Pressure-Induced Topological Phase Transitions in Membranes

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Some highly unusual features of a lipid-water liquid crystal are revealed by high pressure x-ray diffraction, light scattering and dilatometric studies of the lamellar (bilayer L_α) to nonlamellar inverse hexagonal ($H_{\rm II}$) phase transition: (i) The size of the unit cell of the $H_{\rm II}$ phase increases with increasing pressure; (ii) the transition volume, ΔV_{bh}, decreases as the pressure is increased; and (iii) the intensity of scattered light increases as ΔV_{bh} decreases. Data are presented which suggest that this increase is due to the formation of an intermediate cubic phase, consistent with theoretical suggestions of an underlying universal phase sequence.

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Dispersions of biological lipids in water exhibit a rich variety of structurally complex phases as the temperature, pressure, and water content are varied. The lamellar liquid crystalline bilayer (L_α) to inverse hexagonal ($H_{\rm II}$) phase transition has attracted particular interest because it is the simplest of the lyotropic transitions in which both the topology and curvature of the lipid-water interfaces internal to the liquid crystal change discontinuously at the transition [1]. The presence of water and electrostatic effects makes lyotropic dispersions complicated. Insight into understanding such systems first came with the recognition that they form assemblies of two-dimensional liquidlike films which elastically resist lateral extension or compression, or bend away from a desired curvature. Winsor [2] and Helfrich [3] suggested that much of the complexity of the molecular interactions can be lumped into phenomenological parameters such as the curvature of the lipid-water interfaces and the elastic moduli of the lipid layers. The utility of this phenomenological approach toward understanding the lamellar bilayer to nonlamellar transitions has been established by Gruner and co-workers [4] and by Charvolin [5], who suggest that the phase behavior can be understood as a result of a *frustration* between opposing requirements of membranes that have to curl to satisfy curvature requirements on the one hand and to try to maintain constant bilayer thickness on the other.

The idea that many aspects of the global phase behavior can be explained without considering the detailed molecular structure of the molecules involved led Goldstein and Leibler [6] to propose a mean-field model for the well known bilayer chain melt (i.e., gel–liquid-crystalline) phase transition. While this approach has not been extended to nonlamellar phases, the enormous simplification afforded by Helfrich's insight has stimulated recent experimental [1] and theoretical interest in nonlamellar phases [7,8]. A rather general attempt in this direction is the Ginzburg-Landau model of Chen *et al.* [8], which involves a combination of scalar and vector order parameters that is claimed to have, in principle, sufficient complexity to describe not only lamellar and hexagonal phases, but also more geometrically complex phases, such as bicontinuous cubic phases [7,9]. However, the large number of phenomenological parameters involved, as well as a lack of an intuitive physical interpretation of the proposed order parameters, makes the connection to experiments tenuous. In this Letter, we describe some rather unusual phenomena exhibited by excess-water dispersions of electrically neutral lipid, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE; also known as dioleoylphosphatidylethanolamine), which swells in water to form phases with a well-defined water fraction, with a view to stimulating quantitative models.

(1) *Nonlamellar phase transition temperatures are very pressure sensitive.* Optical scattering [10] and x-ray diffraction [11] measurements suggested that the L_α-$H_{\rm II}$ transition temperature may be depressed by surprisingly modest pressures. Experiments were conducted using a rotating anode x-ray source, a point-focused beam line, a thermostated high pressure beryllium x-ray cell, and a two-dimensional x-ray detector [12] which, in combination, allowed rapid surveys of the temperature-pressure structure of small (~ 10 mg) lipid samples. The phases and internal dimensions of the lipid-water liquid crystals were identified by the characteristic x-ray diffraction patterns of unoriented dispersions [13]. The Clausius-Clapeyron coefficient of change of transition temperature with pressure was $dT_{bh}/dP = 44 \pm 3$ K/kbar, a value which is nearly twice as large as for the chain melting transition [14] and which establishes the excess-water L_α-$H_{\rm II}$ transition as one of the most pressure sensitive first order transitions known in a nongaseous system.

(2) The unit cell spacing compressibility is negative. Figure 1 shows that the unit cell spacing, b, of the $H_{\rm II}$ phase increases as pressure is applied. Furthermore, this pressure dependence is remarkably steep: at 20 °C, b increases by nearly 1 Å for every 100 bar increase in pressure, due primarily to the transfer of water from the coexisting water bulk to the liquid crystal [15]. The data yield a value of the isothermal bulk linear compressibility of the Bragg lattice of $\alpha_T = -\frac{1}{b}\left(\frac{\partial b}{\partial P}\right)_T = -1.1 \times 10^{-4}$ bar^{-1}. We define a "generalized Clausius-Clapeyron parame-

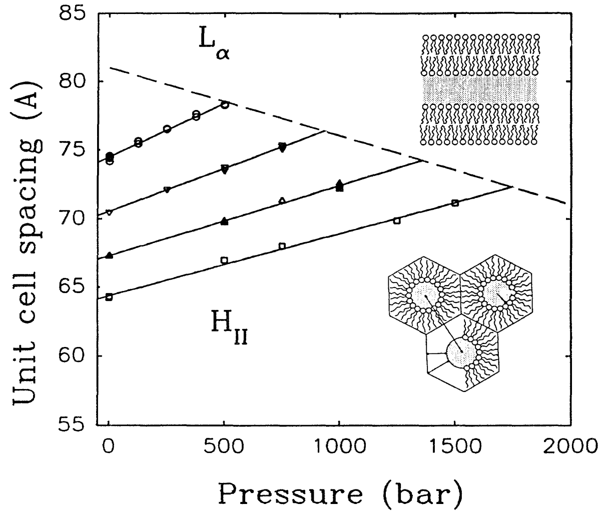

FIG. 1. Unit cell spacing in the H_{II} phase as a function of pressure at various temperatures. Data are shown for aqueous dispersions of high purity DOPE purchased from Avanti Polar Lipids at the temperatures 25 °C (○), 43 °C (▽), 62 °C (△), and 81 °C (□). Sample purity was periodically checked before and after experiments by thin layer chromatography (TLC). The dashed line indicates the *apparent* phase boundary between the H_{II} and L_α phases.

ter," $\frac{dT}{dP} = \frac{\Delta T}{\Delta \xi}\frac{\Delta \xi}{\Delta P}$, that relates the sensitivity of a structural parameter, ξ, to pressure and temperature changes. For the unit cell spacing with $\xi = b$, $\Delta b/\Delta T = 0.22$ Å/K and $\Delta b/\Delta P = 9.6$ Å/kbar, giving for $\frac{dT}{dP} = 44$ K/kbar, which is essentially the same as the Clausius-Clapeyron value for the L_α-H_{II} phase transition given above. This lends support to the suggestion that the phase transition may be understood using models based only on gross structural parameters, without reference to details of the molecular interactions.

Thermodynamic stability arguments show that the apparent negative compressibility in Fig. 1 refers only to the increase in the unit cell size of the two-dimensional hexagonal lattice; the overall specimen volume must decrease with increasing pressure. Almost all of the increase in size with increasing pressure arises from an increase in the radius of the central water core of the hexagonally packed tubes and the resultant influx of bulk water. Electron density reconstruction of the x-ray data show that the truly pressure sensitive aspect of the system is the spontaneous curvature of the lipid layer making up the hexagonally packed tubes [15]. On these liquid crystals, measurement of parameters such as the average volume, length and interfacial area per lipid molecule cannot readily be obtained from x-ray diffractometry alone; therefore we constructed a high pressure (0–3 kbar) dilatometer capable of resolving volume changes of about 0.1 nl/mg in small lipid-water specimens [16]. Figure 2 shows the specific volume as a function of pressure at various temperatures.

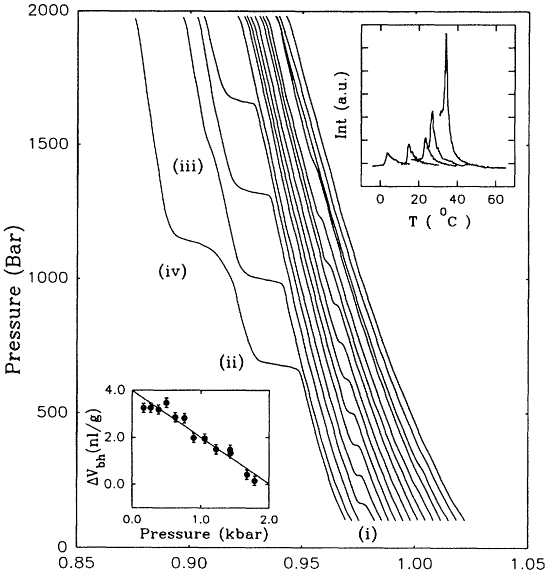

FIG. 2. P-V isotherms for the DOPE-water system. Solid lines indicate experimental data. Isotherms at 5°C intervals from 8 to 83 °C (from left to right) are shown. Inset at lower left shows the transition volume, ΔV_{bh} (in 10^{-9} l/g), for the L_α-H_{II} transition [indicated by (i)] derived from the isotherms using a procedure similar to that of Ref. [22]. Upper inset shows the intensity of light scattered from DOPE water at a scattering vector of $q = 10^{-3}$ nm^{-1} for (left to right) increasing pressures. The increase in light scattering intensity coincides with the decrease in ΔV_{bh} and the peak temperatures match T_{bh} vs pressure.

(3) At least four different pressure sensitive phase transitions are observed in excess-water–DOPE samples (Fig. 2). The isothermal bulk compressibility of the DOPE-water system is about $\beta_T = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_T = +2 \times 10^{-4}$ bar^{-1}, comparable to that of water. Phase transitions are identified in the dilatometry data as discontinuities in the lipid specific volume or as slight changes in the slope of the isotherms signaling a change in compressibility. Figure 2 shows that four distinct transitions corresponding to five phases, which have subsequently been examined via x-ray diffraction [17]. (i) At 13 °C a small volume change occurs at about 200 bar which corresponds to the L_α-H_{II} transition. The transition exhibited hysteresis over a temperature range of about 8 °C. (ii) At 8 °C and ~ 700 bar, a large volumetric change is observed which is identified from the appearance of sharp peaks in the wide angle x-ray scattering (WAXS) as the familiar chain melting (gel to liquid crystalline) transition. The volume change is in agreement with previous thermodynamic studies on lipid systems with phosphatidycholine (PC) headgroups [18]. (iii) At 8 °C and ~ 1000 bar, a phase transition with a small volume change is observed with the position and intensity

of the sharp peaks in the WAXS changing discontinuously, suggesting that the symmetry of the packing of the hydrocarbon chains has been changed. (iv) A large volume change is observed at 8 °C and ~ 1.2 kbar—the system forms a high-pressure phase characterized by five distinct WAXS lines. This suggests that the chains are packed with a high degree of order and that the phase is likely to be a crystalline phase, analogous to ones known in PC systems [18,19].

(4) The transition volume, ΔV_{bh}, enthalpy, ΔH_{bh}, and entropy, ΔS_{bh}, of the L_α-$H_{\rm II}$ transition decrease as the pressure is increased. Interestingly, ΔV_{bh}, and therefore ΔH_{bh} and ΔS_{bh}, become too small to measure with our apparatus at about 80 °C and ~ 1750 bar. The transition volumes involved in these topological transitions are at least an order of magnitude smaller than the volume changes accompanying the chain-melting transition and sub-gel transitions. *This suggests that such phase transitions involve a very delicate balance of competing large energetic contributions such that small changes in overall volume result in large structural transformations.*

(5) Light scattering of the DOPE-water system increased as the transition volume decreased. A conventional high pressure optical cell was constructed out of beryllium copper and equipped with sapphire windows and unsupported area seals. The intensity I of scattered He-Ne laser light ($\lambda = 632$ nm) was measured with a light sensitive diode at an angle corresponding to $q \sim 10^{-3}$ nm^{-1}. A plot of I versus temperature shows a prominent peak at the L_α-$H_{\rm II}$ transition, as identified by both dilatometry and x-ray diffraction (inset, Fig. 2).

(6) A cubic phase occurs between the L_α and $H_{\rm II}$ phases. Because symmetry arguments would appear to rule out the occurrence of a critical point between phases of different symmetry, we searched for evidence of a kinetically hindered intermediate as an explanation for the light scattering anomaly. An excess water-DOPE specimen was allowed to equilibrate at 80 ± 0.05 °C and 1750 ± 3 bar. *In situ* x-ray diffraction patterns of the sample were taken roughly every hour for three days and are shown in Fig. 3. The initial diffraction pattern is characteristic of the hexagonal phase. As time elapses, diffuse scatter becomes prominent concomitant with a decrease in the intensity of the hexagonal diffraction peaks, and additional peaks appear. After 36 h, the diffraction pattern can be indexed to a cubic lattice of $Pn3m/Pn3$ symmetry. The onset of an intermediate cubic phase appears to be associated with very small latent heat, and the slow formation rate suggests that it is kinetically hindered by large activation barriers. The slow kinetics of formation makes it difficult for conventional calorimetric and dilatometric studies to measure the accompanying latent heat and volume changes. The occurrence of a cubic phase suggests that conventional phase diagrams may have to be modified to include the formation of a cubic phase as an intermediate between the L_α and $H_{\rm II}$ phases.

Region of thermodynamic stability of the cubic phase. One possibility is that there is a triple point with the cubic phase only intervening at pressures higher than 1750 bar at 80 °C. A second is that the cubic phase intervenes at all pressures down to 1 bar, but the rate of formation at the lower temperatures is so slow as to be unobservable on a reasonable time scale. We favor the latter view, based on experiments in which we were able to obtain a cubic phase by repeatedly thermally cycling the system across the L_α-$H_{\rm II}$ phase transition at a pressure of 1 bar [20]. Phase transitions between the L_α, $H_{\rm II}$, and bicontinuous cubic phases which have very different topologies must necessarily be accompanied by repeated tearing of

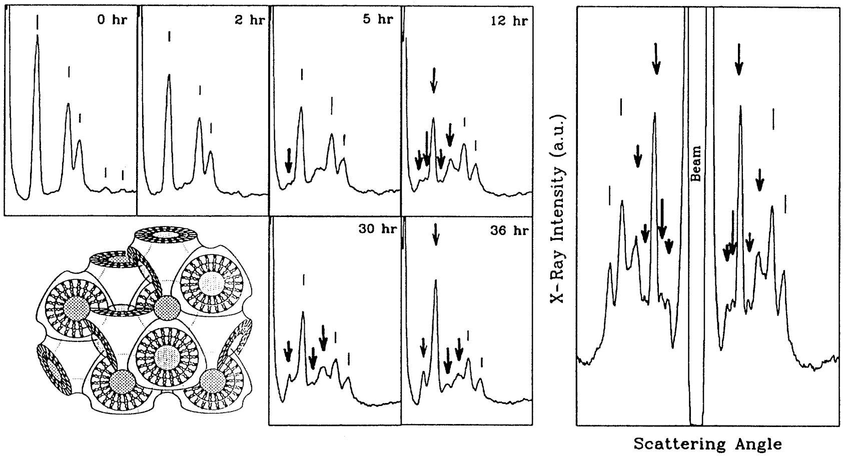

FIG. 3. Evolution of the x-ray diffraction pattern at the times indicated after reaching 1750 bar, 80 °C. The Bragg peaks are initially characteristic of the hexagonal $H_{\rm II}$ phase (indicated by lines). As time elapses, additional Bragg peaks emerge that index to a cubic lattice (bold lines with arrows). The best fit to the observed Bragg peaks corresponds to a lattice of $Pn3m$ (or $Pn3$) symmetry. An example of a bicontinuous lattice with this symmetry is shown schematically in the lower left.

lipid-water interfaces. Repeated cycling across the phase transition region might promote the formation of defects that can nucleate the cubic phase. However, because the mechanism by which such temperature or pressure cycling speeds up the formation of an intermediate phase is not proven, the region of stability of cubic phases remains an open question.

The sequence of phase dimensionality, from 1D lamellar to 3D cubic to 2D hexagonal, underscores the fact that the lattice dimensionality is not a relevant parameter for these topological phase transitions. Anderson, Gruner, and Leibler [7] argued that certain bicontinuous cubic phases provide equilibrium compromises between lamellar and inverse hexagonal phases such that one expects them to always occur between these phases in the phase diagrams. The data presented in this Letter support this picture, and suggest that the underlying universality of this phase sequence may be obscured by the large kinetic barriers. Rather striking support for the notion of an underlying universality comes from the observation diblock copolymers exhibit essentially the same sequence of phases [21], although the microscopic interactions are quite different. It would be interesting to see if the observed negative anisotropic compressibility also carries over to the diblock copolymer systems.

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