Biochimica et Biophysica Acta, 602 (1980) 32—44

BBA 78950

A FOURIER TRANSFORM INFRARED SPECTROSCOPIC STUDY OF THE MOLECULAR INTERACTION OF CHOLESTEROL WITH 1,2-DIPALMITOYL-*sn*-GLYCERO-3-PHOSPHOCHOLINE *

JUNZO UMEMURA **, DAVID G. CAMERON and HENRY H. MANTSCH

Division of Chemistry, National Research Council of Canada, Ottawa K1A OR6 (Canada)

(Received March 3rd, 1980)

Key words: Cholesterol; Phosphatidylcholine; Fourier transform infrared spectra; Melting phenomenon; Ester bond

Summary

The temperature dependencies of the infrared spectra of pure and cholesterol-containing multibilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine were studied using Fourier transform infrared techniques. A comparison of the spectroscopic data showed the retention of a melting phenomenon at 60 mol% cholesterol content, and the retention of some all-*trans* conformations in the liquid-crystalline phase. It is also demonstrated that at temperatures less than 30°C, the cholesterol-containing 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine multibilayers still contain a small amount of pure 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, packed in an orthorhombic subcell lattice. Spectral changes were found in the absorptions characteristic of the phospholipid head groups. The addition of cholesterol results in changes in the ester bands, and demonstrates the induction by cholesterol of non-equivalent ester conformations.

Introduction

The dynamics and conformational properties of lipid-cholesterol systems have been studied extensively by a variety of techniques including X-ray [1,2], neutron diffraction [3], multinuclear NMR [4—7], ESR [8], Raman [9—11] and dispersion infrared [12,13] spectroscopy. Despite this body of literature, the interaction of cholesterol with model and natural membranes at the molecular level is not yet fully understood. We have therefore undertaken a study of temperature variations of the infrared spectra of multibilayers of 1,2-dipal-

*N.R.C.C. Publication No. 18358.

**On leave from The Institute for Chemical Research, Kyoto University, Uji, Kyoto-fu, 611 Japan.

Abbreviation: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

mitoyl-*sn*-glycero-3-phosphocholine (DPPC) containing various amounts of cholesterol, taking advantage of the precision of Fourier transform infrared spectroscopy to monitor changes too small to be observed in earlier dispersion studies [12,13]. The results provide considerable insight into the structure of the hydrophobic region of the bilayer, and demonstrate conformational changes in the head group.

Materials and Methods

Cholesterol and DPPC were obtained from Sigma Chemical Co. and used without further purification. Multibilayer dispersions of DPPC containing 40 and 60 mol% cholesterol were prepared from spectrograde chloroform solutions. 6-, 12- and 25-μm thick samples in BaF_2 cells were prepared and hydrated with double-distilled water using the methods described elsewhere [14]. Samples were annealed for 1 h at 50°C prior to each experiment.

In order to record spectra in the range 0—60°C the cell was placed in a hollow cell mount thermostatically controlled by a flow of ethanol/water. Spectra below 0°C were recorded by placing the cell in a mount in an evacuable chamber, temperature control being achieved by flowing cold nitrogen gas through the mount. The temperature was always reduced to below −10°C before evacuating in order to avoid to possibility of evaporation of water from the sample. In both cells the temperature was monitored by a copper-constantan thermocouple located against the cell windows.

Spectra were recorded on a Nicolet 7199 Fourier transform spectrometer equipped with a mercury-cadmium telluride detector (0—60°C study), a Digilab FTS-11 spectrometer equipped with a mercury-cadmium telluride detector (0—60°C study, −62 and −14°C spectra), and a Perkin-Elmer 180 grating spectrometer (low-temperature monitoring of the CH_2 scissoring peak positions). Grating spectra were recorded at 1.5 cm^{-1} resolution. Interferograms were averaged for 500 scans. On both Fourier transform instruments, an optical retardation of 1 cm was used, apodized with a Happ-Genzel (Nicolet) or triangular (Digilab) function and Fourier transformed to yield a resolution of 0.9 cm^{-1}. The frequencies are accurate to better than ± 0.01 cm^{-1}. Band positions measured from expanded plots are reproducible to better than ± 0.2 cm^{-1}. Infrared difference spectra were recorded and processed as described in detail elsewhere [14]. Neither the absorbance nor the difference spectra were smoothed. As the application of Fourier transform infrared spectroscopy to the study of biomembranes is a relatively recent innovation, an added measure of confidence in the technique is available from the fact that identical results were obtained from both instruments.

Results and Discussion

The following sections discuss the infrared spectra of DPPC multibilayers containing 40 or 60 mol% cholesterol, in comparison with that of pure DPPC multibilayers. The discussion is mainly in terms of the DPPC spectra as the cholesterol spectrum is an order of magnitude weaker. This results from the greater

molecular weight of DPPC and the fact that the DPPC spectrum is intrinsically stronger.

A. Spectral features characteristic of the acyl chains

The CH stretching vibrations. Fig. 1 shows the infrared absorption spectra of DPPC multibilayers containing 40 mol% cholesterol, at four different temperatures. The strong bands at 2920 and 2851 cm^{-1} are due to the antisymmetric and symmetric CH_2 stretching modes of the palmitoyl chains [13,15]. Both show temperature-dependent variations in peak position, peak height and bandwidth. The upper trace in this figure shows a typical difference spectrum, which is used to monitor the temperature-induced changes in the spectral features of this model membrane.

From such difference spectra a parameter, ΔA, can be obtained (see Fig. 1) which, if normalized with respect to the corresponding temperature increment, yields $\Delta A/°C$, a measure of the total change in a particular peak. A plot of $\Delta A/°C$ vs. temperature for the CH_2 symmetric stretching mode of DPPC multibilayers is shown in Fig. 2A. The sharp increase in the rate of change of absorbance at 41.5°C is due to the melting of the palmitoyl chains at the gel-to-liquid crystal phase transition, while the smaller change around 37°C is correlated with a crystal lattice phase change, commonly referred to as the pretransition [15]. The $\Delta A/°C$ plot of pure DPPC may be contrasted with that observed in the cholesterol-containing systems. The presence of 40 mol% cholesterol (Fig. 2B, solid line) drastically increases the width of the phase transition, although the transition is still centered at 41°C. In the presence of 60 mol% cholesterol (Fig. 2B, broken line) there is no indication of a phase transition in the $\Delta A/°C$ plot.

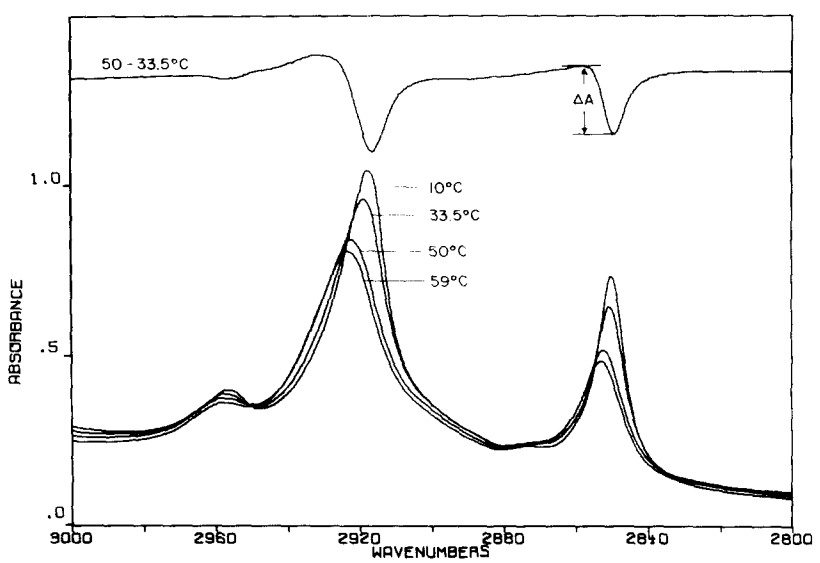

Fig. 1. (Bottom) Infrared spectra of the CH stretching region of fully hydrated DPPC multibilayers (6 μm thick) containing 40 mol% cholesterol at four temperatures. (Top) Representative difference spectrum obtained by subtracting the 33.5°C absorbance spectrum from that at 50°C.

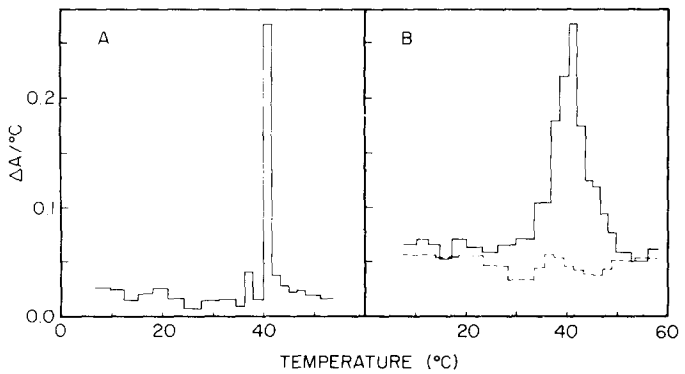

Fig. 2. Rate of change of the absorbance of the symmetric CH_2 stretching mode of the palmitoyl chain methylene groups as a function of temperature. (A) Pure DPPC multibilayers, (B) DPPC multibilayers containing 40 and 60 mol% cholesterol (solid and broken lines, respectively, ordinate scale X12).

Although convenient for general monitoring of phase changes, $\Delta A/°C$ is a complex parameter reflecting concomitant changes in frequency, intensity and bandshape. However, the frequencies and bandwidths of CH stretching modes can be related to specific phenomena at the molecular level: a shift in frequency to the introduction of *gauche* conformers, and changes in bandwidths to variations in the rates of librational motions of the acyl chains [13,15].

The temperature dependencies of the frequencies of the symmetric CH_2 stretching mode in pure DPPC, and in the cholesterol-containing systems are shown in Fig. 3A. The frequency of this band is related to the average number of *gauche* conformers in the system. An all-*trans* conformation shows this frequency at 2849.5 cm^{-1}, while the introduction of *gauche* conformers results in a shift to higher frequency. This point is illustrated in Fig. 3A in the pure DPPC plot (x-x) in which the 3.5 cm^{-1} shift in frequency at the main transition results from the melting of the acyl chains [13,15]. The incorporation of cholesterol into the bilayer results in several effects. The introduction of *gauche* conformers occurs over a wider temperature range in the cholesterol-containing

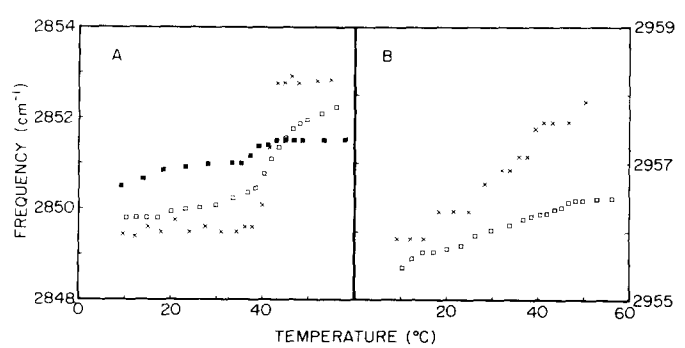

Fig. 3. (A) Temperature dependence of the frequency of the maximum of the CH_2 symmetric stretching vibration in DPPC (x), in DPPC with 40 mol% cholesterol (□) and in DPPC with 60 mol% cholesterol (■). (B) Temperature dependence of the frequency of the asymmetric CH_3 stretching vibration of the terminal palmitoyl methyl groups in DPPC (x) and in DPPC with 40 mol% cholesterol (□).

DPPC multibilayers. This is the same trend as is reflected in the corresponding $\Delta A/°C$ plots. However, whereas no phase transition is apparent in the $\Delta A/°C$ plot of DPPC + 60 mol% cholesterol, the frequency plot in Fig. 3A (□-□) demonstrates one centred at about 38°C.

There are also differences between these systems in the gel and liquid-crystalline phases. In the gel phase, the frequencies follow the order: pure DPPC < DPPC + 40 mol% cholesterol < DPPC + 60 mol% cholesterol while the reverse order is observed in the liquid-crystalline phase. This demonstrates that cholesterol increases the average number of *gauche* conformers in the system in the gel phase, and decreases it in the liquid-crystalline phase. These conclusions were confirmed by measurements of the half-bandwidth of the symmetric CH_2 stretching mode of DPPC. Plots (not shown) similar in form to those of the peak position (Fig. 3A) were obtained, the greatest change being observed in pure DPPC and the least in DPPC containing 60 mol% cholesterol.

These observations are in agreement with the results of ^{2}H-NMR [16] and Raman [9] spectroscopic studies. We also note that the decrease in the transition temperature with increasing cholesterol concentration is consistent with calorimetric data [1].

The band at 2956 cm^{-1} (Fig. 1) is the asymmetric stretching vibration of the terminal methyl groups of the palmitoyl chains and hence provides a monitor of the centre of the bilayer. Plots of frequency vs. temperature in DPPC and DPPC with 40 mol% cholesterol are shown in Fig. 3B. In both plots there is a monotonic increase in frequency with increasing temperature, reflecting increasing librational freedom of the acyl chains in the central area of the bilayer. The melting phenomena are not evident, reflecting the fact that the methyl mode is mechanically decoupled from the methylene mode and hence need not respond to the melting phenomenon [15].

In the gel phase below 20°C the frequencies are nearly the same in both systems, in agreement with the results on the symmetric CH_2 stretching band. However, at higher temperatures the frequency in pure DPPC is higher than in the system with 40 mol% cholesterol. This confirms the conclusion from ^{2}H-NMR studies that the addition of cholesterol 'stiffens' the membrane in the liquid-crystalline phase [4].

The CH_2 scissoring vibrations. The CH_2 scissoring vibrations of the extended acyl chains of DPPC are located in the region of 1480—1460 cm^{-1} and are characteristic of the nature of the acyl chain packing in the gel phase. Between the main transition and the pretransition a single sharp band is observed in the spectrum of DPPC at 1468 cm^{-1}, representative of all-*trans* chains packed in a hexagonal subcell lattice, with no interchain coupling. Reduction of the temperature below the pretransition results in crystal field splitting characteristic of orthorhombic subcell packing [15,17].

Fig. 4A shows the CH_2 scissoring region of DPPC containing 40 mol% cholesterol at 33.5°C (broken line) and at 10°C (solid line). Broadening of the band on reduction of the temperature is evident and the corresponding difference spectrum in Fig. 4A (top) shows two minima, indicating the existence of at least two bands at 10°C. In Fig. 5A, the frequencies of these two minima are plotted as a function of temperature for the three systems. The temperature marking the onset of the splitting decreases with increasing cholesterol concen-

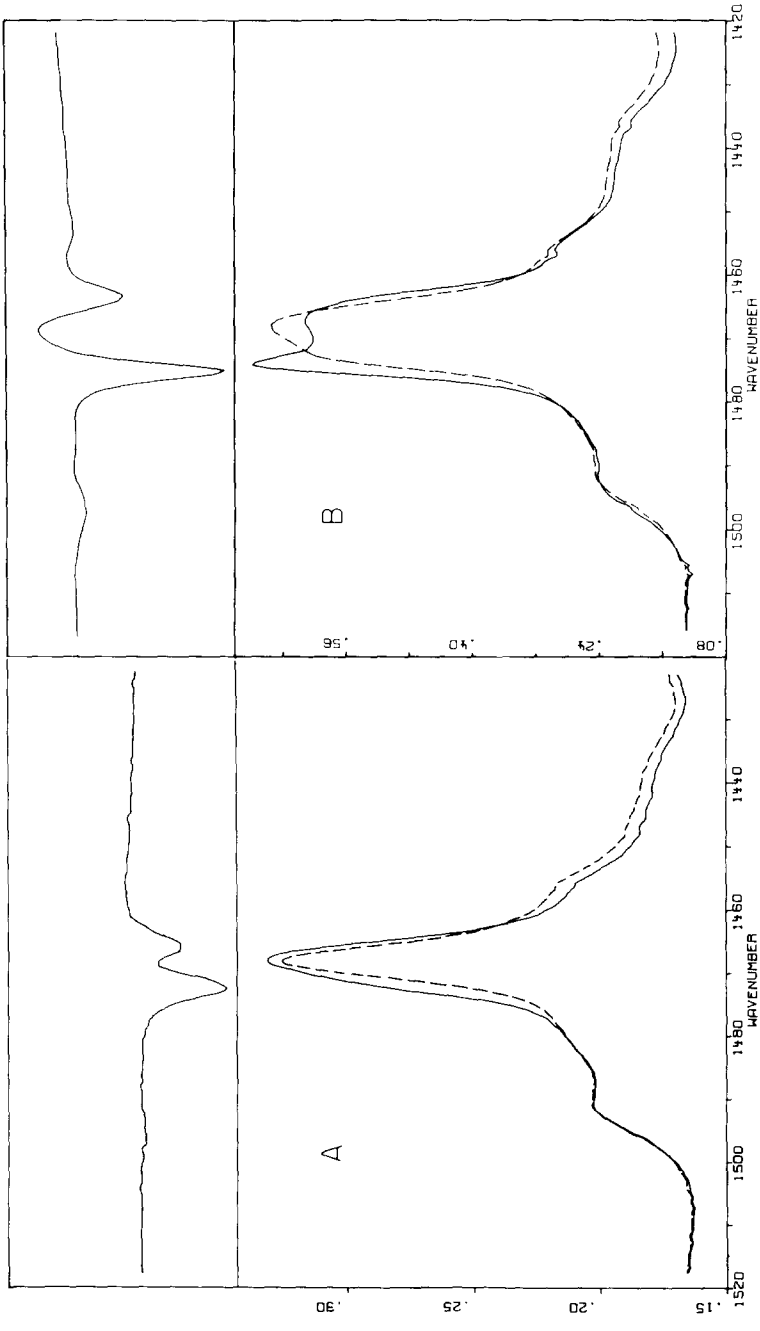

Fig. 4. Infrared (bottom) and corresponding difference spectra (top) in the CH_2 scissoring region of fully hydrated DPPC multibilayers containing 40 mol% cholesterol. (A) At 10°C (———) and 33.5°C (- - - - - -), thickness 6 μm, and (B) at −62°C (———) and −14°C (- - - - - -), thickness 12 μm.

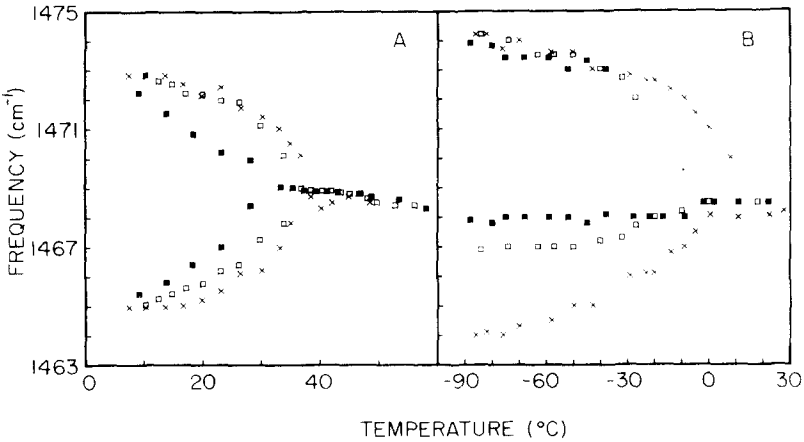

Fig. 5. (A) Temperature-dependent splitting of the acyl chain CH_2 scissoring mode in fully hydrated DPPC multibilayers with no cholesterol (x) and with 40 (□) or 60 mol% cholesterol (■) as obtained from the minima in the difference spectra, recorded from 10 to 60°C. (B) Corresponding splitting of the CH_2 scissoring mode as obtained from the absorption maxima in the infrared spectra recorded from −90 to +20°C. The lowest frequency component in the cholesterol-containing samples is not shown (see text).

tration, from 36°C in pure DPPC to about 30°C in the system with 60 mol% cholesterol. If the temperature of the onset of splitting is compared with the data in Figs. 2B and 3A it is evident that the phase transition, as characterized by the chain-melting phenomenon, occurs only after the collapse of all orthorhombic packing of the acyl chains into hexagonal packing.

Reduction of the temperature below 10°C results in an increase in the magnitude of the high-frequency shoulder (Fig. 4B, broken line) and eventual splitting into two maxima (Fig. 4B, solid line). The frequencies of the CH_2 scissoring peak positions in pure DPPC, and in DPPC containing 40 and 60 mol% cholesterol are plotted as functions of temperature in Fig. 5B. Below −40°C the high frequency band is at the same frequency in all three systems. However, the low frequency maximum is shifted only 0.5—1.0 cm^{-1} in the cholesterol-containing systems, compared to the 4 cm^{-1} shift in pure DPPC.

The explanation of this is apparent from a close examination of the absorption and difference spectra. In the spectrum of pure DPPC at −18°C the CH_2 scissoring band is completely split, with the stronger component at 1472.9 cm^{-1} and a weaker component at 1466.8 cm^{-1}. At 40 mol% cholesterol (Fig. 4B, solid line), the strongest band at −14°C appears at 1467.8 cm^{-1} and corresponds to that observed in hexagonally packed DPPC, while the shoulder at 1473 cm^{-1} corresponds to the high-frequency component of the CH_2 scissoring pair in pure DPPC at this temperature. As the temperature is reduced the central band remains fixed while the high frequency shoulder becomes more distinct. At −40°C it is resolved as shown in Fig. 5B, while at about the same temperature, a low-frequency shoulder is evident. This shoulder is located at about 1467—1464 cm^{-1}, as shown by both the absorbance and difference spectra. Also evident in the difference spectrum is an inflection point at about 1470 cm^{-1}, which is also apparent in Fig. 5A. Such inflections do not occur in the spectra of pure DPPC where only two bands are present.

Confirmation of crystal field splitting was obtained by studying the CH_2 rocking mode at 720 cm^{-1} which is also split in the orthorhombic subcell. As a 10 cm^{-1} splitting can only occur as a result of specific interchain interactions in large domains of fully extended acyl chains, we conclude that in both cholesterol-containing systems two phases exist at all temperatures below 30°C. The predominant phase gives rise to the band at 1468 cm^{-1}, and is a mixed DPPC/cholesterol phase in which there is no crystal field splitting, while the second phase contains orthorhombically packed DPPC. The latter phase is present only as a minor component, especially when it is considered that the scissoring mode from the mixed phase is reduced in intensity relative to that in pure DPPC by virtue of the presence of *gauche* conformers. The smallest amount of orthorhombically packed DPPC is found in the system containing 60 mol% cholesterol.

The present finding of the existence of orthorhombically packed pure DPPC at a cholesterol concentration of 60 mol% is in contrast with earlier results obtained with other techniques. From wide-angle X-ray studies at 20°C [18] and electron-diffraction measurements between −10 and 50°C [19], the characteristics of crystalline acyl chain packing have been reported to disappear at approx. 33 and 50 mol% cholesterol, respectively. However, other authors (Ref. 20 and references contained therein) have reported that, under certain circumstances, cholesterol segregation is not observed at concentrations below 67 mol%, from which we may infer that the crystalline acyl chain packing persists up to this concentration. It would seem that the differences can be attributed partly to the sample-preparation technique [20], and partly to the sensitivity of the individual techniques employed.

The CH_2 wagging band progression. The CH_2wagging band progression is a series of weak, regularly spaced bands in the region 1350—1180 cm^{-1} which results from the wagging of all-*trans* acyl chains. The 1350—1180 cm^{-1} region is dominated by the strong antisymmetric phosphate stretching vibration as illustrated in Fig. 6 for the DPPC system with 60 mol% cholesterol. In the absorbance spectra in the bottom part of this figure, the CH_2 wagging band progression appears as a weak series superimposed on this strong feature. However, the band progression is clearly evident in the difference spectra (shown in the upper traces in this figure) as minima at about 1200, 1223, 1246, 1266, 1285 and 1305 cm^{-1}. These frequencies coincide with those found in the hexagonally packed acyl chains of pure DPPC, and not with those characteristic of orthorhombically packed acyl chains [15]. This confirms that the mixed DPPC/cholesterol phase predominates as in this phase interchain interactions are minimal. However, unlike the situation in pure DPPC, where the CH_2 wagging band progression disappears at the main transition, it is still present in the DPPC/cholesterol systems at temperatures well above the melting transition. This demonstrates the presence of all-*trans* acyl chains in the liquid-crystalline phase, confirming that cholesterol reduces the average number of *gauche* conformers per chain introduced in the main transition. It also indicates the retention of some gel-like structure. As the frequencies of the band progression match those of the hexagonal phase of pure DPPC these ordered chains are located in the DPPC/cholesterol phase, if phase separation is still present above 30—36°C.

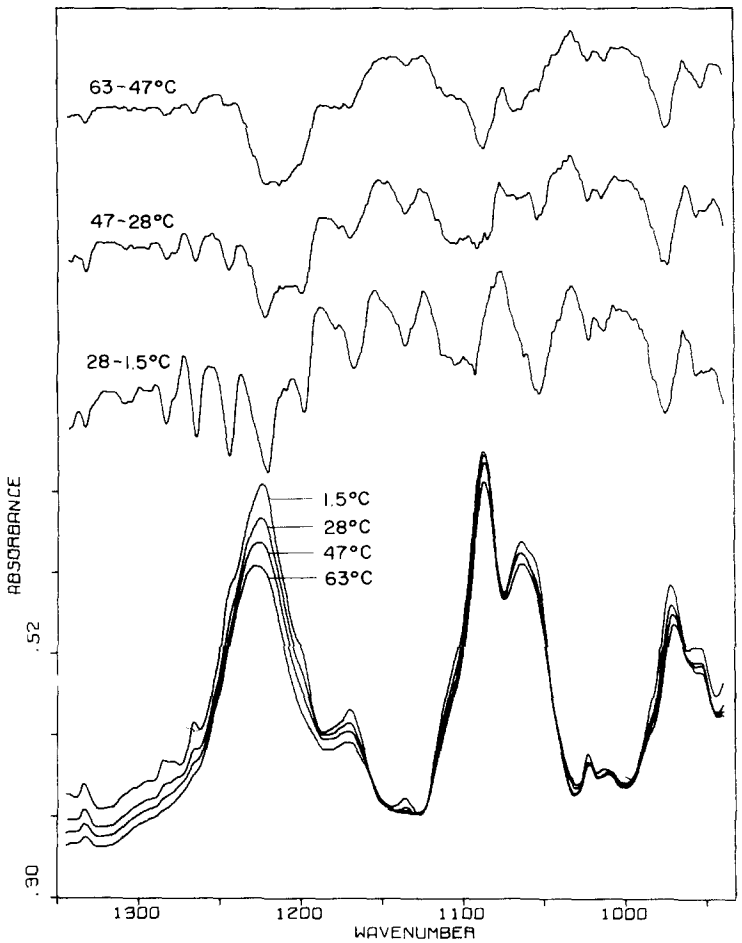

Fig. 6. (Bottom) Infrared spectra in the fingerprint region of DPPC multibilayers (25 μm thick) containing 60 mol% cholesterol at four temperatures. (Top) Corresponding difference spectra (ordinate scale $\times 5$).

B. Spectral features characteristic of the head group

Ester group bands. DPPC contains two ester linkages located at the *sn*-1 and *sn*-2 carbons of the glycerol moiety. Three distinctive absorptions can be expected from each ester group, the weak antisymmetric (1180—1160 cm^{-1}) and symmetric (1070—1060 cm^{-1}) C-O-C stretching modes overlapped by the phosphate modes, and the strong C=O stretching mode located in a region free of any other DPPC or cholesterol bands.

Spectra of the C=O absorptions in DPPC containing 40 and 60 mol% cholesterol are shown in Fig. 7A and B, respectively. In the gel phase the band contours in both systems are highly asymmetric, the asymmetry being greater in the 60 mol% cholesterol/DPPC system. The peak maximum is observed at about 1739 cm^{-1} and a second band is evident at about 1730 cm^{-1}. As the temperature is increased, the 1730 cm^{-1} band is relatively constant while the 1739 cm^{-1} band decreases in intensity and shifts to lower frequency, although two bands are still evident at 63°C in this system.

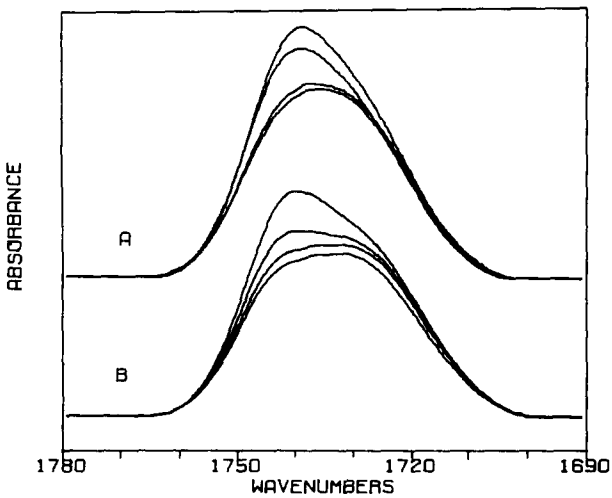

Fig. 7. Infrared spectra in the C=O stretching region of DPPC multibilayers with 40 and 60 mol% cholesterol (A and B, respectively) at four temperatures (1.5, 28, 47 and 63°C in decreasing intensity). The spectra were baseline-corrected.

This behaviour contrasts with that observed in the gel-phase spectrum of pure DPPC in which the band contour of the C=O stretching mode is much more symmetric and the peak maximum is at 1734 cm^{-1}, i.e., 5 cm^{-1} lower in frequency than in the cholesterol-containing systems. In the liquid-crystalline phase of pure DPPC, the band contour is nearly symmetric, as it is in DPPC with 40 mol% cholesterol, but there are still substantial differences as is evident from a comparison of bandwidths, shown as functions of temperature in Fig. 8A. At all temperatures the bandwidth is greatest in pure DPPC, despite the greater separation of the component bands in the cholesterol-containing system. This indicates a restricted freedom of motion of the carbonyl groups in cholesterol-containing DPPC relative to that in pure DPPC.

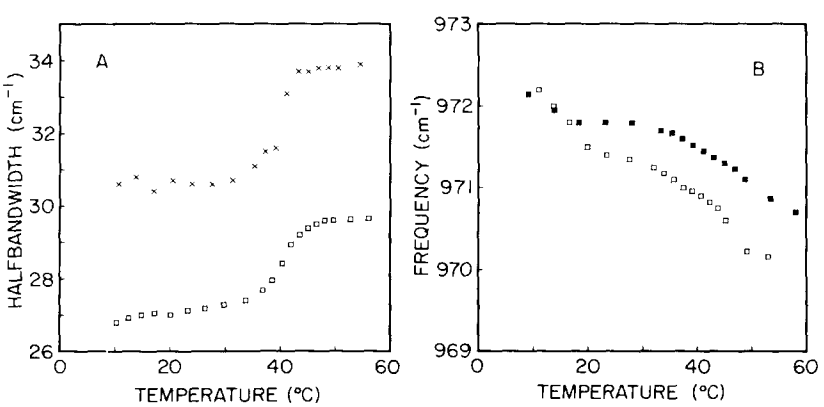

Fig. 8. (A) Temperature dependence of the half-bandwidth of the C=O stretching mode of DPPC (x) and DPPC with 40 mol% cholesterol (□). (B) Temperature dependence of the frequency of the C-N^+ stretching vibration of the choline group in DPPC multibilayers containing 40 (□) and 60 mol% cholesterol (■).

It is evident from the above data that the incorporation of cholesterol into the DPPC membrane induces a non-equivalence of the two carbonyl groups of DPPC. This results in the two maxima in the band contour, neither of which corresponds to the maximum observed in pure DPPC. This non-equivalence extends to the ester C-O-C groups as shown by the detection of two peaks in each of the C-O-C stretching regions at about 1178 and 1170 cm^{-1} (antisymmetric) and 1066 and 1063 cm^{-1} (symmetric) (Fig. 6). As we have demonstrated the presence of pure DPPC in the cholesterol-containing system, these splittings may reflect intra- and intermolecular non-equivalence of the ester groups. The largest splitting of the C=O and C-O-C modes is observed in the sample with 60 mol% cholesterol, which contains the smallest amount of pure DPPC. Therefore, we consider the main cause of this splitting to be intramolecular non-equivalence of the two ester groups. These findings agree with recent ^{2}H-NMR results [7].

It has also been suggested that in hydrated samples, hydrogen bonding occurs between the cholesterol OH group and the DPPC *sn*-1 [5,21] or *sn*-2 [22,23] carbonyl group, hydrogen bonding of the cholesterol OH having been demonstrated to occur in dry films of DPPC/cholesterol mixtures [24]. However, extensive hydrogen bonding between water and carbonyl groups is present in hydrated DPPC multibilayers [25]. Consequently, as we expect the hydrogen bond strength of the cholesterol OH to be similar to that of water, we are unable to observe any resultant effect, especially in the presence of the large changes due to conformational non-equivalence of the ester groups.

We also note that generalized evidence for hydrogen bond formation is available from the strongest cholesterol band in the fingerprint region, the C_3-O stretching mode [26], a weak shoulder at 1055 cm^{-1} in these spectra. In the free state in CCl_4 solution we observed this band at 1051 cm^{-1}, while in the hydrogen-bonded solid phase it shifts to 1056 cm^{-1}. The latter value compares well with the value of 1055 cm^{-1} in the DPPC/cholesterol systems. However, as hydrogen bonding may occur to either the C=O group or to water, this only indicates that the OH is not located in the central area of the bilayer, where such interactions are not expected.

Phosphate group absorptions. The antisymmetric phosphate stretching absorptions of DPPC are located at 1230 and 1088 cm^{-1}, respectively. Although the strongest bands in the fingerprint region, they are almost invariant with temperature. As they are overlapped by the CH_2 wagging band progression and other weak acyl chains modes which do change with temperature, the small temperature dependencies cannot be precisely characterized. However, in itself, the relative invariance of the modes and the exact correspondence of the frequencies with those observed in pure DPPC demonstrates that there is little interaction between the phosphate group and cholesterol. This agrees with the results of NMR studies [4,21], but is in contrast to an earlier infrared study [12] which reports frequencies for the antisymmetric phosphate stretching mode of 1250^{-1} in pure DPPC and 1230 cm^{-1} in DPPC containing 50 mol% cholesterol. As we find a frequency of 1250 cm^{-1} for the phosphate mode in a dry sample of pure DPPC, we attribute their results to improper sample hydration of the pure DPPC. We do note one difference between the mixtures and pure DPPC, that is the slight change in the liquid-crystalline phase

(top difference spectrum, Fig. 6), which is totally absent in pure DPPC.

Choline group. The principal absorption from the choline group is the asymmetric N^+-$(CH_3)_3$ stretching mode at about 970 cm^{-1} (Fig. 6). As is evident in the spectra and in a plot of frequency vs. temperature (Fig. 8B), this band steadily shifts to lower frequency throughout the temperature range 0—60°C.

This contrasts with the behaviour observed in pure DPPC where the band is invariant at temperatures above the pretransition, and is observed in the frequency range 971—970 cm^{-1}. It is generally proposed that the choline group interacts with the phosphate group of an adjacent DPPC molecule. Such bonding should result in a shift to lower frequency due to weakening of the N^+-C bond. Consequently, the order of the frequencies, pure DPPC < DPPC + 40 mol% cholesterol < DPPC + 60 mol% cholesterol, reflects increases in the intermolecular choline-phosphate distances due to the incorporation of cholesterol.

Factors influencing the temperature dependence of this mode in pure DPPC are presently the subject of study by us; it appears to be related to the change in acyl packing in the gel phase. If this is the case, then the behaviour in the cholesterol/DPPC systems may result from the fact that in the liquid-crystalline phase the mixtures are still changing, both in the hydrophobic region and, as shown by the difference spectra in the region of the phosphate bands, in the hydrophilic regions, whereas in pure DPPC the liquid-crystalline phase is relatively static. However, the cause and effect relationship is still not established.

Conclusions

The results of this study confirm the previously reported conclusions that the incorporation of cholesterol into DPPC multibilayers results in a population of *gauche* conformers in the gel phase and decreases the average number of *gauche* conformers in the liquid-crystalline phase. They also demonstrate the high symmetry of the melting phenomenon as observed by various parameters, and show that a small phase transition is still present at 60 mol% cholesterol content. The presence of this transition has not been previously reported.

The results of the studies of the CH_2 scissoring and CH_2 wagging modes demonstrate several points. Firstly, at all temperatures below 30°C, mixed-phase systems are present in DPPC containing 40 and 60 mol% cholesterol, one phase being pure DPPC packed in an orthorhombic subcell lattice and the major phase being a mixture of DPPC and cholesterol. Due to the highly ordered nature of the orthorhombic phase, we would also postulate that the *gauche* conformers are present only in the mixed phase. A pure DPPC phase has not been previously reported at cholesterol concentrations as high as 60 mol%.

In the liquid-crystalline phase, we do observe a CH_2 wagging band progression at the frequencies characteristic of fully extended acyl chains in a hexagonal lattice; this progression is not present in the spectra of pure DPPC. Consequently, these data suggest that the rigidity of DPPC induced by the addition of cholesterol results from the retention of some all-*trans* conformations in the immediate environment of the cholesterol molecule.

The principal data related to the head group are those characteristic of the ester linkage. At all temperatures they demonstrate the induction of a non-equivalent conformation of the two ester groups as a result of the addition of

cholesterol. The phosphate and choline absorptions are almost the same as those of pure DPPC, suggesting that the interaction between cholesterol and these functional groups is minimal.

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