $T = 18\,^\circ\mathrm{C}$ in a partially polymerized vesicle, indicated by the arrow. This vesicle contains other lyposomes including an already wrinkled one. Notice (a) the floppy (fluctuating) vesicle before wrinkling, (b) the spherical tense shape resulting from wrinkling, and (d),(e) the tearing of the membrane and expulsion of vesicular content, shown by the passage of internal vesicles through a hole in the membrane. The time lapse between (b) and (f) is 30 sec. (The bar denotes 10 μm.)

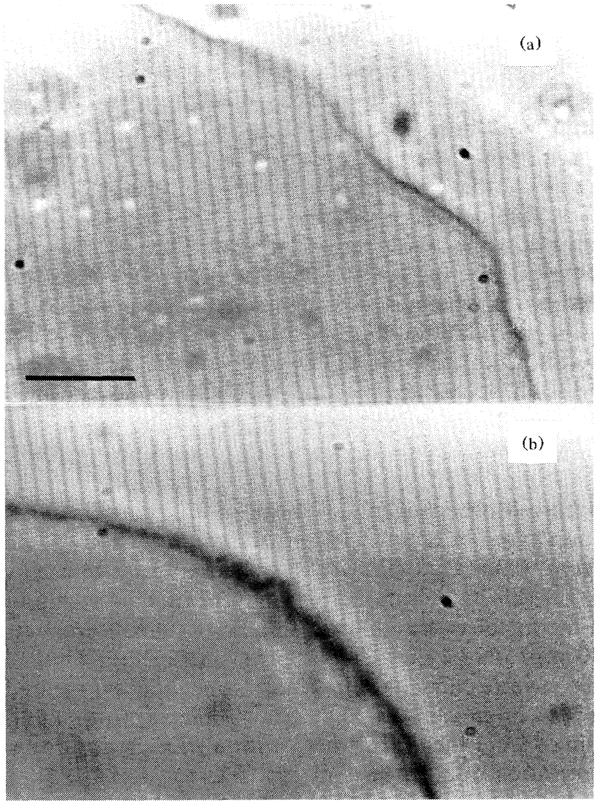

FIG. 2. (a) Detail of the membrane of a very large (≈ 180 μm) unilamellar vesicle before wrinkling. The membrane is flacid and fluctuating. (b) Same as (a), but after wrinkling. The membrane is rigid. Its surface is highly convoluted as noticeable from the small rigid bumps visible near the center of the figure. Unlike smaller vesicles, this one did not tear. (The bar denotes 10 μm.)

py fluctuating state (Fig. 3). A similar wrinkling-unwrinkling transition (but without tearing of the membrane and expulsion of the vesicular content) has been reported by Sackmann *et al.* [1] in polymerized vesicles made up of a mixture of diacetylenic phospholipids and dimyristoylphosphatidylcholine (DMPC). That seems to indicate that the wrinkling transition is a robust phenomenon, not too sensitive to the particularities of the system under study.

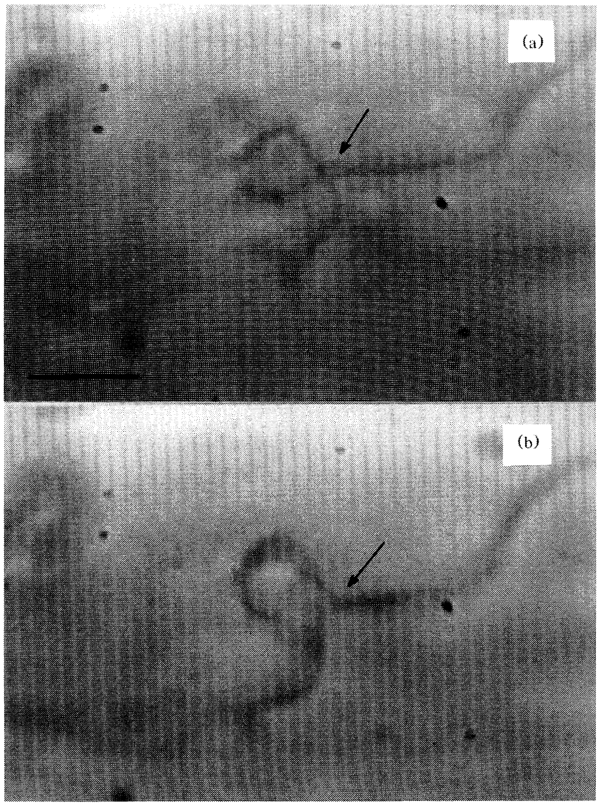

FIG. 3. Snapshots of the unwrinkling of the neck (indicated by the arrow) of a purselike pocket in the very large unilamellar vesicle of Fig. 2 as it is heated to 50 °C (the interior of the vesicle is in the upper part of the figures). In (a) the neck is almost closed whereas a few minutes later, (b), it has opened. Eventually the pocket will disappear. (The bar denotes 10 μm.)

In the following we shall sketch our theoretical interpretation of these observations (details will be published elsewhere [7]). The formation of cylindrical structures from unpolymerized phospholipids below T_m implies that this low-temperature phase has a high curvature (>2 μm^{-1}). Partial polymerization will create "roof-tile"-like 2D polymer patches of high spontaneous curvature. Upon heating, the tubules revert to fluid vesicles, in which membrane the polymer nets are free to diffuse. Cooling these vesicles below T_m results in crystalline (or hexatic) membranes where the embedded randomly distributed polymer nets induce an asymmetry between the layers of the membrane, resulting in a random spontaneous local curvature. It is thus natural to look for an explanation of the wrinkling transition in terms of a theory for crystalline membranes with quenched disorder. A microscopic Hamiltonian for such membranes is [3]

$$H=-\sum_{\langle i,j\rangle}\kappa_{ij}\mathbf{n}_i\cdot\mathbf{n}_j+\sum_{\langle\alpha,\beta\rangle}V(|\mathbf{r}_\alpha-\mathbf{r}_\beta|)-\sum_{\langle i,j\rangle}\mathbf{D}_{ij}\cdot(\mathbf{n}_i\times\mathbf{n}_j)\,, \qquad (1)$$

where $\mathbf{r}_\alpha$ denotes the position of the αth vertex, $\mathbf{n}_i$ is the normal to the ith triangular plaquette, κ_{ij} is the local bending rigidity, $V(r)$ is a tethering potential between nearest-neighbor vertices (contributing to the elastic compression and shear), $\mathbf{D}_{ij}$ is a random vector, and the summation is over nearest neighbors. The first two terms (when $\kappa_{ij}=\kappa$) were introduced previously [3]. The last one is new and represents the contribution of a quenched random local curvature term to the Hamiltonian. Notice that in principle the bending rigidity is also a quenched random variable (with $[\kappa_{ij}]_{\text{av}}>0$), since the bending rigidity of polymerized and unpolymerized regions could be different. In the long-wavelength limit this Hamiltonian reduces to the usual Landau-Lifshitz elastic theory of crystalline membranes [3] with random bending rigidity $[\kappa(\mathbf{x})]$ and spontaneous curvature $[c_{ij}(\mathbf{x})]$:

$$H=\int d^Dx\left[\tfrac{1}{2}\kappa(\mathbf{x})(\nabla^2 f)^2+c_{ij}(\mathbf{x})\partial_i\partial_j f\right]+(\text{in-plane elastic terms})\,,$$

where $f(\mathbf{x})$ is a small vertical displacement of the membrane from the flat configuration ($\mathbf{n}\approx\nabla f$). Equation (1) can be simulated on a computer, but is difficult to analyze. A simplifying assumption often made in the study of disordered systems is to consider the mean-field approximation:

$$H=-\sum_{\{i,j\}}\kappa_{ij}\mathbf{n}_i\cdot\mathbf{n}_j-\sum_{\{i,j\}}\mathbf{D}_{ij}\cdot(\mathbf{n}_i\times\mathbf{n}_j)\,, \qquad (2)$$

where the summation is over all distinct pairs of normals (spins). This model is the mean-field model of a Heisenberg ferromagnet with random couplings and Dzyaloshinskii-Moriya interactions [8]. In the absence of disorder ($\kappa_{ij}=\kappa/N$ and $\mathbf{D}_{ij}=0$), it exhibits a crumpling transition from an ordered ferromagnetic (or flat) phase to a high-temperature disordered (or crumpled) phase, which is *quantitatively* correct for membranes of inner dimension $D\geq 4$ (and outer dimension $d=D+1$). However, since the effect of the tethering potential in Eq. (1) is to introduce long-range interactions between the normals [3], it turns out that this mean-field model is still *qualitatively* valid for membranes in $D=2$, which in the absence of self-avoiding interactions also exhibit a crumpling transition, albeit with a different critical behavior than for $D\geq 4$.

In the presence of disorder (either in κ_{ij}, $\mathbf{D}_{ij}$, or both) Eq. (2) possesses a low-temperature spin-glass phase

[7,8] characterized by randomly frozen normals (spins). Although the mean-field approximation [Eq. (2)] is only expected to be correct for $D \geq 4$, the phase diagram derived from it may still be valid at $D < 4$. We are therefore tempted to identify the spin-glass phase with the observed low-temperature wrinkled phase, which is also characterized by randomly frozen normals. A related model in $D=2$ has been studied by Rubinstein, Shraiman, and Nelson who also pointed out the existence of a reentrant transition from a ferromagnetic ordered phase to a (spin-glass) disordered phase [9]. Let us finally remark that the wrinkling transition reported here may be an extension to 2D polymers (membranes) of the folding transition observed in 1D heteropolymers (e.g., proteins), where spin-glass models have also been postulated as an explanation [10]. However, the size of the objects under study and the existence of an equivalent of the magnetic field (the surface tension) may allow for a more precise comparison between experiments and spin-glass models of the wrinkling transition in 2D systems (membranes) than in 1D systems (proteins).

Admittedly, our experimental results and theoretical model raise many more questions than they solve. Is disorder important in real ($D=2$) membranes? If there is a glass transition, what is its lower critical dimension? How do self-avoiding interactions modify the theoretical results? How does one explain the fact that the observed transition is first order? Nevertheless, these observations and their interpretation (*if valid*) suggest that the release of the content of lyposomes could be controlled by embedding in their membrane some local constraints (in the form of polymerized diacetylenes, for example). Vesicular release may then be triggered by a modification of the physicochemical properties of the lyposomal environment.

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Note added.—After this work was submitted, we received a preprint by Nelson and Radzihovsky [11], where a spin-glass transition was also predicted for a model of a membrane with quenched randomness.

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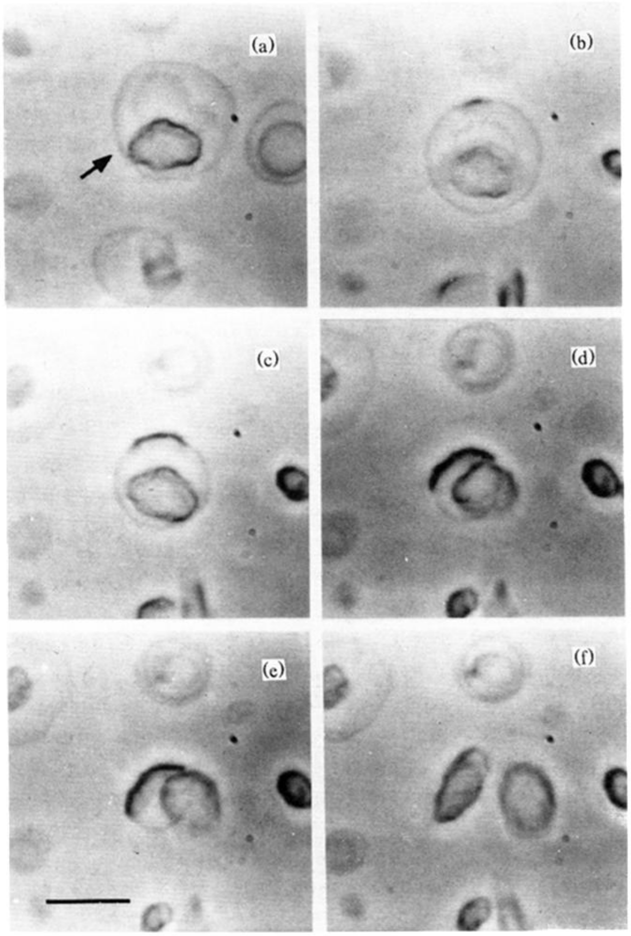

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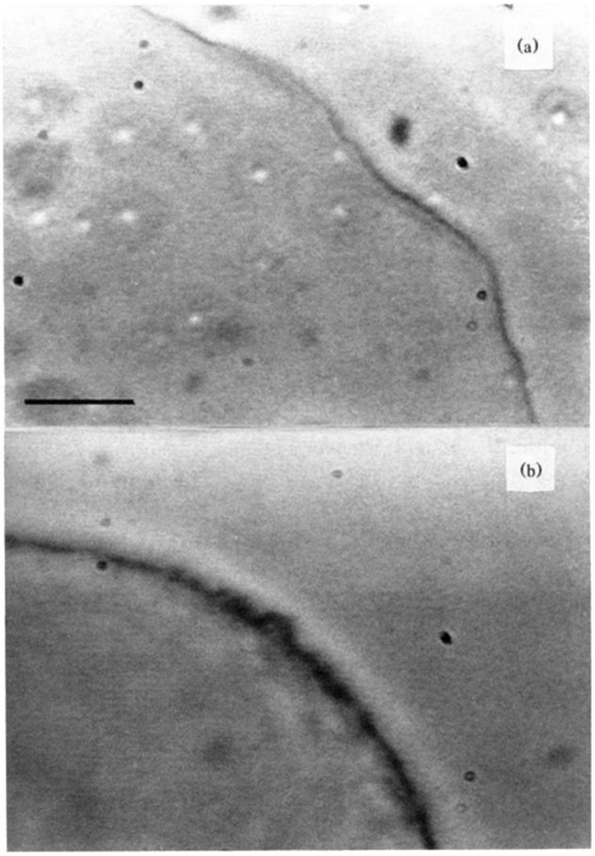

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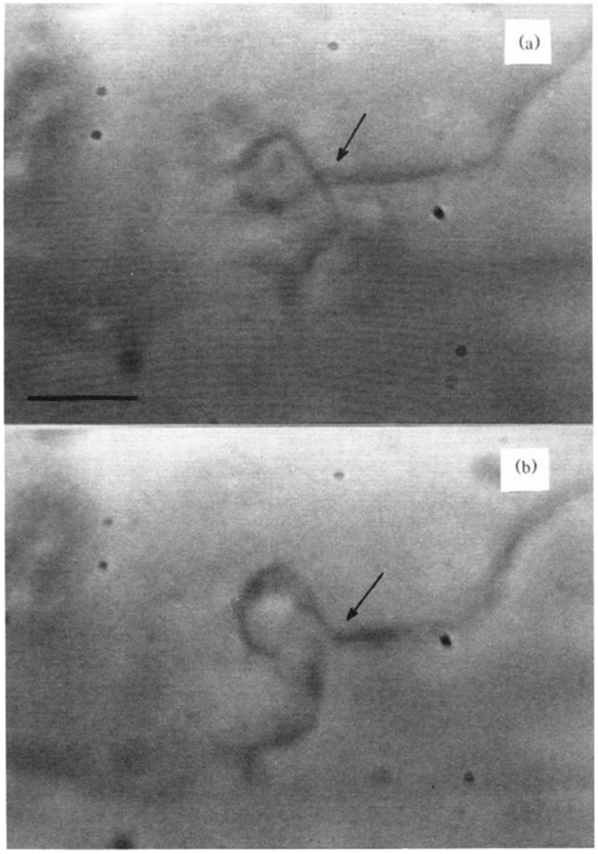

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