Rarely Observed Phase Transitions in a Novel Lyotropic Liquid Crystal System

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This Letter presents neutron diffraction data from a novel, biologically relevant, lyotropic membrane system which is highly alignable (≤1.0° mosaic) in a magnetic field and gives rise to a number of well-defined Bragg reflections. The system, composed of two different phosphorylcholine lipids, undergoes a rare nematic → smectic phase transition upon doping the system with paramagnetic ions (e.g., 2.7 wt % Tm^{3+}). In addition, the isotropic phase occurs at a lower temperature than the smectic phase, in contrast to other lyotropic systems and in contrast to the phase behavior predicted by the McMillan model [Phys. Rev. A **4**, 1238 (1971)] of smectic ordering. [S0031-9007(96)02290-9]

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Liquid crystals are classified as being either thermotropic or lyotropic [1] depending on the way by which the mesophase is obtained. In thermotropic liquid crystals, the mesophase is formed by the intermolecular interactions while the driving force in lyotropic systems is the hydrophobic effect.

Two classes of structures formed by both thermotropic and lyotropic liquid crystals are the so-called nematic (threadlike) and smectic (soaplike) phases, which differ primarily in their dimensional order [2]. The nematic phase has ordering in 1D, the lowest of all the liquid crystal classes, while the smectic phase exhibits 2D order. Experimental evidence for the nematic → smectic phase transition in a variety of thermotropic liquid crystal systems is commonly reported (e.g., [3]). However, the same cannot be said of lyotropic systems. To the best of our knowledge, the nematic → smectic transition has only been observed, as a function of temperature, in the caesium perfluoro-octanoate (CsPFO)/water [4] and the ternary decylammonium chloride (DACL)/water/ammonium chloride systems [5].

Lipids are amphiphilic molecules of fundamental importance to biology as they constitute one of the main components of biological membranes. In addition, lipid/water systems form a variety of interesting structures (e.g., lamellar, cubic, hexagonal, "ripple," etc.) which, for a variety of reasons, have recently been the focus of both experimental [6–8] and theoretical interest [9]. One such structure, consisting of phospholipids and small amounts of detergent [10], is the disk-shaped micelle or so-called "bicelle" previously observed in a variety of single-chain amphiphile systems (e.g., [4,5,11]). In this magnetically alignable system, the detergent serves to stabilize the edge of the bilayer leaving the two surfaces of the unilamellar bilayer populated, for the most part, with lipid molecules [10].

A more pertinent system for biologists has been the substitution of the detergent by a short chain (e.g., dihexanoylphosphatidylcholine, DHPC) phosphatidylcholine [12]. This magnetically alignable bilayered micelle system has proved to mimic the essential physical properties of biological membranes [13] while remaining stable over a wide range of temperature, *p*H, and ionic strength [12]. Both lipid/detergent and lipid/lipid bicelle systems align preferentially with their bilayer normals perpendicular to the applied magnetic field and have proved able to incorporate a variety of membrane peptides and proteins under physiologically relevant conditions [10,12]. Further characterization of the lipid/detergent system using small-angle x-ray scattering resulted in the observation of only one broad (FWHM $\Delta Q \simeq 0.1$ Å^{-1}) diffraction maximum [14] whose intensity was distributed over 360° with the meridional direction experiencing two times more scattering intensity than the equatorial direction [15]. It was therefore demonstrated that although these aggregates possess long-range orientational order [10,12] they nevertheless lack positional order [14], typical of a lyotropic nematic phase.

In this Letter we present results for a novel system in which doping DMPC/DHPC bicelles with selected paramagnetic lanthanide ions (e.g., Tm^{3+}) cause the system to undergo a rarely observed (for lyotropic systems) nematic → smectic transition. Moreover, the bicelles now align with their bilayer normals parallel (Fig. 1) to the applied magnetic field [16] instead of perpendicular to the field prior to the addition of the paramagnetic ion [10,12]. Our neutron data reveal highly aligned (mosaic of ≤1.0°) stacks of liquid-crystalline bilayers which give rise to distinct higher-order Bragg reflections ($n = 5$) indicative of a smectic mesophase. In addition, a transition from the isotropic phase to the smectic lyophase occurs with increasing temperature and in marked contrast to the CsPFO/water [4] and DACL/water/ammonium chloride [5] systems.

The bicelle system is composed of a 3.2:1 molar ratio of DMPC/DHPC (Avanti Polar Lipids, Birmingham, AL)

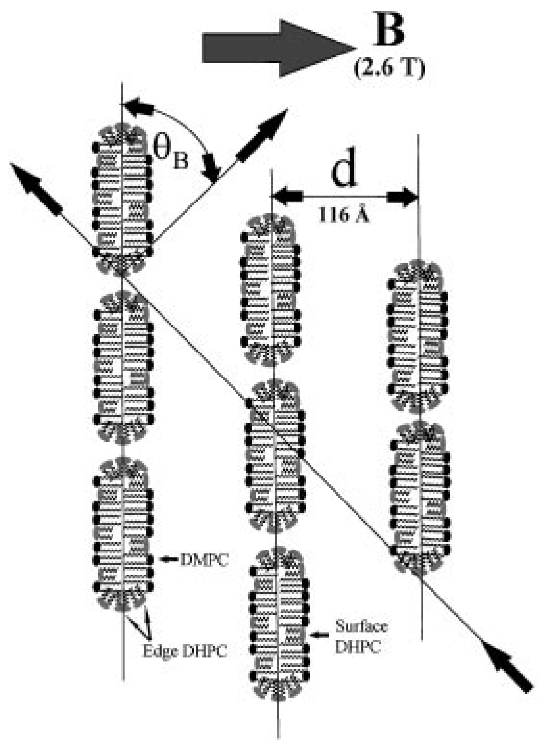

FIG. 1. Schematic of the diffraction geometry used to study magnetically aligned unilamellar DMPC/DHPC bilayers containing Tm^{3+}. The liquid-crystalline lipid bilayers are highly aligned (mosaic $\leq 1°$) with their bilayer normals parallel to the magnetic field ($\mathbf{B} = 2.6$ T). θ_B is the Bragg angle while the repeat distance of $\simeq$116 Å is denoted by d.

and thulium chloride hexahydrate (Aldrich Chem. Co., Milwaukee, WI) suspended in high purity D_2O such that the composition of D_2O/lipid is 3.3:1 by weight while the DMPC/Tm^{3+} molar ratio is 7.5:1. The transparent liquid mixture was placed in a thin-walled aluminum cylinder (diameter = 0.5 c m, length = 6 cm) and was aligned using a horizontal 2.6 T field supplied by a superconducting magnet. The experiments were carried out at the NRU reactor, Chalk River Laboratories, using the C5 and N5 triple-axis spectrometers which have a thermal flux of $\approx 5.4 \pm 0.3 \times 10^9$ neutrons $cm^{-2}\,s^{-1}$ at the monochromator position. Neutrons of wavelength 2.37 Å were selected using the (002) reflection of a pyrolytic-graphite monochromator (mosaic of $\simeq 0.4°$) while a graphite filter was used to eliminate higher-order neutrons. The instrumental resolution for the C5 spectrometer was defined by three slits leading to an overall calculated ΔQ (FWHM) of 0.0033 $Å^{-1}$. ΔQ was the result of finite beam collimation while the contribution of $\Delta\lambda/\lambda$ remained small ($\Delta Q = 0.00032n$ $Å^{-1}$) even at the largest scattering angles. The N5 spectrometer was set up using Soller collimators for both the incident and diffracted beams yielding an overall calculated ΔQ of 0.0083 $Å^{-1}$. This factor of 2.5 coarser resolution and a wider incident beam (compared to C5) resulted in the N5 instrument having a tenfold increase in scattered neutron intensity when compared to the C5 spectrometer.

Figure 1 shows a schematic of a thulium doped magnetically aligned stack of unilamellar bilayers. The morphology of the oblate lipid bilayers, as depicted in Fig. 1, is consistent with nuclear magnetic resonance (NMR) studies which suggest that the bilayer aggregate consists of a disk-shaped patch rich in DMPC and which is edge stabilized primarily by the short-chained DHPC lipid [12].

In the absence of a magnetic field and at a temperature of 315 ± 1 K the DMPC/DHPC/Tm^{3+} bicelle system forms a poorly aligned (mosaic $\approx 90°$) smectic phase having a repeat distance of 120 Å and giving rise to only two Bragg reflections [Fig. 2(a)]. Application of the magnetic field

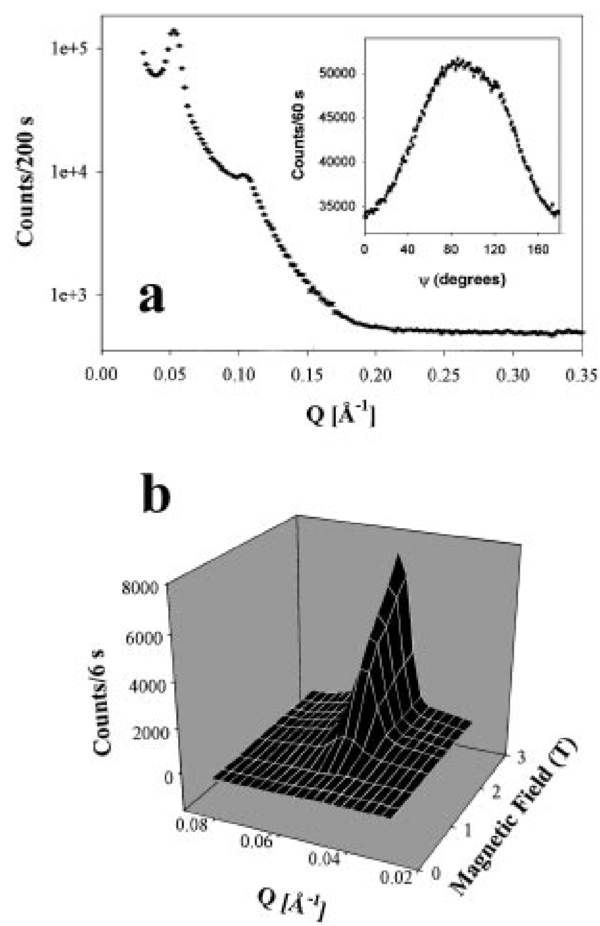

FIG. 2. (a) Q scan at a temperature of 315 ± 1 K and no applied magnetic field of the bicelle system containing Tm^{3+} ions using the N5 spectrometer. The inset contains the rocking scan of the first-order peak and corresponds to a mosaic of $\approx 90°$. (b) Q scans of the first-order diffraction ($n = 1$) maximum plotted as a function of magnetic field using the C5 spectrometer. At a field of ≈ 1 T, unilamellar lipid bilayers started rapidly to align with their bilayer normals parallel to the applied magnetic field. At ≤ 1 T no distinct Bragg reflection is discernable due to poor counting statistics. The lyotropic smectic phase exists in the presence or absence of a magnetic field. The magnetic field was ramped at a rate of 0.067 T/min from 0 to 1.5 T and from 1.5 to 2.6 T at a rate of 0.133 T/min.

did not seem to have any effect on the intensity of the first-order Bragg peak until a field of ≈ 1 T was reached [Fig. 2(b)]. At that point, the system began to rapidly align with the most intense Bragg reflection occurring at the highest magnetic field (2.6 T). At a constant magnetic field of 2.6 T the peak intensity remained unaltered over a period of days. Removal of the magnetic field caused the system to relax back to its initial state (mosaic $\approx 90°$) giving rise to a diffraction pattern similar to that presented in Fig. 2(a).

In Fig. 3(a) we present the diffraction pattern obtained from the aligned unilamellar bilayer stacks. The repeat spacing d was found to be 116 Å at 315 ± 1 K and 2.6 T. It is interesting to note that in the absence of Tm^{3+} but the presence of the magnetic field (2.6 T), the system is characterized by a single broad peak [Fig. 3(b)] resulting from bilayers lacking positional order and similar to that

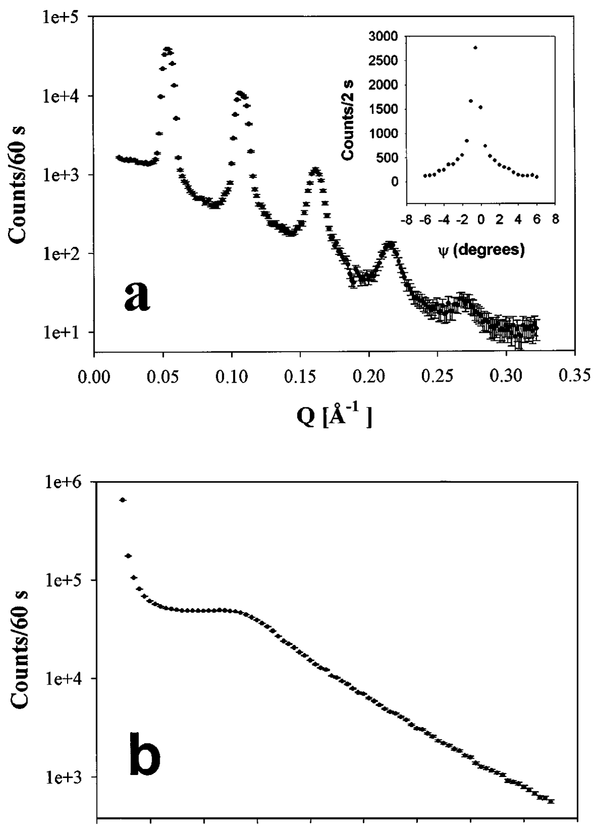

FIG. 3. (a) Diffraction pattern from the DMPC/DHPC unilamellar bilayer stack system containing Tm^{3+} at 315 ± 1 K and 2.6 T obtained using the C5 spectrometer. The FWHM of the Bragg peaks increases linearly with increasing scattering angle indicative of a 2D "fluid" system. The diffraction geometry was such that at $2\theta_B = 0$ the incident beam was $\perp$ to **B**. (b) Q scan of the DMPC/DHPC system in the absence of Tm^{3+} and under conditions of 315 K and 2.6 T. Note the absence of Bragg reflections present in the lyotropic smectic phase (a). At $2\theta_B = 0$ the incident beam was $\parallel$ to **B**.

observed in an analogous lipid/detergent system [14]. In the absence of both the paramagnetic ion and the applied magnetic field the scattering pattern was again similar to that presented in Fig. 3(b). Thus the high-quality diffraction pattern ($n = 5$, mosaic $\leq 1.0°$) shown in Fig. 3(a) is indicative of an aligned sample with a well-defined interlayer spacing. Consequently, the addition of a paramagnetic causes the bilayers to undergo a phase transition from a lyotropic nematic [Fig. 3(b)] to a lyotropic smectic [Fig. 3(a)] while the applied magnetic field acts to align the smectic phase [Figs. 2(b) and 3(a)]. Finally, we observe that the Bragg peaks are lying on a rapidly decreasing background which is adequately accounted for by the small-angle scattering resulting from slightly misaligned disks having a "contrast" thickness of $\simeq 25$ Å. This is a reasonable hydrophobic thickness for DMPC bilayers in the L_α phase [17].

From the diffraction pattern presented in Fig. 3(a), we observe that as a function of increasing Q the Bragg reflections decrease rapidly in intensity and broaden (FWHM) linearly with n, characteristic of 2D and 3D systems. Lipid bilayer systems are generally characterized as 2D structures with no interbilayer correlations [6,8,18]. After accounting for the instrumental resolution, we observe peak widths which vary as $[(\Delta Q_{\mathrm{length}})^2 + (n \times \Delta Q_{\mathrm{strain}})^2]^{1/2}$ giving an out-of-plane correlation length ($D = 2\pi/\Delta Q_{\mathrm{length}}$) of $\simeq 1400$ Å. The peak widths due to $\Delta Q_{\mathrm{strain}}$ (fluctuations in d) are consistent with L_α phase bilayers in which rapid molecular reorientations along with fluctuations of the membrane as a whole are taking place [19].

To a very good approximation, both the Bragg and total scattering intensities are unaffected by the orienting paramagnetic thulium ion. The proportions of $\sigma_{\mathrm{coh}\ Tm^{3+}}/\sigma_{\mathrm{coh\ lipid}}$ and of $\sigma_{\mathrm{paramag}\ Tm^{3+}}/\sigma_{\mathrm{incoh\ lipid}}$ are about 3×10^{-4} and 2×10^{-4}, respectively. Hence no additional corrections need be applied to structural or inelastic measurements when using this system.

Figure 4(a) shows a series of temperature-dependent Q scans of the first-order Bragg reflection at 2.6 T. Below 305 K no distinct diffraction maximum is observable. This is consistent with the isotropic spectrum (*sn*-1 chain of DMPC perdeuterated) obtained using deuterium quadrupole echo NMR at 8.5 T [Fig. 4(b)] and which differs from the NMR spectrum of the smectic lyophase [Fig. 4(c)] at 317 K. In contrast, as a function of increasing temperature, both the CsPFO/water [4] and DACL/water/ammonium chloride [5] systems undergo a smectic to isotropic phase transition predicted by the theoretical model described by McMillan [20]. Such a model of smectic ordering used to describe the CsPFO/water [4] and DACL/water/ammonium chloride [5] systems does not hold true for the DMPC/DHPC/water system.

In summary, we have shown that the biologically relevant DMPC/DHPC/water system undergoes, upon the addition of Tm^{3+}, a rarely observed nematic $\rightarrow$ smectic

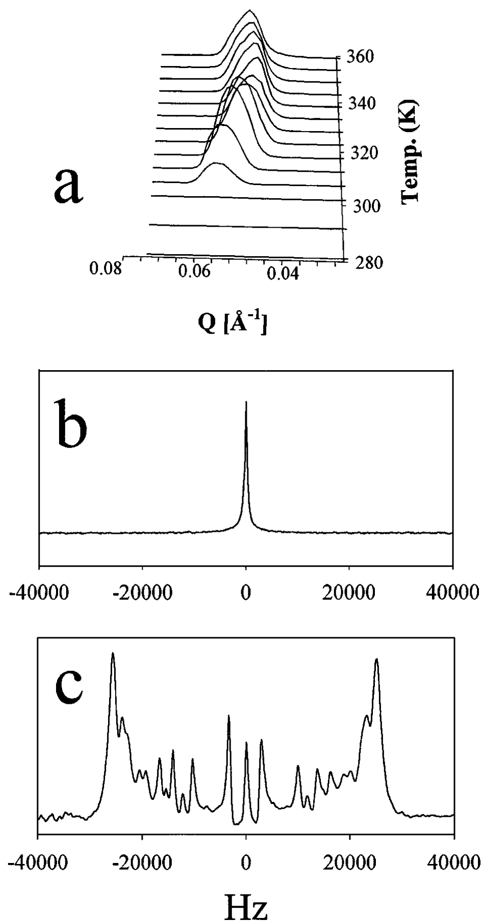

FIG. 4. (a) Q scans of the first-order Bragg reflection as a function of temperature at a magnetic field of 2.6 T. The onset of Bragg scattering occurs at temperatures above 305 K while the repeat spacing d changes as a function of temperature. There was a 30 min. equilibration period between successive scans. ^{2}H-NMR spectra of perdeuterated *sn*-1 chain DMPC in the bicelle system containing Tm^{3+} at (b) 285 K and (c) 317 K.

transition. This transition, although common in thermotropic systems, has to the best of our knowledge been observed, as a function of temperature, in only two other lyotropic systems. In addition, the present system differs from the two others by having an isotropic phase occurring at a lower temperature than the smectic phase. Finally, application of an external magnetic field aligns the smectic layers (mosaic of $\leq 1.0°$) giving rise to multiple Bragg peaks necessary for detailed structural studies when the system is used as a substrate to align a variety of macromolecules.

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