Biochimica et Biophysica Acta, 735 (1983) 387–396
Elsevier

BBA 71884

THE THERMOTROPIC PHASE BEHAVIOR OF *N*-METHYLATED DIPALMITOYLPHOSPHATIDYLETHANOLAMINES *

HECTOR L. CASAL and HENRY H. MANTSCH

Division of Chemistry, National Research Council of Canada, Ottawa, Ontario, K1A 0R6 (Canada)

(Received June 3rd, 1983)

Key words: N-Methyl phosphatidylethanolamine; Calorimetry; Infrared spectroscopy; Differential scanning calorimetry; Phase transition

The thermal phase behavior of the mono- and di-*N*-methylated derivatives of DPPE has been investigated by DSC and infrared spectroscopy. It is shown that these partially *N*-methylated DPPE derivatives present endothermic phase transitions at 48.2 and 58.0°C. The thermodynamic properties of these gel to liquid-crystalline transitions are comparable to those of DPPE and DPPC with no evidence for a pretransition in either of these *N*-methylated lipids. The progressive methyl substitution of the ethanolamine group of DPPE does not have a constant effect on reducing the transition temperature. The spectral properties of the gel phases of DPPE, DPMePE, $DPMe_2PE$ and DPPC (or $DPMe_3PE$), suggest that there is a progressive increase in the acyl chain tilt angle from DPPE to DPPC, probably induced by the differences in the cross-sectional area of the headgroups which lead to small differences in the glycerol moiety.

Introduction

Among the lipid components of biological membranes, phosphatidylcholines (PC) and phosphatidylethanolamines (PE) have been and are most extensively studied with regard to their thermotropic phase behavior [1]. Metabolically, phosphatidylcholines can be produced via direct methylation of the amino group of phosphatidylethanolamine by the methyl group donor *S*-adenosylmethionine [2]; this yields two intermediaries which contain one and two methyl groups in the ethanolamine moiety, respectively. Since the physical state of biological membranes is regulated not only through changes in the nature of the lipid acyl chains but also through changes in the headgroup [1], it is quite plausible that the partially methylated PE may serve a role in the regulation of membrane properties. In fact, Axelrod and coworkers [3] have studied the possible influence of PE methylation on receptor-related membrane events. Besides, it is unknown why the PC synthesis via sequential methylation of PE is of crucial importance in certain mammalian organs such as the liver, in contrast to the insignificance of that pathway to other organs [4].

There are substantial differences between the phase behavior of phosphatidylethanolamines and that of phosphatidylcholines [5,6]. In the first place, the temperatures of the corresponding gel to liquid-crystalline phase transitions (i.e. the melting of the acyl chains) are higher for PE than for PC with the same acyl chains. Secondly, PC with saturated acyl chains are highly hygroscopic compounds, while the corresponding PE are difficult to hydrate. The differences between PC and PE are particularly evident in the properties of the gel

* Issued as NRCC publication No. 22508.

Abbreviations: PE, phosphatidylethanolamine(s); MePE, *N*-methyl phosphatidylethanolamine; Me_2PE, *N*,*N*-dimethyl phosphatidylethanolamine; PC, phosphatidylcholine(s); DP, dipalmitoyl; DM, dimyristoyl; T_m, temperature of the gel to liquid-crystalline phase transition; DSC, differential scanning calorimetry.

phases. Thus, PC with saturated acyl chains show a pretransition in the gel phase which has not been observed in the corresponding PE. Saturated PE, on the other hand, show phase transitions involving poorly hydrated gels [7–10], which are not observed in the highly hygroscopic PC. Finally, aqueous gels of saturated PC are metastable and prolonged incubation at low temperatures results in a structural rearrangement with greatly altered acyl chain packing and head group dehydration [11–15].

In the present study we have used DSC and infrared spectroscopy to characterize the phase behavior of two *N*-methylated PE which are part of a homologous series with respect to methyl substitution, i.e., DPPE, DPMePE, $DPMe_2PE$ and DPPC (or $DPMe_3PE$).

Experimental

Materials. High purity samples of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine (DPPE) were obtained from Sigma Chemical Co. (St. Louis, MO). 1,2-Dipalmitoyl-*sn*-glycero-3-phospho-*N*-methylethanolamine (DPMePE) and 1,2-dipalmitoyl-*sn*-glycerol-3-phospho-*N*,*N*-dimethylethanolamine ($DPMe_2PE$) were obtained from Calbiochem-Behring Corp. (La Jolla, CA). All samples were pure by thin-layer chromatography and were lyophilized prior to use, as PE are known to occur in different polymorphs [7].

Calorimetry. Samples for calorimetry were prepared from 3 mg of the solid lipid in 1 ml of 0.1 M sodium phosphate buffer (pH 7.0). These aqueous dispersions were immersed for several minutes in an acetone/solid CO_2 bath, allowed to thaw and then vortexed at room temperature. This cycle was repeated three times whereby the sample temperature was never allowed to exceed room temperature (approx. 22°C). The samples were then equilibrated for 1 h in the calorimeter at 5°C and a first trace was recorded from 5 to 70°C. After this measurement, the samples were cooled in the calorimeter (over a period of approx. 45 min), and allowed to equilibrate for 1 h at 5°C. A second calorimetric trace was then recorded over the same temperature range. Calorimetric data were obtained with a Microcal MC-1 instrument operating with a heating rate of 1 K/min. Enthalpies were calculated from peak areas determined by weight and transition temperatures were determined from the midpoints of the corresponding endotherms.

Infrared spectroscopy. Samples (80% H_2O wt/wt) for infrared spectroscopy were prepared as described previously [12] using double distilled water. To ensure complete hydration the lipid-water mixtures were heated above the corresponding T_m, vortexed while hot and cooled to approx. 5°C; this cycle was repeated three times. All experiments were started within 1 h of sample preparation. The temperature was increased in steps of 2 K with a waiting period of 15 min between consecutive spectra which corresponds to a constant heating rate of 0.125 $K \cdot min^{-1}$.

Infrared spectra were recorded on a Digilab FTS-11 Fourier transform infrared spectrometer equipped with a mercury cadmium telluride detector. Automated temperature experiments were carried out as described elsewhere [16,17]. For each temperature, 300–400 scans were averaged, with a maximum optical retardation of 0.5 cm; the interferograms were triangularly apodized, zero-filled once, and Fourier transformed to yield a resolution of 2 cm^{-1} and an encoding interval of 1 cm^{-1}. Frequencies were determined by measuring the centre of gravity [18] of the topmost 2 cm^{-1} wide segment of the CH_2 symmetric stretching bands and the topmost 6 cm^{-1} wide segment of the C = O stretching bands. Full widths at nine tenths peak height for the CH_2 scissoring bands were measured relative to linearly interpolated baselines extending from 1400 to 1520 cm^{-1} [18].

Results

Microcalorimetry

The physical method most commonly employed in the study of lipid phase transitions is differential scanning calorimetry [5], a technique which yields transition temperatures as well as thermodynamic data. We have measured, under strictly comparative conditions, the excess specific heat as a function of temperature for aqueous suspensions of DPPE, DPMePE, $DPMe_2PE$ and DPPC. The purpose of the calorimetric measurements described herein was to establish whether the par-

tially *N*-methylated PE show polymorphic phase behavior similar to that observed recently for DPPE and for other PE with saturated acyl chains [7–10] which involves concomitant hydration and acyl chain melting.

The excess heat capacity for aqueous dispersions of $DPMe_2PE$ and DPMePE as a function of temperature is shown in Fig. 1. The solid traces show a first scan obtained immediately after sample preparation (see Experimental), while the dashed traces show a repeat scan obtained without removing the samples from the calorimeter. It is clear from Fig. 1 that for each of the two compounds the two scans gave identical transitions which are in good agreement with the transition temperatures reported by Vaughan and Keough [19]. The transition temperatures, transition widths and ΔH values are listed in Table I where we have also included the thermodynamic data for DPPE and DPPC. While we found no evidence of a pretransition in $DPMe_2PE$ or DPMePE, we note that the specific heat curves for the partially methylated PE are asymmetric with the low-temperature side being broader. This could be taken to indicate that there is a pretransition, very close to the main transition temperature; however, the specific heat curves for several saturated PE are also asymmetric [5,10].

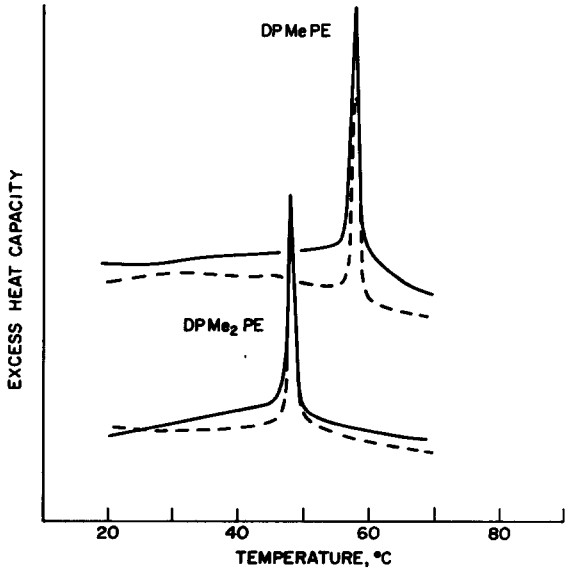

Fig. 1. Calorimetric transition curves of aqueous dispersions of DPMePE and $DPMe_2PE$. The solid traces represent scans obtained immediately after sample preparation while the dotted traces show repeat scans (see Experimental for details).

TABLE I

PROPERTIES OF THE MAIN PHASE TRANSITION OF AQUEOUS DISPERSIONS OF DPPE, DPMePE, $DPMe_2PE$, AND DPPC

Lipid	t_m (°C)	ΔH (kcal/mol)	$\Delta T_{1/2}$ (K)	ΔT_m (K)
DPPE	63.4	8.8 ± 0.2 [a]	1.2	5.4
DPMePE	58.0	8.5 ± 0.5	1.1	9.8
$DPMe_2PE$	48.2	8.5 ± 0.5	0.9	6.7
DPPC	41.5	8.5 ± 0.2 [a]	0.6	

[a] These values were obtained under identical conditions as those for DPMePE and $DPMe_2PE$ (Fig. 1), and agree with those of Wilkinson and Nagle [10].

In Table I we have also included the difference in transition temperature (ΔT_m) attained by each methyl substitution. The first methyl group substitution from DPPE to DPMePE reduces T_m by only 5.4 K while the second and third methyl group substitution reduces T_m by 9.8 and 6.7 K, respectively.

Infrared spectra

General characteristics. While calorimetry is the method of choice for measuring thermodynamic properties of lipid phase transitions, spectroscopic methods have provided a detailed picture of lipid structure and dynamics at the molecular level [20]. Infrared spectroscopy in particular is now well established as a powerful technique for these studies. For a detailed analyses of the infrared spectra of membrane lipids the reader is referred to Refs. 7, 21–23 (and references therein).

Salient spectral features which are common to the infrared spectra of aqueous dispersions of DPPE, DPMePE, $DPMe_2PE$ and DPPC are the C-H stretching bands in the 2800–3000 cm^{-1} region, the ester carbonyl stretching bands at 1730 cm^{-1}, the CH_2 and CH_3 deformations (1350–1500 cm^{-1}), and the phosphate stretching bands at 1090 and 1220 cm^{-1}. The four spectra differ with respect to bands due to vibrations of the substituted amino group. The spectra of DPPE, DPMePE and $DPMe_2PE$ show a number of broad bands between 2500 and 2700 cm^{-1}, associated with N-H stretching vibrations [21,22], which are

absent in the spectrum of DPPC. N-H bending vibrations are expected in the 1600 cm^{-1} region; thus the spectrum of DPPE clearly shows the symmetric $\overset{+}{N}H_3$ bending band at 1560 cm^{-1}, a band which is obscured in the spectra of the partially methylated PE because of the strong bending mode of water at 1640 cm^{-1}. The spectral region between 930 and 1030 cm^{-1} contains bands associated with C-N stretching vibrations which are dependent on the number of methyl groups on the amino group; the symmetric and antisymmetric CNC stretching modes of the $\overset{+}{N}(CH_3)_3$ group give bands at 930 and 970 cm^{-1}, which shift to 946 and 994 cm^{-1} in the case of the $\overset{+}{N}H(CH_3)_2$ group and to 958 and 1032 cm^{-1} in the case of the $\overset{+}{N}H_2CH_3$ group; evidently, these bands are not observed in the spectrum of DPPE.

Temperature dependence of the infrared spectra. Here we present a detailed description of the temperature-induced changes in the infrared spectra of the four lipids. The temperature-dependence of aqueous DPPC has been described in detail previously [23] and is included here for the purpose of comparison. PE with saturated acyl chains, on the other hand, present a complex polymorphic phase behavior in the gel phase [7–10,24] and the phase transition involving the acyl chain melting of DPPE and that of other saturated PE is currently under investigation in our laboratory; the data on aqueous DPPE are included here to allow comparison with the three methylated PE.

Fig. 2 shows the temperature dependence of the C-H stretching region in the spectrum of $DPMe_2PE$ which is representative for that of all four lipids. Common to the spectra of other compounds with extended methylene chains are the strong bands at 2920 and 2850 cm^{-1} which are the antisymmetric and symmetric CH_2 stretching modes; the weaker bands ar 2960 and 2870 cm^{-1} originate from the CH_3 groups in the acyl chains. The spectra in Fig. 2 encompass the temperature range of the gel to liquid-crystalline phase transition for this lipid (see Table I). There are marked differences between the spectra recorded at temperatures below and above T_m. All bands are broader above T_m and have lost considerable height; at T_m the peak maximum shifts to higher frequencies. Similar behavior is shown by the other compounds.

The detailed temperature dependence of the

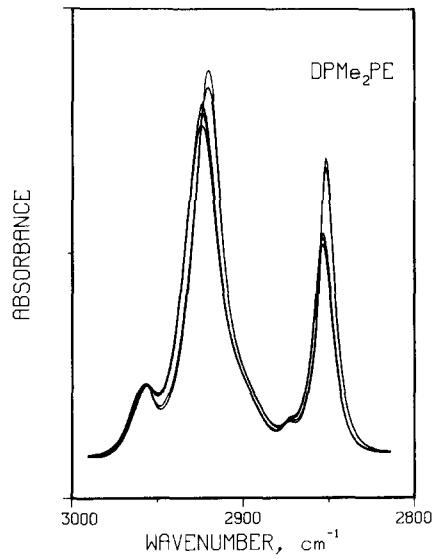

Fig. 2. Temperature dependence of the C-H stretching bands in the infrared spectrum of $DPMe_2PE$. Spectra were recorded at 40, 44, 48, 52 and 56°C; peak heights decrease with increasing temperature.

frequency of the CH_2 symmetric stretching mode in the spectra of these four compounds is shown in Fig. 3. At $T_m - 30$ K the frequency values for the CH_2 symmetric stretching bands are: 2851, 2850, 2849.5, 2848.5 cm^{-1} for DPPC, $DPMe_2PE$, DPMePE and DPPE, respectively. While the frequency of this band increases slightly with increasing temperature, the gel to liquid-crystalline transition is marked by an abrupt upward shift of the frequency of the CH_2 stretching modes [23,25,26] which is a consequence of the introduction of conformational disorder in the acyl chains. For the four compounds described herein, these frequency shifts are not of the same magnitude; the values for DPPC, $DPMe_2PE$ and DPMePE are comparable (1.8, 2.1, and 2.2 cm^{-1} respectively), whereas DPPE shows a larger increase in frequency at T_m of 4.5 cm^{-1}. A similar trend was observed for the frequency of the CH_2 antisymmetric stretching bands at 2920 cm^{-1}, the shifts being 2.8, 2.9., 3.2 and 5.2 cm^{-1} for DPPC, $DPMe_2PE$, DPMePE and DPPE, respectively.

While changes in the frequency of the CH_2 stretching modes are associated with the melting transitions and the introduction of *gauche* conformers, the CH_2 scissoring mode is very sensitive

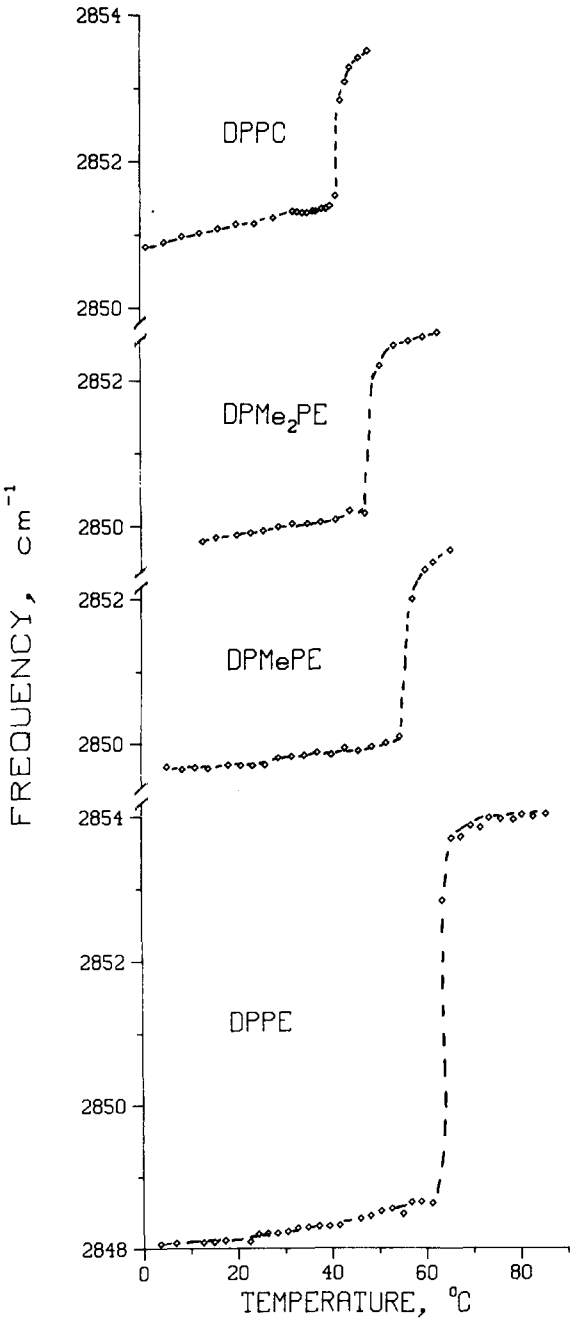

Fig. 3. Temperature dependence of the frequency of the CH_2 symmetric stretching bands in the spectra of DPPE, DPMePE, $DPMe_2PE$ and DPPC.

to changes in the chain packing arrangements and to intermolecular interactions. Fig. 4 shows the 1400–1500 cm^{-1} region of the infrared spectra of DPPE, DPMePE, $DPMe_2PE$ and DPPC; the gel

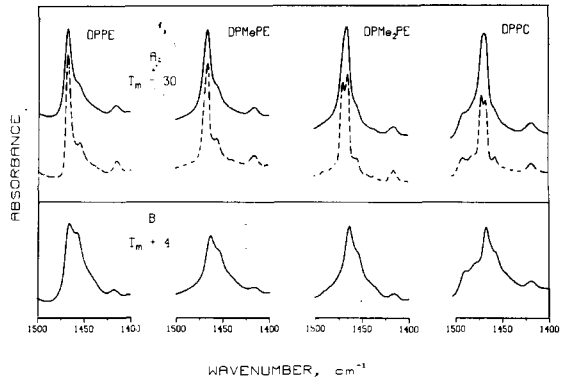

Fig. 4. The 1400-1500 cm^{-1} region of the infrared spectra of DPPE, DPMePE, $DPMe_2PE$ and DPPC. (A) Gel phase spectra recorded at $T_m - 30$ K; the dashed traces show the same spectra after deconvolution using 4 cm^{-1} with Lorentzian lines [27]. (B) Liquid-crystalline phase spectra recorded at $T_m + 4$ K.

phase spectra in Fig. 4A were all recorded at the same reduced temperature, i.e., 30 K below the corresponding chain melting transition temperature, while the liquid-crystalline phase spectra in Fig. 4B were recorded at $T_m + 4$ K. The dashed traces in Fig. 4A show the spectra following Fourier self deconvolution, a procedure which reduces the intrinsic linewidth of each component band [27].

From a comparison of Figs. 4A and 4B the effect of transition to the liquid-crystalline phase is evident; in all four spectra the CH_2 scissoring band at around 1468 cm^{-1} shows considerable loss of intensity while the band at 1458 cm^{-1} gains intensity in the liquid-crystalline phase. The latter band, which is associated with *gauche* conformers, follows a specific pattern; the band is clearly not very strong in the spectra of DPPC, but increases in intensity with decreasing methyl substitution. In fact, in the spectrum of DPPE at $T_m + 4$ K (Fig. 4B) it is as intense as the CH_2 scissoring band at 1468 cm^{-1}. The isolated band at 1420 cm^{-1}, a CH_2 bending mode of CH_2-CO_2 groups, broadens and shifts to higher frequency at T_m, a behavior previously observed for DPPC [23].

Another pattern is observed in the gel phase spectra in that the width of the CH_2 scissoring band at 1468 cm^{-1} increases as the number of methyl groups increases. The deconvoluted spectra in Fig. 4A reveal that the increased width is due to the existence of a two band system in, at least the

spectra of DPPC and $DPMe_2PE$. The appearance of two bands for the CH_2 scissoring mode in the spectra of gel phase lipids has been observed in a variety of systems [23,24]. The CH_2 scissoring mode is split in two components in the spectra of *n*-alkanes and polyethylene when the subcell is orthorhombic. This splitting arises from intermolecular vibrational coupling due to the crystal field [28] and, because of its intermolecular nature it is temperature dependent; therefore, the frequency separation of the two components decreases with increasing temperature. The deconvoluted spectra of DPMePE and DPPE at $T_m - 30$ K do not reveal the presence of two bands, however, in the spectrum of DPPE two band components appear at lower temperatures [24].

Evidence for the presence of a two band system is also obtained by measuring the width of the CH_2 scissoring band at 9/10 peak height, data shown in Fig. 5. With decreasing temperature in the gel phase the two component bands shift further apart and the values of the overal bandwidth increases; again, this is particularly evident in the plots for DPPC and $DPMe_2PE$. In all four cases the minimum value for the bandwidth, attained just prior to T_m, is 3 cm^{-1}, while the chain melting transition is marked by an abrupt increase in the width of this band due to the introduction of gauche conformers [23].

The C-N stretching bands of methyl substituted PE which appear in the spectral region below 1100 cm^{-1} (vide supra) are also sensitive to the chain melting transitions at T_m. We find that the frequencies of the antisymmetric and symmetric C-N stretching bands decrease by 1.5 cm^{-1} in the spectra of DPMePE and $DPMe_2PE$, which is comparable to the change observed in the spectrum of DPPC at T_m [29]; these bands are absent in the spectrum of DPPE.

Finally, a comparison of the thermal phase behavior of the members of this homologous series can also be obtained by examining the temperature dependence of headgroup vibrational modes; in particular, the ester carbonyl stretching bands originating from the interfacial bilayer region proved to be excellent monitors of thermal reorganizations in lipid bilayers [12]. The temperature dependence of the frequency of the C=O stretching modes of DPPC and $DPMe_2PE$ are

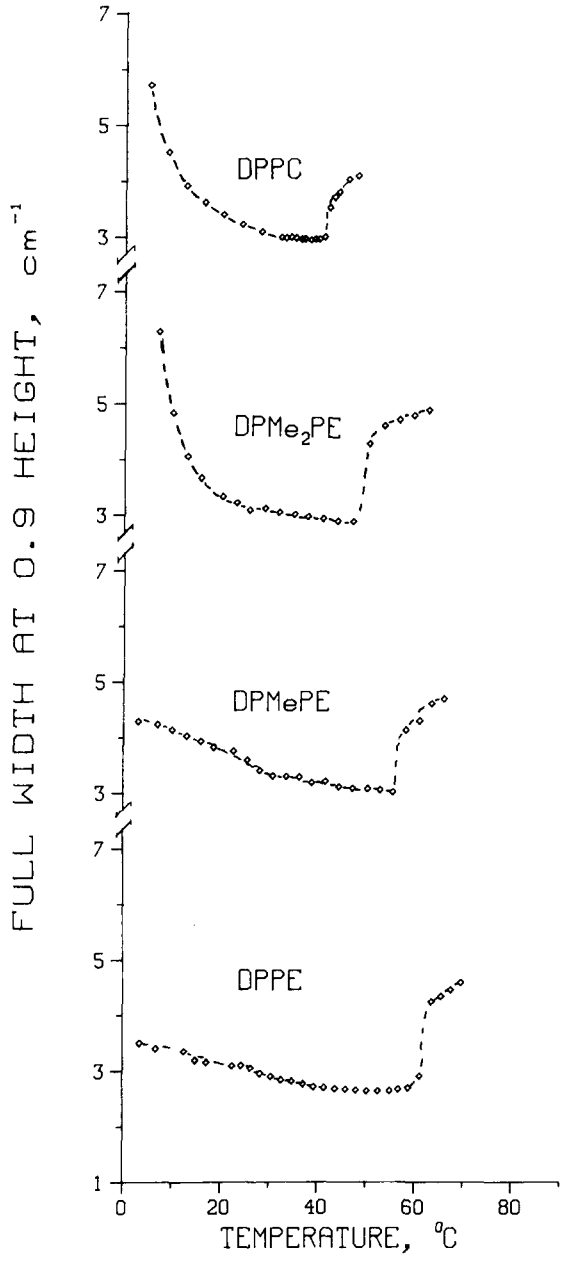

Fig. 5. Temperature dependence of the full width at 9/10 peak height of the CH_2 scissoring bands in the spectra of DPPE, DPMePE, $DPMe_2PE$ and DPPC. In all cases the values of the bandwidth were determined after subtraction of a baseline extending from 1400 to 1520 cm^{-1}.

shown in Fig. 6; these plots give an indication of the frequency of the overall C=O stretching band contour. The plot for DPPC shows shifts at two temperatures, 35 and 41.5°C which correspond to

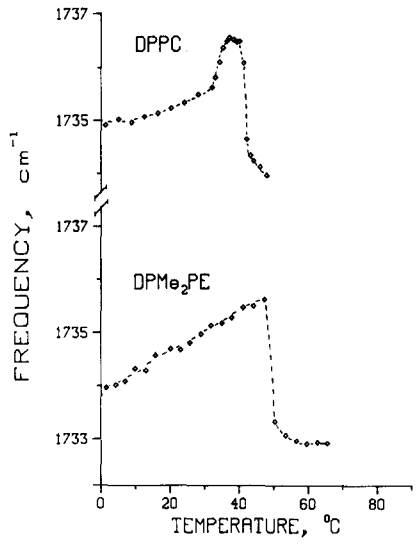

Fig. 6. Temperature dependence of the frequency of the C = O stretching band contours in the spectra of $DPMe_2PE$ and DPPC.

the pretransition and to T_m, respectively; at the pretransition the frequency increases by 0.8 cm^{-1}, while at T_m there is a decrease in frequency of about 2.5 cm^{-1}. The plot for $DPMe_2PE$, which is similar to that of DPMePE and DPPE, shows only a monotonic increase with increasing temperature in the gel phase, followed by an abrupt decrease at T_m.

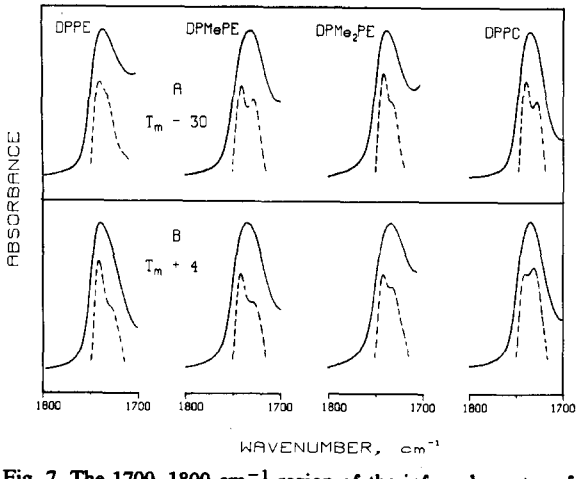

Fig. 7. The 1700–1800 cm^{-1} region of the infrared spectra of DPPE, DPMePE, $DPMe_2PE$ and DPPC. (A) Gel phase spectra recorded at $T_m - 30$ K. (B) Liquid-crystalline phase spectra recorded at $T_m + 4$ K; the dashed traces represent the results of deconvolution using 22 cm^{-1} wide Lorentzian lines [27].

TABLE II

PEAK HEIGHT RATIOS OF THE α (1742 cm^{-1}) AND β (1729 cm^{-1}) BANDS IN THE SPECTRA OF DPPE, DPMePE, $DPMe_2PE$ and DPPC

Values of α/β were determined from deconvoluted spectra (see Fig. 7) measuring the peak heights from a linearly interpolated baseline extending from 1710 to 1750 cm^{-1}.

Lipid	α/β ratio at	
	$T_m - 30$ K	$T_m + 4$ K
DPPE	1.16	1.81
DPMePE	1.18	1.34
$DPMe_2Pe$	1.20	1.17
DPPC	1.21	0.93

It is known, however, that the C = O stretching band contour is composed of at least two bands, which have been assigned to the *sn*-1 and *sn*-2 ester carbonyl bands [30]. In Fig. 7 we show the 1700–1800 cm^{-1} region in the spectra of DPPE, DPMePE, $DPMe_2PE$ and DPPC; the gel phase spectra in Fig. 7A were recorded at the same reduced temperature, i.e. $T_m - 30$ K, while the liquid crystalline spectra in Fig. 7B were recorded at $T_m + 4$ K. For each case we include a dashed trace showing the spectra after Fourier self deconvolution. This allows the clear observation of a two-band system for the C = O stretching mode in all cases. The two component bands are at 1742 ± 1 cm^{-1} (band α) and at 1729 ± 1 cm^{-1} (band β) and correspond to the *sn*-1 and *sn*-2 ester groups, respectively [30]. The experimental values of the peak height ratio α/β in the gel phase and in the liquid-crystalline phase of the four lipids are summarized in Table II. These values follow a trend in both phases; in the gel phase the α/β ratio increases slightly as the number of methyl groups increases, whereas it decreases considerably in the liquid crystalline phase. Although the peak height ratio of the *sn*-1 and *sn*-2 ester carbonyls cannot be directly correlated with a given conformation of the headgroup, it has been shown to be sensitive to the hydration state [12,31] as well as to the nature of the acyl chain packing in these lipids (unpublished results from our laboratory).

Discussion

The purpose of this study was to determine the nature of the phase transition in the partially

N-methylated DPPC and to establish whether they show polymorphic phase behavior comparable to that of DPPE or to that of the fully N-methylated DPPC.

The DSC and infrared spectroscopic data presented herein clearly demonstrate the existence of a phase transition at 48.2°C in $DMPe_2PE$ and at 58.0°C in DPMePE, in good agreement with the calorimetric data of Vaughan and Keough [19]. Since the thermodynamic properties and the infrared spectral changes observed at these transition temperatures are comparable with those observed for a large number of phospholipids at the acyl chain melting phase transition [5], we conclude that the nature of this transition is also a gel to liquid-crystalline phase transition.

The changes detected in the infrared spectra as a function of temperature indicate that the temperature-induced structural rearrangements of the four lipid molecules are comparable. The gel to liquid-crystalline phase transition at T_m is marked by the introduction of *gauche* conformers (chain melting), which is reflected in the infrared spectra as a shift to higher frequencies of the CH_2 stretching modes; the larger change observed in the case of DPPE (Fig. 3) could be taken to indicate that there are more *gauche* bonds per chain introduced in the liquid-crystalline phase of DPPE than is the case of the partially or fully *N*-methylated PE. In fact, recent results of Wilkinson and Nagle [10] indicate that the number of *gauche* bonds introduced when DMPE melts is twice the number found for DMPC (Δn_g is 4.2 for DMPE and 2.1 for DMPC). If we assume that the same trend holds for DPPC and DPPE, one could rationalize the larger frequency shift of DPPE as arising primarily from an increased *gauche* population, though our current understanding of the relationship between chain conformation and CH_2-stretching frequencies does not allow for an unambiguous assignment of the number of *gauche* bonds. However, the fact that the frequency shifts for $DPMe_2PE$ and DPMePE compare closer to those of DPPC indicates that the properties of the partially *N*-methylated PE are closer to those of DPPC than to those of DPPE.

The introduction of conformational disorder at T_m is also manifest in the temperature dependence of the CH_2 scissoring mode; at T_m there is an abrupt increase in the bandwidths and a decrease in the frequencies. The frequency shifts of the ester carbonyl stretching bands at T_m, while different for each lipid, are of the same magnitude and nature. This observation is in accord with recent conclusions derived from NMR experiments [32–34] comparing the temperature dependence of the geometry of the glycerol backbone in saturated PE and PC. The behavior of the peak height ratios of the two ester carbonyl bands in Table II, however, reveals subtle differences and suggests that there is a trend in the conformational change of the interface region of these four lipids at the corresponding gel to liquid-crystalline phase transition.

In our DSC measurements we found no evidence for a phase transition similar to that observed in DPPE and in other PE with saturated acyl chains, involving the concomitant hydration and chain melting [7–10]. On the other hand, samples of DPMePE and $DPMe_2PE$ when annealed at temperatures around 0°C for up to 4 days revealed no effects of a gel phase metastability similar to that observed upon incubation of gels of DPPC and other PC with saturated acyl chains [11–15]. Thus, it appears that the partially *N*-methylated PE DPMePE and $DPME_2PE$ present a phase behavior which is intermediate between that of DPPE and that of DPPC. The subtle differences of their phase behavior are due to the different degree of methylation of the ethanolamine group and consequently due to differences in their hydrogen-bonding capabilities.

In the recent calorimetric and dilatometric comparison of the phase behavior of saturated PC and PE Wilkinson and Nagle [10] found that the higher transition temperatures of PE compared to those of PC with identical acyl chains can be explained on the basis of the smaller volume of the PE headgroup; these authors have calculated that the headgroup volume is 246 $Å^3$ for PE and 344 $Å^3$ for PC [10,35]. Thus, the hydrogen bonding between amino groups and water molecules and/or other amino or phosphate groups in the PE impose a greater constraint than those involving the choline groups in the PC which are void of hydrogens.

The effect of gradual replacement of hydrogen atoms by methyl groups is more marked in the

transition temperatures when the first methyl group is replaced (see Table I), which must be a consequence of the very different hydrogen bonding capabilities of the moieties $-\overset{+}{N}H_3$, $-\overset{+}{N}H_2(CH_3)$, $-\overset{+}{N}H(CH_3)_2$ and $-\overset{+}{N}(CH_3)_3$. The fact that the ΔT_m values do not follow a pattern indicates that the headgroup volume alone does not determine the transition temperatures directly. The transition enthalpies for PE and PC with the same acyl chains are practically the same, 8.5 kcal/mol for DPPC and 8.8 kcal/mol for DPPE [5]. The ΔH values for $DPMe_2PE$ and DPMePE are thus comparable to those of DPPC and DPPE and therefore in keeping with the conclusions drawn when comparing the phase behavior of PE and PC [10,35].

With the transition temperatures of DPMePE and $DPME_2PE$ falling between those of DPPE and DPPC the effect of the headgroup could be rationalized as follows. As the number of methyl groups gradually increases, the cross-sectional area of the molecules increases, thus increasing the interchain distances. The balance between the headgroup forces and those of the acyl chains is affected due to increased hydrogen bonding in the polar region of the bilayer.

The absence of a noticeable pretransition, even in the case of the dimethyl derivative $DPMe_2PE$, indicates differences in the acyl chain packing pattern of the gel phase. The pretransition involves mainly a change in the packing arrangement [23] with possibly, the introduction of a very small number of *gauche* conformers [36]. The acyl chains in the gel phase of DPPE are not tilted [37] while a tilt angle of about 35° has been measured in the gel phase of DPPC, which diminishes at the pretransition [38,39]. Thus, the data obtained from the behavior of the CH_2 scissoring mode for $DPMe_2PE$ and DPMePE compared to that in DPPC suggest that the $P_{\beta'}$ phase [38] is not attained in these two partially *N*-methylated PE. Furthermore, the relative intensities of the two components of the CH_2 scissoring bands are determined by the shape of the subcell [24,28,40]. The trend observed for the CH_2 scissoring band contour in Fig. 4A suggests that the shapes of the subcells for DPPE, DPMePE, $DPMe_2PE$ and DPPC are different. The most ready explanation for this trend in the subcell shape is differences in the angles of tilt of the acyl chains with respect to the bilayer normal. As we already pointed out these angles are very different for PE and PC [37]. However, the exact tilt angles or nature of the acyl chain packing cannot be established from the infrared spectra alone; X-ray diffraction measurements are needed to provide this information. Yet, the trend observed for the temperature behavior of the frequencies and bandwidths of the CH_2 scissoring modes strongly suggests that there is an angle of tilt in $DPMe_2PE$ and DPMePE, and that this angle decreases gradually in the homologous series DPPC, $DPMe_2PE$, DPMePE and DPPE.

Finally, we also advance the suggestion that the differences in headgroup volume and hydrogen-bonding characteristics, are 'transmitted' to the interface region and thus influence the ratio of the peak height of the two ester carbonyl groups. Thus, the results from Table II could be correlated with the trend observed for the CH_2 scissoring bands and therefore also be related to the angle of tilt of the acyl chains with respect to the bilayer normal.

References

1 Quinn, P.J. (1980) Prog. Biophys. Mol. Biol. 38, 1–104
2 Lands, W.E.M. and Crawford, C.G. (1976) in The Enzymes of Biological Membranes (Martonosi, A., ed.), Vol. 2, pp. 3–85, Plenum Press, New York
3 Axelrod, J. and Hirata, F. (1981) Ann. N.Y. Acad. Sci. 373, 51–53
4 Thompson, G.A., Jr. (1980) The Regulation of Membrane Lipid Metabolism, p. 98, CRC Press, Boca Raton
5 Mabrey, S. and Sturtevant, J.M. (1978) Methods Membrane Biol. 9, 237–274
6 Chapman, D., Urbina, J. and Keough, K.M. (1974) J. Biol. Chem. 249, 2512–2521
7 Mantsch, H.H., Hsi, S.C., Butler, K.W. and Cameron, D.G. (1983) Biochim. Biophys. Acta 728, 325–330
8 Chang, H. and Epand, R.M. (1983) Biochim. Biophys. Acta 728, 319–324
9 Seddon, J.M., Harlos, K. and Marsh, D. (1983) J. Biol. Chem. 258, 3850–3854
10 Wilkinson, D.A. and Nagle, J.F. (1981) Biochemistry 20, 187–192
11 Chen, S.C., Sturtevant, J.M. and Gaffney, B.J. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5060–5063
12 Cameron, D.G. and Mantsch, H.H. (1982) Biophys. J. 38, 175–184
13 Ruocco, M.J. and Shipley, G.G. (1982) Biochim. Biophys. Acta 684, 59–66
14 Ruocco, M.J. and Shipley, G.G. (1982) Biochim. Biophys. Acta 691, 309–320
15 Fuldner, H.H. (1981) Biochemistry 20, 5707–5710

16 Cameron, D.G. and Charette, G.M. (1981) Appl. Spectrosc. 35, 224–225
17 Cameron, D.G. and Jones, R.N. (1981) Appl. Spectrosc. 35, 448
18 Cameron, D.G., Kauppinen, J.K., Moffatt, D.J. and Mantsch, H.H. (1982) Appl. Spectrosc. 36, 245–250
19 Vaughan, D.J. and Keough, K.M. (1974) FEBS Lett. 47, 158–161
20 An excellent recent compilation is: Membrane Spectroscopy (Grell, E., ed.) (1981) Springer, Berlin
21 Fringeli, U.P. (1977) Z. Naturforsch. 32C, 20–45
22 Fringeli, U.P. (1981) Biophys. J. 34, 173–187
23 Cameron, D.G., Casal, H.L. and Mantsch, H.H. (1980) Biochemistry 19, 3665–3672
24 Cameron, D.G., Gudgin, E.F. and Mantsch, H.H. (1981) Biochemistry 20, 4496–4500
25 Mantsch, H.H., Martin, A. and Cameron, D.G. (1981) Biochemistry 20, 3138–3145
26 Dluhy, R.A., Mendelsohn, R., Casal, H.L. and Mantsch, H.H. (1983) Biochemistry 22, 1170–1177
27 Kauppinen, J.K., Moffatt, D.J., Mantsch, H.H. and Cameron, D.G. (1981) Appl. Spectrosc. 35, 271–276
28 Snyder, R.G. (1979) J. Chem. Phys. 71, 3229–3235
29 Casal, H.L. (1981) PhD Thesis, University of Ottawa
30 Bush, S.F., Levin, H. and Levin, I.W. (1980) Chem. Phys. Lipids 27, 101–111
31 Mantsch, H.H., Cameron, D.G., Tremblay, P.A. and Kates, M. (1982) Biochim. Biophys. Acta 689, 63–72
32 Blume, A., Rice, D.M., Wittebort, R.G. and Griffin, R.G. (1982) Biochemistry 21, 6220–6230
33 Wittebort, R.G., Schmidt, C.F. and Griffin, R.G. (1981) Biochemistry 20, 4223–4228
34 Wittebort, R.G., Blume, A., Huang, T.H., Das Gupta, S.K. and Griffin, R.G. (1982) Biochemistry 21, 3487–3502
35 Nagle, J.F. and Wilkinson, D.A. (1978) Biophys. J. 23, 159–175
36 Levin, I.W. and Bush, S.F. (1981) Biochim. Biophys. Acta 640, 760–766
37 McIntosh, T.J. (1980) Biophys. J. 29, 237–246
38 Janiak, M.J., Small, D.M. and Shipley, G.G. (1979) J. Biol. Chem. 254, 6068–6078
39 Tardieu, A., Luzzatti, V. and Reman, F.C. (1973) J. Mol. Biol. 75, 711–733
40 Snyder, R.G. (1961) J. Mol. Spectrosc. 7, 116–144