Biochimica et Biophysica Acta, 689 (1982) 63–72
Elsevier Biomedical Press

BBA 71235

PHOSPHATIDYLSULFOCHOLINE BILAYERS

AN INFRARED SPECTROSCOPIC CHARACTERIZATION OF THE POLYMORPHIC PHASE BEHAVIOR *

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(Received February 2nd, 1982)

Key words: Infrared spectroscopy; Thermotropism; Membrane structure; Phosphatidylsulfocholine; Phase transition

The thermal response of aqueous dispersions of phosphatidylsulfocholines (dimyristoyl-, dipalmitoyl- and distearoyl-) was studied by Fourier transform infrared spectroscopy. Comparison with that of the corresponding phosphatidylcholines showed several close resemblances, including the observation in the gel phase of a 'pretransition' and of a 'subtransition'. The similarity in the thermotropic phase behavior of these two lipid classes is consistent with the total replacement of phosphatidylcholine by phosphatidylsulfocholine in certain marine diatoms.

Introduction

Lipid/water dispersions are widely used as model systems in studies of structure-function relationships of biological membranes. While the effect of the fatty acyl chain on membrane 'fluidity' is well recognized and has been extensively studied (Refs. 1–3 and references therein), attention is now shifting towards the effect of the polar head group on the properties of membranes (Refs. 4–7 and references therein), as it is at the level of the head group that membranes interact with the environment.

The most commonly studied head groups are those of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. Recently, a new class of phospholipids was discovered, the phosphatidylsulfocholines, in which the trimethylammonium function of the phosphocholine moiety is replaced by a dimethylsulfonium group (see inset in Fig. 1). These phospholipids constitute the major membrane component of certain marine diatoms [8,9]; in fact, in *Nitzschia alba*, a nonphotosynthetic diatom, the usual phosphatidylcholines are replaced entirely by this structural analogue [9]. Such findings raise the question of the role played by the sulfonium analogues as membrane components in comparison with that of phosphatidylcholines. Two of us (M.K. and P.A.T.) have therefore synthesized a homologous series of phosphatidylsulfocholines [10], the physical properties of which were studied by differential scanning calorimetry, steady-state fluorescence polarization and electron spin resonance [11], and by osmometry [12]. DPPSC containing a monodeuteromethylsulfonium group was also investigated by ^{2}H- and ^{31}P-NMR [13]. No further physico-

* Issued as NRCC Publication No. 20054.

Abbreviations: DMPC, DPPC and DSPC, 1,2-dimyristoyl-, 1,2-dipalmitoyl- and 1,2-distearoyl-3-*sn*-phosphatidylcholine, respectively; DMPSC, DPPSC and DSPSC: 1,2-dimyristoyl-, 1,2-dipalmitoyl- and 1,2-distearoyl-3-*sn*-phosphatidylsulfocholine, respectively; T_m, temperature of the gel-to-liquid crystalline phase transition, (m denotes acyl chain melting); T_p, temperature of the pretransition; T_s, temperature of the subtransition.

chemical studies of synthetic or natural lipids containing this head group have been reported.

However, the closely related phosphatidylcholines have been studied in detail, and three phase transitions were identified for fully hydrated samples. The highest temperature transition, and the one involving the greatest enthalpy change, is the acyl chain melting phase transition, generally referred to as the main, or gel-to-liquid crystalline transition. A second phase transition, which occurs several degrees below the main transition, is broader and has an enthalpy change approx. one-sixth that of the main transition [14]. This so-called pretransition is a solid-solid phase change and has been characterized as a change in the unit cell in which the acyl chains are packed [15,16]. More recently, a third phase transition was found in phosphatidylcholines [17]; referred to as the subtransition, it is only observed after extensive incubation of the sample at low temperature, and occurs at a temperature T_s, lower than that of the pretransition, T_p, or that of the main transition, T_m. X-ray diffraction [18,19] and infrared studies [20] have shown that the subtransition is another solid-solid phase transition which involves a change in the acyl chain packing and in the conformation of the head group.

Whereas all phospholipids exhibit a gel-to-liquid crystalline phase transition, the pretransition and subtransition have only been observed in phosphatidylcholines. It was thus of interest to establish whether the phosphatidylsulfocholines, so closely related structurally to the phosphatidylcholines, exhibit a similar polymorphism in the gel phase. The present report is a detailed infrared spectroscopic study of the thermotropic phase behavior of aqueous dispersions of DMPSC, DPPSC and DSPSC.

Experimental Procedure

Materials

DMPSC, DPPSC and DSPSC were synthesized as previously described [10] and purified by TLC using chloroform/methanol/water (65:35:5) followed by acetone precipitation. All samples were chromatographically, optically and analytically pure. Aqueous bilayer dispersions were prepared by adding approx. 30 mg of ^2H_2O or double-distilled H_2O to approx. 2 mg of the dry sulfocholine in a glass ampoule. The ampoule was sealed and heated in a water bath to about 5°C above T_m, then cooled to temperatures well below T_m. The heating-cooling cycle was repeated three times. The gel was then assembled into a Harrick-type infrared cell with CaF_2 or BaF_2 windows; the cell thickness was 6, 12 or 25 μm. The formation of a typical multibilayer structure was previously demonstrated by electron microscopy [10], and the large excess of water or ^2H_2O was evident from the intense infrared absorption in the region of the O–H or O–^{2}H stretching vibrations.

Spectra

Infrared spectra were obtained on a Digilab FTS-11 Fourier transform infrared spectrometer fitted with a high-sensitivity mercury cadmium telluride detector. Interferograms were obtained with a maximum optical retardation of 0.5 cm. Typically, 600 scans were co-added, triangularly apodized and zero-filled once to yield a final resolution of 2 cm^{-1} and an encoding interval of 1 cm^{-1}. For temperature control a cell mount was used through which thermostatically controlled ethanol/water was circulated; temperatures were stable to within ± 0.05°C [21]. The temperature was increased in steps of 2°C with a waiting period of 16 min between runs which, if converted to a constant heating rate such as used in DSC measurements, would correspond to a heating rate of 0.125°C/min. The operation of regulating the temperature and recording individual spectra was completely under the control of the spectrometer computer [22]. The incubation experiment with DPPSC was performed at 5°C on a Digilab FTS-15 Fourier transform instrument equipped with a wide-range mercury cadmium telluride detector and 1000 scans were accumulated for each spectrum. The sample for this experiment was prepared in a 50 μm thick cell fitted with BaF_2 windows.

Data processing

Frequencies were determined by computing centers of gravity of the bands. For sharp bands, such as the C–H stretching bands, the top three data points were used (i.e., the topmost 2 cm^{-1} wide segment); for broad bands, such as the C = O

stretching band, the top nine data points were used. Bandwidths were determined relative to linearly interpolated baselines, the widths being computed at various fractions of the peak height. The overall capability of measuring temperature-induced changes in frequency and bandwidth was substantially better than 0.1 cm^{-1} [23]. Infrared difference spectra were generated by subtracting low temperature (or shorter time) from high temperature (or longer time) spectra and normalizing with respect to the corresponding temperature (or time) interval, i.e., $\Delta A = (A_T - A_{T-1})/\Delta T$, where T may be temperature or time.

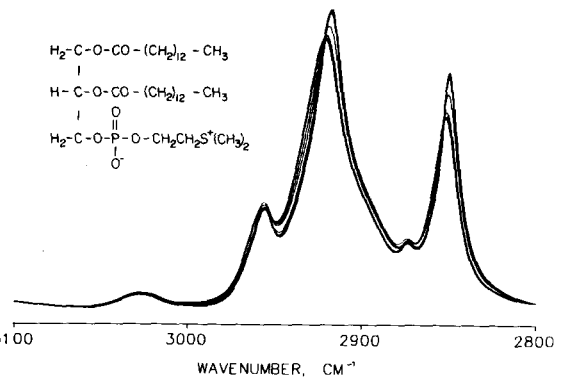

Fig. 1. Temperature dependence of the C–H stretching bands of DMPSC. Shown are ten individual spectra recorded between 10 and 30°C; peak heights decrease with increasing temperature. Inset: structural formula of DMPSC.

Results

Main transition and pretransition

The frequencies and assignments of the major infrared active modes of DMPSC are given in Table I, for both the gel and the liquid-crystalline phases. Virtually identical spectra were obtained for DPPSC and DSPSC except for the frequencies of the chain length-dependent CH_2 wagging band progression, a series of weak, regularly spaced bands in the 1350–1200 cm^{-1} region which are observed only in the gel phase. The spectra are also similar to those of the corresponding phosphatidylcholines, apart from the sulfocholine methyl bands at 3023 and 1435 cm^{-1}, which replace the choline methyl bands at 3040 and 1490 cm^{-1}. Absorption bands of major diagnostic value

TABLE I

FREQUENCIES OF SELECTED INFRARED ABSORPTION BANDS OF DMPSC AQUEOUS BILAYERS IN THE GEL PHASE (AT 12°C) AND IN THE LIQUID-CRYSTALLINE PHASE (AT 30°C)

sh, shoulder. The strong absorption due to the excess water obscures most DMPSC bands below 1000 cm^{-1}.

Frequency (cm^{-1})		Assignment	
12°C	30°C		
3023.1	3024.1	$-S^+(CH_3)_2$	asymmetric CH_3 stretching
2955.1	2956.0	$-(CH_2)_nCH_3$	asymmetric CH_3 stretching
2918.1	2922.8	$-(CH_2)_n-$	antisymmetric CH_2 stretching
2872.7	2872.3	$-(CH_2)_nCH_3$	symmetric CH_3 stretching
2850.0	2852.3	$-(CH_2)_n-$	symmetric CH_2 stretching
1735.7	1733.1	$-CO_2CH_2-$	C=O stretching
1467.8	1467.4	$-(CH_2)_n-$	CH_2 scissoring
1435.4	1436.1	$-S^+(CH_3)$	asymmetric CH_3 deformation
1418.5	1419.9	$-CO_2CH_2-$	αCH_2 scissoring
1378.4	1378.5	$-(CH_2)_nCH_3$	symmetric CH_3 deformation
1343.7	1342.3	$-(CH_2)_n-$	CH_2 wagging
1229.7	1230.3	$-OPO_2O-$	antisymmetric PO_2^- stretching
1170.1	1180.0	$-CO_2CH_2-$	C–O–C asymmetric stretching
≈1114 sh	≈1114 sh	$-(CH_2)_nCH_3$	C–C stretching
1084.3	1083.0	$-OPO_2O-$	symmetric PO_2^- stretching
1046.7	1048.6	-COC(P)	C–O stretching

are the C–H stretching, the CH_2 scissoring, and the C = O stretching bands.

Illustrated in Fig. 1 is the temperature dependence of the infrared spectrum of DMPSC in the 3100–2800 cm^{-1} region. Changes in frequency, peak height and bandwidth are evident; these changes are detailed in Fig. 2 which shows the temperature dependences of the frequency and width (measured at 3/4 peak height) of the symmetric CH_2 stretching band near 2850 cm^{-1}. The corresponding plots for DPPSC and DSPSC are similar and are not shown here. Identical temperature dependences were observed in data obtained from the antisymmetric CH_2 stretching bands near 2920 cm^{-1}. The C–H stretching band parameters of the investigated phosphatidylsulfocholines exhibit thermotropic behavior similar to that of the corresponding phosphatidylcholines. There are slight changes with temperature throughout the gel and liquid-crystalline phases. Major changes in both frequency and bandwidth are observed at T_m, reflecting the increase in conformational disorder of the acyl chains on transition to the liquid-crystalline phase. In Fig. 2 we also note a minor change in frequency at 24°C, about 3 degrees before the major change at T_m; this results from the pretransition in DMPSC which has also been detected in DSC studies [11]. With DPPSC and DSPSC, we were unable to observe any evidence of a distinct pretransition, in agreement with the results of the DSC study.

Information regarding the interchain interactions in the gel phase can be obtained from the CH_2 scissoring band near 1468 cm^{-1}. This band results from *trans* segments of the acyl chains, and both the frequency and number of components are dependent on the form of packing of the lipids. Fig. 3A shows the 1480–1420 cm^{-1} region of the spectrum of DPPSC at different temperatures between 5 and 30°C; the region is dominated by the strong CH_2 scissoring band contour. On transition from the gel to the liquid-crystalline phase there is an abrupt decrease in the height of this band, due

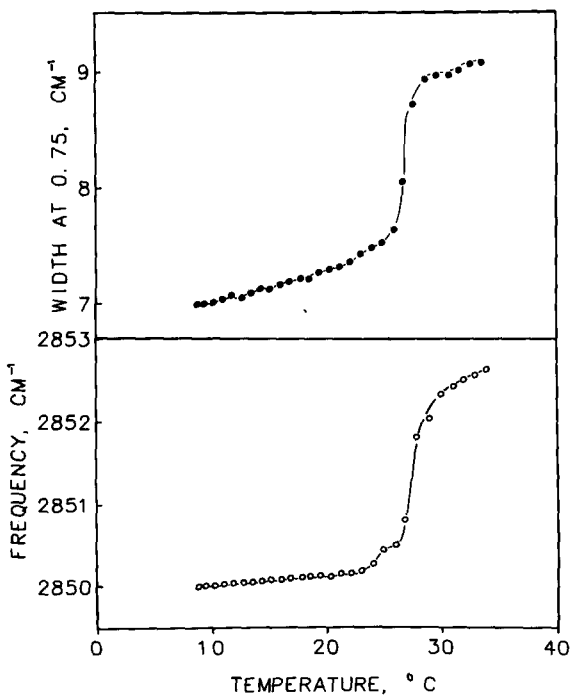

Fig. 2. Frequency (○) and full width at 3/4 peak height (●) of the symmetric CH_2 stretching vibration of DMPSC as a function of temperature.

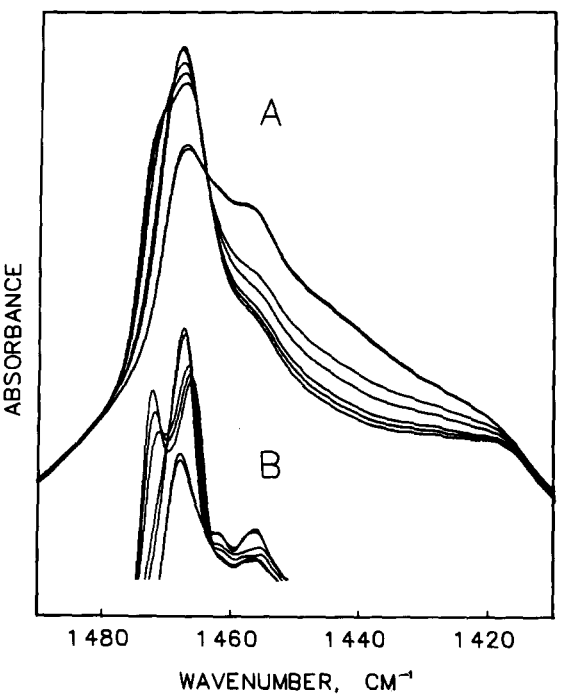

Fig. 3. (A) Temperature dependence of the contour of the CH_2 scissoring band of DPPSC. (B) Same spectra as in A following self-deconvolution with a Lorentzian line of 6 cm^{-1} half-width and resulting in reduction of the bandwidths by a factor of 1.8 [24,25]. The splitting decreases with increasing temperature and spectra are shown at: 5, 8, 11, 30, 33, 49 and 51°C.

to the loss of extended CH_2 *trans* segments. From the spectra in the gel phase it is apparent that at lower temperatures the band is split into two components; as the temperature is increased, the magnitude of the splitting decreases. This is more clearly demonstrated in Fig. 3B, which shows the spectra following self-deconvolution. At 5°C the factor group splitting is 7 cm^{-1} (1472 and 1465 cm^{-1}) and decreases progressively as the temperature is increased, until at temperatures near T_m only a single band at 1468 cm^{-1} is observed. The splitting of this band can also be deduced from the existence of two minima in the corresponding difference spectra (see Experimental Procedure) while the progressive nature of this splitting is reflected in the width of this band, especially when measured close to the peak maximum. Normally, on increasing the temperature, the width of an infrared band increases due to the increased amplitudes of the various molecular motions; thus, at T_m there is a steep increase in the width of this band. However, throughout the gel phase of DMPSC, DPPSC and DSPSC, the width (measured at nine-tenths peak height) of the CH_2 scissoring band decreases progressively as the temperature increases to within a few degrees of T_m. This reflects the continuously decreasing factor group splitting, behavior similar to that observed in the phosphatidylcholines where factor group splitting exists at all temperatures below T_p.

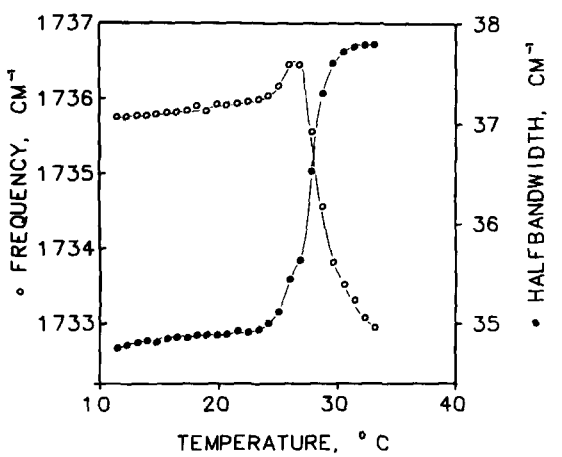

Fig. 4. Temperature dependence of the peak maximum (○) and of the band width at half-height (●) of the C=O stretching band of DMPSC/water dispersions.

Further confirmation of similarities in the thermotropic behavior of choline and sulfocholine lipids comes from a study of the head group vibrations. While absorption bands of the phosphosulfocholine moiety are rather insensitive to the phase changes at T_m and T_p, the bands resulting from the *sn*-1 and *sn*-2 ester linkages show sizable changes. These changes are most evident in the C=O stretching band near 1735 cm^{-1}, as illustrated for DMPSC in Fig. 4. With increasing temperature the peak maximum shifts to higher frequency at T_p, remains constant between T_p and T_m and shifts to a considerably lower frequency at T_m. The bandwidth remains constant in the gel phase, shows a small increase at T_p and then a large increase at T_m. This behavior is identical to that observed in phosphatidylcholines at T_p and T_m [16] except that we found no evidence of a pretransition in the plots derived from the DPPSC and DSPSC spectra.

From the asymmetry of the C=O stretching band contour (Fig. 5A), this band would appear to be comprised of at least two components. This was confirmed by a deconvolution of the band, and the resultant spectra are shown in Fig. 5B. At all temperatures the C=O stretching band consists of two major components which at temperatures

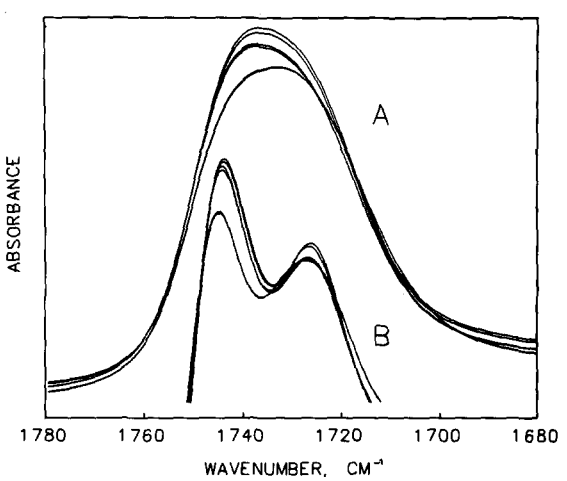

Fig. 5. (A) Infrared spectra of DMPSC in the C=O stretching region at temperatures below T_p (top two spectra at 15 and 20°C), between T_p and T_m (middle two spectra at 26 and 27°C) and above T_m (bottom two spectra at 29 and 31°C). (B) Same spectra as in A after Fourier self-deconvolution with a Lorentzian line of 20 cm^{-1} half-width resulting in a reduction of the band width by a factor of 2.5.

below T_p are found at 1743 and 1725.5 cm^{-1}. At T_p both components show a small shift in frequency to 1744 and 1726 cm^{-1}, respectively, and the low-frequency component also decreases in intensity. At T_m neither band shifts in frequency; however, the 1744 cm^{-1} component shows a significant decrease in peak height. The changes observed in the two band components thus explain the overall changes in frequency and width plotted in Fig. 4. The C = O stretching band contour in the spectra of the other sulfonium analogs also consists of two major band components. The changes observed in the relative intensities of the CH_2 and C = O stretching bands (not shown) parallel those observed in the spectra of the corresponding phosphatidylcholines (unpublished results).

Subtransition

Fig. 6 shows the 1850–1150 cm^{-1} region of the infrared spectrum of DPPSC after 6 h (A) and after 5 days (B) at 5°C. Superimposed is the difference spectrum (B − A) which reflects the changes resulting from the incubation. Major changes are evident in acyl chain modes such as the methylene scissoring band (approx. 1470 cm^{-1}) and the CH_2 wagging band progression (1350–1190 cm^{-1}). Upon incubation at 5°C one observes a general narrowing of all of these bands, and an increase in the peak height, indicative of a reduction of the mobility of the acyl chains.

The behavior of the CH_2 scissoring band contour on incubation is shown in Fig. 7. Incubation at 5°C results in an increase in the magnitude of factor group splitting, and a better resolution of the two component bands. After 6 days the two band components are at 1473 and 1463 cm^{-1} and are of almost equal intensity. The spectra closely

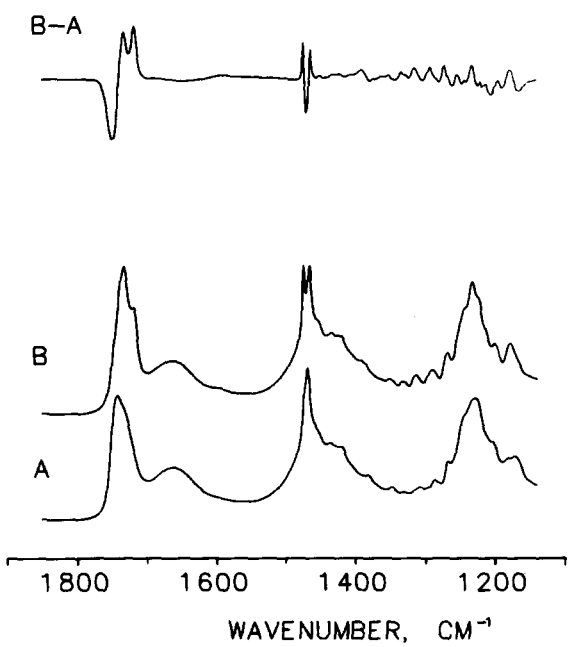

Fig. 6. Infrared spectra in the 1850–1150 cm^{-1} region of a 50 μm thick sample of DPPSC, hydrated with ^2H_2O and kept in BaF_2 cell at 5°C for 6 h (A) and for 6 days (B). Top: corresponding infrared difference spectrum.

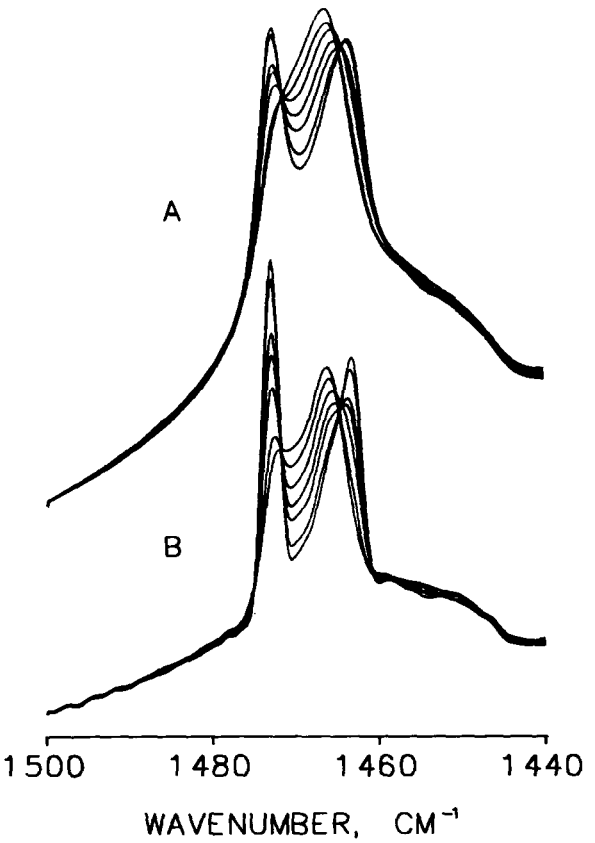

Fig. 7. (A) Infrared spectra of the CH_2 scissoring band of DPPSC after annealing at 5°C for 0.25, 1, 2, 3, 4, 5 and 6 days. The peak at 1473 cm^{-1} increases and the 1466 cm^{-1} component decreases and gradually shifts to 1463 cm^{-1} with longer incubation periods. (B) Same spectra as in A following Fourier self-deconvolution with a Lorentzian line of 3 cm^{-1} half-width resulting in a reduction of the bandwidth by a factor of 1.6.

resemble those of *n*-alkanes, fatty acids or esters when packed in an orthorhombic subcell, indicating a high degree of interchain coupling and a rigid packing of the acyl chains. These spectra contrast with those observed when DPPC is incubated at 2°C [20]; in the latter case, the lower frequency band (approx. 1462 cm^{-1}) is almost completely absent and the spectra resemble those of triclinically packed *n*-alkanes.

Large changes are also evident in the bands associated with the ester linkage, i.e., the C = O stretching band at 1735 cm^{-1} and the antisymmetric C–O–C stretching band at 1170 cm^{-1}. Fig. 8A shows in detail the changes induced in the C = O stretching band by incubation at 5°C. From Fig. 8B, which shows the spectra following self-deconvolution, it can be seen more clearly that the C = O stretching band contour changes progressively with time, and that following 6 days of incubation it is comprised of four narrow bands at 1744, 1737, 1730 and 1715 cm^{-1}. In fact, the C = O stretching band contour of the annealed sample resembles that observed in the spectrum of poorly hydrated or crystalline DPPSC (unpublished data), suggesting that the incubation results in a decrease in hydrogen bonding of water to the C = O group. Similar conclusions were reached in the case of DPPC [18–20].

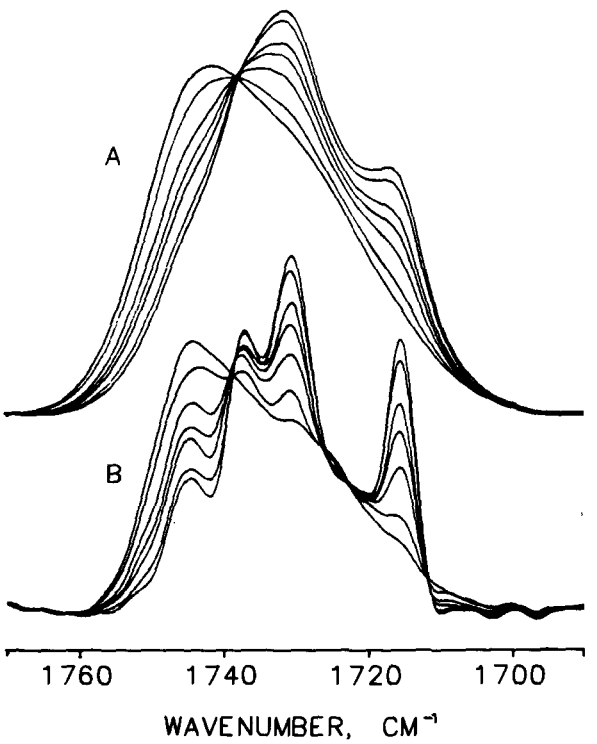

Fig. 8. (A) Infrared spectra of the C=O stretching band of DPPSC after annealing at 5°C for 0.25, 1, 2, 3, 4, 5 and 6 days. The peak at 1743 cm^{-1} decreases and those at 1732 and 1715 cm^{-1} increase with longer incubation periods. There is an isosbestic point at 1738 cm^{-1}. (B) Same spectra as in A following Fourier self-deconvolution with a Lorentzian line of 8.5 cm^{-1} half-width resulting in a reduction of the bandwidth by a factor of 2.2.

The kinetics of the conversion of DPPSC on incubation are shown in Fig. 9, which displays the normalized time-dependent changes in the C = O stretching band. The rate of change which is initially slow, increases with incubation time, reaches a maximum between 1 and 2 days, and decreases thereafter. The changes are not completed within the time scale of this experiment and the kinetics are slightly different from those observed in DPPC [20], although both systems exhibit a maximum rate of change after 1–2 days of incubation.

When a previously incubated sample of DPPSC is heated through the temperature range 5–50°C, one observes abrupt spectral changes at 20°C; above this temperature the spectral changes are the same as those of samples which were not incubated. This abrupt reversion to a nonincubated gel phase occurred at 16°C in DPPC which had been incubated at 2°C [20]; thus, the

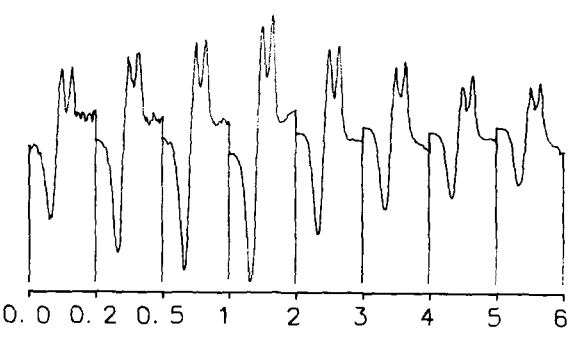

Fig. 9. Infrared difference spectra in the region of the C=O stretching bands (1780–1680 cm^{-1}) recorded during incubation at 5°C. Spectra are normalized with respect to the corresponding time interval.

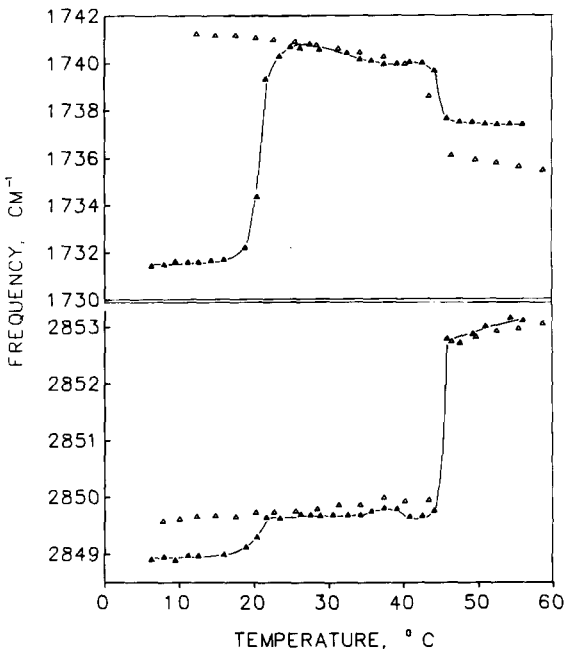

Fig. 10. (A) Temperature dependence of the peak maximum of the C=O stretching band of DPPSC multibilayers without (△) and with previous annealing for 6 days at 5°C (▲). (B) Temperature dependence of the symmetric CH_2 stretching frequency of DPPSC multibilayers without (△) and with previous annealing for 6 days at 5°C (▲).

change observed in DPPSC at 20°C can be identified as the subtransition. The abruptness of the subtransition at T_s is illustrated in Fig. 10. The change of frequency of the carbonyl band at 20°C reflects a reversion to the broad asymmetric carbonyl band characteristic of the nonincubated gel phase, and suggests water penetration and hydrogen bonding to the C=O moiety at this phase transition. The small increase in the frequency of the CH_2 stretching band at T_s, along with an increase in bandwidth (not shown here), may indicate the introduction of some conformational disorder into the acyl chains on reversion to the nonincubated gel phase.

Discussion

The infrared data presented demonstrate that, in general terms, the saturated 1,2-diacyl-3-*sn*-phosphatidylsulfocholines exhibit polymorphic phase behavior similar to that of the analogous phosphatidylcholines, especially when contrasted with other lipid systems. All the spectral changes observed in the phosphatidylsulfocholines at T_m are similar to those observed in the phosphatidylcholines at T_m; their spectra in the liquid crystalline phase are practically identical. In the gel phase, DMPSC exhibits a pretransition similar to that of DMPC while DPPSC and DSPSC exhibit a subtransition similar to those of DPPC and DSPC.

A detailed examination of the data, however, reveals a number of minor differences between phosphatidylcholines and phosphatidylsulfocholines. From a thermotropic point of view, the transition temperatures T_m and T_s are 4–5°C higher in the corresponding phosphatidylsulfocholines, the pretransition is only resolved in DMPSC compared to its observation in all phosphatidylcholines, and T_p and T_m are only 3 degrees apart in DMPSC whereas they are 10 degrees apart in DMPC. A comparison of transition temperatures is shown in Table II.

From a spectroscopic point of view, the main differences between phosphatidylcholines and phosphatidylsulfocholines are found in the spectra of the gel phase, particularly when one considers the subtransition. Both the phosphatidylcholines and phosphatidylsulfocholines exhibit a continuous decrease in the degree of interchain interaction as the temperature is raised throughout the gel phase. However, the shape of the CH_2 scissoring band contours differs between the two systems.

TABLE II

TRANSITION TEMPERATURES FOR PHOSPHATIDYLSULFOCHOLINES AND PHOSPHATIDYLCHOLINES

Transition temperatures were obtained from infrared spectroscopic data. Values in parentheses are from DSC measurements [11,14,17].

Lipid	T_m (°C)	T_p (°C)	T_s (°C)
DMPSC	27.0 (26.5)	24.0 (24.0)	[a]
DMPC	24.0 (23.6)	14.5 (14.4)	[a]
DPPSC	45.5 (45.5)	[b]	20.0
DPPC	41.5 (41.1)	35.0 (34.8)	16.0 (17.5)
DSPSC	60.0 (60.5)	[b]	≈25
DSPC	54.5 (54.2)	50.4 (50.0)	(21.2)

[a] Not determined.
[b] No evidence.

Thus, if one discusses the spectra in terms of decreasing temperature, in the case of the phosphatidylsulfocholines the 1472 cm^{-1} band is evident as a weak shoulder which builds up as the temperature is further reduced (Fig. 3); only after considerable incubation does it attain a peak height greater than that of the 1462 cm^{-1} band (Fig. 7). In the case of the phosphatidylcholines, the CH_2 band contour first broadens to a flat-topped peak (Fig. 1 in Ref. 16), and the 1472 cm^{-1} band is always stronger than the 1462 cm^{-1} band. Major differences are evident following incubation. The spectrum of DPPSC exhibits a 10 cm^{-1} factor group splitting with two strong bands at 1473 and 1463 cm^{-1} (Fig. 7), in contrast to the corresponding DPPC spectra (Fig. 6B in Ref. 20), which exhibit only one strong band, near 1472 cm^{-1}. In terms of the simplest subcell in which the acyl chains can pack, the spectra of DPPSC resemble those expected from an orthorhombic subcell while those of DPPC resemble those expected from a triclinic subcell. We also note that, although in both systems the C = O stretching bands indicate a loss of bound water upon incubation, which is in agreement with X-ray diffraction studies [18,19], the band contours are quite different; DPPC spectra have only two main components at frequencies different from any of the four band components observed in the DPPSC spectra.

In a descriptive sense, the phosphatidylsulfocholines exhibit behavior intermediate between that of phosphatidylcholines and that of phosphatidylethanolamines, which are difficult to hydrate and have higher melting transition temperatures. Thus, the main transition temperatures of the phosphatidylsulfocholines are elevated relative to those of the phosphatidylcholines, although they are well below those of the corresponding phosphatidylethanolamines. Furthermore, the pretransition could only be observed for DMPSC, in contrast to its presence in all phosphatidylcholines, and its absence in all phosphatidylethanolamines. However, the factor group splitting is similar in all three phosphatidylsulfocholines, indicating that in both DPPSC and DSPSC either the pretransition has merged with the main transition, or lies close to it in temperature. Detailed DSC studies, such as those that resolved the two transitions in mixed-chain phosphatidylcholines [14], should help settle this question.

In phosphatidylcholines the acyl chains are tilted with respect to the plane of the bilayer surface [6] and the pretransition has been shown to involve a change in this angle of tilt; phosphatidylethanolamines, where the acyl chains are normal to the bilayer surface, do not exhibit a pretransition. Thus, the cause of differences between the phosphocholines and phosphosulfocholines could possibly be sought in different head group interactions in the gel phase and a different tilt angle of the acyl chains. Space-filling molecular models indicate that the polar head group of the sulfocholine lipids occupies a larger swept-out volume than that of the corresponding phosphocholines; replacement of the $-N^+(CH_3)_3$ group by $-S^+(CH_3)_2$ was estimated to result in a 20% increase in this volume [11]. This conclusion is supported by a comparison of the ^{31}P-NMR spectra of DPPSC and DPPC [13]. The lower values of the phosphorus chemical shift anisotropy for DPPSC indicate a larger amplitude of motion for its phosphate moiety compared to that of DPPC. The larger volume of the phosphosulfocholine head group could lead to increased interactions between the positively charged region of one molecule and the negatively charged phosphate residues of neighboring molecules. In the phosphatidylethanolamines, the ethanolamine groups link adjacent phosphate ribbons into a very compact, rigid head group network. In the phosphatidylcholines, the phosphate groups are linked to ribbons by water molecules and the distance between individual phosphate ribbons is considerably larger than in the phosphatidylethanolamines [6]. Thus, in the phosphatidylsulfocholines the head group could be not as compact as in phosphatidylethanolamines, but more rigid than in phosphatidylcholines. The strongest indication of a correlation between phosphatidylsulfocholines and phosphatidylethanolamines comes from the CH_2 scissoring band contours. In DPPSC the relative intensities of the two components, observed throughout the gel phase and during incubation, resemble those observed in studies of DPPE, but differ from those observed in DPPC. As the relative intensities of the two components of the CH_2 scissoring band are determined by the three-dimensional packing of the acyl chains, the DPPSC spectra may indicate the

existence of a tilt angle in phosphatidylsulfocholine bilayers similar to, but smaller than, that in phosphatidylcholine bilayers. X-ray diffraction studies would aid considerably in the resolution of the sources of these differences.

In summary, the present findings confirm a general similarity in the physical properties of lipids with phosphosulfocholine or phosphocholine head groups which explains why the sulfonium analogues are able to substitute functionally for the ubiquitous phosphocholines in certain natural membranes. However, there are also subtle differences, especially in the structure and the thermotropic behavior of the gel phase, which could prevent such a functional substitution in other membranes.

Acknowledgement

This work was supported in part by a grant to M.K. from the Natural Sciences and Engineering Research Council of Canada.

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