Vol. 94, No. 2, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1980 Pages 638-645

Raman Studies of Lipid Interactions at the Bilayer Interface: Phosphatidyl choline - Cholesterol

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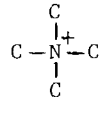
Received April 8,1980

SUMMARY: The Raman C=O stretch region of phospholipids is shown to be a sensitive probe of molecular interactions in the interface region. We report here the effect of cholesterol on the Raman C=O stretch and Raman symmetric C_4N^+ stretch of aqueous dispersions of dipalmitoyl phosphatidyl choline (DPPC). Addition of cholesterol causes a shift of some intensity from the single strong 1737 cm^{-1} C=O stretch to lower frequency - producing a second strong C=O stretch band at 1720 cm^{-1}. Cholesterol methyl ether does not produce this splitting. Evidence is presented which suggests that the C=O frequency shift is due to a conformation change in the acyl region of DPPC induced by the presence of cholesterol. Addition of cholesterol causes no change in the Raman symmetric C_4N^+ stretch band of DPPC - suggesting that the effect of cholesterol does not extend to the head group region.

Introduction: Raman spectroscopic studies of phospholipid structure and interactions have until now concentrated on the C-H stretching region (2800-3000 cm^{-1}) and the skeletal C-C stretching region (1000-1150 cm^{-1}), which provide information about side chain order. The effect of cholesterol on phospholipid side chain fluidity over the entire transition range has been demonstrated by Raman spectroscopic studies of the side chain carbon-carbon stretching region (1000-1150 cm^{-1}) by Lippert and Peticolas (1). It is important to extend the utility of Raman spectroscopic studies of phospholipids to include interactions and structure in the acyl interface and polar head group region as well. We have observed that the Raman C=O stretch frequencies (2) and the Raman symmetric  stretch (3) frequency in dipalmitoyl phosphatidyl choline (DPPC) change dramatically upon dispersion of anhydrous crystalline DPPC in water (4). We have, therefore, explored the possibility of using these vibrational bands as probes to study

0006-291X/80/100638-08$01.00/0

phospholipid interactions in the interface region. We report now results of a Raman study of phospholipid-cholesterol interactions which demonstrate that the Raman C=O stretch region can indeed be used as an important, sensitive new probe of molecular interactions in the phospholipid interface region.

Materials and Methods: Cholesterol methyl ether and cholesterol (99+% pure) were purchased from Sigma.

Samples were prepared by dissolving the phosphatidyl choline and cholesterol (or cholesterol methyl ether) in a 1:1 molar ratio in chloroform. The chloroform was removed under vacuum and the dried mixture was dispersed in excess water. Samples were incubated at temperatures above the lipid phase transition until "swelling" was complete. Samples were centrifuged for ten minutes in a clinical centrifuge to remove some excess water.

The spectra were measured with a laser Raman spectrometer at Wayne State University equipped with a Coherent Radiation CR8 argon ion laser, Spex 14018 double monochromator with spatial filter and a cooled RCA c3104 photomultiplier tube. Laser power at the sample was approximately 200mw. A spectral band pass of 4 cm^{-1} and pen period of 10 sec. were used.

Results and Discussion: The Raman spectra of the aqueous dispersion of dipalmitoyl phosphatidyl choline (DPPC/H_2O) and of the aqueous dispersion of DPPC and cholesterol (DPPC/cholesterol/H_2O) are compared in the carbonyl stretch and choline C-N^+ stretch regions in figures 1a and 1b. The temperature of both samples was 19°C, which is below the mid-temperature of the gel-to-liquid crystal phase transition.

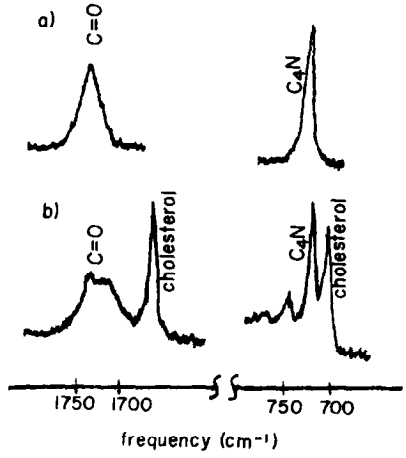


Figure 1: The Raman C=O stretch and C_4N^+ stretch bands of dipalmitoyl phosphatidyl choline/H_2O dispersions: a) without cholesterol b) with cholesterol at 19° C.

The most striking difference between the Raman spectra of gel phase DPPC/H_2O and DPPC/cholesterol/H_2O in figure 1 occurs in the carbonyl stretch region (1720-1740 cm^{-1}). The DPPC/H_2O Raman spectrum has one strong C=O stretch band at 1737 cm^{-1} with a 30 cm^{-1} band width. The samples prepared with cholesterol have <u>two</u> overlapping Raman bands in the C=O stretch region. The higher frequency band is found at 1737 cm^{-1}, the C=O stretch frequency of the sample with no cholesterol present. The lower frequency band is found at approximately 1720 cm^{-1}. The total integrated intensity (measured by planimeter) over the two bands in the DPPC/cholesterol/H_2O sample is equal to that of the single C=O stretch band in the DPPC/H_2O Raman spectrum.

To determine if the cholesterol -OH group is required for the splitting of the DPPC carbonyl stretch band, a sample containing cholesterol methyl ether was measured. The Raman spectrum of the mixture DPPC/cholesterol methyl ether/H_2O has only one C=O stretch band at 1737 cm^{-1} with a 30 cm^{-1} bandwidth, as in the spectrum of DPPC/H_2O. Therefore, it appears that the -OH group on the cholesterol may be required for the effect observed in the C=O stretch region of the Raman spectrum.

There are two possible interpretations of the shift of some intensity from the 1737 cm^{-1} C=O stretch band to a new band appearing at approximately

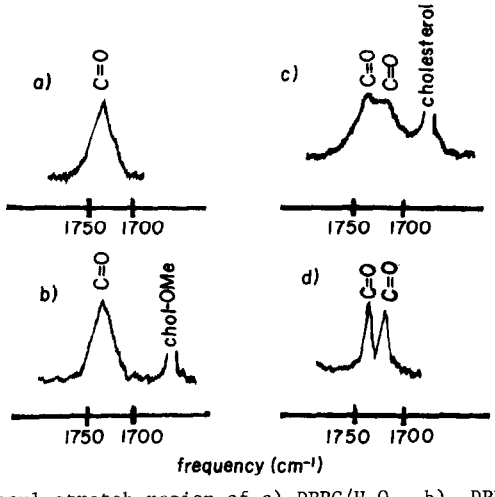


Figure 2: Raman carbonyl stretch region of a) DPPC/H_2O b) DPPC/cholesterol methyl ether/H_2O and c) DPPC/cholesterol/H_2O dispersions and of d)crystalline DPPC. All spectra were measured at 19° C.

1720 cm^{-1} when cholesterol is present in the DPPC aqueous dispersion: 1) One is that in some acyl linkages a conformational change is induced for which the C=O stretch occurs at a lower frequency. 2) The other is that a change in the hydrogen-bonding environment occurs at the C=O group of DPPC when cholesterol is introduced. Both possibilities are discussed below, beginning with the hydrogen-bonding model.

In order for hydrogen bonding to cause the observed 17 cm^{-1} downward frequency shift in the DPPC carbonyl stretch, it would seem that the hydrogen-bonding environment of some DPPC carbonyl groups would have to change significantly - probably from an environment which offers no hydrogen-bonding possibility to one in which hydrogen bonding with cholesterol is possible. It seems likely that the DPPC carbonyl groups would hydrogen bond to water in a pure DPPC/H_2O system and that if cholesterol added to the system were to hydrogen bond with the DPPC C=O groups, the hydrogen-bonding effect of cholesterol would not be so different from water as to cause a 20 cm^{-1} shift in the C=O frequency. Thus, it seems unlikely to us that hydrogen bonding with cholesterol causes the change in the DPPC carbonyl stretch region. However, we are investigating this possibility in work in progress.

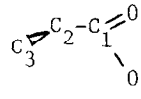
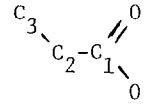
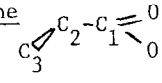
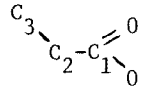
The second possibility is that cholesterol allows or induces a conformational change in the C=O region which lowers the C=O stretch frequency. Evidence has been presented which demonstrates that the C=O stretch frequency is dependent on geometry in phospholipids (2). Brown, Brown and Person (2) have proposed that the C=O stretch frequency is sensitive to rotation about the acyl C_2-C_1 bond and that the gauche  conformer C=O stretch is about 10-20 cm^{-1} higher frequency than the trans  conformer C=O stretch. If the two C=O stretching frequencies of the DPPC/cholesterol/H_2O samples are compared to those of crystalline DPPC (see Table 1), it can

Table 1. Frequencies (ν) and bandwidths (BW) of the dipalmitoyl phosphatidyl choline C=O stretch and C_4N^+ symmetric stretch in the Raman spectra of various DPPC mixtures at 19°C

	C_4N^+ symmetric stretch of choline	
	$\nu(cm^{-1})$	$BW(cm^{-1})$
DPPC/H_2O	718	14
DPPC/cholesterol/H_2O	718	14
DPPC/cholesterol methyl ether/H_2O	718	14
crystalline DPPC	710	11
	acyl C=O stretch (higher frequency)	
	$\nu(cm^{-1})$	$BW(cm^{-1})$
DPPC/H_2O	1737	30
DPPC/cholesterol/H_2O	1737	30
DPPC/cholesterol methyl ether/H_2O	1737	30
crystalline DPPC	1737	12
	acyl C=O stretch (lower frequency)	
	$\nu(cm^{-1})$	$BW(cm^{-1})$
DPPC/H_2O	-----	
DPPC/cholesterol/H_2O	1720	~50
DPPC/cholesterol methyl ether/H_2O	-----	
crystalline DPPC	1720	12

be seen that they are very similar. Since 1) we believe the doublet in crystalline DPPC is due to the presence of two acyl linkage conformers (a gauche conformer in the β chain and a trans conformer in the γ chain), and 2) the frequencies of the C=O "doublet" in DPPC/cholesterol/H_2O are the

same as those of the crystalline DPPC doublet, it seems likely that the two C=O stretches in the Raman spectrum of DPPC mixed with cholesterol are due to a conformational change in DPPC in which rotation about some acyl C_2-C_1 bonds has occurred, bringing these acyl conformers from a gauche  conformation to a trans  conformation. As discussed in reference 9, such a rotation at temperatures below the mid-transition temperature may cause the entire chain to rotate about its axis, thereby changing the packing array of the side chains from the stronger interchain interaction of the "tilted" chain-planes arrangement to the less strong interactions of the "parallel" chain-planes (2,5).

In figure 3, the Raman C=O stretch regions for two DPPC/cholesterol/H_2O samples with different cholesterol-DPPC ratios are compared. The frequency-shifted C=O stretch intensity appearing at 1720 cm^{-1} is greater for the sample in which the cholesterol-DPPC molar ratio is 1.0 (figure 3a) than that for which the molar ratio is 0.5 (figure 3b). The intensity ratio of the 1720 cm^{-1} band to the 1737 cm^{-1} band appears to be approximately proportional to the molar ratio of cholesterol to DPPC, indicating that the cholesterol effect on DPPC conformation is probably a local one.

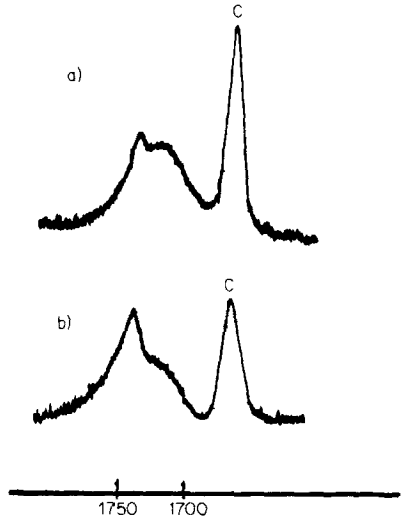


Figure 3: The Raman C=O stretch region for samples with varying cholesterol-DPPC ratios a) 1 to 1 cholesterol-DPPC ratio. b) 0.5 to 1 cholesterol-DPPC ratio. C denotes a cholesterol band.

Considerable broadening of the lower C=O stretch Raman band occurs in the presence of cholesterol (see Table 1). We believe the broadening is due to increased freedom of motion in the acyl linkage region since the C=O stretch frequency is very sensitive to conformation. This result suggests that the Raman C=O stretch bandwidth may be used to monitor freedom of motion and that the presence of cholesterol greatly enhances freedom of motion in the acyl linkage region.

The 720 cm^{-1} C_4N^+ symmetric stretch of the choline head group is not appreciably altered by the presence of cholesterol. The C_4N^+ symmetric stretch frequencies and bandwidths are approximately the same for the DPPC/H_2O samples with and without cholesterol. (See Table 1.) Since we observe that the C_4N^+ symmetric stretch Raman band of the DPPC/H_2O sample is at 10 cm^{-1} higher frequency and is about 10 cm^{-1} broader than that of the crystalline DPPC sample, we believe that the C_4N^+ stretch is sensitive to environment or to geometry. Thus, we feel that the most likely explanation for the similarity observed in the C_4N^+ stretch Raman band in DPPC/H_2O samples with and without cholesterol is that the cholesterol has little affect on the amine region of the head group. This conclusion is in agreement with interpretations of ^2H nmr (6) and ^{31}P nmr (7) studies of lipid head group-cholesterol interactions.

Thus, the results show that cholesterol affects the frequency and bandwidth of the Raman C=O stretch but not the C_4N^+ symmetric stretch of DPPC in aqueous dispersion. The appearance of a second strong C=O stretch upon addition of cholesterol suggests that cholesterol induces a conformational change at the acyl linkage in about half of the side chains in a 1 to 1 molar mixture of cholesterol and DPPC. This conformation change and the cholesterol-lipid interaction can be monitored by measuring the relative intensities of the C=O stretch bands at 1740 cm^{-1} and 1720 cm^{-1}. The results show that the Raman carbonyl stretch region in phospholipid samples affords a sensitive probe of geometry, freedom of motion and molecular interactions in the interface region.

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