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THE MELTING TRANSITION IN SINGLE AND MULTIBILAYER PHOSPHOLIPID TUBULES

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Abstract The melting transition of phospholipid-based microcylindrical tubules, has been investigated for single and multibilayer tubules, using high-field, high-resolution magnetic birefringence and precision microcalorimetry techniques. Measurements of the magnetically induced optical palarizability anisotropy and the heat capacity, reveal that the transition of multibilayer tubules is discontinuous, consistent with the melting behavior of crystalline lipid phases. Single bilayer tubules exhibit a melting transition that conforms with hexatic-smectic phase transitions.

INTRODUCTION

Amphiphilic lipid molecules, when dissolved in water, self-assemble into various morphologies such as bilayer membranes and spherical vesicles, so that their oily hydrocarbon tails are not in contact with the water that surrounds them. In 1984, Yager and coworkers[1] showed that the chiral molecules of certain phospholipids can self-assemble into a fascinating microstructure, the tubule, that is a hollow cylinder with open ends, formed by helically wrapped, chiral, phospholipid bilayers. Tubules

have lengths that vary between a few and about one hundred microns, and inner diameter that is typically about 0.5 μm. The phospholipid walls consist from one, up to one hundred bilayers with bilayer thickness ~ 6 nm. Tubular microstructures have also been found in biological organisms[2].

A wide variety of technological applications have been suggested for the tubules[3]. The combination of their microscale size, their highly anisotropic morphology, their hollowness and the mechanical rigidity of their membrane-walls, make them promising candidates for electro-optical, pharmaceutical and biological applications. Even though the importance of molecular self-assembly has been widely recognized, the underlying mechanisms are still not well understood. So, tubules are also studied as a paradigm for understanding molecular self-assembly. Recently theoretical and experimental progress has been made towards explaining the chiral tubular architecture.

From the theoretical point of view, it is now widely believed, that an intrinsic bending force due to molecular chirality, along with the onset of 2D order are responsible for driving tubule formation[4]. The proposed theories are based on simple single bilayer tubule-models. Experimentally though, most quantitative studies of tubules are concerned with multibilayer ones. The mechanisms that govern multibilayer tubular formation may be far more complicated than in the single bilayer case. In this article, high-resolution, high-field magnetic birefringence and calorimetric measurements are presented, for the melting of phospholipid tubules. The data demonstrate important differences between the single and multiple bilayer systems. These differences are related to the molecular order within the membrane-walls. The melting of multibilayer tubules is consistent with the melting behavior of crystalline lipid phases. On the other hand, the melting behavior of single bilayer tubules is more consistent to that of hexatic phases.

The molecules of the chiral diacetylenic phospholipid 1,2-bis(tricosa-10,12-diynotl)-*sn*-glycero-3-phosphocholine, ($DC_{8,9}PC$) in pure water or water/alcohol solutions and temperatures below the chain-melting temperature T_m form tubules. At T_m, the hydrocarbon tails melt (from all-*trans* to *cis* transition) and the tubules

transform into a closed spherical morphology, the vesicles. Caffrey et al.[5], studied multibilayer tubules of ($DC_{8,9}PC$) formed in pure water solution. Their crystallographic measurements indicate that in the tubular morphology, the chain-frozen lipid phase in the membrane walls is the L_c' phase. The L_c' phase is the most ordered among the lipid phases and it was recently demonstrated, for hydrated multilamellar systems, that it is characterized by a distorted hexagonal sublattice of the tilted hydrocarbon chains and a commensurate molecular superlattice[6]. Even though interbilayer correlations for the in-plane order were found to be very weak, the phase possesses strong crystalline characteristics. It must be noted that in the case of the tubules such a superlattice was not observed. For temperatures above T_m, multilamellar vesicles (MLV's) are formed. The chain-melted phase of the bilayers was identified as L_α, a non-tilted smecticlike phase with liquidlike in-plane order. The $L_c' \rightarrow L_\alpha$ transition is first order, described by sharp heat capacity anomalies[5].

Thomas and coworkers[7], studied multibilayer tubules of ($DC_{8,9}PC$), assembled in ethanol/water solutions. Their synchrotron x-ray diffraction results reveal that while in the MLV topology, the phase of the phospholipid membranes is the L_α, in the tubular morphology the tilted hydrocarbon chains are packed is the $L_{\beta'}$ phase. There are three different $L_{\beta'}$ lipid phases known so far[8]. In all three, the chains are packed in a distorted hexagonal lattice. The distinction between them is based on the direction of the chain tilt towards the nearest, $L_{\beta I}$, or next-nearest neighbor chain, $L_{\beta F}$, or at an angle between these two, $L_{\beta L}$. In the tubule experiment, as well as in the original work by Sirota et al[8]. on freely suspended, hydrated lipid membranes, the x-ray diffraction measurements could not reveal whether the in-plane positional order of the $L_{\beta'}$ phase is quasi long-range so that the membrane is a 2D crystallite, or a 2D hexatic with short range positional correlations. The $L_{\beta'} \rightarrow L_\alpha$ phase transition was clearly shown to be reversible and first order

The molecules of ($DC_{8,9}PC$) in methanol/water solutions and concentrations under 2 mg/ml of lipid in the 85/15 percent in volume fraction solution, self-assemble into single bilayer tubules[9]. This tubular system is very important. First, it

corresponds to the proposed theoretical models for tubule formation and second, it approximates wrapped 2D-like membranes in dilute solutions and may thus be ideal for studying critical phenomena and the cylinder - sphere topological transformation. Since x-ray diffraction experiments are almost impossible for this very dilute system, high-resolution, high-field magnetic birefringence and calorimetric measurements were carried out, which probe respectively the morphological transformations and the bilayer phase transitions.

EXPERIMENTAL

The samples were prepared by dissolving the lipid in methanol at 65 ^{o}C, adding water at the same temperature and subsequently cooling the system at room temperature. This technique yields essentially 100% efficiency for tubule formation. Moreover the tubules produced this way have very uniform dimensions (60 μm length, 0.5 μm inner diameter and 6 nm lipid bilayer thickness). For lipid concentrations $\rho < 2$ mg/ml, single bilayer tubules are produced, while for concentrations $\rho \sim 4$ mg/ml, all the tubules have walls with 3 to 5 bilayers.

The high-field magnetic birefringence experiments were carried out at the Francis Bitter National Magnet Lab, at the Massachusetts Institute of Technology. Samples were loaded into sealable cells (316 stainless steel with fused silica windows, 3.2 mm optical path length). Sample cells were contained in a specially designed insert for a 33 mm vertical bore Bitter magnet, with fields up to 17.5 T. Although the molecules of ($DC_{8,9}PC$) have only a weak diamagnetic anisotropy, the cumulative effect of the supramolecular structure enables the tubules to align completely in fields 10 T. They align with the long axes parallel to the field direction.

Earlier magnetic birefringence studies[10] have shown that tubules can be treated as hard rods, the field-induced orientational ordering of which yields a measurement of the maximum induced refractive index anisotropy Δn_{max}. This quantity is proportional to the optical polarizability anisotropy of the tubule, which is determined

by the anisotropic cylindrical tubule shape rather than the individual molecular anisotropy. As it was shown in Ref. 10, from the analysis of Δn versus H data, measurements of the magnetic susceptibility $\Delta\chi$ can be extracted. $\Delta\chi$ is directly related to the molecular properties and thus the molecular ordering of the lipids within the tubule walls.

RESULTS AND DISCUSSION

First, the high precision differential scanning calorimeter measurements, which are sensitive to thermal fluctuations of the molecular order in the tubule walls, are presented. The specific heat in excess of background, ΔC_p for slow (~ 0.5 K/hour) heating of single and multibilayer tubule samples is displayed in Fig. 1. The ΔC_p peak for the single bilayer case is broad and rounded, with a non-vanishing tail

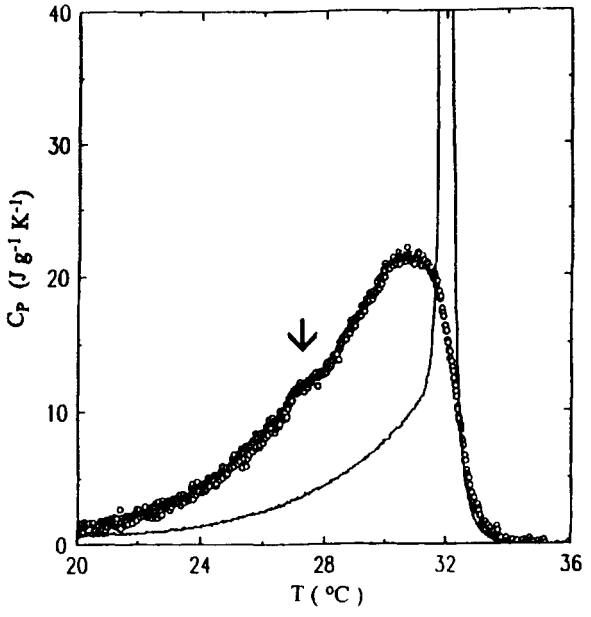

FIGURE 1 Heat Capacity measurements for the melting transition of single (open circles) and multibilayer (solid lines) tubules. The arrow indicates a weak pretransitional C_p anomaly.

indicating the presence of significant thermal fluctuations as much as ten degrees below T_m. The roundness of the peak in the region around Tm (± 1K) makes it impossible to describe the data by conventional power law expressions.

Since the purity of all the samples was verified, it is unlikely that impurities are responsible for the broadening of the peak for the single bilayer sample. The shape and size of the anomaly are also independent of the heating and cooling rates through the transition, for rates between 0.2 K/hour and 10 K/hour. It is thus unlikely that slow molecular kinetics are responsible for the observed broadening. The asymmetric shape of this broad anomaly is very similar, although much broader, to that for the (hexatic) smectic-I - smectic-A liquid crystal transition (i.e. a weakly first order transition)[11].

In contrast, the peak for the multibilayer tubule samples is sharp, with ΔC_{pmax} at T = 31.93 °C equal to 345 J K^{-1} g^{-1} off the scale of Fig. 1. The sharpness of this peak is consistent with the discontinuous first order $L_{c'} \rightarrow L_\alpha$ and $L_{\beta'} \rightarrow L_\alpha$ phase transitions reported in other multibilayer tubule systems[12]. The arrow in Fig. 1 indicates a weak reproducible C_p anomaly at 26.6 °C which may be associated with either a transition into the $P_{\beta'}$ or ``ripple'' phase[13], or a transition between two $L_{\beta'}$ phases with different molecular tilt directions with respect to the 2D bond direction, as it was mentioned earlier.

In Fig. 2, data are presented, for a tubule sample that contained single (95%) and multibilayer (5%) tubules. There is a broad ΔC_p peak at 31.1 °C and a sharp one at 32.0 °C. The broad peak can readily be identified as the melting transition of single bilayer tubules. The sharp ΔC_p anomaly must be associated with the melting of multibilayer ones. This can be established by calculating the total enthalpy ΔH of the peaks. ΔH = 10.3 J g^{-1} for the sharp anomaly, which is 8% of the total enthalpy ΔH = 126 J g^{-1}.

In Fig. 3, the specific magnetic birefringence $\Delta n_{max}/\rho$ at 17.5 T (where ρ is the lipid concentration), is presented as a function of temperature, for two samples of different ρ, containing single bilayer tubules and one containing multibilayer ones.

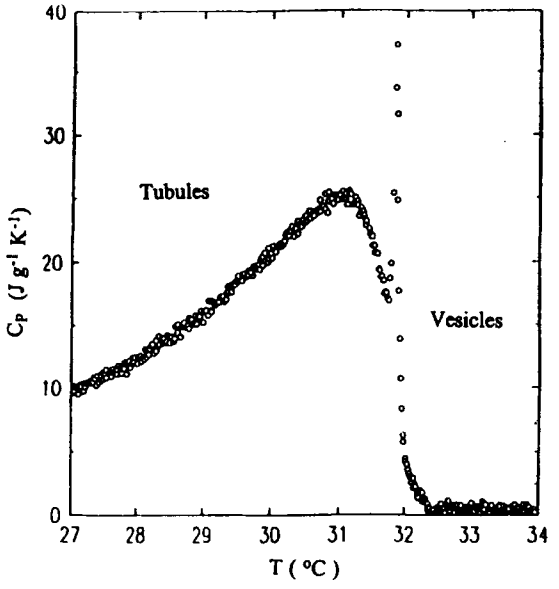

FIGURE 2 Heat Capacity measurements for a tubule sample that contains 95% single and 5% multibilayer tubules

The data were obtained as the samples were heated through T_m. It can be seen that in the case of single bilayer tubules, $\Delta n_{max}/\rho$ decays continuously to zero as the temperature is raised towards T_m. For the multibilayer sample, $\Delta n_{max}/\rho$ is constant for about eight degrees and goes to zero discontinuously at T_m. For temperatures higher than T_m, $\Delta n_{max}/\rho$ is zero since all three systems were composed of optically isotropic spherical vesicles that showed no measurable magnetic response.

The apparent differences between the two systems, i.e. the flexibility of the single bilayer tubules, the pretransitional behavior and the continuity in the single bilayer melting transition, versus the rigidity and the discontinuity shown by the multibilayer tubules, suggest that the in the case of single bilayer tubules the transition should be associated with a hexatic - fluid one, while for the multibilayer ones to a crystalline - fluid transition.

Finally, the molecular susceptibility $\Delta\chi$ as a function of temperature, data are presented in Fig. 4 for single bilayer tubules. The arrow indicates an anomaly in the temperature dependence of $\Delta\chi$ that is in agreement with the weak pretransiotional

C_p peak observed for this sample. Since the $\Delta\chi$ data are sensitive to the molecular ordering within the tubule walls, the observed anomaly should once again be associated with a phase transition between two $L_{\beta'}$ or between the $L_{\beta'}$ and the $P_{\beta'}$ or "ripple" phase.

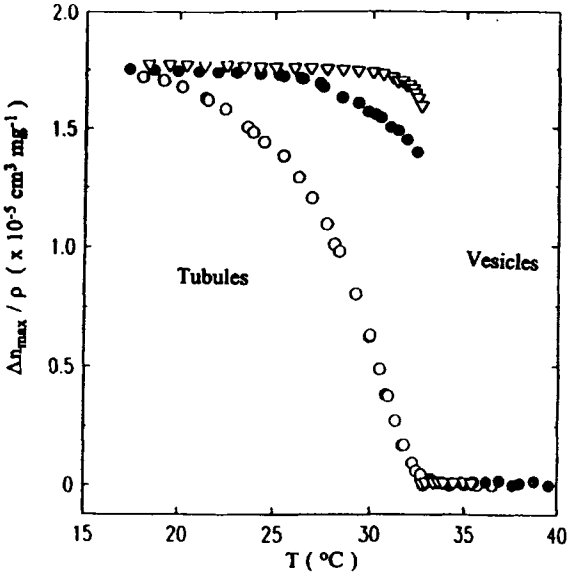

FIGURE 3 Specific magnetic birefringence measurements for single (open circles) and multibilayer (filled circles and triangles) tubules. The multibilayer samples have different lipid concentrations.

The magneto-optic and thermal measurements presented here support the same conclusion. There are important differences in the melting behavior of single and multibilayer tubules. Both techniques show clearly discontinuous melting of multibilayer tubules, which is expected for three dimensional crystalline phases and a weakly first order melting behavior of single bilayer tubules which is consistent with melting of a hexatic phase. The differences between the two systems may be associated with the gradual disappearance of crystalline order as the tubule walls become thinner.

Multibilayer tubular systems exhibit either the $L_{c'}$ or the $L_{\beta'}$ phase. Both phases have strong crystalline characteristics. Yet a complete structural analysis of these phases in tubules does not exist and little is known about interbilayer interactions.

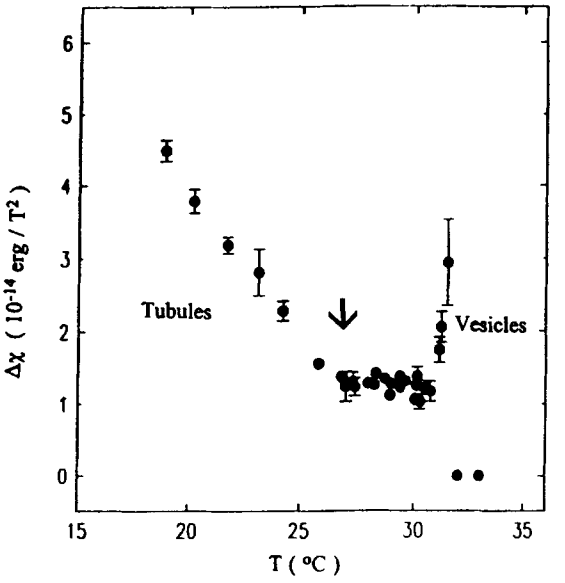

FIGURE 4 Magnetic susceptibility measurements near the melting transition of single bilayer tubules. The arrow indicates a possible pretransition.

Whether these phases are hexatics with strong translational in-plane correlations, is yet to be determined. The heat capacity and magnetic susceptibility measurements demonstrate that in the case of single bilayer tubules a pretransition takes place at temperatures below the chain-melting temperature. This transition is either between two $L_{\beta'}$ or between the $L_{\beta'}$ and the $P_{\beta'}$ or "ripple" phase.

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