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Melting of Phospholipid Tubules

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The melting of lipid-based microcylinders (tubules) has been investigated for systems with single
and multiple bilayer walls using high field, magnetic birefringence, and precision microcalorimetry.
The pretransitional behavior of both the magnetic birefringence and the specific heat is very different
in tubules with a single bilayer wall from that of tubules with multiple bilayers. [S0031-9007(96)
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Among the most fascinating self-assembled supramolecular structures is the tubule, a hollow cylinder formed by
helically wrapped bilayers of chiral amphiphilic molecules [1]. The highly anisotropic cylindrical shape and the
Crystaline molecular order of the tubule walls make tubules a promising candidate for a wide variety of technological
applications that range from electro-optical through pharmaceutical to biological [2–4]. Tubules are also being
studied as a paradigm for understanding molecular self-assembly [5]. Even though the importance of molecular
self-assembly has been widely recognized [5–7], our knowledge of the underlying mechanisms is far from
being complete. In the case of self-assembled tubules, theoretical [8–12] and experimental [13,14] progress has
recently been made toward explaining the chiral tubular architecture. While the existing theories of tubule forma-
ation are for those whose walls are a single bilayer thick, quantitative measurements to date have been carried out
only on tubules with multibilayer walls. The principal result of this Letter is our discovery that the melting behav-
ior of single bilayer tubules is very different from those with thicker walls. The melting of multibilayer tubules
was found to be discontinuous, as would be expected for the melting of a crystalline phase. The melting of single
bilayer tubules was found to be continuous with a broad heat capacity anomaly that should be associated to a tilted-
hexatic–smectic phase transition.

The diacetylenic molecules of 1,2-bis(tricosa-10,12-
diynoyl-sn-glycero-3-phosphocholine (DC8,9PC), in pure
water or alcohol/water solution and at temperatures below
the chain melting temperature \(T_m\), form tubules. In the
tubular morphology, the alcyl chains of the lipid favor
a tilted crystallinelike (\(L_{\alpha}\)) packing when the tubules
are formed in pure water [15], or a tilted, possibly
hexatic, packing (\(L_{\beta'}\)) when formed in ethanol/water [14].
Above \(T_m\) the lipid bilayers melt into a fluid lamellar phase (\(L\alpha\)), and the cylindrical tubules transform into
spherical vesicles. Recent investigation of the effects of
the alcohol chain length on tubule formation [16] revealed
that tubules formed in methanol/water mixtures can have
walls that consist of a single lipid bilayer, corresponding
to theoretical models for tubule formation. Tubules with a
single bilayer wall may also approximate two-dimensional
membranes in dilute solution and be ideal for studying
critical phenomena and melting in such systems [17], as
well as cylinder to sphere topological transformations.

We present here a series of high field magnetic birefrin-
gence and calorimetric measurements which probe, respecti-
vively, the morphological transformations and the bilayer
phase transitions of DC8,9PC tubules in methanol/water so-
lutions. At a lipid concentration, \(\rho\), less than 2 mg cm\(^{-3}\)
the majority, \(\sim95\%\), of the tubules formed have single bi-
layer walls. With \(\rho > 4\) mg cm\(^{-3}\), most of the tubules
have from two to four bilayers in the walls [18]. The
DC8,9PC tubules were grown in methanol/water solutions
85/15 in volume fraction. The samples were prepared by
dissolving the lipid in methanol at 65 °C, adding water at the
same temperature and subsequently cooling the system
to room temperature. This technique yields essentially
100% efficiency for tubule formation. Moreover, the
tubules produced this way have very uniform dimensions
(60 \(\mu\)m length, 0.5 \(\mu\)m inner diameter, and 6 \(\mu\)m lipid bi-
layer thickness), independent of \(\rho\) [16].

Although the molecules of DC8,9PC have only a weak,
diamagnetic anisotropy, the cumulative effect of the
supramolecular structure enables the tubules to align com-
pletely in fields \(\leq 10\) T. They align with the long axes
parallel to the field direction [19]. Earlier magnetic birefrin-
gence studies [19,20,21] have shown that tubules can be

treated as hard rods, the field-induced orientational or-
dering of which yields a measurement of the maximum
induced refractive index anisotropy \(\Delta n_{\text{max}}\). This quantity
is proportional to the optical polarizability anisotropy of
the tubule, which is determined by the anisotropic cylin-
drical tubule shape rather than the individual molecular
anisotropy.
The birefringence measurements were carried out in fields up to 17.5 T, with an optical path length in the sample of 3.2 mm, and the apparatus could measure $\Delta n$ with a resolution $1 \times 10^{-8}$. In order to minimize possible field effects on the phase behavior of the phospholipid molecules, the data were collected in the following manner: At any given temperature, the magnetic field was ramped up to 17.5 T, $\Delta n_{\text{max}}$ was measured, and the field was ramped down before the temperature was changed. In Fig. 1, $\Delta n_{\text{max}}/\rho$ at 17.5 T as a function of temperature $T$ is shown as solid circles for a sample with phospholipid concentration $\rho = 1.0$ mg cm$^{-3}$. The data were obtained as the sample was heated through $T_m \sim 32$ °C. It can be seen that $\Delta n_{\text{max}}$ decays continuously to zero as the temperature is raised. For temperatures higher than $T_m$, $\Delta n_{\text{max}}$ is zero since the system was composed of optically isotropic spherical vesicles that showed no measurable magnetic response.

Figure 1 also displays the maximum specific birefringence $\Delta n_{\text{max}}/\rho$ for two other lipid concentrations $\rho = 1.5$ and 4.0 mg cm$^{-3}$. For temperatures under 20 °C, all three samples have the same $\Delta n_{\text{max}}/\rho$ value, indicating that for each $\rho$, all of the lipid participates in tubule formation. However, while the 1.0 and 1.5 mg cm$^{-3}$ data overlap for all temperatures, the data for the 4.0 mg cm$^{-3}$ sample behave very differently, with very weak pretransitional features. In this sample, $\Delta n_{\text{max}}$ is constant for almost eight degrees before it starts decreasing. For temperatures just below $T_m \sim 32.3$ °C, its value is still 80% of the value at room temperature; then at $T = T_m$, $\Delta n_{\text{max}}$ goes to zero discontinuously.

We attribute the difference in the magnetic birefringence data for $\rho = 1.0$ and 1.5 mg cm$^{-3}$ from those for $\rho = 4.0$ mg cm$^{-3}$ to structural differences within the tubule walls. In contrast to the more dilute concentrations, the $\rho = 4.0$ mg cm$^{-3}$ system consists entirely of tubules that have walls comprised of from two to four lipid bilayers [18]. The order within the multibilayer walls is reported to be either tilted crystallinelike in pure water [15] or tilted $L_{\beta'}$ with strong translational correlations in ethanol and water [14]. In either case, it appears that the molecular packing of the acyl chains, combined with interactions between the bilayers, produces relatively rigid multilayer tubules that show little pretransitional behavior. As can be seen in Fig. 1, $\Delta n_{\text{max}}(T)$ is similar for multibilayer tubules in pure water and methanol/water; this suggests that multilayer tubules have strong crystalline correlations regardless of the solvent.

Theoretical calculations indicate that a single membrane that can buckle in three dimensions to form a hollow cylinder cannot possess true long-range positional order, but at most bond-orientational order [22,23]. The flexibility of single bilayer tubules should thus be comparable to that of hexatic rather than crystalline phases, and the pretransitional behavior observed as $T \rightarrow T_m$ for the $\rho = 1.0$ and 1.5 mg cm$^{-3}$ samples should be associated with a hexatic to fluid transition.

In order to elucidate further the differences between single bilayer and multibilayer tubules, we carried out a high precision differential scanning calorimetric study, which is sensitive to thermal fluctuations of molecular order in the tubule walls. The specific heat in excess of background, $\Delta C_p$, for slow ($\sim 0.5$ K h$^{-1}$) heating of the $\rho = 1.0$ and 4.0 mg cm$^{-3}$ samples is displayed in Fig. 2. The $\Delta C_p$ peak for $\rho = 1.0$ mg cm$^{-3}$ is broad and rounded with a nonvanishing tail, indicating the presence of significant thermal fluctuations as much as ten degrees below $T_m$. The roundness of the peak in the region $T_m \pm 1$ K makes it impossible to describe the data by conventional power law expressions. The arrow in the figure 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, or a transition between two $L_{\beta'}$ phases with different molecular tilt directions with respect to the 2D bond direction [24].

It may be noteworthy that the asymmetric shape of the broad $C_p$ anomaly is very similar to that for the hexatic smectic-$I$ to smectic-$A$ liquid crystal transition in bulk samples [25], but the tubule peak is broader. In contrast, the peak for the 4.0 mg cm$^{-3}$ sample is sharp, with $\Delta C_p^{\text{max}}$ at $T = 31.9$ °C equal to 345 J g$^{-1}$ K$^{-1}$, off the scale of Fig. 2. The sharpness of this peak is consistent

![Figure 1](image-url)
The arrow indicates the location of a weak $T$ anomaly for the 4.0 mg cm$^{-3}$ sample is far above the range of the plot at 345 J g$^{-1}$ K$^{-1}$. The total enthalpy is $\Delta H = 128 \pm 5$ J g$^{-1}$ for $\rho = 1.0$ mg cm$^{-3}$ and $137 \pm 6$ J g$^{-1}$ for $\rho = 4.0$ mg cm$^{-3}$.

with the discontinuous first-order $L_a$ to $L_\alpha$ [15] and $L_{\beta'}$ to $L_\alpha$ [14,26] phase transitions reported in other multibilayer tubule systems. While the total enthalpy change of our two samples is similar, ($\Delta H = 137 \pm 6$ J g$^{-1}$ for 4.0 mg cm$^{-3}$, and $128 \pm 5$ J g$^{-1}$ for 1.0 mg cm$^{-3}$), it is larger ($170$ J g$^{-1}$) for similar concentrations of multibilayer tubules in ethanol [26], which indicates somewhat stronger correlations in ethanol/water systems. As all the samples are of comparable purity, it is unlikely that impurities are responsible for the broad peak for the single bilayer sample. The shape and size of the peak are independent of the heating and cooling rates through the transition, for rates between 0.2 and 10 K/h. It is thus unlikely that slow molecular kinetics are responsible for the observed broadening. The broadening of the $C_p$ peak for single bilayer tubules is, however, reminiscent of the crossover from three- to two-dimensional behavior for phase transitions in thin liquid crystal films [27].

We turn now to our results for the intermediate lipid concentration $\rho = 1.5$ mg cm$^{-3}$ displayed in Fig. 3 which shows a broad peak at 31.1 °C and a sharp one at 32.0 °C. The broad peak is similar to that for $\rho = 1$ mg cm$^{-3}$ and can readily be identified as the melting transition of single bilayer tubules. The sharp peak anomaly can be associated with the melting of multibilayer ones. To establish this, we scaled the data for the 4.0 mg cm$^{-3}$ concentration to match the $\Delta C_p^{\text{max}}$ value for the sharp peak of the 1.5 mg cm$^{-3}$ sample and shifted the temperature scale up by 0.1 °C to match the transition temperatures. The scaled data, shown as the solid line in Fig. 3, fit the high temperature peak remarkably well. By calculating the total enthalpy $\Delta H$ of the scaled peak, we find $\Delta H = 10.3 \pm 0.5$ J g$^{-1}$, which is 8% of the total enthalpy ($\Delta H = 126 \pm 6$ J g$^{-1}$). This agrees well with electron microscopy measurements [16] where the number of multibilayer tubules for $\rho = 2$ mg cm$^{-3}$ was found to be 5% of the total. The measurements presented in Fig. 3 show the melting for noninteracting single and multibilayer tubules at the same time, and are unique in this respect in the study of lipid bilayers.

Multibilayer tubular systems exhibit either a $L_a$ or a $L_{\beta'}$ phase. Both phases have strong crystalline characteristics [14,15]. Especially the $L_a$ phase, which is shown to be characterized by a molecular superlattice and a hydrocarbon chain sublattice [28]. Yet a complete structural analysis of these phases in tubules does not exist and little is known about interbilayer interactions. Whether these phases are hexatics with strong translational in-plane correlations is yet to be determined. The magneto-optic and thermal measurements presented here support the same conclusions. Both techniques show melting behavior of single bilayer tubules, which is consistent with melting of a hexatic phase. Both techniques show discontinuous melting of multibilayer tubules, which is expected for three-dimensional crystalline phases. The differences between the two systems may be associated with the gradual disappearance of crystalline order as the tubule walls become thinner. Our results suggest that tubular morphologies can exist in either hexatic or crystalline phases of lipids, so that neither the development of bond orientational or positional order is primarily responsible for tubule formation, but rather that tilting of the chiral phospholipid molecules is the driving event [29]. Single bilayer tubules seem to be better candidates for description by current theories. In future studies of the formation and
melting of tubules, it will be important to ascertain if the tubules are formed from single or multiple bilayers.

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[21] The data for \( \rho = 4.0 \text{ mg cm}^{-3} \) differ from those of Ref. [20] because the earlier measurements did not allow enough equilibration time after temperature changes.