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RAMAN SPECTROSCOPIC STUDY OF AN INTERDIGITATED LIPID BILAYER DIPALMITOYLPHOSPHATIDYLCHOLINE DISPERSED IN GLYCEROL

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Dipalmitoylphosphatidylcholine (DPPC) dispersed in perdeuterated glycerol was investigated in order to determine the effects on the Raman spectra of hydrocarbon chain interdigitation in gel-phase lipid bilayers. Interdigitated DPPC bilayers formed from glycerol dispersions in the gel phase showed a decrease in the peak height intensity I_{2850}/I_{2880} ratio, for the symmetric and asymmetric methylene CH stretching modes, respectively, as compared to non-interdigitated DPPC/water gel-phase dispersions. The decrease in this spectral ratio is interpreted as an increase in chain-chain lateral interactions. Spectra recorded in the 700–740 cm^{-1} CN stretching mode region, the 1000–1200 cm^{-1} C–C stretching mode region and the 1700–1800 cm^{-1} C=0 stretching mode region were identical for both the interdigitated and non-interdigitated hydrocarbon chain systems. At low temperatures the Raman peak height intensity ratios I_{2935}/I_{2880} were identical for the DPPC/glycerol and DPPC/water dispersions, indicating that this specific index for monitoring bilayer behavior is insensitive to acyl chain interdigitation. The increase, however, in the change of this index at the gel-liquid crystalline phase transition temperature for the DPPC/glycerol dispersions implies a larger entropy of transition in comparison to the non-interdigitated DPPC/water bilayer system.

Introduction

Raman spectroscopy provides a valuable technique for describing the structures of lipid aggregates. While conformational changes of the glycerol backbone and headgroup regions are conveniently monitored through changes in spectral frequencies and intensities, most attention has been focused on spectral features reflecting lipid acyl-chain lattice packing and *trans-gauche* isomerization. For example, orthorhombic and hexagonal gel-phase and liquid-crystalline hydrocarbon chain structures can be unambiguously identified from distinctive spectral transitions in the CH stretching (2800–3100 cm^{-1}), CH_2 deformation (1400–1500 cm^{-1}) and C–C stretching (1000–1200 cm^{-1}) mode regions; for the melted chain phases, micellar and liquid crystalline vesicular states may also be differentiated by both their characteristic spectra and states of turbidity [1–3].

Recent investigations have established the existence and possible biological importance of lipid bilayers in which the hydrocarbon chains of opposing monolayers are interdigitated [4–8]. Raman spectra of compounds known to form interdigitated bilayers, such as asymmetric phosphatidylcholines, have shown a decrease in the peak height intensity ratio (I_{2850}/I_{2880}) involving the 2850 cm^{-1} methylene symmetric stretching modes and

Abbreviation: DPPC, dipalmitoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol.

the 2880 cm^{-1} methylene asymmetric stretching modes [6]. The significance of the intensity change is complicated, however, by the possibility of modifications in subcell packing. Recent X-ray diffraction experiments have demonstrated, for example that the acyl chains of the interdigitated asymmetric phoshatidylcholine also pack in an orthorhombic subcell (Huang, C., personal communication); the lateral chain-chain interactions reflected by this packing arrangement are typically represented by a lower I_{2850}/I_{2880} intensity ratio than that for chains occupying a hexagonal lattice [9]. X-ray diffraction experiments have demonstrated that dipalmitoylphosphatidylcholine (DPPC) forms interdigitated bilayers when dispersed in glycerol instead of water and that the chains do not pack in orthorhombic subcells [7]. DPPC-glycerol dispersions thus provide a system for establishing the spectral features of well-characterized interdigitated lipid bilayers.

Experimental

DL-Dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG) were obtained from Sigma Chemical Co., recrystallized from ethanol, and lyophilized from chloroform at 10^{-5} torr for 48 h. Perdeuterated glycerol (99 atom% deuterium) was obtained from Merck Isotopes Ltd. DPPC dispersions were prepared by suspending DPPC (30 wt.%) in either perdeuterated glycerol or distilled water at 60–70 °C for 6–8 h, with repeated mechanical mixing. The dispersions were then sealed in Kimex glass capillary tubes (1.25 mm inside diameter), and allowed to equilibrate at room temperature for 2–3 days. Aqueous DPPG multilayers were analogously prepared.

Spectra were obtained on a Spex Ramalog 6 Raman spectrometer, which has been described previously [10]. In this study, as well as in previous studies on phospholipids reported from this laboratory, the parallel polarized component of the scattered radiation, as opposed to the total intensity, was recorded. One to 40 signal-averaged scans, obtained at a rate of 1 cm^{-1}/s, were recorded for each spectrum. Excitation intensities (514.5 nm) at the sample were 200 mW in order to avoid the effects of sample heating on the apparent phase-transition temperature. Since neither perdeuterated glycerol nor water exhibits significant Raman scattering in the 1400–1500 cm^{-1} CH_2 deformation region, the 2800–3100 cm^{-1} CH stretching mode region, or the 1700–1800 cm^{-1} C=O stretching mode region, no background substraction was required to establish the DPPC spectra in these spectral intervals. Subtraction of the background due to glycerol was, however, performed in the 1000–1200 cm^{-1} C–C stretching mode region.

Results and Discussion

A comparison of the recorded spectra in the 2800–3100 cm^{-1} CH stretching mode region at 0 °C for gel-phase DPPC/glycerol and DPPC/water dispersions is shown in Fig. 1. The peak height intensity ratio I_{2850}/I_{2880} of 0.65 for DPPC-glycerol dispersions is markedly less than the value of 0.75 for DPPC-water dispersions. The decrease in this spectral intensity ratio indicates either increased interactions between neighboring acyl chains, as reflected by a broadening and concomitant decrease in intensity of the methylene symmetric stretching modes at 2850 cm^{-1}, or an increase in the 2880 cm^{-1} feature as a consequence of additional contributions from the underlying Fermi resonance background arising from an increased fraction of *trans* chain segments [2,9,11]. The appearance of identical spectral ratios for the various C–C stretching mode features at approximately 1065, 1101 and 1132 cm^{-1} implies the same intrachain order for the two DPPC/glycerol and DPPC/water systems and precludes *trans/gauche* conformational changes from affecting the intensity ratios in the C–H stretching mode regions. No difference exists in the two systems, however, in the I_{2935}/I_{2880} ratio, an index also used to monitor bilayer behavior [1]. Since isotope dilution experiments have suggested that the former ratio I_{2850}/I_{2880} is more sensitive to intermolecular effects than the latter (O'Leary and Levin, unpublished data), the similarity between the I_{2935}/I_{2880} indices for interdigitated and noninterdigitated systems is not surprising. Spectra taken in the 1400–1500 cm^{-1} CH_2 deformation region show no evidence of the approx. 1420 cm^{-1} vibrational feature characteristic of hydro-

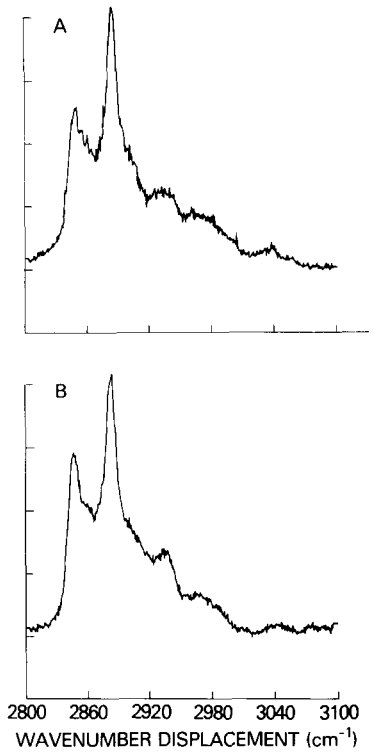

Fig. 1. Raman spectra of (A) DPPC/perdeuterated glycerol and (B) DPPC/water dispersions in the 2800–3100 cm^{-1} CH stretching region at 0 °C.

carbon chains packed in orthorhombic subcells [12]; the 1700–1800 cm^{-1} C=0 stretching mode region also fails to display the spectral pattern characteristic of phospholipids with orthorhombically packed acyl chains [13]. The frequency of the totally symmetric choline C–N stretching mode at 717 cm^{-1} is identical in the DPPC/glycerol and DPPC/water dispersions, suggesting that the headgroup conformation is the same in the two systems.

Temperature profiles constructed using both the I_{2850}/I_{2880} and I_{2935}/I_{2880} intensity ratios are shown in Fig. 2A and B for DPPC dispersed in glycerol and water respectively. The I_{2850}/I_{2880} index clearly differentiates the two systems for the temperature interval in the gel phase from 0 °C to T_m at 41 °C. The profile for the DPPC/water system displays the bilayer pretransition at 35.5 °C, while that for the DPPC/glycerol system shows the effect of the melting of the deuterated glycerol solvent from approx. 22 °C to the gel to liquid-

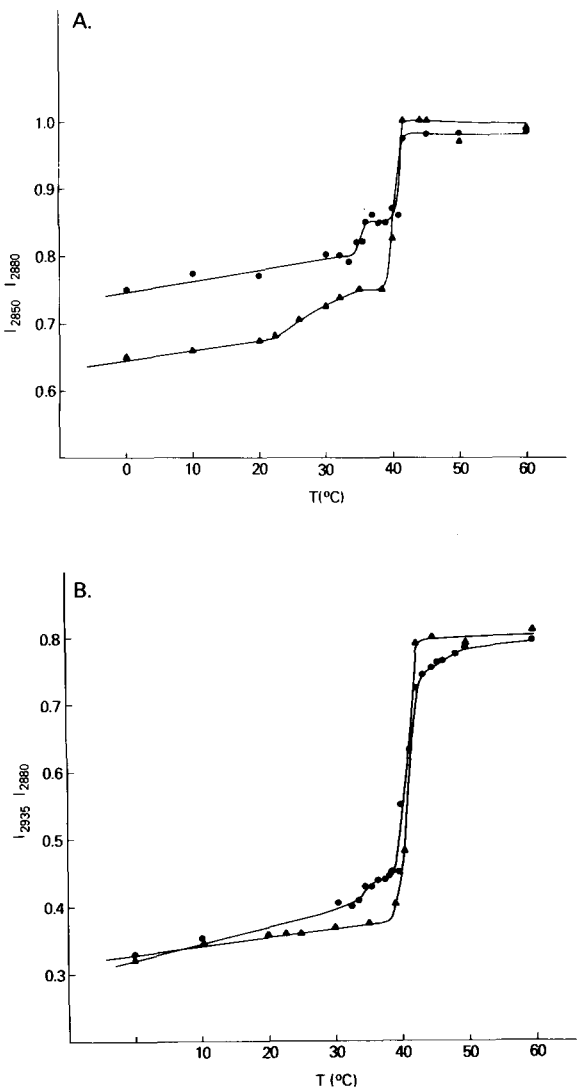

Fig. 2. Temperature profiles for DPPC/perdeuterated glycerol (▲) and DPPC/water (●) dispersions derived from the I_{2850}/I_{2880} (A) and I_{2935}/I_{2880} (B) peak height intensity ratios. The gel to liquid-crystalline phase transition for both systems is 41 ± 0.5 °C.

crystalline phase transition at approx. 41 °C.

The change in the I_{2935}/I_{2880} ratio between the gel and liquid-crystalline states at T_m is greater for the DPPC/glycerol system than for the DPPC/water system. Since this difference is correlated to the entropy change for the 41 °C phase transition [10], we estimate an enthalpy of meltin of 11.6 kcal/mol for the interdigitated

DPPC/glycerol system compared to the measured value of 8.6 kcal/mol for DPPC/water. This estimate agrees approximately with the calorimetric value of approx. 10.5 kcal/mol reported by McDaniel et al. [7] for DPPC/glycerol dispersions.

The I_{2850}/I_{2880} intensity index may be used to clarify further the question regarding the apparent interdigitation of acyl chains in dipalmitoylphosphatidylglycerol (DPPG) multilayers. Although the acyl chains in the gel phase of DPPG were originally believed to be interdigitated [14], Ranck and Tocanne [15] recently suggested that interdigitation was induced by the presence of an impurity, perhaps choline, in the earlier study. A comparison of the I_{2850}/I_{2880} intensity indices for pure DPPC and DPPG aqueous dispersions at various gel-phase temperatures yields the same ratios for both systems at various temperatures. Thus, at 25, 0 and $-196\,^{\circ}C$, we record values of 0.80, 0.70 and 0.51 for both the DPPC and DPPG multilayers. (At $-196\,^{\circ}C$ both systems pack in the orthorhombic form as evidenced by the marker at 1420 cm^{-1} in the methylene deformation region (data not shown).) Fig. 3 shows the comparison of the 2800–3000 cm^{-1} C–H stretching region at $0\,^{\circ}C$ for DPPC and DPPG. We conclude from these results that, for temperatures greater than $0\,^{\circ}C$, pure DPPG multilayers are not interdigitated in the gel phase. In addition, at $25\,^{\circ}C$ the I_{1100}/I_{1130} and I_{1130}/I_{1065} peak height intensity ratios for the DPPC and DPPG dispersions are also identical, indicating no change in the acyl chain *trans/gauche* conformational composition between the two systems.

In summary, we have demonstrated that at a fixed temperature the Raman peak height intensity ratio I_{2850}/I_{2880} is lower for interdigitated DPPC/perdeuterated glycerol dispersions than for non-interdigitated DPPC/water dispersions. This confirms our earlier findings [5,6] and indicates that this ratio can be used to determine whether the acyl chains of opposing lipid monolayers are interdigitated. The reliability of this ratio for determining chain interdigitation depends upon the chains being packed in a hexagonal lattice and on knowledge of the I_{2850}/I_{2880} ratio for a non-interdigitated dispersion of the same or a closely related lipid system. The ability to determine the presence of acyl-chain interdigitation by Raman spectroscopy may prove useful in exploring the bilayer effects of non-lipid perturbants [8] and solvent changes.

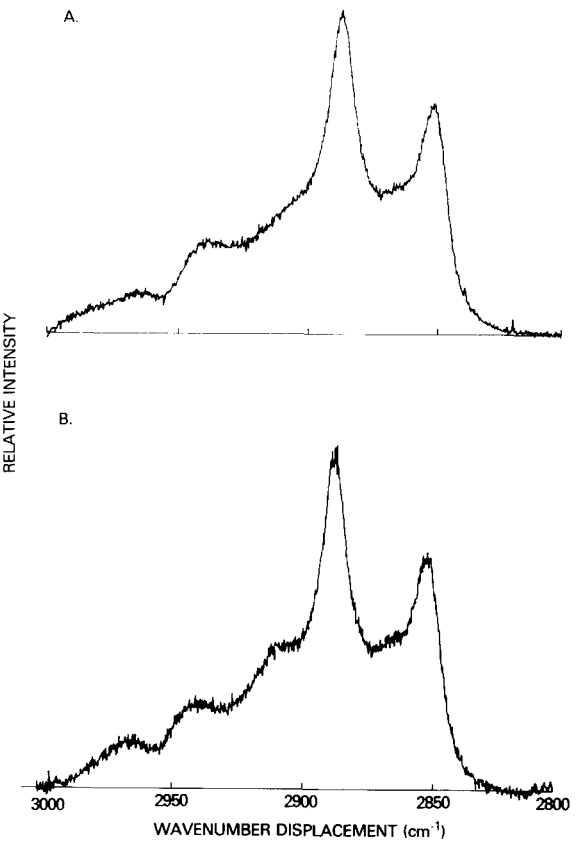

Fig. 3. Comparison of the C–H stretching mode regions for (A) DPPC and (B) DPPG multilayers at $0\,^{\circ}C$. The I_{2850}/I_{2880} peak height intensity ratio for both systems is 0.70, confirming that pure DPPG gel-phase multilayers are not interdigitated [15].

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