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Study of the Structure of *N*-Acyldipalmitoylphosphatidylethanolamines in Aqueous Dispersion by Infrared and Raman Spectroscopies†

Denis Lafrance,‡ Didier Marion,§ and Michel Pézolet*,‡

Centre de Recherche en Sciences et Ingénierie des Macromolécules, Département de Chimie, Université Laval, Cité Universitaire, Québec, Canada G1K 7P4, and Laboratoire de Biochimie et Technologie des Protéines, Institut National de la Recherche Agronomique, rue de la Géraudière, B.P. 527, 44026 Nantes Cédex 03, France

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ABSTRACT: The effect of the headgroup chain length on the structure and on the thermotropic behavior of *N*-acyldipalmitoylphosphatidylethanolamines (*N*-acyl-DPPEs) has been studied by infrared and Raman spectroscopies. The results show that the *N*-acyl-DPPEs can be divided in two classes depending on the *N*-acyl chain length. When the *N*-acyl chain contains 10 carbon atoms or more, it penetrates into the bilayer while it remains at the level of the glycerol backbone for shorter *N*-acyl chains. For both classes of *N*-acyl-DPPEs, the rotation of the lipid chains in the liquid-crystalline phase is hindered by the presence of the *N*-acyl group. In addition, the disruption of the hydrogen bonds between the amino and phosphate groups by N-acylation of the amino group results in an increase of the hydration of the phosphate group compared to that in DPPE. The hydration occurred at both the phosphate and amide group levels; the phosphate group is more hydrated for phospholipids with long *N*-acyl chains while in the case of short-chain derivatives both the phosphate and amide groups are hydrated. This higher degree of hydration coupled with the immobilization of the lipid molecule may contribute to the bilayer stabilizer role of *N*-acyl-PEs since hydration is an important factor in bilayer stability.

The *N*-acylphosphatidylethanolamines are a minor lipid component of seeds (Bomstein, 1965; Dawson et al., 1969; Hargin & Morrison, 1980), microorganisms (Hazlewood & Dawson, 1975; Clarke et al., 1976), and some vertebrate tissues (Matsumoto & Miwa, 1973; Gray, 1976; Somerharju & Renkonen, 1979; Epps et al., 1980; Natarajan et al., 1985, 1986). It has been shown that *N*-acyl-PEs[1] are authentic components of membranes (Matsumoto & Miwa, 1973) and not a product made by transacylation during extraction of lipids as suggested by Wren and Merryfield (1965). *N*-Acyl-PEs are mainly produced as a result of a degenerative change or cell injury. In wheat grain, for example, they are synthesized during dehydration of the endosperm in which *N*-acylethanolamine phospholipids account for more than 50% of the total phospholipids of dehydrated tissue (Hargin & Morrison, 1980). The precise role of these lipids is still unknown although it has recently been reported that they might be the precursor of *N*-acylethanolamine, a derivative produced by phospholipase D activity, which has potential protective effects against cell injury (Epps et al., 1982; Parinandi & Schmid, 1988). However, *N*-acetylethanolamine phospholids are normally present in some tissues (Natarajan et al., 1985) and during some period of the life cycle of microorganisms

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‡ Université Laval.

§ Institut National de la Recherche Agronomique.

[1] Abbreviations: DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol; *N*-acyl-PE, 1,2-diacyl-*sn*-glycero-3-phospho-*N*-acylethanolamine; *N*-oleoyl-DOPE, 1,2-dioleoyl-*sn*-glycero-3-phospho-*N*-oleoylethanolamine; *N*-C4-DPPE or *N*-butyryl-DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*N*-butyrylethanolamine; *N*-C6-DPPE or *N*-caproyl-DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*N*-hexanoylethanolamine; *N*-C8-DPPE or *N*-capryl-DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*N*-octanoylethanolamine; *N*-C10-DPPE or *N*-capryl-DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*N*-decanoylethanolamine; *N*-C12-DPPE or *N*-lauryl-DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*N*-dodecanoylethanolamine; *N*-C16-DPPE or *N*-palmitoyl-DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*N*-hexadecanoylethanolamine; ^{31}P NMR, phosphorus-31 nuclear magnetic resonance.

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(Ellingson, 1980) or plants (Wilson & Rinne, 1974), suggesting that these phospholipids should have a proper role in membrane function.

The elucidation of the function of these molecules in cell physiology requires the determination of their structure and physical properties in membranes. It is evident that N-acylation of aminophospholipids modifies the membrane properties since this reaction results not only in the addition of a hydrophobic moiety but also in the formation of an acidic headgroup due to the loss of the free amino group. However, there have been relatively few studies of the physical properties and the thermotropic behavior of these lipids in aqueous dispersion. In a recent work, Newman et al. (1986) have found that, depending on the nature of the acyl chain involved, the fluidity within membrane domains could be potentially affected by N-acylation of PE. In addition, they have found that N-acylation may have an overall bilayer stabilizing effect since unsaturated phosphatidylethanolamines, which are nonbilayer lipids, are converted to acidic bilayer lipids on N-acylation with a saturated fatty acid.

More recently, Akoka et al. (1988) have used ^{31}P nuclear magnetic resonance spectroscopy to investigate the structure of saturated and unsaturated *N*-acyl-PEs in aqueous dispersions. They have proposed that when the *N*-acyl chain is long enough, it penetrates deeply into the hydrophobic part of the membranes and is parallel to the bilayer normal. This restricts the headgroup motion, giving rise to a powder spectrum governed by a nonaxially symmetric tensor just a few degrees below the temperature of the gel to liquid-crystalline phase transition, and increases the temperature of the gel to liquid-crystalline transition of unsaturated PEs. For unsaturated PEs, this penetration increases the temperature of the lamellar to hexagonal II transition, even for *N*-oleoyl-DOPE. Furthermore, they have suggested that intermolecular hydrogen bonds between adjacent amide groups might participate in restriction of headgroup motion. For short *N*-acyl chains, penetration does not occur so that anchoring of the headgroup is not possible and intermolecular hydrogen bonds might be prevented. These authors have proposed that the *N*-acyl chain of the lipids penetrates into the bilayer when the number of carbon atoms is greater than six.

In this study, we have investigated the effect of the headgroup chain length on the structure and thermotropic behavior of *N*-acyl-DPPEs using infrared and Raman spectroscopies. Vibrational spectroscopy proved to be very effective in investigating the molecular organization of phospholipids since it provides useful structural information on both the fatty backbone and the polar headgroup of the phospholipids and does not require the addition of any probe that could possibly perturb the system under investigation. Finally, these techniques are sensitive to hydrogen bonding at the phosphate and amide levels, which has been suggested to play an important role in the physical properties of *N*-acyl-PEs and, therefore, on the biological function of these molecules.

Materials and Methods

Materials. *N*-Acyl-DPPEs used were synthesized and purified as described by Akoka et al. (1988). Dipalmitoylphosphatidylethanolamine was purchased from Avanti Polar-Lipids Inc. and was used without further purification.

Aqueous dispersions of DPPE and its *N*-acyl derivatives were prepared by vortexing 10 or 5% (wt) lipid dispersions, depending whether the samples were prepared for Raman or infrared measurements, respectively, in H_2O, D_2O, or 100 mM phosphate buffer (pH 7.0). Then, they were submitted to four or five cycles of heating at 10 °C above the phase transition temperature, vortex shaking, and cooling to 0 °C. The pH of the dispersions was measured with a microelectrode (Microelectrodes Inc.) and adjusted to pH 7.0, if necessary. For Raman experiments, samples were transferred into glass capillary tubes in which they were centrifuged. The white pellet was used to obtain the spectra. For infrared measurements, 20 μL of the pellet was transferred, when hot, between two CaF_2 windows separated with a 6-μm Mylar spacer.

Raman Measurements. Samples were illuminated with the 514.5-nm line of an argon ion laser (Spectra Physics Inc., Model 2020) at a power of about 200 mW at the sample. Spectra were recorded with a computerized Spex Model 1400 double monochromator (Savoie et al., 1979) with a spectral resolution of 5 cm^{-1}. The monochromator was calibrated with a neon discharge lamp, and the frequencies cited later are believed to be accurate to ±2 cm^{-1} for sharp peaks. Capillaries containing the samples were placed in a thermoelectrically regulated sample holder (Pézolet et al., 1983) whose temperature was monitored at ±0.2 °C with a copper–constantan thermocouple. Spectra were recorded digitally with an integrating period of 2 s and a frequency increment of 2 cm^{-1}.

Infrared Measurements. Spectra were recorded with a Bomem DA3-02 Fourier transform infrared spectrophotometer with a mercury–cadmium–telluride detector and a germanium-coated KBr beam splitter. Routinely, 1000 scans taken with a maximal optical retardation of 0.5 cm were coadded, triangularly apodized, and Fourier transformed to yield a resolution of 2 cm^{-1}. The infrared cell containing the CaF_2 windows was placed in a thermoelectrically regulated sample holder as described for Raman measurements.

Spectra in the PO_2^- stretching region were corrected for the water background by subtraction of appropriate polynomial functions. In the carbonyl and amide I regions, spectra were corrected by subtraction of the solvent spectrum. Spectra in the ester carbonyl region were also Fourier deconvolved with a 20 cm^{-1} wide Lorentzian line and a *K* factor of 1.8 (Kauppinen et al., 1981).

Results

Raman Spectroscopy. For the study of lipid conformation and thermotropism, the Raman signals arising from the C–C (950–1200 cm^{-1}) and C–H (2750–3100 cm^{-1}) stretching vibrations are the most interesting. Figure 1 shows the acyl chain C–C stretching mode region of the Raman spectrum of a *N*-C16-DPPE multilamellar dispersion. Below the phase transition of the lipid (continuous line), three bands clearly emerge at 1064, 1098, and 1130 cm^{-1}. The 1064- and 1130-cm^{-1} bands have been assigned to C–C vibrations of chain segments of the trans conformation (Schachtschneider & Snyder, 1963; Snyder, 1967). The 1098-cm^{-1} band arises from a combination of the C–C stretching mode of gauche conformers, the C–C stretching mode of trans conformers, which is only visible at low temperature (Schachtschneider & Snyder, 1963), and the PO_2^- symmetric stretching mode of the headgroup (Levin & Bush, 1981). When the sample is heated above the phase transition temperature (dotted line), there is a marked increase of the intensity of the 1098-cm^{-1} band, which shifts to 1080 cm^{-1}, while the 1064- and 1130-cm^{-1} bands decrease in intensity. Both changes are due to the decrease of the number of trans bonds at higher temperature and to the increase of conformational disorder.

Since the C–C stretching mode region provides direct information about the intramolecular order of the acyl chains of phospholipids, the intensity ratio of the band near 1090 cm^{-1} to the one near 1130 cm^{-1} can be used as a sensitive probe of the intramolecular order of the acyl chains in the bilayer, since

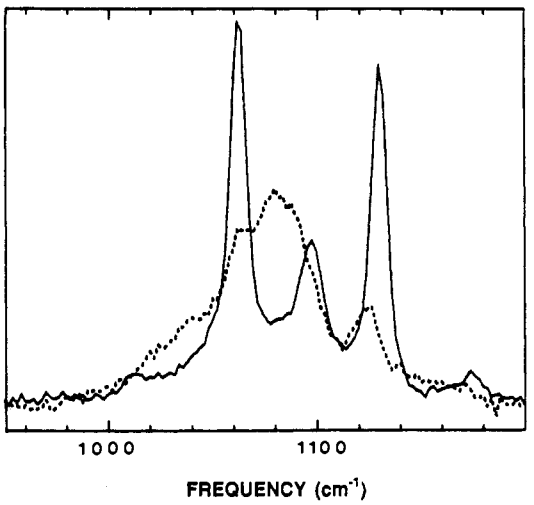

FIGURE 1: Raman spectra in the C–C stretching mode region of a dispersion of *N*-C16-DPPE at 20 °C (solid line) and 70 °C (dotted line).

Table I: Transition Temperatures Estimated by Raman and Infrared Spectroscopies for DPPE and *N*-Acyl-DPPEs

lipid	T_m (°C) this study *a*	T_m (°C) this study *b*	T_m (°C) Akoka et al. (1988)
DPPE	62	62	
N-butyryl-DPPE (*N*-C4)	31	31	
N-caproyl-DPPE (*N*-C6)	32	32	34
N-caprylyl-DPPE (*N*-C8)	18	18	
N-capryl-DPPE (*N*-C10)	26	28	
N-lauryl-DPPE (*N*-C12)		46	48
N-palmitoyl-DPPE (*N*-C16)	58	64	65

[a] In water. [b] In 100 mM phosphate buffer solution (pH 7.0).

it increases with the growing population of gauche conformers (Lippert & Peticolas, 1971; Spiker & Levin, 1975; Yellin & Levin, 1977a).

Figure 2a illustrates the effect of temperature on the h_{1090}/h_{1130} intensity ratio for both DPPE and *N*-acyl-DPPEs. As can be seen in this figure, except for *N*-C16-DPPE, the gel to liquid-crystalline transition of most *N*-acyl-DPPEs, especially for *N*-C10-DPPE, is less cooperative than that of DPPE, and its midpoint temperature is lowered (Table I). In the gel phase, the intramolecular order of the acyl chains is rather similar for DPPE and *N*-C4- and *N*-C16-DPPE. On the other hand, the effect of the headgroup *N*-acyl chain on the conformational disorder of the acyl chains of the lipid seems to be more important for the *N*-C8- and *N*-C10-DPPEs. In the liquid-crystalline phase, it seems that the presence of the *N*-acyl chain increases the intramolecular order of the lipid acyl chains. However, it is important to emphasize that the *N*-acyl chain also contributes to the spectrum, so that interpretation of the acyl chain results is more delicate.

The C–H stretching mode region of a *N*-C16-DPPE multilamellar dispersion is shown in Figure 3. Below the phase transition of the lipid (continuous line), two bands clearly emerge from the broad background at 2845 and 2880 cm^{-1}, corresponding to the symmetric and antisymmetric C–H stretching mode vibrations of the acyl chain methylenes, respectively (Gaber & Peticolas, 1977; Spiker & Levin, 1975). The two weaker bands at 2930 and 2954 cm^{-1} are due to the symmetric and asymmetric C–H stretching modes of the terminal methyl groups (Spiker & Levin, 1975; Hill & Levin, 1979).

When the dispersion of phospholipids undergoes the gel to liquid-crystalline phase transition, the intensity of the 2930-

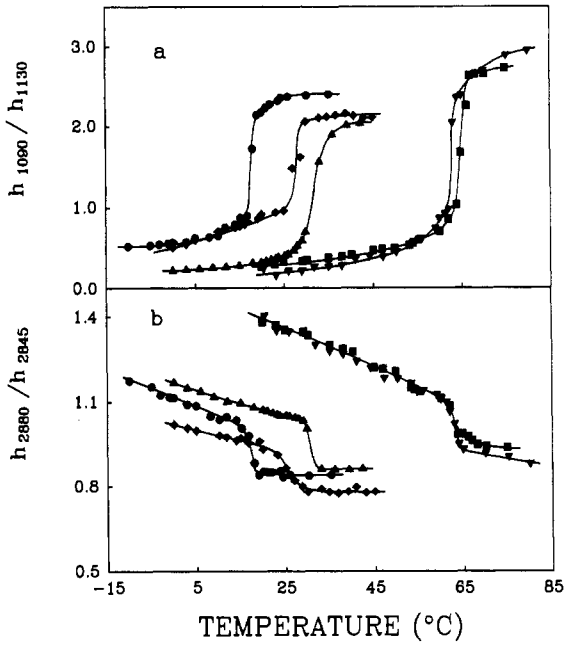

FIGURE 2: Temperature profiles of DPPE (▼), *N*-C4-DPPE (▲), *N*-C8-DPPE (●), *N*-C10-DPPE (◆), and *N*-C16-DPPE (■) derived from (a) the h_{1090}/h_{1130} and (b) the h_{2880}/h_{2845} peak height intensity ratios in the Raman spectra.

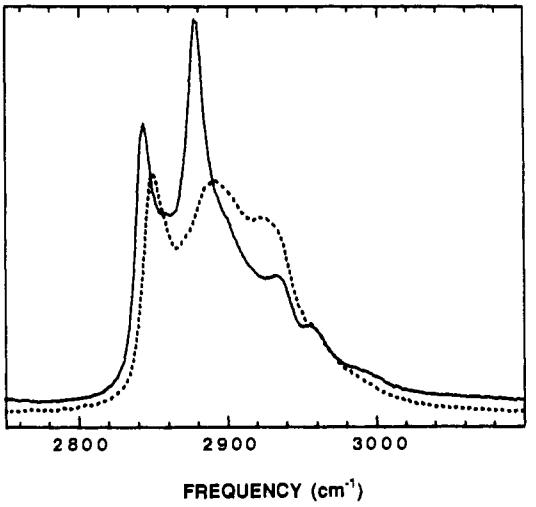

FIGURE 3: Raman spectra in the C–H stretching mode region of a dispersion of *N*-C16-DPPE at 20 °C (solid line) and 70 °C (dotted line).

cm^{-1} band increases (dotted line). This change results in part from an underlying infrared-active methylene antisymmetric stretching mode that becomes Raman active when the chain symmetry is lowered (Bunow & Levin, 1977; Huang et al., 1982) and also from a change in the Fermi resonance interaction between the symmetric C–H stretching mode of the terminal methyl groups and the first overtone of the asymmetric CH_2 bending mode (Hill & Levin, 1979; Snyder et al., 1980; Huang et al., 1982). Molecular disordering also affects the 2880-cm^{-1} feature considerably. When the temperature is increased, this band shifts to higher frequency, broadens, and decreases in intensity. This later change is partially due to the lowering of the intensity of the broad underlying background, which arises from inter- and intramolecular Fermi resonance interactions between the methylene symmetric C–H stretching mode and the different binary combinations of the methylene bending fundamentals (Snyder et al., 1978; Snyder & Scherer, 1979).

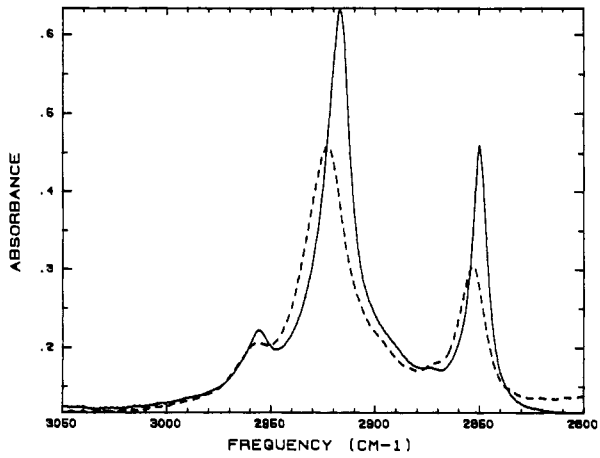

FIGURE 4: Infrared spectra in the C–H stretching mode region of a dispersion of *N*-C16-DPPE at 20 °C (solid line) and 70 °C (dotted line).

It has previously been shown that the h_{2880}/h_{2845} intensity ratio is particularly well suited for study of the thermotropic behavior of phospholipid bilayers (Snyder et al., 1980; Yellin & Levin, 1977b). This ratio monitors essentially the overall disorder of the lipid acyl chain matrix (Dasseux et al., 1984; Bunow & Levin, 1977) but is also quite sensitive to the intermolecular vibrational coupling and the lateral packing of the acyl chains (Gaber & Peticolas, 1977; Snyder et al., 1978).

Figure 2b shows the temperature profiles derived from the h_{2880}/h_{2845} ratio of DPPE and *N*-acyl-DPPEs. The midpoint temperature and cooperativity of the transitions detected from this spectral index are in very good agreement with those obtained from the C–C region. As can be seen for all *N*-acyl-DPPEs, except *N*-C16-DPPE, the molecular vibrational coupling between the acyl chains is lower than that of DPPE below the phase transition temperature. In the liquid-crystalline phase, DPPE and all of the *N*-acyl-DPPEs except *N*-C10, show a similar level of vibrational coupling.

Infrared Spectroscopy. The spectral features associated with C–H (2800–3100 cm^{-1}), C=O (1600–1780 cm^{-1}), and phosphate (950–1330 cm^{-1}) stretching vibrational modes in the infrared spectra of phospholipids are of major diagnostic value since they can provide valuable structural and conformational information about the thermotropic changes that occur in the acyl chain, interfacial, and headgroup regions of the lipid molecules, respectively (Casal & Mantsch, 1984). These spectral regions are analyzed below for DPPE and its *N*-acyl derivatives.

(A) C–H Stretching Mode Region. Figure 4 shows the acyl chain C–H stretching mode region of *N*-C16-DPPE below and above the lipid phase transition temperature. This spectral region is dominated by two strong bands at 2920 and 2850 cm^{-1}, assigned to the methylene antisymmetric and symmetric stretching modes, respectively (Asher & Levin, 1977; Cameron et al., 1980). Weaker bands due to the asymmetric and symmetric stretching modes of the terminal methyl group are also observed near 2950 and 2870 cm^{-1}, respectively (Cameron et al., 1980; Casal & Mantsch, 1984). The two methylene bands exhibit the same behavior as the temperature is increased: they become broader and they shift to higher frequency. Earlier studies have assigned the bandwidth increase to the augmentation of the rotational mobility of the acyl chains and the frequency shift to the introduction of gauche conformers in the acyl chains at high temperature (Cameron et al., 1980; Asher & Levin, 1977).

Figure 5 shows the temperature profiles derived from the frequency and bandwidth of the symmetric methylene

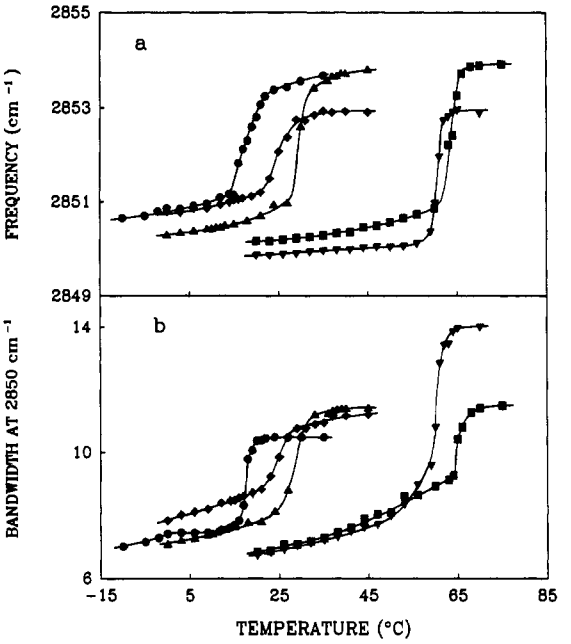

FIGURE 5: Temperature profiles of DPPE (▼), *N*-C4-DPPE (▲), *N*-C8-DPPE (●), *N*-C10-DPPE (◆), and *N*-C16-DPPE (■) derived from (a) the frequency and (b) the bandwidth at 65% of the height of the band due to the methylene symmetric mode.

stretching mode band near 2850 cm^{-1}. Similar results were obtained with the band due to the antisymmetric stretching mode, but with more dispersion because of overlapping vibrational modes. These infrared results are in very good agreement with the Raman results presented in the preceding section. The phase transition temperature of each lipid is the same for both techniques (Table I), and the transition is less cooperative for most of the *N*-acyl-DPPEs. Figure 5a shows that below the phase transition temperature the acyl chains of all *N*-acyl-DPPEs have more gauche conformers than DPPE, while in the liquid-crystalline phase they seem to have a lower conformational disorder for DPPE and *N*-C10-DPPE than for the other *N*-acyl-DPPEs. On the other hand, Figure 5b reveals that, in the liquid-crystalline phase, the bandwidth of the 2850-cm^{-1} feature of all *N*-acyl-DPPEs is lower than that of DPPE, indicating that the rotational mobility is lower for the *N*-acyl lipids.

(B) Carbonyl Stretching Mode Region. This spectral region for the *N*-acyl-DPPEs includes the ester carbonyl vibrational modes (from 1710 to 1780 cm^{-1}) as well as the amide I vibration of the headgroup *N*-acyl chain (from 1580 to 1680 cm^{-1}). In order to obtain more information from the ester carbonyl region, the spectra were first corrected for the water contribution and then deconvolved as described under Materials and Methods. Under these conditions, two main features are clearly seen around 1740 and 1725 cm^{-1} (Figure 6) and have been assigned to stretching vibrations of the *sn*-1 and *sn*-2 ester carbonyl groups, respectively (Bush et al., 1980; Mushayakarara & Levin, 1982; Wong et al., 1985). The *sn*-1 carbonyl group having the more hydrophobic environment is probably not hydrogen bonded and has the highest vibrational frequency while the *sn*-2 carbonyl, which is closer to the polar bilayer surface and is most likely hydrogen bonded with water, absorbs at lower frequency (Blume et al., 1988; Wong & Mantsch, 1988).

As seen in Figure 6a, at temperatures below the gel to liquid-crystalline phase transition, the intensity of the low-frequency component is much lower for DPPE than for *N*-acyl-DPPEs. We believe that this result indicates that the *sn*-2 carbonyl group is located in a more polar environment, and

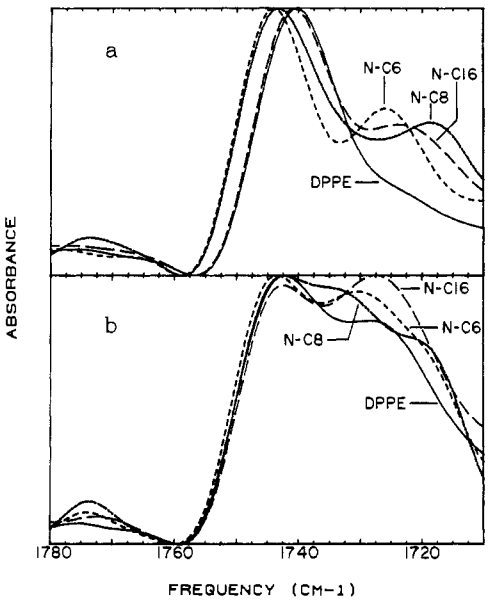

FIGURE 6: Fourier deconvolved infrared spectra of dispersions of DPPE (—), *N*-C6-DPPE (- - -), *N*-C8-DPPE (—··—), and *N*-C16-DPPE (– –) in the C=O stretching mode region in (a) the gel phase (30 °C below the transition temperature) and (b) the liquid-crystalline phase (5 °C above the transition temperature).

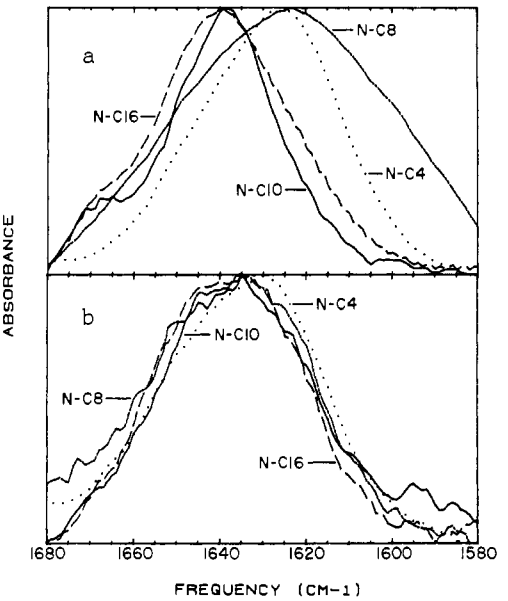

FIGURE 7: Infrared spectra of dispersions of *N*-C4-DPPE (···), *N*-C8-DPPE (—··—), *N*-C10-DPPE (—), and *N*-C16-DPPE (– –) in the amide I region in (a) the gel phase (30 °C below the transition temperature) and (b) the liquid-crystalline phase (5 °C above the transition temperature).

thus probably more exposed to the solvent, in *N*-acyl-DPPEs than in DPPE. On the other hand, the high-frequency component of the carbonyl band of *N*-C6- and *N*-C8-DPPEs is 5 cm^{-1} higher in frequency than those of DPPE and *N*-C16-DPPE. A similar shift has been observed for complexes of DPPG with polymyxin B (Babin et al., 1987) and melittin (M. Lafleur, I. Samson, and M. Pézolet, unpublished results) and has been assigned to a lower dielectric constant or polarity of the environment of the ester C=O group of the *sn*-1 chain (Babin et al., 1987). For the short-chain *N*-acyl-DPPEs, the frequency shift might also be due to a similar effect due to the presence of the *N*-acyl chains. In the liquid-crystalline phase (Figure 6b), the low-frequency component is stronger in intensity for all lipids investigated, and its frequency is also slightly higher than that in the gel phase. Finally, the high-frequency component has the same frequency for all lipids.

Another region of interest in the infrared spectrum of the *N*-acyl-DPPEs is the amide I region, which ranges from 1580 to 1680 cm^{-1}. The amide I band of the *N*-acyl-DPPEs, which is normally observed in protein spectra and arises from the C=O stretching vibration coupled with an out-of-phase C–N stretching mode and an in-plane N–H bending mode (Miyazawa et al., 1958), is reproduced in Figure 7. For *N*-acyl-DPPEs with short *N*-acyl chains below their phase transition temperature (Figure 7a), the frequency of the amide I vibration, which appears at 1625 cm^{-1}, indicates that for these lipids the amide group is exposed to water and that the hydrogen bonds are almost as strong as those in completely hydrated *N*-methylacetamide (Chen & Swenson, 1969). In addition, the broadness of the amide I band shows that there is a wide distribution of hydrogen-bond strength, especially for *N*-C8-DPPE for which the amide I band is 60 cm^{-1} wide.

For *N*-acyl-DPPEs with longer *N*-acyl chains, the amide I band is centered at 1640 cm^{-1}, thus showing that the amide group is not as exposed to the solvent as for *N*-acyl-DPPEs with shorter chains. In fact, the frequency of the amide I band is very close to that of self-associated *N*-methylacetamide in which both the NH and CO groups formed intermolecular hydrogen bonds (Lozé et al., 1978). In addition, the width of the amide I band indicates that the distribution of hydrogen-bond strength is much narrower in this case. Finally, a smaller shoulder appears near 1670 cm^{-1} for *N*-C10- and *N*-C16-DPPE (Figure 7a). According to Lozé et al. (1978), such a frequency for an amide I vibration could be due to amide bonds with only the NH or the CO group involved in intermolecular hydrogen bonding. Since the 1670-cm^{-1} feature is stronger in the spectrum of *N*-C10-DPPE, it seems that more amide bonds are partly self-associated in this lipid, compared to *N*-C16-DPPE.

Figure 7b shows that the notable changes of the shape and position of the amide I band occur when the lipid passes into the liquid-crystalline phase. For *N*-acyl-DPPEs with short *N*-acyl chains, the amide I band shifts to higher frequency as the hydrogen bonds of the solvated amide groups become weaker at elevated temperature. On the other hand, for DPPEs with long *N*-acyl chains, the profile of the amide I band suggests that this band is composed of two components. Band-fitting analysis of the amide I band of *N*-C16-DPPE (data not shown) has revealed that these components are located around 1650 and 1630 cm^{-1}, that is, at the frequency of the amide I vibration of self-associated and solvated amide bonds, respectively.

(*C*) *Phosphate Stretching Mode Region.* This spectral region (Figure 8) is dominated by two main features near 1225 and 1075 cm^{-1} that have been assigned to PO_2^- antisymmetric and symmetric stretching modes, respectively (Shimanouchi et al., 1964; Akutsu et al., 1975; Goni & Arrondo, 1986; Sen et al., 1988). For acyl chains in the trans conformation, a series of small bands due to the acyl chain methylene wagging progression (Cameron et al., 1980; Casal & Mantsch, 1984) is superimposed on the PO_2^- antisymmetric stretching mode, making the interpretation of this region more difficult. The assignment of the other bands of the 1000–1330-cm^{-1} region is given in Table II.

Below the phase transition temperature, the band due to the PO_2^- antisymmetric stretching mode is clearly lower in frequency for *N*-acyl-DPPEs than for DPPE. However, the presence of the wagging progression bands precludes the precise determination of the maximum of this band. On the other hand, in the liquid-crystalline phase, it is free from

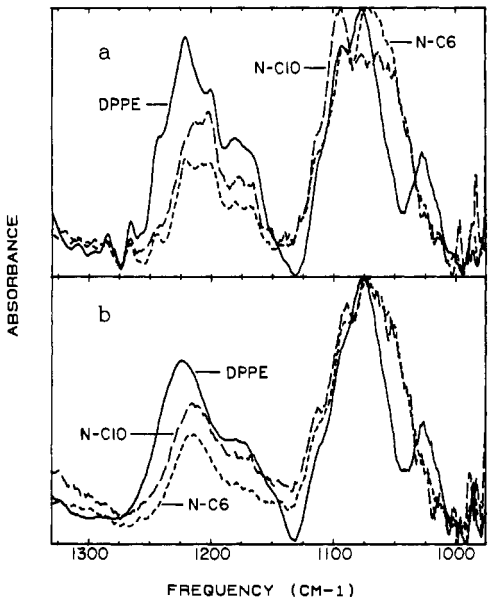

FIGURE 8: Infrared spectra of dispersions of DPPE (—), *N*-C6-DPPE (---), and *N*-C10-DPPE (--) in the phosphate stretching mode region in (a) the gel phase (30 °C below the transition temperature) and (b) the liquid-crystalline phase (5 °C above the transition temperature).

Table II: Assignment of the Infrared Spectrum of DPPE in the Gel Phase for the 950–1330-cm^{-1} Spectral Region[a]

dispersion (cm^{-1})	assignment
1309 (w)	CH_2 wagging progression bands
1285 (w)	
1267 (w)	
1243 (sh)	
1199 (w)	
1181 (w)	
1224 (vs)	ν_{as} PO_2^-
1170 (m)	CH_2 wagging and ν_{as} C–O–C
1148 (sh)	
1111 (sh)	ν C–C (trans)
1090 (s)	
1075 (vs)	ν_{sym} PO_2^-
1064 (sh)	ν_{sym} C–O–C
1030 (m)	ν_{as} C–C–N^+
1027 (sh)	C–O–P–O–C

[a] ν, stretching vibration; vs, very strong; s, strong; m, medium; w, weak; sh, shoulder.

spectral interference and appears at 1224, 1216, and 1213 cm^{-1} for DPPE and its *N*-C6 and *N*-C10 derivatives, respectively. It is well-known that the frequency of this vibration is quite sensitive to the degree of hydration of the phosphate group (Fringeli & Günthard, 1981). For example, it has been observed at 1222 cm^{-1} for totally hydrated DPPE and at 1229 cm^{-1} for the dry lipid (Sen et al., 1988). Therefore, the N-acylation of DPPE leads to an increase of the degree of hydration of the phosphate group of the lipid, this effect being more pronounced for *N*-acyl-DPPE with longer *N*-acyl chains.

This conclusion is further supported by the band due to the PO_2^- symmetric stretching mode. This band, which appears near 1075 cm^{-1} for DPPE, shifts down to 1065 cm^{-1} and becomes much broader for all *N*-acyl-DPPEs, showing that the phosphate groups are involved in stronger hydrogen bonds in these lipids (Sen et al., 1988). For *N*-C10- and *N*-C16-DPPE in the gel phase (Figure 8a), the absorption near 1070 cm^{-1} is even smaller compared to that of *N*-acyl-DPPEs with shorter *N*-acyl chains. Since normal-mode calculations have shown that the PO_2^- vibrations are not sensitive to the conformation of the phosphate group (Shimanouchi et al., 1964), this spectral change cannot be assigned to a reorganization of the lipid headgroup. On the other hand, since the symmetric C–O single-bond stretching mode of the ester groups also contributes to the absorption in this spectral region (Akutsu et al., 1981), it is likely that the decrease of intensity around 1070 cm^{-1} is due to a conformational change of the glycerol backbone for *N*-C10- and *N*-C16-DPPEs. However, this particular conformation seems to occur only in the gel phase.

Discussion

The results obtained by infrared and Raman spectroscopies clearly emphasize the strong dependence of the thermotropic behavior of *N*-acyl-PEs on the length of the *N*-acyl chain. For the *N*-acyl-DPPEs investigated, except for *N*-C16-DPPE, the phase transition temperature is much lower than that of DPPE, and there is a marked decrease of the lateral interactions of the acyl chains. These effects cannot be associated with a modification of the molecular organization of the lipid assembly since previous ^{31}P NMR results have shown that all *N*-acyl-DPPEs form multilamellar liposomes. In addition, the infrared bands due to the methylene scissoring modes (data not shown) indicate that the chain packing symmetry is hexagonal for DPPE as well as for all the *N*-acyl derivatives.

In fact, the *N*-acyl-DPPEs can be divided in two classes according to the length of the *N*-acyl chain. When the *N*-acyl chain contains eight carbon atoms or less, it is too short to reach the hydrophobic core of the bilayer, thus leading to an increase of the headgroup volume. The steric hindrance between phospholipids at the bilayer surface is then at the origin of the decrease in temperature of the gel to liquid-crystalline transition from *N*-C4- to *N*-C8-DPPE. From the Raman bands associated with the CH_2 groups of the acyl chains, it is clear that above eight carbon atoms the *N*-acyl chain can penetrate deeply into the bilayer and restore a close molecular packing which results in an increase in the transition temperature from *N*-C10- to *N*-C16-DPPE. In the gel phase, *N*-C16-DPPE shows a slightly higher conformational disorder, most likely because the *N*-acyl chain has to be bended to penetrate into the bilayer. Furthermore, *N*-C10-DPPE displays the transition with the lowest cooperativity, probably because the chain is not as well anchored into the bilayer as that for *N*-C16, the depth of penetration of the chain varying from one molecule to another. These results are in agreement with the conclusions of Akoka et al. (1988) except that the limiting length for the *N*-acyl chain from which the thermotropic behavior of the *N*-acyl-DPPEs is modified is now believed to be eight carbon atoms instead of six found by these authors.

The width of the infrared band due to the methylene asymmetric C–H mode (Figure 5b) shows that the mobility of the acyl chain of all *N*-acyl-DPPEs in the liquid-crystalline phase is much lower than that of DPPE. This result is in good agreement with the temperature dependence of the ^{31}P NMR T_1 relaxation time in the liquid-crystalline phase, which demonstrates that the activation energy of the headgroup rotation is greater than in most phospholipids (Akoka et al., 1988). The immobilization of DPPE by N-acylation is probably at the origin of the bilayer stabilizing effect of the *N*-acyl chain that has been suggested by some authors and that leads to an increase of the hexagonal (H_{II}) phase transition temperature (Akoka et al., 1988; Newman et al., 1986).

The ester group carbonyl region of the infrared spectra of the *N*-acyl-DPPEs in the gel phase (Figure 6a) also shows clearly that these lipids can be separated into two classes according to the length of the *N*-acyl chain. For *N*-C4- to *N*-C8-DPPEs, the ester carbonyl vibration of the *sn*-1 chain is 5 cm^{-1} higher than that of the corresponding bands of DPPE

and *N*-acyl-DPPEs with longer *N*-acyl chains. We believe that this shift in frequency is due to the fact that when the *N*-acyl chain is too short to penetrate into the hydrophobic core of the bilayer, it is folded in the interfacial region near the *sn*-1 carbonyl, which is a less polar environment. On the other hand, the relative intensity of the two components of the carbonyl band reveals that the proportion of hydrogen-bonded carbonyls is higher for all *N*-acyl-DPPEs, suggesting that the *sn*-2 carbonyl is in a more polar environment for these lipids and is probably hydrogen bonded with water (Blume et al., 1988; Wong & Mantsch, 1988). In the liquid-crystalline phase, the environment of the *sn*-1 carbonyl group is similar for all lipids investigated, and more carbonyl groups are hydrogen bonded, in agreement with the results obtained for most phospholipids above the gel to liquid-crystalline phase transition temperature (Blume et al., 1988).

The results obtained from the phosphate group vibrations also demonstrate that N-acylation of DPPE affects the degree of hydration of the lipid. It is well-known that phosphatidylethanolamines are less hydrated than phosphatidylcholines because they can form intermolecular hydrogen bonds between the PO_2^- and NH_3^+ groups (Hauser et al., 1981; Akutsu et al., 1981). The above results show that the disruption of this intermolecular hydrogen-bond network by N-acylation of the amino group leads to an increase in the degree of hydration of the lipid headgroup. This effect, which is observed in both the gel and liquid-crystalline phases, is more pronounced for *N*-C10- and *N*-C16-DPPEs than for *N*-acyl-DPPEs with shorter chains. Therefore, when the *N*-acyl chain is well embedded into the bilayer, as it is the case when the acyl chain contains 10 carbon atoms or more, the headgroup region is more exposed to the solvent. This conclusion is further supported by the effect of ionic strength of the phase transition temperature of *N*-acyl-DPPEs (Table I): the phase transition temperature is shifted down by 6 and 2 °C when *N*-C16- and *N*-C10-DPPEs are dispersed in water instead of 100 mM phosphate buffer, while it is not affected for *N*-acyl-DPPEs with shorter chains. Therefore, when the *N*-acyl chain is not folded into the bilayer, it seems to limit the penetration of the solvent in the headgroup region.

The amide I vibration of the amide group introduced by N-acylation of DPPE also reveals that the arrangement of the headgroup region changes with the length of the *N*-acyl chain and also with the physical state of the bilayer. For *N*-acyl-DPPE with short *N*-acyl chains in the gel state, the low frequency of the amide I band suggests that the amide group is hydrogen bonded with water and is thus located near the bilayer surface. In addition, the profile of the amide I band shows that a certain conformational inhomogeneity exists at the headgroup level for *N*-C8-DPPE, the *N*-acyl chain being too short to penetrate into the bilayer but also too hydrophobic to remain at the headgroup level. This heterogeneity might also explain why the phase transition temperature shifts from around 30 °C for *N*-C4- and *N*-C6-DPPEs to 18 °C for the *N*-C8 derivative (Table I).

For *N*-acyl-DPPEs with longer *N*-acyl chains, the spectral characteristics of the amide I band in the gel phase suggest that intermolecular hydrogen bonds between amide groups of adjacent molecules may exist when the *N*-acyl chain is anchored into the bilayer. Although our results do not prove unambiguously the existence of such intermolecular hydrogen bonds, the presence of a band due to partly self-associated amide groups for *N*-acyl-DPPEs with long *N*-acyl chains supports our hypothesis. Interactions between amide groups have also been proposed by Akoka et al. (1988) to account for the decrease of the headgroup rotational motion detected by ^{31}P NMR spectroscopy. In the liquid-crystalline phase, the amide group of *N*-acyl-DPPEs with longer *N*-acyl chains appears to be more exposed to the solvent. This result as well as the intensity of the band at 1070 cm^{-1} due to the symmetric C–O stretching mode of the glycerol backbone suggests that the *N*-acyl chain is partly released from the bilayer when *N*-acyl-DPPEs with long *N*-acyl chains undergo their gel to fluid phase transition. This may increase the volume of the hydrophobic part of the molecule and explain why the lamellar to hexagonal transition occurs just after the gel to liquid-crystalline transition for unsaturated *N*-acyl-PEs such as *N*-oleoyl-DOPE (Akoka et al., 1988).

In conclusion, N-acylation of phosphatidylethanolamines, which leads to the disruption of the intermolecular hydrogen bonds between the phosphate and amino groups, increases the degree of hydration of the polar headgroup of these lipids. The hydration is felt at both the phosphate and amide group levels: the phosphate groups are more hydrated for *N*-acyl-DPPEs with long chains while in the case of the short-chain derivatives both the phosphate and amide groups are hydrated. This difference is mainly due to the fact that *N*-acyl chains containing at least 10 carbon atoms are embedded into the acyl chain region of the bilayer. The higher degree of hydration, coupled with the immobilization of the lipid molecule, may contribute to the bilayer stabilizer role of *N*-acyl-PEs since hydration is an important factor in bilayer stability (Kleman et al., 1977; Boni et al., 1984).

From the results of the present work, it is possible that the bilayer stabilizing effect of N-acylation might protect membranes from dehydration as it occurs in wheat albumen during the last stage of grain maturation. However, the motion of *N*-acyl-PEs being considerably reduced in regard to the motion of corresponding diacyl-PEs, the activity of membrane enzymes, and therefore the function of the whole membrane, might be impaired. For example, it has been shown that the activity of Ca^{2+}-ATPase is completely inactivated when PEs are acetylated (Knowles et al., 1975). However, it is important to take into account that these lipids are a part of the total membrane lipids and that an explanation of their role has to consider interactions with other phospholipids. The study of binary mixtures of *N*-acyl-PE and diacyl phospholipids should bring new information about the role of *N*-acyl phospholipids on the properties of biological membranes.

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