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Raman spectroscopy of selectively deuterated dimyristoylphosphatidylcholine: studies on dimyristoylphosphatidylcholine-cholesterol bilayers

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Dimyristoylphosphatidylcholine (DMPC), selectively deuterated in the *sn*-2 chain at the 3, 6, and 10 positions is used to probe DMPC-cholesterol interactions in multilamellar dispersions. Using the Raman spectral linewidths of the 2100 cm^{-1} C^2H_2 stretching modes as an index of membrane disorder, we demonstrate that cholesterol tends to order, or increase the number of *trans* carbon-carbon bonds within the DMPC acyl chain near the headgroup region at all temperatures. At low temperatures, cholesterol disorders the acyl chains near the methyl termini by inducing *gauche* conformers; cholesterol orders the entire chain at higher temperatures. These determinations are qualitatively consistent with conclusions drawn from deuterium nuclear magnetic resonance studies, but specifically reflect acyl chain *trans/gauche* isomerization on the 10^{-12}–10^{-13} s vibrational time scale.

Deuterated lipids have proved to be extremely valuable as nuclear magnetic resonance (NMR) probes of biomembrane structure [1–5]. The interpretation of deuterium NMR quadrupole splittings provides, in many cases, unambiguous information about the orientational characteristics of particular C^2H_2 units within the acyl chain regions of phospholipid bilayers; the T_1 relaxation time yields dynamic information in the millisecond to microsecond time scale. As a result, deuterated lipids have been exploited in understanding the difference between the *sn*-1 and *sn*-2 chains of phosphatidylcholines [1] and in determining the 'fluidity gradient' of both pure phosphatidylcholine and phosphatidylcholine-cholesterol bilayers [2–5]. Although the use of selectively deuterated lipid probes in the infrared and Raman spectroscopy of membrane systems has been proposed [6–9], the vibrational characteristics of these molecules have not been exploited to the same extent as the magnetic resonance spectrum. The vibrational frequencies and linewidths of the deuterated methylene modes may, in principle, be influenced both by lateral packing characteristics of lipid acyl chains and by acyl chain *trans/gauche* isomerization effects which complicate the interpretation of the observed spectral changes [10,11]. In addition, since the methylene spectral characteristics are influenced by electronic induction effects from the acyl chain carbonyl groups, the proximity of the C^2H_2 group to the phospholipid head group becomes important in assessing the observed frequencies and intensities. For example, the Raman spectral C^2H_2 stretching mode region of all-*trans* 2-[3,3-$^2H_2$]dimyristoylphosphatidylcholine (DMPC) differs markedly from that of all-*trans* 2-[10,10-$^2H_2$]DMPC [9]. While these factors complicate the interpretation of changes in the 2100 cm^{-1} C^2H_2 stretching mode region of the Raman spectrum, useful information can nevertheless be obtained. In this paper we clarify the dependence of the 2100 cm^{-1} C^2H_2 stretching mode Raman

spectral features on lipid acyl chain packing and *trans/gauche* isomerization. We then demonstrate for liposomal dispersions the use of the Raman spectra of selectively deuterated DMPC in studying the influence of cholesterol on *trans/gauche* conformational changes in various acyl chain regions.

Dimyristoylphosphatidylcholines (DMPC), selectively deuterated at the 3, 6 and 10 positions of the *sn*-2 chain, were prepared and purified as described previously [12]. Nondeuterated DMPC was obtained from Avanti Polar Lipids and was used without further purification. Cholesterol was obtained from Sigma Chemical Company, twice recrystallized from ethanol, and lyophilized for 24 h at 10^{-5} torr.

A sample of polycrystalline isotopically diluted 2-[6,6-$^2H_2$]DMPC was prepared by codissolving 33 mol% deuterated and 67 mol% nondeuterated DMPC in choroform, drying under flowing nitrogen gas, and lyophilizing for 24 h at 10^{-5} torr. Cholesterol/DMPC mixtures were prepared by first codissolving the two compounds in chloroform in a 1:1 mole ratio, and then drying and lyophilizing as before. The pure lipids and lipid-cholesterol mixtures were then hydrated with 75 wt% water at 40°C while being mechanically agitated.

TABLE I

RAMAN FREQUENCIES ν AND LINEWIDTHS $\Delta\nu_{1/2}$ AND $\Delta\nu_{3/4}$ (cm^{-1}) FOR SYMMETRIC (S) AND ANTISYMMETRIC (A) C^2H_2 STRETCHING MODES OF 2-[6,6-$^2H_2$]DMPC AT 0 AND 30°C

	Pure		Diluted	
	S	A	S	A
Anhydrous				
ν	2094	2171	2093	2172
$\Delta\nu_{3/4}$	12	13	12	15
$\Delta\nu_{1/2}$	18	24	20	27
Hydrated, 0°C				
ν	2094	2171	2093	2172
$\Delta\nu_{3/4}$	11	14	11	13
$\Delta\nu_{1/2}$	18	26	18	26
Hydrated, 30°C				
ν	2096	2183	2098	2181
$\Delta\nu_{3/4}$	21	26	23	24
$\Delta\nu_{1/2}$	34	46	34	47

The Raman spectrometer and temperature control of the sample chamber have been described previously [13]. An argon ion laser provided ap-

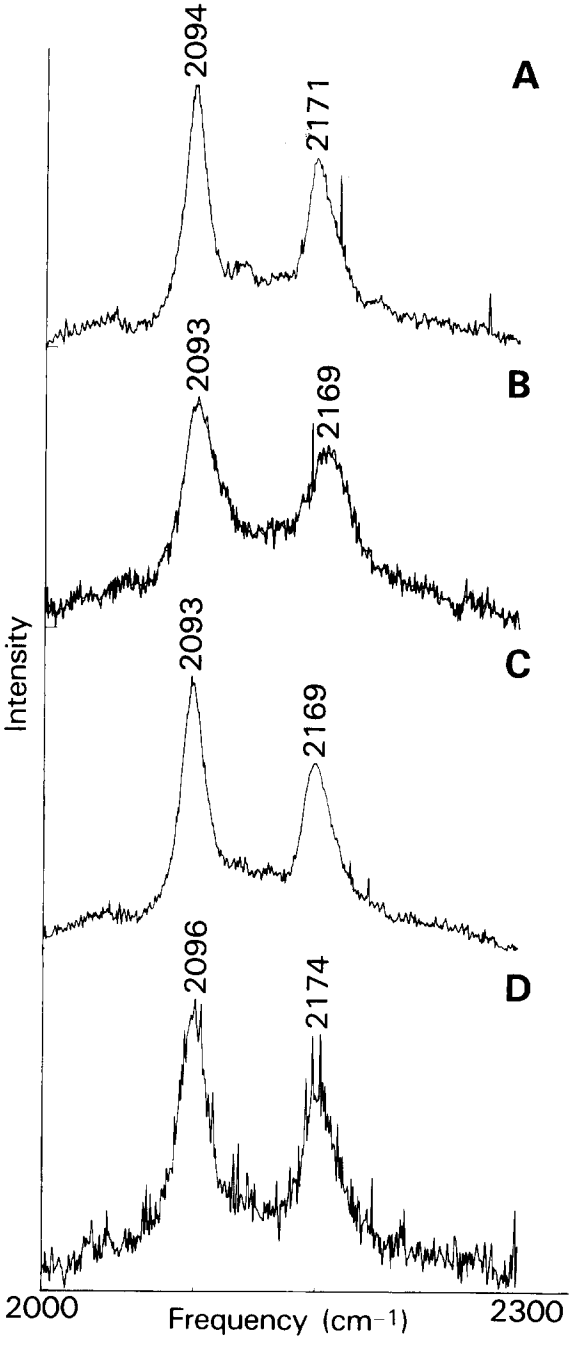

Fig. 1. Raman spectra of hydrated 2-[6,6$^2H_2$]DMPC dispersions at 5°C (A) and 30°C (B), contrasted with those of 2-[6,6-$^2H_2$]DMPC-cholesterol (1:1 mole ratio) dispersions at the same temperatures (C and D).

proximately 400 mW of 514.5 nm radiation at the sample. Spectra from 2000–2300 cm^{-1} were acquired at 5 cm^{-1}resolution; spectral frequencies are reported to ± 2 cm^{-1}. For each compound, mixture and temperature examined, twelve to sixteen h of signal-averaged spectra were acquired at a scanning rate of 1 cm^{-1}/s. For pure DMPC dispersions, spectra were acquired at 5 and 30°C; for DMPC-cholesterol dispersions they were acquired at 5, 17, and 30°C (these temperatures have been corrected for local heating of the sample by the laser radiation). Linewidths at either half maximum or three quarters maximum relative to a baseline drawn between 2000 and 2300 cm^{-1} are reported to ± 1 cm^{-1}. Differences in the baseline definitions result in the small quantitative differences between our results and those reported by Bansil et al. [9] for the lipid dispersions not containing cholesterol.

Table I demonstrates the effect in the 2000–2300 cm^{-1} C^2H_2 stretching mode region of diluting 2-[6,6-$^2H_2$]DMPC to 33 mol% in unlabeled DMPC. Linewidths of the fundamental modes at both half ($\Delta\nu_{1/2}$) and three quarters height ($\Delta\nu_{3/4}$) were determined, since the latter are less strongly affected by errors which might arise from subtraction of a pure DMPC overtone at 2185 cm^{-1}. The linewidths of the symmetric C^2H_2 stretching mode feature at 2094 cm^{-1} are essentially unchanged, while the linewidths of the asymmetric C^2H_2 feature of the anhydrous lipid at 2172 cm^{-1} are increased very slightly on dilution with unlabeled DMPC. There is a significant increase in the widths of both the symmetric and the asymmetric stretching mode features when the dispersion is heated to a temperature above that of the gel to liquid-crystalline phase transiton, however, as seen in Fig. 1. Similar effects are seen for 2-[3,3-$^2H_2$]- and 2-[10,10-$^2H_2$]DMPC dispersions and are summarized in Table II. The symmetric C^2H_2 stretching mode linewidth of 2-[3,3-$^2H_2$]DMPC changes little on melting. In contrast, both the asymmetric and the symmetric modes of 2-[6,6-$^2H_2$]DMPC and 2-[10,10-$^2H_2$]DMPC, and the asymmetric C^2H_2 stretching mode of 2-[3,3$^2H_2$]DMPC show large increases in linewidth upon melting. This suggests intrinsic insensitivity of the 2-[3,3$^2H_2$]DMPC symmetric C^2H_2 stretching mode linewidth to chain melting. These results are comparable to those reported by Bansil et al. [9].

Presumably, a 67% isotopic dilution of the selectively deuterated species should exert a greater effect on lateral chain-chain interactions than the gel to liquid-crystalline phase transition, since the latter is associated with a membrane volume increase of only about 3% and an area increase of about 20% [14]. The small change in the linewidths of the C^2H_2 stretching modes seen on isotopic dilution, in contrast to the significant increase in the linewidths observed on bilayer melting, provides convincing evidence that these Raman spectral features are primarily sensitive to intramolecular *trans/gauche* isomerization rather than to lateral chain-chain interactions involving vibrational dephasing or motional broadening [15]. This result is not surprising, since the deuterated *sn*-2 chains have already been 50% isotopically diluted by normal *sn*-2 chains; Mendelsohn and Koch [10]

TABLE II

RAMAN FREQUENCIES (HALFWIDTHS, cm^{-1}) FOR SYMMETRIC (S) AND ASYMMETRIC (A) C^2H_2 STRETCHING MODES OF AQUEOUS DISPERSIONS OF SELECTIVELY DEUTERATED DMPC

T (°C)	[3,3-$^2H_2$]-		[6,6-$^2H_2$]-		[10,10-$^2H_2$]-	
	S	A	S	A	S	A
No cholesterol						
5	2117(40)	2201(46)	2094(18)	2171(26)	2094(22)	2173(32)
30	2115(44)	2202(55)	2096(34)	2183(46)	2100(36)	2176(43)
50% cholesterol						
5	2115(39)	2200(45)	2093(21)	2169(28)	2097(29)	2178(35)
17	2115(40)	2199(53)	2097(22)	2175(27)	2096(32)	2179(35)
30	2114(40)	2199(52)	2096(27)	2174(34)	2098(32)	2179(38)

have shown that changes in isotopic dilution in the 0.1 to 0.9 mole fraction range have little or no effect on the linewidths of perdeuterated hexadecane.

Incorporation of 50 mol% cholesterol into lipid bilayer dispersions has dramatic effects on Raman spectra of all three of the isotopically labeled species. As shown in Table II, at 5°C cholesterol increases the linewidths of the symmetric and the asymmetric C^2H_2 features for both the 2-[6,6-$^2H_2$]DMPC and the 2-[10,10-$^2H_2$]DMPC systems and increases the linewidth of the asymmetric feature for the 2-[3,3-$^2H_2$]DMPC molecule. Cholesterol has no effect on the linewidth of the symmetric stretching feature for this compound; since the symmetric stretching feature of 2-[3,3-$^2H_2$]DMPC changes relatively little on melting, we believe this reflects intrinsic insensitivity of this vibrational feature to *trans/gauche* isomerization. In contrast, cholesterol narrows the C^2H_2 stretching region features for all species at 30°C, which is above the transition temperature, 24°C, for pure multilamellar dispersions of DMPC. Clearly the 'fluidizing' effect of cholesterol on low-temperature dispersions and the 'ordering' effect on high-temperature dispersions are reflected by the C^2H_2 stretching mode linewidths as they are in deuterium nuclear magnetic resonance quadrupole splittings.

Comparison of C^2H_2 stretching mode linewidths at several temperatures, as shown in Table II, suggests that the number of *gauche* isomers near the 3 position of the *sn*-2 chain changes little as the temperature is increased from 5 to 30°C in the presence of 50 mol% cholesterol. In contrast, there is an increase in the relative disorder at both the 6 and 10 positions of the *sn*-2 chains as the temperture is increased over this range. The relative increase in linewidth resulting from cholesterol incorporation is greater at the 10 position than at the 6 position for all three temperatures examined. The data indicate, therefore, that the effect of 1 : 1 mole ratios of cholesterol in broadening the gel to liquid-crystalline phase transition varies as one moves along the lipid chain, such that near the polar headgroup region the acyl chains are ordered in comparison with those of the pure DMPC bilayer, at all temperatures, while nearer the chain termini the bilayer is disordered at low temperatures and ordered at high temperatures, as compared to bilayers composed of pure DMPC. This is precisely the picture which emerges from deuterium NMR studies [2], which do not, however, directly monitor acyl chain *trans/gauche* isomerization.

In summary, we have demonstrated that Raman spectroscopy of selectively deuterated phospholipids can provide information on the effects of cholesterol at various levels in the lipid bilayer. The conclusions agree qualitatively with those obtained by deuterium nuclear resonance; since the two techniques measure different molecular properties, reflecting the time scales of the respective experiments, quantitative comparison is not possible. Although the utility of the technique is limited by the relatively long times required to acquire interpretable vibrational spectra of isotopically dilute deuterated methylene modes, Raman spectroscopy of selectively deuterated phospholipids should be useful in elucidating details of the effects of other perturbants, such as polypeptides and steroids, on phospholipid structure.

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