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A LOW-TEMPERATURE STRUCTURAL PHASE TRANSITION OF 1,2-DIPALMITOYL-*sn*-GLYCERO-3-PHOSPHOCHOLINE BILAYERS IN THE GEL PHASE *

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A new thermotropic phase transition, at −30°C and atmospheric pressure, was found to occur in the gel phase of aqueous DPPC dispersions. The Raman spectral changes at this phase transition are similar to those observed in the gel phase of DMPC dispersions at −60°C. The thermotropic phase transition at −30°C is equivalent to the barotropic GII to GIII phase transition observed in DPPC at 1.7 kbar and 30°C. It is shown that the rate of the large angle interchain reorientational fluctuations decreases gradually with decreasing temperature, and that the orientationally disordered acyl chain structure of the GII phase is extended into the GIII phase of DPPC. The interchain interaction, arising from the damping of the reorientational fluctuations, increases with decreasing temperature in the GII gel phase as well as in the GIII gel phase.

Introduction

In a high-pressure Raman spectroscopic study of aqueous 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) bilayers we found a new structural phase transition at 2.6 kbar and ambient temperature, which we have assigned to a GII to GIII gel-gel phase transition [1]. Based on the thermodynamics of this first-order phase transition we predicted a similar structural phase transition in the gel phase of DMPC at low temperature, and indeed found it at −60°C at atmospheric pressure [2].

More recently we also investigated the high-pressure Raman spectra of aqueous 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) bilayers, and found a similar gel-gel phase transition at 1.7 kbar and 30°C [3]. According to the thermodynamics of this transition and the analogy with DMPC, this pressure-induced gel-gel phase transition is also expected to occur in aqueous DPPC at low temperatures and atmospheric pressure. As the DPPC is one of the most popular model biomembrane systems, its thermotropic phase behaviour has attracted many studies and thus a search for this low temperature phase transition in DPPC is certainly warranted.

Although the temperature dependence of the Raman [4] and infrared [5] spectra of aqueous DPPC bilayers have been studied at temperatures as low as −100°C, this low-temperature gel-gel phase transition has not been observed. However, Yellin and Levin [4] had observed an inflection point at −40°C in the plots of various Raman band intensity ratios, which was attributed to the onset of the hydrocarbon *gauche-trans* isomerization in the gel phase of DPPC bilayers. Also, a change in slope around this temperature was observed in the temperature-dependent splitting of the CH_2 scissoring band in the infrared spectra of gel phase DPPC bilayers [5], which, however, was

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Abbreviations; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

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not commented upon. Herein we have performed a detailed study of the temperature dependence of the frequencies and widths of various Raman bands of DPPC/water dispersions in the gel phase, with the hope to detect a phase transition at low temperature and atmospheric pressure which would correspond to that observed at 1.7 kbar at ambient temperature. In the search of this phase transition we were considerably aided by technical improvements with our Raman spectroscopic systems which enabled us to detect discontinuities in frequencies of less than 1 cm^{-1} [1,2].

Experimental

High purity L-DPPC was obtained from Sigma Chemical Co., and was used without further purification. A sample of gel phase DPPC containing 42 wt% double distilled water was prepared by heating the DPPC/water mixture in a closed vial to about 60°C, vortexing the heated sample and allowing it to cool to room temperature. The heating-cooling cycle was repeated three times. At room temperature, the DPPC sample was placed in a quartz capillary and mounted in a home-made low-temperature system cooled by boiling liquid nitrogen. Raman spectra were measured upon decreasing the temperature from about 20°C to about −100°C. Two chromel-alumel thermocouples were taped on the capillary tube as close as possible to the laser beam transit. One thermocouple served to control the temperature of the nitrogen gas while the second was used to measure the temperature of the sample. Temperature control was maintained within ±1 K.

The Raman spectra were obtained using the 514.5 nm argon line from a CRL model 12 argon ion laser, a Spex Model 14018 double monochromator having 1800 line/mm holographic gratings and a cooled RCA C31034 photomultiplier. The intensity of the laser beam was approx. 150 mW at the sample. The temperature increase due to the local heating by the laser beam was estimated to be 2 K. The data acquisition system and the data treatment have been described previously [1].

Results

At ambient pressure and temperature aqueous DPPC dispersions are in the gel phase [6,7]. We have measured the Raman spectra of such gel phase DPPC dispersions over a temperature range of over 100 K by decreasing the temperature from

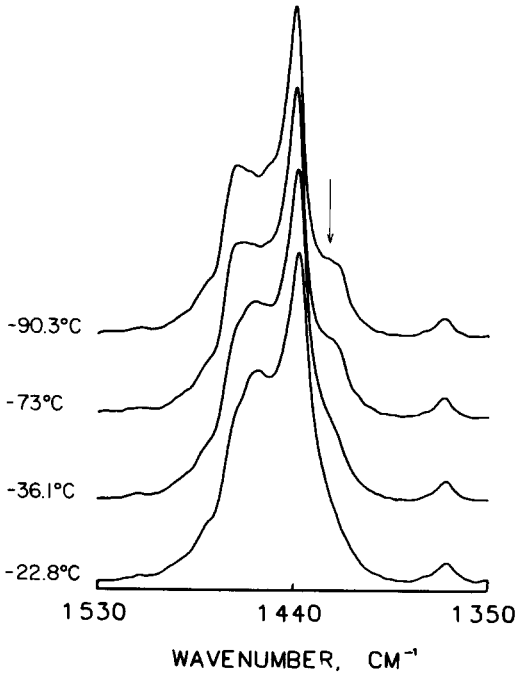

Fig. 1. Representative Raman spectra of aqueous DPPC dispersions in the gel phase in the 1350–1530 cm^{-1} region.

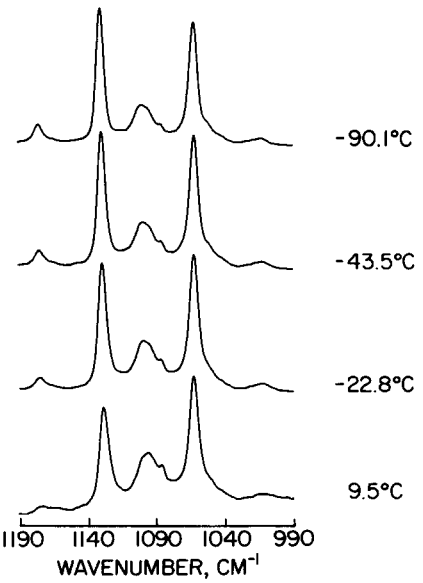

Fig. 2. Typical Raman spectra of aqueous DPPC dispersions at several temperatures in the region of the C–C skeletal stretching bands.

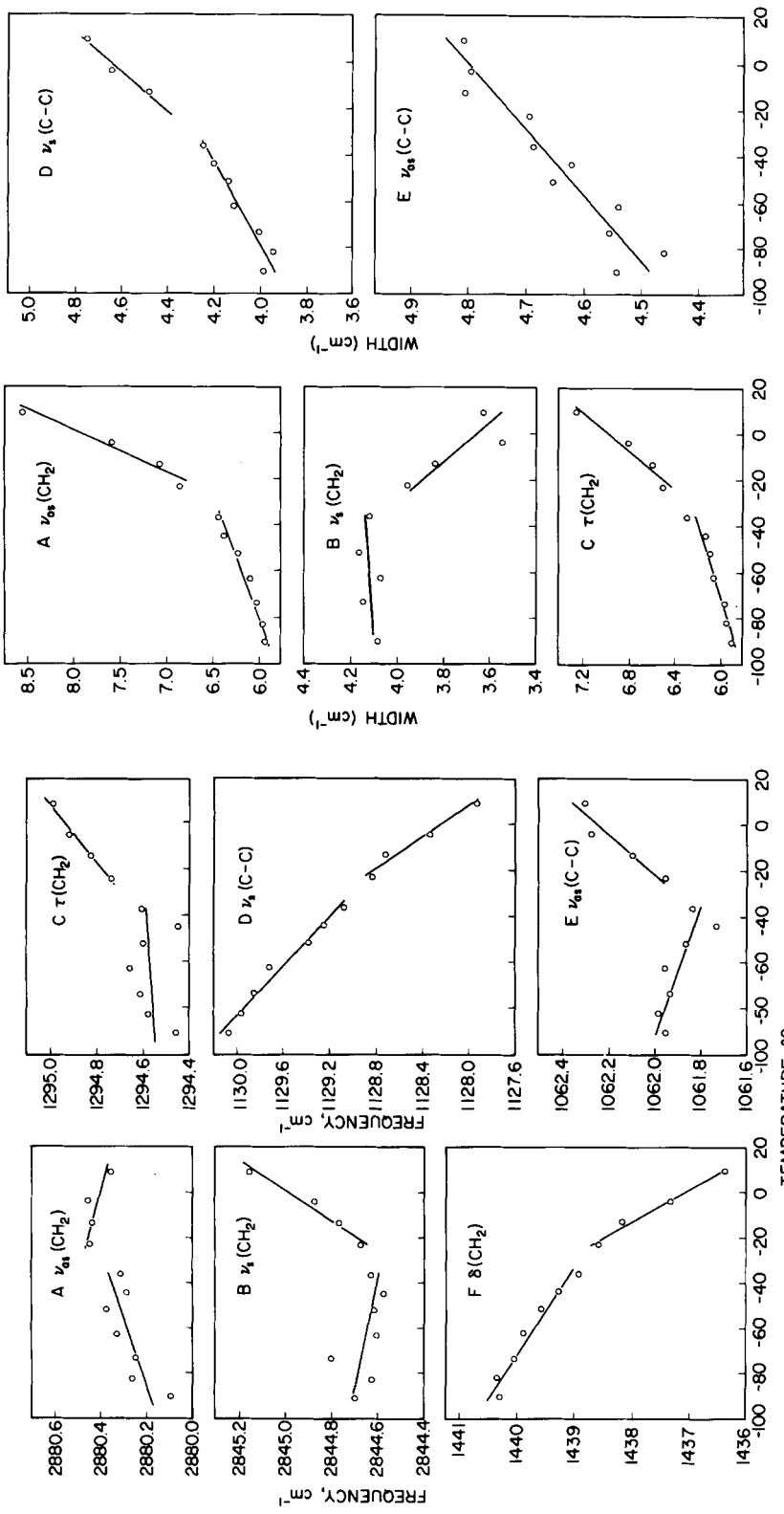

Fig. 3. Temperature dependence of the frequencies of selected Raman bands of aqueous DPPC disperisons in the gel phase. Panels A and B, antisymmetric and symmetric CH_2 stretching bands; C, CH_2 twisting band; D and E, symmetric and antisymmetric C–C stretching bands; F, CH_2 scissoring band.

Fig. 4. Temperature dependence of the widths of selected Raman bands of aqueous DPPC dispersions in the gel phase. Panels A through E refer to the same bands as described in Fig. 3. Widths are measured at different peak heights: 0.8(A), 0.95(B), 0.5(C) and 0.75(D and E).

20°C to about −100°C; the frequency regions recorded were 1000–1530 cm^{-1} and 2750–3050 cm^{-1}. The main features in the Raman spectra of DPPC in these regions are similar to those of DMPC [1,2]. Representative Raman spectra in the CH_2 bending and the skeletal C–C stretching regions are shown in Fig. 1 and Fig. 2, respectively. The Raman spectra at temperatures above −30°C are similar to those of the GII gel phase of DPPC at pressures below 1.7 kbar, whereas the spectra at temperatures below −30°C resemble those of the GIII gel phase at pressures above 1.7 kbar [3]. Furthermore, the changes observed in the Raman spectra of DPPC dispersions at −30°C are similar to those observed in DMPC dispersions at −60°C [2].

The correlation field band near 1410 cm^{-1} which is a characteristic band observed in the GIII gel phase of DMPC bilayers below −60°C [2] or at pressures above 2.6 kbar [1], and in the GIII phase of DPPC bilayers above 1.7 kbar [3], appears in DPPC immediately below −30°C at atmospheric pressure as seen in Fig. 1.

The temperature-dependent changes of a number of Raman bands are shown in Fig. 3 and Fig. 4. Discontinuities near −30°C are observed in the temperature dependences of the frequencies and widths of these vibrational modes. The shifts in frequency at −30°C, however, are smaller than those observed at −60°C in DMPC dispersions for the same vibrational modes [2]. The width of the depolarized and weakly polarized Raman bands decreases with decreasing temperature throughout the entire gel phase (Fig. 4 A, C, D and E). The magnitude of the decrease is smaller in the gel phase below −30°C, but is considerably larger than that in the gel phase of DMPC below −60°C [2]. On the contrary, the width of the symmetric CH_2 stretching band, a strongly polarized Raman band, increases with decreasing temperature, the magnitude of increase being smaller in the gel phase below −30°C compared to that observed in the gel phase above −30°C (Fig. 4B).

A popular parameter, the ratio of the peak heights between the antisymmetric and the symmetric CH_2 stretching bands, H2880/H2845, is plotted against temperature in Fig. 5. This ratio increases with decreasing temperature over the whole temperature range from 20 to −100°C; the rate of increase in this ratio is smaller below −30°C and a change of slope is observed near −30°C. In the present work we also observed a change in the relative peak heights of the symmetric and antisymmetric C–C stretching bands in the gel phase of DPPC. As shown in Fig. 2, at temperatures above −30°C the band corresponding to the ν_s(C–C) mode at 1128 cm^{-1} is higher than that of the ν_{as}(C–C) mode at 1062 cm^{-1}, whereas below −30°C the situation is reversed; at −30°C, the peak heights of the ν_{as}(C–C) and ν_s(C–C)

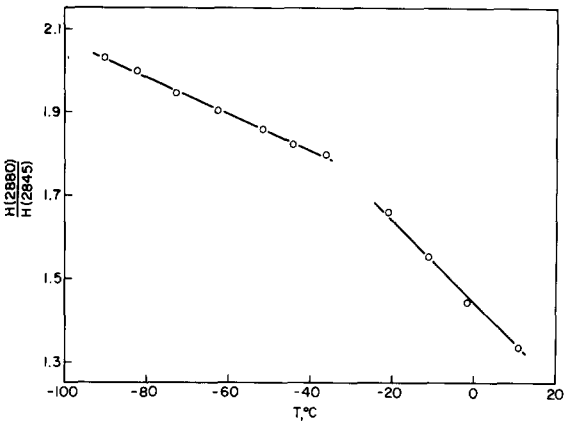

Fig. 5. Temperature dependence of the peak height ratio between the antisymmetric and symmetric CH_2 stretching bands of DPPC dispersions in the gel phase.

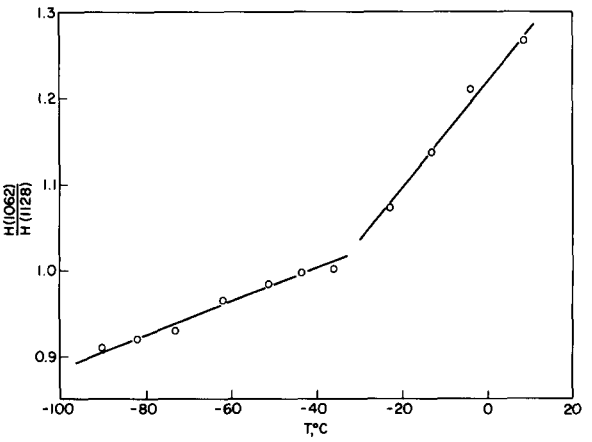

Fig. 6. Temperature dependence of the peak height ratio of the antisymmetric and symmetric C–C stretching bands of aqueous DPPC.

bands are about the same. The peak-height ratio between the ν_{as}(C–C) and ν_s(C–C) bands is plotted against temperature in Fig. 6; a change of slope at −30°C in this plot is evident.

Discussion

The polymorphism of fully hydrated phosphatidylcholines was studied extensively revealing the existence of a number of different phases (Refs. 4–14 and references therein). One of the most thoroughly investigated systems, hydrated multibilayer assemblies of DPPC, exhibit a gel-to-liquid-crystalline phase transition at 41.5°C, the main or acyl chain melting phase transition, a gel-gel phase transition at 35°C, generally referred to as the pretransition and another, more recently discovered phase transition around 16°C, observed only after extensive incubation of the sample at temperatures around 0°C.

The results of the present study clearly show that yet another structural phase transition occurs in aqueous DPPC bilayers at −30°C. As the dT/dP value for the transition between the liquid-crystalline and the gel phases of DPPC bilayers is almost identical with that of this transition in DMPC bilayers [15], one may assume, to a first approximation, that the dT/dP values of the GII to GIII gel-gel transitions of these two phospholipid systems are also identical, i.e. 34.6 K/kbar. Thus, a simple arithmetic calculation shows that the thermal phase transition in DPPC bilayers, which would correspond to the barometric transition at 1.7 kbar, is expected at −28.8°C at ambient pressure, a value which agrees surprisingly well with the present experimental results.

The appearance of the correlation field component of the CH_2 bending mode near 1410 cm^{-1} immediately below −30°C, along with the change in the spectral features in the CH_2 stretching region at this temperature indicate that this phase transition is associated with a change in the packing pattern of the acyl chains and suggests that it involves a structural change from a distorted hexagonal acyl chain packing at temperatures above −30°C, which we refer to as the GII gel phase, to a phase with an orthorhombic subcell structure at temperature below −30°C, which we refer to as the GIII gel phase [16]. The final word on the structure of the GIII phase of DPPC, however, has to await the results of X-ray diffraction measurements. The spectral changes at this temperature-induced phase transition in the gel phase of DPPC are similar to those observed at the pressure-induced structural phase transition at 1.7 kbar [3], and resemble those observed in the thermotropic phase transition of DMPC bilayers at −60°C [2]. Thus, the approximate phase boundary between the GII and GIII phases of DPPC can be drawn as shown in Fig. 7.

It is known that broadening of the anisotropic (depolarized) component of Raman bands of condensed phase systems is largely due to reorientational fluctuations [17,18]. Thus, the decrease in width of all the depolarized and weakly polarized bands with decreasing temperature observed in the gel phase of DPPC (Fig. 4 A, C, D and E), indicates that the large-angle reorientational fluctuations of the acyl chains in the gel phase are gradually damped as the temperature decreases. The width of the highly polarized symmetric CH_2 stretching band (Fig. 4B), on the other hand, increases with decreasing temperature; this indicates that the vibrational relaxation due to the interchain interaction increases with decreasing temperature, since the width of this band is unaffected

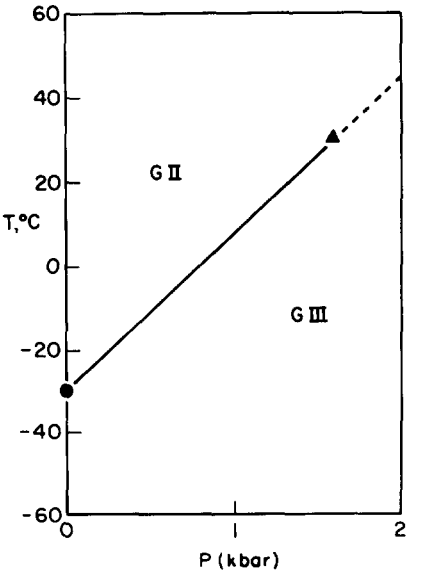

Fig. 7. Approximate phase boundary between the GII and GIII phases of aqueous DPPC dispersions.

by reorientational fluctuations, but is strongly affected by interchain interactions [19,20]. The increase in interchain interaction with decreasing temperature, due to the damping of the reorientational fluctuations, is also evident from the temperature dependence of the peak height ratio $H2880/H2845$ in Fig. 5, a quantity which has been associated with the relative magnitude of interchain interactions in long chain hydrocarbons [21–23].

It is interesting to comment on the chain length dependence of this low-temperature phase transition observed so far in DPPC and DMPC bilayers. Although the acyl chains in DPPC are only slightly longer than those in DMPC, by two methylene groups, there are a number of intriguing differences in the temperature effect on the gel phase of these two systems.

First, the transition temperature between the GII and GIII phases of aqueous DPPC is 30°C higher than that of aqueous DMPC bilayers [2]. This difference in temperature parallels the differences between the critical temperatures of the main phase transitions (i.e. approx. 17°C) and that of the pretransitions (i.e. approx. 21°C) of these two systems. It is interesting to note that the differences in the transition temperatures between the two systems increases in going from the high- to the low-temperature transitions.

Secondly, while the width of all depolarized and weakly polarized bands decreases with decreasing temperature in the GIII phase of DPPC and DMPC, the rate of decrease is slightly larger in the DPPC system than that in the DMPC system, which suggests a slightly more orientationally-disordered structure of the GIII phase in DPPC compared to that of the GIII phase in DMPC.

Thirdly, the width of the highly polarized $\nu_s(CH_2)$ band in the GIII phase of DMPC decreases with decreasing temperature, whereas it is almost constant in the GIII phase of DPPC (compare Fig. 4B with Fig. 2B in Ref. 2). The decrease in the width of this mode in the GIII phase of DMPC has been attributed to a small change in the interchain interaction with temperature and to a significant contribution of the intramolecular anharmonicity [2]. The anharmonic contribution to this mode is expected to be larger in the GIII phase of DPPC than that in the GIII phase of DMPC, because the onset of this phase in DPPC (at -30°C) starts at a higher temperature than that in DMPC (-60°C). Therefore, a larger change in the width of this band is expected to occur in the GIII phase of DPPC than that in the GIII phase of DMPC. The fact that the width of this highly polarized band does not change considerably with temperature in the GIII phase of DPPC indicates that the interchain interactions continue to increase as the temperature decreases, and thus dominate the anharmonic effect in this phase. This conclusion is consistent with the temperature effect on the $H2880/H2845$ ratio, which increases with decreasing temperature in the GIII phase of DPPC, whereas it remains more or less constant with temperature in the GIII phase of DMPC (compare Fig. 5 with Fig. 3 in Ref. 2).

Fourth and lastly, the frequency discontinuities observed at the critical temperature between the GII and GIII phases of DPPC are smaller than those observed at this critical temperature in DMPC. As the shifts in frequency between the two gel phases are mainly the result of a change in interchain interactions [1], the smaller frequency shifts found at the critical temperature in DPPC indicate that the difference in the interchain interaction between the GII and the GIII phases of DPPC is smaller than that between the GII and GIII phases of DMPC. This is the result of the higher magnitude of the orientational disorder of the acyl chains in the GIII phase of DPPC, as indicated by the steeper slope in the temperature dependence of the width of the depolarized and weakly polarized bands in DPPC compared to that in DMPC.

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