Gauging the Effect of Impurities on Lipid Bilayer Phase Transition Temperature

Monique M. Koan and G. J. Blanchard*

Department of Chemistry, Michigan State University, East Lansing, Michigan 48824-1322

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We report on the gel-to-fluid phase transition behavior of unilamellar vesicles formed with 1,2-dimyristoyl-*sn*-phosphatidylcholine (14:0 DMPC). We have interrogated the gel-to-fluid transition temperature of these bilayer structures using the chromophore perylene incorporated in their nonpolar region. We observe a discontinuous change in the reorientation time of perylene sequestered within the bilayer at the known melting transition temperature of 14:0 DMPC, 24°C. The perylene reorientation data reveal a local viscosity of 14.5 ± 2.5 cP in the gel phase, and 8.5 ± 1.5 cP in the fluid phase. We have also incorporated small amounts of 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine (14:1 DMPC) into these unilamellar vesicles and find that the melting transition temperature for these bilayers varies in a regular manner with the amount of 14:1 DMPC present. These data demonstrate that very little "contaminant" is required to cause a substantial change in the gel-to-fluid transition temperature, even though these contaminants do not alter the viscosity of the bilayer sensed by perylene, either above or below the melting transition.

Introduction

Phospholipid bilayers have attracted a great deal of interest because of their central role in cellular function and the potential for the use of model bilayer systems in chemical and biological sensing applications. As our understanding of phospholipid bilayers has developed, the structural and compositional complexity of this family of molecular assemblies has become more apparent. It is now known that natural bilayer structures contain 100 or more constituents,[1] and for simple model systems comprised of as few as two or three different species, phase separations have been observed.[2−6] While phase segregation remains to be seen for biologically derived bilayers, it is likely that these compositionally heterogeneous structures are characterized by small, fluid domains that are beneath our ability to resolve. Our interest in phospholipid bilayers lies in their potential utility as supported bilayer membranes, a structural motif useful in the creation of selective chemical sensing systems based on biological molecules such as enzymes and transmembrane proteins. We are investigating the fluidity and phase segregation behavior of model bilayer structures in an effort to optimize these films for incorporation of selected biomolecules in their active form. We focus in this work on the composition dependence of bilayer fluidity.

Phospholipids self-assemble spontaneously to form a bilayer structure with the hydrophobic tails directed toward the center, and the headgroups exposed to the aqueous phase. Phase transitions have been seen in a variety of bilayers, and it is thought that at the so-called gel-to-fluid melting transition temperature, T_m, the hydrocarbon tails undergo a change from being predominantly trans to a structural configuration characterized by a significant number of trans-gauche conformers.[7] The characteristic phase transition temperature (T_m) for a phospholipid depends sensitively on the aliphatic chain length and the presence and location of any unsaturations in the aliphatic chain(s), and such phase transitions have been detected by a number of techniques, ranging from calorimetry[8] to fluorescence spectroscopy[9] and spin labeling.[10] For temperatures below T_m, the lipid aliphatic tails are said to exist in the gel phase, a comparatively well-ordered state where translational mobility of the bilayer constituents is thought to be limited. Above the phase transition temperature, the phospholipid aliphatic tails exist in a more disordered fluid phase,[11] characterized by more motional freedom and consequently, more lateral freedom. Lipid bilayers may exist in the gel phase or the fluid phase depending on the temperature, pressure, extent of hydration, and the structural identity of the phospholipids.[12] This phase transition has received a great deal of research attention, both experimental and theoretical, because the phase transition temperature is related to the energetics of lipid−lipid interactions within the bilayer, and phospholipid phase transitions are considered to be important in regulating the activities of membrane-associated proteins.[13,14]

There is a significant body of literature focused on measuring T_m for selected phospholipid bilayer systems.[11−25] We have undertaken the present study for two reasons. The first is to evaluate the utility of fluorescent probe molecules sequestered within the nonpolar regions of lipid bilayers to detect phase transitions. The second purpose of this work is to evaluate the effect of impurities within the bilayers on the characteristic T_m for the resulting system. Both of these issues are of great potential importance because of the information content of the fluorescence experiments in addition to the phase transition temperature. While there has been a great deal of work focused on understanding structural phase transitions in bilayer systems, there remains the issue of the characteristic length scale over which the change in organization at the phase transition is seen. By working with systems containing controlled amounts of impurities, and comparing microscopic viscosity data to phase transition temperatures, we can assess whether the change in organization associated with a phase transition is reflected accurately by molecular-scale changes in the nonpolar region of the bilayer on the molecular scale.

For these experiments we use the chromophore perylene. Perylene is a planar polycyclic aromatic hydrocarbon with a well-characterized linear optical response. Its nonpolar nature

* To whom correspondence should be addressed. E-mail: blanchard@chemistry.msu.edu.

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makes it well-suited to incorporation within the acyl chain region of lipid bilayers. In this work, we focus on the rotational motion of perylene within the bilayer nonpolar region as a function of temperature. Not only is rotational diffusion more sensitive to local environment than fluorescence lifetime, but the details of the rotational motion provide a gauge of the bilayer viscosity. While perylene has been used previously in the study of bilayer systems, our direct picosecond time-resolved measurements afford more detail than has been available from either steady state or phase-resolved fluorescence measurements.[26,27]

The phase transition behavior of phospholipids bilayers from the gel phase to the fluid phase has been studied in great detail previously.[15−25,28] The phase transition temperatures of many phospholipids are well-documented, especially for those phospholipids containing no unsaturations. For some systems, even more specific information is known, such as changes in partial specific molar volume, for example.[28] However, there are comparatively fewer reports on the effect of "impurities" on T_m for a phospholipid bilayer. The work we report here, using 1,2-dimyristoyl-*sn*-phosphatidylcholine (14:0 DMPC) and 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine (14:1 DMPC) as the phospholipid and "impurity", respectively, demonstrates that even well-ordered phospholipid bilayers are characterized by a viscosity of 14.5 ± 2.5 cP in the gel phase and 8.5 ± 1.5 cP in the fluid phase. When 14:1 DMPC is introduced to a 14:0 DMPC bilayer, we observe a dramatic decrease in the gel-to-fluid transition temperature from 24 °C for the pure 14:0 DMPC bilayer, with a gradual approach to a new phase transition temperature of ca. 4 °C for concentrations of 14:1 DMPC on the order on 1 mol %. These findings point to the relatively small amount of impurity required to disrupt the phase transition temperature of a bilayer, even though the local viscosity of the bilayers did not sense a corresponding change in order.

Experimental Section

Vesicle Preparation. Phospholipids 1,2-dimyristoyl-*sn*-phosphatidylcholine (14:0 DMPC) and 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine (14:1 DMPC) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL) and used as received. Both lipids are >99% pure, with the details of the analyses being provided on Avanti's web site (www.avantilipids.com). Perylene was purchased from Sigma-Aldrich (Milwaukee, WI) and used without further purification. For each sample, the lipids and the perylene probe were mixed in selected ratios so that the final concentrations of the samples were 2.9×10^{-4} M lipid and 1×10^{-5} M perylene. The chloroform solvent was evaporated and an appropriate volume of Tris buffer (Sigma-Aldrich) was added to each mixture so that the lipid concentration was 1 mg/mL. The buffer (10 mM, pH 7.8) was prepared with purified water from a Milli-Q Plus water purification system (Millipore, Bedford, MA). The mixtures were processed five times through a freeze−thaw−vortex cycle to ensure complete mixing of the constituents. Each cycle was comprised of freezing the solution by immersion in liquid nitrogen for 5 min, followed by thawing via immersion in 60 °C water (5 min), and concluded with vortexing (approximately 2 min). After freeze−thaw−vortex processing, the solutions were extruded once through two polycarbonate membrane filters with 400 nm pore diameter, using a miniextruder (Avanti Polar Lipids Inc.). The initially extruded vesicle suspension was then extruded 11 times through two polycarbonate membranes (Avanti Polar Lipids Inc.) with a nominal pore diameter of 100 nm. Extrusions were performed at room temperature. The resulting solution contained vesicles with a size distribution centered around 100 nm diameter (Figure 1).[29−34]

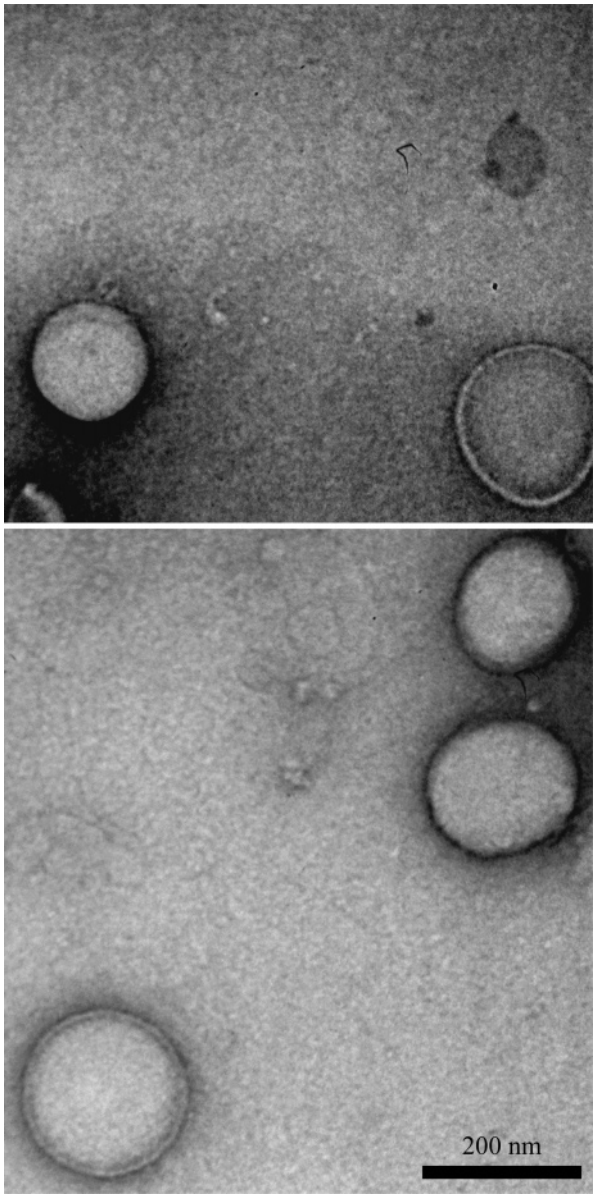

Figure 1. Transmission electron microscopic images of a unilamellar vesicle used in this work. The scale bar for both images is shown in the lower right corner.

Time-Resolved Fluorescence Measurements. Time-domain polarized fluorescence intensity decays were acquired over a range of temperatures with a time-correlated single-photon counting (TCSPC) system. This system has been described in detail elsewhere, and we briefly recap its salient features here.[35,36] The light source is a CW mode-locked Nd:YAG laser (Coherent Antares 76-s) that produces 100 ps 1064 nm pulses at 76 MHz repetition rate. The third harmonic of the Nd:YAG laser output is used to excite a cavity-dumped dye laser (Coherent 702-2) operated with Stilbene 420 dye (Exciton) at ca. 430 nm. The output of this laser is ca. 25 mW average power, with 5 ps pulses at a 4 MHz repetition rate. Sample fluorescence is detected with a microchannel plate−photomultiplier tube (MCP-PMT, Hamamatsu R3809U) and the electronics used to temporally resolve the fluorescence transients were a constant fraction discriminator (CFD, Tennelec 454) and a time-to-amplitude converter/biased amplifier (TAC, Tennelec 864). The collection wavelength and polarization were computer controlled using LabVIEW 7.0 code. The fluorescence lifetime data were collected at 54.7° with respect to the vertical excitation

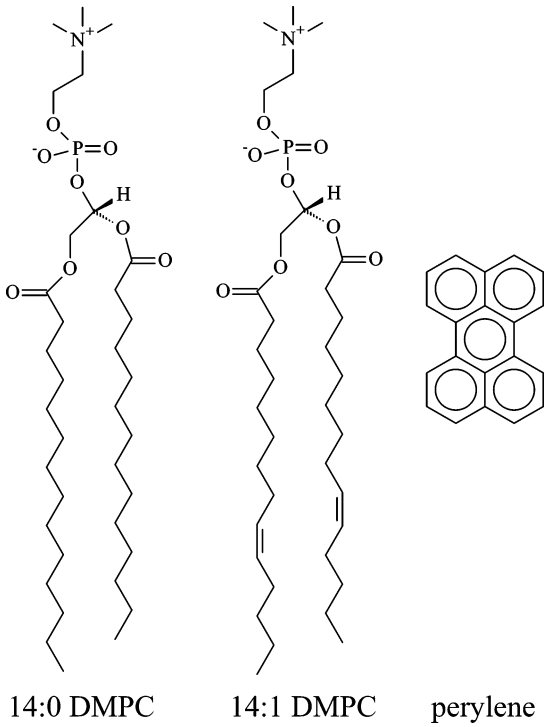

Figure 2. Structures of 14:0 DMPC, 14:1 DMPC, and perylene.

polarization, while the reorientation data were collected at polarizations parallel (0°) and perpendicular (90°) to the vertically polarized incident light. The temperature of the sample was regulated to ±0.1°C with a water-circulating bath (Neslab RTE-110) connected to a cooling jacket that holds the sample cuvette. All samples were allowed to thermally equilibrate for 10 min prior to data acquisition.

Steady-State Spectroscopy. Steady-state excitation and emission spectra (not shown) were acquired for our vesicle samples for the purpose of characterizing the perylene band positions. We used a Spex Fluorolog 3 emission spectrometer for all measurements, set to a spectral band-pass for both excitation and emission monochromators.

Results and Discussion

As noted above, there are two major goals of this work. The first is to demonstrate the feasibility of using a chromophore imbedded in the nonpolar region of a lipid bilayer structure to sense the gel-to-fluid phase transition temperature. The second purpose of this work is to achieve a more quantitative understanding of the effect that an impurity has on the phase transition temperature of vesicles comprised primarily of the phospholipid 14:0 DMPC. The chosen "impurity" for this series of experiments is 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine, a 14-carbon phospholipid that closely resembles 14:0 DMPC, but contains one cis-unsaturation at the 9-position in each acyl chain (14:1 DMPC, Figure 2). This choice of "impurity" was intended to introduce irregularities in the bilayer structures in a relatively well-controlled manner. We consider the utility of perylene as an optical probe of bilayer phase transitions first.

The gel-to-fluid transition temperature of pure 14:0 DMPC has been established to be 24 °C,[37] and we find that perylene exhibits a discontinuous change in its rotational diffusion dynamics in DMPC unilamellar vesicles at this temperature (Figure 3). Phenomenologically, perylene functions as a phase

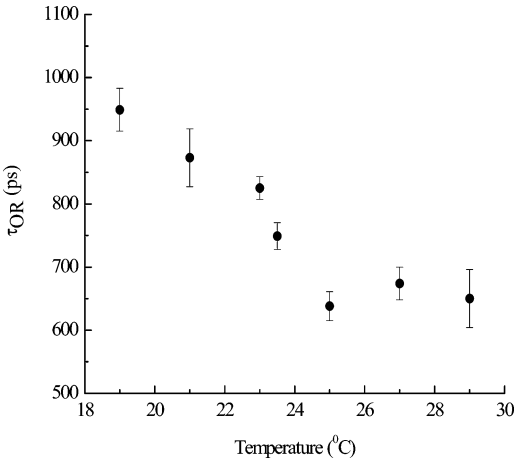

Figure 3. Reorientation time of perylene in unilamellar vesicles comprised of 14:0 DMPC as a function of temperature. There is a discontinuous change in τ_{OR} at the gel-to-fluid phase transition temperature. Error bars are $\pm 1\sigma$ for at least four individual determinations at each temperature.

transition probe likely because the chromophore is sequestered within the portion of the bilayer structure that is thought to undergo the greatest structural change at the point of phase transition from the gel to the fluid phases. It is fair to consider that the perylene itself may be responsible for some perturbation of the bilayer structure. While this possibility must be considered for any fluorescent probe experiment, we believe that the perturbation imposed on the bilayer by perylene is modest. Our reasons for this assertion are that, with perylene, we observe the same phase transition temperature for 14:0 DMPC that has been measured by other methods.[37] With the addition of relatively small amounts of 14:1 DMPC, we observe changes in the phase transition temperature for the bilayer system. Thus, even if the perylene does perturb the bilayer structure, it is constant for all of our measurements and the changes in phase transition temperature associated with the introduction of DMPC are manifested by large changes in the phase transition temperature. Apparently, the planar structure of the perylene molecule can be accommodated within the nonpolar bilayer region with less disruption than the presence of cis defects in the chains themselves.

In addition to the ability of perylene to sense the phase transition temperature of 14:0 DMPC, the measurements we perform provide other valuable information on the nonpolar interior region of these bilayers. We determine transient fluorescence anisotropy dynamics through the induced optical anisotropy function, $R(t)$ (eq 1)

$$R(t) = \frac{I_{||}(t) - I_{\perp}(t)}{I_{||}(t) + 2I_{\perp}(t)} \tag{1}$$

where $I_{||}(t)$ and $I_{\perp}(t)$ are the emission intensities polarized both parallel and perpendicular to the incident vertically polarized excitation pulse. The chemical information contained in these experiments resides in the decay functionality of $R(t)$, and this information differs for chromophores that exist in free solution versus those confined within a membrane structure. In general, a chromophore can produce an anisotropy decay with up to five exponential components, but owing to symmetry considerations and experimental signal-to-noise limitations, it is highly unusual to observe more than two decay components in $R(t)$. These decay components can be related to the Cartesian components

TABLE 1: Viscosities of 14:0 DMPC Unilamellar Vesicles as a Function of 14:1 DMPC Concentration[a]

	gel phase			fluid phase		
mol % 14:1 DMPC	τ_{OR} (ps)	T (K)	η (cP)	τ_{OR} (ps)	T (K)	η (cP)
0.0	825 ± 18	296	15.0 ± 0.3	638 ± 23	298	9.0 ± 0.1
0.3	646 ± 38	282	11.9 ± 0.7	408 ± 67	283	7.1 ± 1.2
0.7	850 ± 43	277	14.5 ± 0.7	453 ± 37	278	7.7 ± 0.6
1.0	767 ± 73	277	13.0 ± 1.2	588 ± 60	278	10.0 ± 1.0
1.5	1061 ± 26	271	17.6 ± 0.4	539 ± 5	273	9.0 ± 0.1

[a] Viscosities were determined from τ_{OR} data with use of eq 2.

of the rotational diffusion constant, D, and to the orientation(s) of the excited and emitting transition dipole moments relative to the molecular structure.

The reorientation dynamics of perylene are well-understood in a variety of environments. The excited and emitting transition dipole moments of perylene are known to reside along its long in-plane axis, which is typically assigned as the x-axis. In this scheme, the short in-plane axis is designated y and the z-axis is perpendicular to the chromophore π-system plane. For this molecule, a single-exponential anisotropy decay is taken to indicate perylene reorienting as a prolate rotor ($D_x > D_y = D_z$) and a two-component anisotropy decay is indicative of reorientation as an oblate rotor ($D_z > D_x = D_y$). We find experimentally that perylene reorients as a prolate rotor, rendering the recovered time constant $\tau_{OR} = 6D_z^{-1}$.[38] It is significant for several reasons that we recover a single-exponential anisotropy decay for all measurements. First, our earlier work on perylene in *n*-alkane bulk solvents revealed a change in the effective rotor shape of the chromophore as the length of the alkane exceeded the length of the perylene long axis.[38] The fact that we do not observe this same change suggests that the perylene may reside in a region that is not spanned continuously by discrete aliphatic chains. Such a finding is consistent with perylene residing near the center of the nonpolar region of the bilayer. The second reason that this finding is significant is that the reorientation time constants both above and below the phase transition are directly comparable to one another, allowing the straightforward extraction of local viscosity information (vide infra). While the use of perylene as a probe of bilayer microviscosity has been questioned before,[27] we believe that perylene is reporting accurately on microscale changes in analogous molecular environments across the gel-to-fluid phase transitions in these bilayers, and that on both sides of the phase transition, the probe sweeps out the same ellipsoidal volume of rotation. At the very least, the relative changes in local environment across the bilayer phase transition are reflected accurately in the perylene reorientation data. We consider next the relationship between the recovered reorientation time constant and the local environment of the chromophore.

The modified Debye−Stokes−Einstein equation relates the measured reorientation time to the quantities of interest.[39−41]

$$\tau_{OR} = \frac{\eta V f}{k_B T S} \qquad (2)$$

This model has been discussed extensively before, and we will not attempt a detailed review here. The quantities of interest here are η, the viscosity of the medium surrounding the reorienting chromophore, V, the solute hydrodynamic volume, f, a solvent−solute frictional interaction coefficient, and S, a shape factor to account for the nonspherical shape of the reorienting chromophore. This model has been shown to be in reasonable agreement with experimental data for a wide variety of systems. While this model assumes a continuum medium surrounding the reorienting moiety, which is clearly not an accurate description because the molecular motion we sense is averaged over many configurations of the local environment, the approximation of a continuum is phenomenologically reasonable. Using the calculated hydrodynamic volume of perylene (225 Å^3) and the experimental reorientation data, we can extract information on the viscosity of the lipid bilayers. These data lie in the range of ca. 14.5 cP for the gel phase and ca. 8.5 cP for the fluid phase (Table 1). These values are broad averages over a range of experimental conditions, but they are nonetheless important because they provide significant insight into the fluidity of these bilayer structures, and the influence that structural disorder has on this property.

To place these values in context, there has been a good deal of interest in the viscosity of bilayers because this property is a gauge of membrane fluidity and thus the mobility of membrane constituents. Our understanding of bilayer structures has evolved substantially over time, and with it has come a range of estimates for the viscosity of bilayers. Initially it was thought that bilayers were characterized by a viscosity of ca. 100 cP,[1] with subsequent estimates being revised downward. A recent estimate of plasma membrane viscosity was that it was similar to "crocodile fat on a warm summer's day".[1] Absent accurate viscosity data on warm crocodile fat, we chose to quantitate the viscosity of the bilayer structures directly. The viscosities we recover from our measurements are in a range one would expect for either a long chain alkanol such as nonanol or decanol (8.7−10.5 cP)[42] or an extensively hydrogen-bonded liquid such as ethylene glycol (ca. 16 cP).[43] Our findings, based on the fluorescence anisotropy data we report here, are consonant with other literature reports. X-ray diffraction experiments[44,45] and simulation studies[11] have shown that phospholipids in the gel phase pack less tightly than a crystal, but still exhibit short-range order. In the gel phase, the lipids are situated in a nominally hexagonal lattice pattern and the fatty acid chains are extended fully.[11] Our use of a nonpolar chromophore places the probe in a location ideally suited to the detection and characterization of changes in bilayer structure associated with the phase transition.

With the ability to detect phase transitions in bilayer structures using perylene as an optical "sensor," we turn our attention to the effect of impurities on the phase transition temperature and local organization of DMPC bilayers. To evaluate this issue, we have made samples containing comparatively small amounts of 14:1 DMPC as an impurity (0.3, 0.7, 1.0, and 1.5 mol %), and have measured the transition temperatures of the vesicle solutions using perylene reorientation. We show the temperature-dependent reorientation data in graphical form in Figures 4 and 5, for 14:0 DMPC vesicles containing varying amounts of 14:1 DMPC. We find that there is a regular progression in T_m,

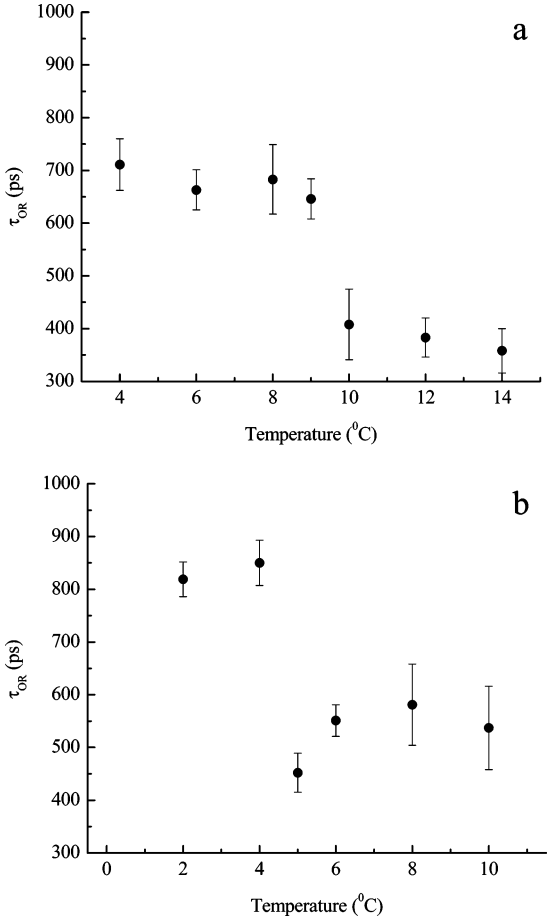

Figure 4. Reorientation time of perylene in 14:0 DMPC unilamellar vesicles containing (a) 0.3 mol % 14:1 DMPC and (b) 0.7 mol % 14:1 DMPC, as a function of temperature. Error bars are $\pm 1\sigma$ for at least four individual determinations at each temperature.

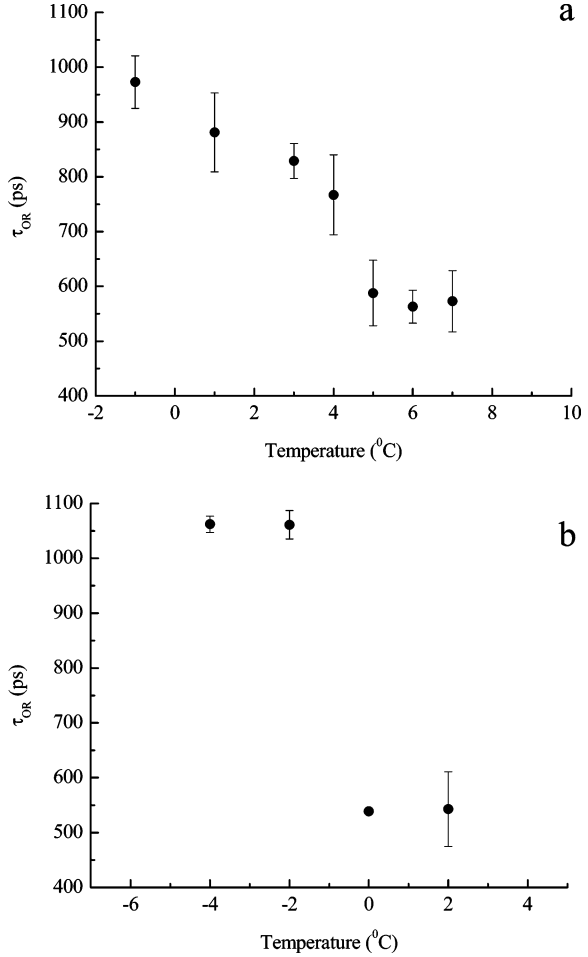

Figure 5. Reorientation time of perylene in 14:0 DMPC unilamellar vesicles containing (a) 1.0 mol % 14:1 DMPC and (b) 1.5 mol % 14:1 DMPC, as a function of temperature. Error bars are $\pm 1\sigma$ for at least four individual determinations at each temperature.

decreasing with increasing amounts of 14:1 DMPC (Figure 6). For 0.3 mol % 14:1 DMPC, we observe a 15 °C decrease in T_m, indicating that even relatively small amounts of a structural impurity can give rise to marked changes in the organization of bilayers. We note that experiments on bilayers comprised of 5 mol % DMPC did not yield a phase transition temperature in the range we can access (down to −15 °C). Given that as few as three molecules per thousand can disrupt the organization of a bilayer substantially, it is fair to question whether the viscosity of the bilayers changes with the addition of 14:1 DMPC. As shown in Table 1, we recover viscosities that are possibly slightly different than those for vesicles of pure 14:0 DMPC, but the fact that we are measuring the reorientation times at substantially different temperatures makes a direct comparison of viscosity values for the different vesicles difficult. That said, for all of the viscosity data we report, the fluid phase viscosities lie in the range of 8.5 ± 1.5 cP and the gel phase viscosities are in the range of 14.5 ± 2.5 cP. These are measurably different values for the two phases, but there appears to be no discernible trend in either phase with respect to the amount of 14:1 DMPC present. Thus, even though the longer range organization that is characterized by T_m is compromised by the presence of 14:1 DMPC, the local environment(s) sensed by perylene in either the gel or fluid phases is not influenced measurably by the presence of the structural impurity.

The perylene reorientation data provide information on the effective local viscosity of the DMPC bilayer. This information can be used to estimate the translational diffusion coefficient,

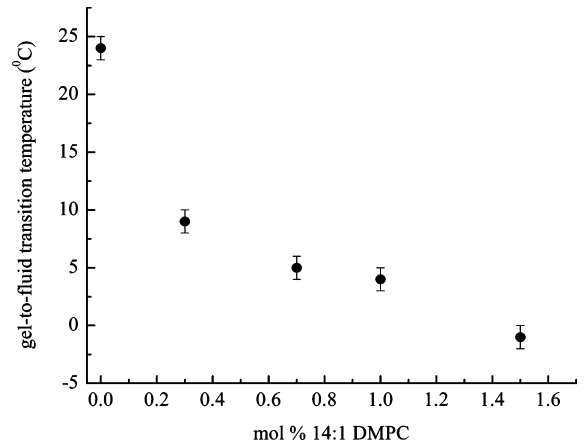

Figure 6. Variation of measured T_m in 14:0 DMPC unilamellar vesicles as a function of 14:1 DMPC concentration.

D_T, of perylene in these systems according to the Stokes−Einstein equation,[46]

$$D_T = \frac{k_B T}{6\pi R\eta} \tag{3}$$

where R is the radius of the diffusing species and η is the viscosity. To estimate R, we use the hydrodynamic volume of

TABLE 2. Calculated Translational Diffusion Coefficients for Perylene in 14:0 DMPC Unilamellar Vesicles as a Function of 14:1 DMPC Concentration[a]

	gel phase			fluid phase		
mol % 14:1 DMPC	T (K)	η (cP)	D_T (μm^2/s)	T (K)	η (cP)	D_T (μm^2/s)
0.0	296	15.0 ± 0.3	38 ± 1	298	9.0 ± 0.1	64 ± 1
0.3	282	11.9 ± 0.7	46 ± 3	283	7.1 ± 1.2	77 ± 11
0.7	277	14.5 ± 0.7	37 ± 2	278	7.7 ± 0.6	70 ± 5
1.0	277	13.0 ± 1.2	41 ± 4	278	10.0 ± 1.0	54 ± 5
1.5	271	17.6 ± 0.4	30 ± 1	273	9.0 ± 0.1	59 ± 1

[a] Viscosities were determined from τ_{OR} data with use of eq 2, and D_T values were calculated according to eq 3.

perylene (225 Å^3) and presume a spherical shape, yielding $R = 3.77$ Å. We present the calculated values of D_T for our systems in Table 2. The data show that for all of these systems, there is an increase of a factor of ca. 1.5 in D_T across the phase transition, and that, even with the addition of 14:1 DMPC, D_T changes only modestly. It is, of course, important to consider how our values of D_T ($\sim$40 μm^2/s below T_m, $\sim$65 μm^2/s above T_m) compare to other reports of D_T. Our values for D_T are in excellent qualitative agreement with those reported for tethered fluorophores in phospholipid monolayers, where D_T values between 15 and 110 μm^2/s were reported, depending on the compression applied to the phospholipid LB monolayer.[47]

We provide the calculated D_T values in Table 2 with a couple of caveats. The first is that our bilayer structures could be a heterogeneous system, because we do not know the extent to which the 14:1 DMPC tends to aggregate within the bilayers. In this limit, the translational diffusion coefficient we report would not be an accurate representation of the frustrated translational diffusion that would occur in a heterogeneous system. The other caution is that both eqs 2 and 3 were derived for a solute contained within a continuum solvent, and for rotational diffusion measurements, there can be significant deviation from model predictions for cases where the solute and solvent molecules become similar in size. This deviation reflects a complex solvent−solute frictional interaction that must involve molecular-scale steric and dipolar processes. The microscale viscosities we infer from the reorientation data could, in principle, be off by a scaling constant, rendering our D_T values uniformly high or low, but the scaling constant should be essentially the same for all measurements. The change in D_T across the phase transition is thus reflected accurately in our reported results. With this frame of reference, we believe that the relatively modest changes in D_T across the phase transition for our systems is more consistent with a change in the extent of organization within the bilayer than with any substantial changes in free volume within the bilayer.

Conclusions

We have evaluated the utility of fluorescence anisotropy as a means of characterizing gel-to-fluid phase transitions in phospholipid bilayer structures. We find that perylene reorientation is a sensitive measure of the bilayer phase transition and that the incorporation of impurities has a substantial and measurable effect on this phase transition temperature. We have incorporated perylene, a nonpolar polycyclic aromatic hydrocarbon, into lipid bilayer structures consisting of pure 14:0 DMPC and in bilayers containing 14:0 DMPC with 0.3, 0.7, 1.0, and 1.5 mol % of 14:1 DMPC over a range of temperatures surrounding the phase transition for these systems. Despite this mesoscopic structural effect, the local environment of the perylene probe is not affected substantially by the addition of 14:1 DMPC. Our viscosity data above and below the phase transition temperature provide quantitative information on the fluidity of this family of bilayer structures.

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