

the fluorescence excitation spectrum of jet-cooled molecules. Figure 3 shows the laser-induced fluorescence excitation spectrum of 9-anthroic acid in the region of S_1 origin. The most striking feature of the spectrum is the appearance of a long progression in a low-frequency ($\sim 47\text{ cm}^{-1}$) vibration that can only be assigned to the torsion of the carbonyl group (around the bond joining it to the aromatic ring). About 12 members of the $v'' = 0$ progression can be seen in the spectrum. Methyl 9-anthroate also exhibits a long progression in a low-frequency ($\sim 36\text{ cm}^{-1}$) mode (Figure 3). These results, which are similar to those for 9-phenylanthracene,¹⁰ demonstrate that the excited state is strongly displaced along the torsional coordinates relative to S_0 . The large

Stokes shift and the diffuseness of the dispersed fluorescence (not shown) also indicate that the S_1 torsional potential is substantially displaced with respect to S_0 , and that the major contributions to the Franck-Condon factors for the emission come from the vibrational levels high up in the S_0 torsional potential. Analyses of the observed spectra are consistent with the nearly perpendicular S_0 molecule transforming into nearly coplanar S_1 molecule.⁸

Details of these and related work will be presented in the near future.

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Registry No. 9-Acetylanthracene, 784-04-3; 9-anthroic acid, 723-62-6; methyl 9-anthroate, 1504-39-8.

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Fluorescence Detection of Gel-Gel Phase Transitions in DMPC Vesicles at High Pressures

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The fluorescent probe dipyrrenylpropane has been used to monitor pressure-induced phase transitions of dilute DMPC dispersions at different temperatures in the 0.1–340-MPa pressure range. The phase boundary of the main transition (liquid crystalline \rightarrow gel I), the pretransition (gel I \rightarrow gel II), and the subtransition (gel II \rightarrow gel III) are characterized by $dT/dP = 0.24$, 0.18, and 0.20 K MPa⁻¹ and $\Delta V = 17$, 2.2, and $\sim 3.2\text{ cm}^3\text{ mol}^{-1}$, respectively.

Introduction

The fluorescence of dipyrrenylpropane (DPyP) is a familiar indicator of the main lipid bilayer phase transition of synthetic phospholipids¹ and biological membranes.² The liquid crystalline to gel transition (LC \rightarrow GI) significantly modifies the microfluidity of the bilayer; consequently, the dynamics of intramolecular excimer formation are well suited for the detection of fluid-solid transitions. But fluidity is not a useful concept in describing gel-gel transitions, so the utility of this probe is not obvious for the latter application. However, Zachariasse et al.¹ showed that the excimer/monomer intensity ratio was sensitive to the pretransition (GI \rightarrow GII) as well as the main transition of DPPC. In our recent studies of pressure-induced micellar phase changes, we observed in the case of cetyltrimethylammonium chloride that the monomer/excimer intensity ratios of solubilized DPyP possess multiple discontinuities.³ The present report demonstrates that DPyP is indeed a sensitive monitor of pressure-induced gel-gel transitions in dimyristoylphosphatidylcholine (DMPC) vesicles.

The allotropic gel phases are studied by many methods, but differential scanning calorimetry (DSC) has been particularly useful in identifying the transition temperatures T_i and the enthalpies ΔH of the main transition (LC \rightarrow GI), the pretransition (GI \rightarrow GII), and the subtransition (GII \rightarrow GIII) of synthetic phospholipids.⁴ Wong⁵ has reviewed the results of Raman scattering as a means for observing pressure-induced gel-gel

transitions, thus offering a direct comparison with the present work, in which the bilayer is perturbed by a foreign molecule. The present study furnishes the first evidence that DPyP fluorescence intensity ratios are useful in obtaining pressure-induced transition profiles of gel-gel phase changes.

Experimental Section

The high-pressure apparatus⁶ and techniques for handling encapsulated fluid samples^{7,8} have been described. The probe 1,3-di(1-pyrenyl)propane (DPyP) was used as received from Molecular Probes, Inc.³ Synthetic dimyristoyl-L- α -phosphatidylcholine (DMPC) was used as purchased from Sigma Chemical Co. ($\sim 98\%$ purity).

The labeled vesicles are freshly prepared before each pressure experiment. An aliquot of DPyP-chloroform stock solution is added to DMPC in a 5.0-mL flask. Solvent evaporation leaves a 1:1800 DPyP/DMPC film which is dissolved in 0.05 M KCl/0.01 M Tris buffer (pH 7.7) to yield a 1.78 mM DMPC dispersion containing about 1 μM probe. The dilute aqueous dispersion is sonicated under nitrogen at 40 °C and centrifuged to give multilamellar vesicles.⁹ The N₂-purged sample is transferred to a quartz capsule in a N₂-filled glovebag. The temperatures are regulated to $\pm 0.2\text{ °C}$, and the isothermal measurements are made with increasing pressures up to 340 MPa.

Results and Discussion

The effect of pressure on the DPyP fluorescence spectra is shown in Figure 1 for DMPC dispersions at 10.0 °C. Compression of the gel phase reduces the excimer signal ($\lambda = 500\text{ nm}$) more

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TABLE I: Thermodynamic Parameters of DMPC Phase Transitions

| | dT/dP , K MPa ⁻¹ | | T_i , °C | ΔH , kcal mol ⁻¹ | ΔV , cm ³ mol ⁻¹ | |
|------------|-------------------------------|---|--------------------|-------------------------------------|--|--------|
| | this work | lit. | | | this work | ref 18 |
| LC → GI | 0.24 | 0.17, ^a 0.215, ^b 0.259 ^c | 23.59 ^d | 5.03 ^d | 17 | 18 |
| GI → GII | 0.18 | | 14.4 ^d | 0.83 ^d | 2.5 | 1.7 |
| GII → GIII | 0.20 | | | 1.0 ^e | ~3.2 ^f | |

^aReference 11. ^bReference 12. ^cReference 13. ^dReference 14. ^eReference 15. ^f $T_i = -12$ °C at 1 atm; extrapolated from present results (Figure 3).

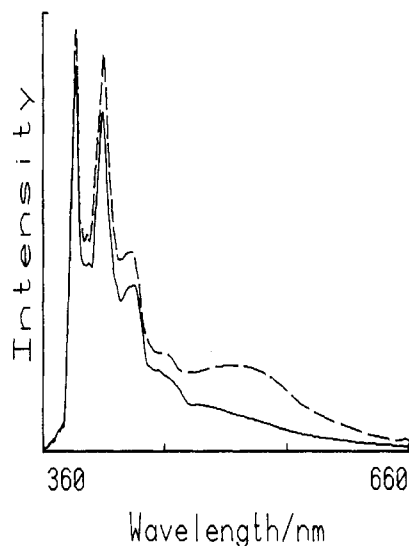


Figure 1. Fluorescence spectra of DPyP solubilized in DMPC vesicles at 10.0 °C and pressures of 0.1 (---) and 300 MPa (—).

strongly than the monomer signal ($\lambda = 379$ nm) of this bichromophoric molecule. Pressure experiments at 10.0, 20.0, and 30.0 °C with DPyP-labeled multilamellar vesicles show discontinuous changes in scattering, at the excitation wavelength of 325 nm, and in fluorescence intensities of monomer (I_M) and excimer (I_E). The excimer intensity and 325-nm scattered light are most sensitive to pressure in the LC phase and at the LC → GI boundary, while I_M is more pressure sensitive to gel-gel transitions. Consequently, the ratio I_M/I_E is best for profiling pressure-induced lipid transitions and is plotted in Figure 2. As we will show in a future report, the intensity ratio is a correct measure of environmental fluidity in the liquid crystalline phase but not in the gel phases.⁹ The trend shown in Figure 2 is an increase for I_M/I_E for phases stable at progressively higher pressures, but this trend does not establish a correspondence between bilayer packing and excited-state kinetics.

In Figure 2, the inflection points are indicated by vertical lines a through d and identified with the transition pressures P_i . The transitions at 30.0 °C are located at 21, 75, and 210 MPa. At this same temperature Wong, et al.¹⁰ report $P_i = 15, 100$, and 260 MPa, respectively. The pressure dependence of the main transition of DMPC has also been studied by fluorescence depolarization.¹¹⁻¹³ Their results for the linear slope dT/dP , using $T_i(1 \text{ atm}) = 23.59$ °C,¹⁴ give transition pressures P_i (MPa) = 39,¹¹ 30,¹² and 25 at 30 °C.¹³ Thus our results for the main transition are bracketed by those inferred from Raman and from fluorescence depolarization measurements and well within uncertainties expected for these microheterogeneous media.

With respect to gel-gel transitions at the higher pressures, the agreement between Raman scattering and our I_M/I_E results is also satisfactory. A striking feature of the probe response is the narrow

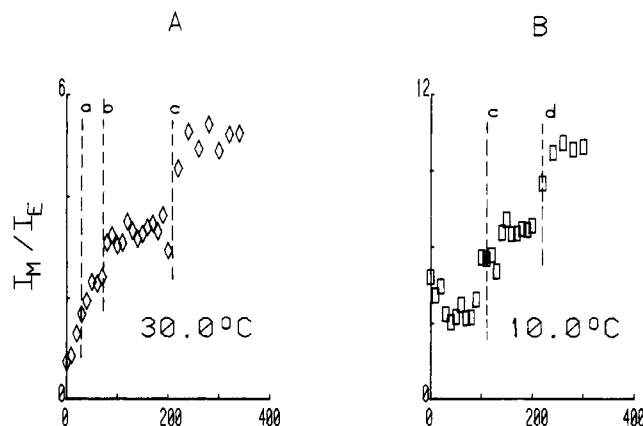


Figure 2. Pressure dependence of I_M/I_E in DMPC vesicles at 30.0 (A) and 10.0 °C (B). The phase transitions are a (LC → GI), b (GI → GII), c (GII → GIII), and d (GIII → GIV).

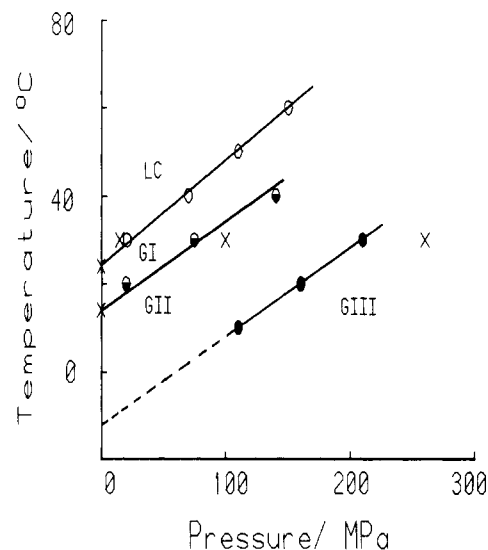


Figure 3. The T-P phase diagram of DMPC. The phase boundaries are LC → GI (○), GI → GII (◐) and GII → GIII (●). Literature values (×) are from ref 14 at 1 atm and from ref 10 at elevated pressures.

width of the gel-gel transitions relative to the main LC → GI transition. The origin of this trend is unknown, but may be related to a significant difference in pressure hysteresis for the fluid-solid vs. solid-solid transformations. An additional gel IV phase is stable above 220 MPa at 10.0 °C (the d boundary in Figure 2). Its location at higher temperatures could not be distinguished from the observed scatter in the I_M/I_E ratios at fixed pressure intervals. The subtransition (GII → GIII) appears sharper than the pre-transition (GI → GII), possibly due to its greater separation from neighboring transitions. The original I_M/I_E values are recovered after release of the applied pressure.

Additional confidence in identifying I_M/I_E discontinuities with phase changes comes from a comparison of the P_i results at different temperatures. The shift in P_i with T is of the expected magnitude for the main transition of phospholipids,^{13,16} as shown

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in Table I. The new thermodynamic information provided by this work is the dT/dP values for the pretransition and subtransition (Table I). The three phase boundaries in the P, T diagram shown in Figure 3 have similar slopes. In the case of the $G_I \rightarrow G_{II}$ transition, the 1-atm intercept is in full agreement with T_i observed by DSC.¹⁴ The $G_{II} \rightarrow G_{III}$ transition extrapolates to $T_i = -12^\circ\text{C}$ at 1 atm, which is considerably higher than the value inferred from Raman scattering.¹⁷

The Clausius-Clapeyron equation $dT/dP = T_i\Delta V/\Delta H$ is used to calculate the volume changes ΔV accompanying first-order phase transitions from measured dT/dP , T_i , and ΔH values. Table I shows that both enthalpy and volume changes are much smaller between gel phases compared to the main $LC \rightarrow G_I$ transition. Independent densitometry measurements yield ΔV values for the main and pretransition which agree well with the present work (Table I).¹⁸

Conclusion

The fluorescence intensity ratio method has been used to study the thermotropic behavior of dilute DMPC dispersions. Intramolecular excimer formation appears more sensitive than fluorescence depolarization to gel-gel phase transitions.¹¹⁻¹³ The observed high-pressure phase boundaries are in agreement, where comparison can be made at 30°C , with the results from Raman scattering.^{5,10} As in Raman,^{5,10,19} the I_M/I_E discontinuities show

that the $G_{II} \rightarrow G_{III}$ transition is more clearly defined than the $G_I \rightarrow G_{II}$ transition, consistent with the assignment of a major structural transition to the $G_{II} \rightarrow G_{III}$ subtransition and a minor step increase in trans conformers to the $G_I \rightarrow G_{II}$ pretransition.¹⁵ We conclude that the perturbation induced by the probe molecule is negligible with respect to the location of structural bilayer transformations. The structural identity of the different phases must come from other methods, but the determination of their boundaries and potentially, the transition kinetics, will be a convenient application of intramolecular excimer probes.

The salient results is that dT/dP for gel transitions are similar in value to the main ($LC \rightarrow G_I$) transition in DMPC. Therefore, low enthalpy transitions are accompanied by low volume changes. This generalization should serve as a useful guide for the mapping of phospholipid phase diagrams, especially at low temperatures and high pressures.

The molecular mechanism responsible for I_M and I_E changes at gel-gel transitions is unknown. The fluidity perspective is not applicable for gel polymorphism, despite contrary discussion.^{1,20} It is equally evident that the dynamic behavior of intramolecular excimers contains additional information about conformational trapping and quenching. However, important clues about structural rearrangements cannot be obtained from the excited-state kinetics until tested in homogeneous fluid, liquid crystalline, and crystalline phases.

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The Proton Affinity of CS

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We have studied the reactivity of HCS^+ ions with several molecules at 300 K using a SIFT apparatus. The measured rate coefficients for the proton-transfer reactions of HCS^+ with $\text{C}_2\text{H}_5\text{OH}$ and CH_3SH are appreciable fractions of their respective collisional rate coefficients and from this we deduce that the proton affinity of CS is $188.2 \pm 1 \text{ kcal mol}^{-1}$. This value differs from the literature value by some 13 kcal mol^{-1} but is in close agreement with a very recent theoretical value.

Introduction

The selected ion flow tube (SIFT) has become a most valuable experimental tool for the study of ion-neutral reactions under thermal conditions. Since its inception we, and others, have exploited it to determine the rate coefficients and product ion distributions for a large number of reactions,¹ including many considered to be important in the synthesis of interstellar molecules.² SIFT experiments are also being performed to provide thermochemical data relating to gas-phase ions (see below). Much of the available data is being used in detailed ion-chemical schemes which can now correctly model the relative densities of many interstellar molecular species.^{3,4} However, a long-standing problem has been the inability of the schemes to correctly model the observed HCS^+/CS abundance ratio. In considering this problem (which we now believe to be resolved⁵), we carried out

TABLE I: Experimental Rate Coefficients, k , Measured at 300 K for the Proton-Transfer Reactions of HCS^+ with a Series of Molecules, M , and Collisional Rate Coefficients, k_c , Calculated for 300 K Using the ADO Theory^a

| M | PA (M), ^b kcal mol ⁻¹ | k , cm ³ s ⁻¹ | k_c , cm ³ s ⁻¹ |
|-----------------------------------|--|--|--|
| NH ₃ | 204.0 | 2.0×10^{-9} | 1.82×10^{-9} |
| CH ₃ COCH ₃ | 196.7 | 2.4×10^{-9} | 2.40×10^{-9} |
| C ₂ H ₅ OH | 188.3 | 8.2×10^{-10} | 1.75×10^{-9} |
| CH ₃ SH | 187.4 | 4.5×10^{-10} | $\approx 1.6 \times 10^{-9}$ |
| HCN | 171.4 | $< 1 \times 10^{-12}$ | 2.62×10^{-9} |
| H ₂ S | 170.2 | $< 1 \times 10^{-12}$ | 1.35×10^{-9} |

^a Reference 18. Also HCS^+ does not react with H_2CO , H_2O , CH_4 , CO_2 , CO , O_2 , N_2 , and H_2 . ^b Literature values.⁷

a SIFT study of the reactions of HCS^+ with several known interstellar molecules. This study showed that HCS^+ is a relatively unreactive ion, a result which is consistent with its relatively large abundance in interstellar clouds. Moreover, the observation that

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