Differential Scanning Calorimetry of Chain-Melting Phase Transitions of *N*-Acylphosphatidylethanolamines

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ABSTRACT Phosphatidylethanolamines in which the polar headgroup is *N*-acylated by a long-chain fatty acid (*N*-acyl PEs) are present in many plasma membranes under normal conditions, and their content increases dramatically in response to membrane stress in a variety of organisms. The thermotropic phase behavior of a homologous series of saturated *N*-acyl PEs, in which the length of the *N*-acyl chain is equal to that of the *O*-acyl chains attached at the glycerol backbone, has been investigated by differential scanning calorimetry (DSC). All fully hydrated *N*-acyl PEs with even chain lengths from C-12 to C-18 exhibit sharp endothermic chain-melting phase transitions in the absence of salt and in 1 M NaCl. Cooperative chain-melting is demonstrated directly by the temperature dependence of the electron spin resonance spectra from probe phospholipids bearing a spin label group in the acyl chain. The calorimetric transition enthalpy and the transition entropy obtained from DSC depend approximately linearly on the chain length with incremental values per CH_2 group that exceed those of normal diacyl phosphatidylethanolamines, but to an extent that underrepresents the additional *N*-acyl chain. A thermodynamic model is constructed for the chain-length dependences and end effects of the calorimetric quantities, which includes a deficit proportional to the difference in *O*-acyl and *N*-acyl chain lengths for nonmatched chains, as is found and justified structurally for mixed-chain diacyl phospholipids. From data on the chain-length dependence of *N*-acyl diC_{16}PEs, it is then deduced that the *N*-acyl chains are less well packed than the *O*-acyl chains and, from the data on the matched-chain *N*-acyl PEs, that the *O*-acyl chain packing is similar to that in normal diacyl PEs. The gel-to-fluid phase transition temperatures of the *N*-acyl PEs in the absence of salt are practically the same as those of the normal diacyl PEs of the corresponding chain lengths, although the transition enthalpies and entropies are appreciably greater, indicating entropy-enthalpy compensation. In 1 M NaCl, the transition temperatures are 3–4.5° higher than in the absence of salt, representing the contribution of the electrostatic surface potential of the *N*-acyl PEs.

INTRODUCTION

N-Acyl phosphatidylethanolamines (*N*-acyl PEs) are naturally occurring phospholipids present in such diverse species as microbes (Hazlewood and Dawson, 1975; Clarke et al., 1976), plants (Bomstein, 1965; Aneja et al., 1969; Dawson et al., 1969; Hargin and Morrison, 1980), as well as vertebrates (Matsumoto and Miwa, 1973; Gray, 1976; Somerharju and Renkonen, 1979; Epps et al., 1980; Natarajan et al., 1985, 1986). Biochemical studies have shown that the content of *N*-acyl PEs increases dramatically when the parent tissue is subjected to a condition of stress, such as wounding or a degenerate change, for example, dehydration of the endosperm in seeds (reviewed in Schmid et al., 1990). It has been postulated that the *N*-acyl PEs probably serve to produce *N*-acylethanolamines (NAEs), which then act as neurotransmitters or second messengers (Schmid et al., 1990, 1996). Consistent with this hypothesis, long-chain *N*-acylethanolamines were also reported to accumulate in the infarcted areas of canine myocardium (Epps et al., 1980, 1982) and in ischemic rat brain (Natarajan et al., 1986). *N*-Acylethanolamines have been shown to be produced by enzymatic degradation of the *N*-acyl PEs, and a phospholipase D activity that catalyses this degradative reaction has been identified in mammalian and plant tissues (Schmid et al., 1983; Chapman et al., 1995). *N*-Arachidonylethanolamine (anandamide) has been shown to act as an endogenous ligand of the cannabinoid receptor (Devane et al., 1992), whereas *N*-oleoylethanolamine was reported to inhibit ceramidase activity (Sugita et al., 1975).

More recently, it has been shown that *N*-acyl egg-PE and *N*-palmitoyl DPPE (*N*-16 DPPE) stabilize liposomes (Domingo et al., 1993; Mercadal et al., 1995). The latter also stabilizes liposomes in the presence of human serum (Mercadal et al., 1995), indicating that the *N*-acyl PEs may be useful in designing drug delivery systems.

In view of their presence in biological membranes and their proposed roles in the defense mechanisms to fight

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Abbreviations used: PE, phosphatidylethanolamine; *N*-acyl PE, *N*-acyl phosphatidylethanolamine; DLPE, DMPE, DPPE, and DSPE, 1,2-dilauroyl-, 1,2-dimyristoyl-, 1,2-dipalmitoyl- and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; *N*-12 DLPE, 1,2-dilauroyl-*sn*-glycero-3-(*N*-lauroyl) phosphoethanolamine; *N*-14 DMPE, 1,2-dimyristoyl-*sn*-glycero-3-(*N*-myristoyl)phosphoethanolamine; *N*-16 DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-(*N*-palmitoyl)phosphoethanolamine; *N*-18 DSPE, 1,2-distearoyl-*sn*-glycero-3-(*N*-stearoyl)phosphoethanolamine; *N*-16 DOPE, 1,2-dioleoyl-*sn*-glycero-3-(*N*-palmitoyl)phosphoethanolamine; *N*-18:1_c DOPE, 1,2-dioleoyl-*sn*-glycero-3-(*N*-oleoyl)phosphoethanolamine; 5-PCSL, 1-acyl-2-[5-(4,4-dimethyloxazolidine-*N*-oxyl)stearoyl]-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; TLC, thin-layer chromatography; HPLC, high-pressure liquid chromatography; ESR, electron spin resonance.

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stress or injury, as well as their potential use in developing drug delivery systems, it is important to investigate the membrane properties of *N*-acyl PEs. In particular, it is necessary to investigate their phase behavior in the pure state as well as in mixtures with other membrane lipids, such as phosphatidylcholine and phosphatidylethanolamine, and to characterize the structures formed by these compounds under a variety of conditions. Previous studies employing ^{31}P-NMR spectroscopy and freeze-fracture electron microscopy have shown that *N*-acyl DPPEs with acyl chain lengths from 2 to 18 C atoms form lamellar structures when dispersed in aqueous buffers, both in the gel phase as well as in the liquid-crystalline phase (Akoka et al., 1988; Domingo et al., 1995). Freeze-fracture electron microscopic studies have revealed that *N*-lauroyl DLPE also forms liposomes in aqueous media (Newman et al., 1986).

Regarding the physical studies on *N*-acyl PEs, there are two interesting aspects that attract attention. One is the question of the minimum *N*-acyl chain length required for it to be anchored in the hydrophobic interior of the membrane. It has been shown that, for *N*-acyl PEs with constant *O*-acyl chain length (16 C atoms) and varying *N*-acyl chain length, the *N*-acyl chain extends into the bilayer interior when the *N*-acyl chain length is equal to or greater than 10 C atoms, whereas for shorter chain lengths, the *N*-acyl chain is in contact with the aqueous environment (Akoka et al., 1988; Lafrance et al., 1990). Differential scanning calorimetric studies on *N*-acyl derivatives of dipalmitoyl phosphatidylethanolamine in which the *N*-acyl chain length was varied systematically are also consistent with the above results (Akoka et al., 1988; Domingo et al., 1995).

The second question concerns the contribution of the *N*-acyl chain to the thermodynamics of the chain-melting phase transitions of the *N*-acyl PEs. In particular, it would be interesting to know for *N*-acyl PEs with matched *N*- and *O*-acyl chains whether the contributions of the *N*-acyl chain to the transition enthalpy and transition entropy differ from, or are similar to, those of the *O*-acyl chains. This aspect is determined directly by the way in which the *O*-acyl and *N*-acyl chains are packed in the aqueous *N*-acyl PE membrane assemblies. It is best addressed by systematic investigation of the chain-length dependence of the calorimetric properties, because the effects of lateral chain packing can then be distinguished from those of transverse packing and other end effects. Therefore, we have investigated the phase behavior of a homologous series of *N*-acyl PEs of identical *N*- and *O*-acyl chain lengths by using differential scanning calorimetry. The results obtained suggest that the average contribution from each CH_2 group of the *N*-acyl chain to the transition enthalpy and entropy is considerably smaller than that of the CH_2 groups of the *O*-acyl chains. The chain-melting transition temperatures of the *N*-acyl PEs, however, are almost identical to those of the corresponding parent diacyl PEs, despite the fact that the overall transition enthalpies and entropies are greater. These observations delineate the thermodynamic consequences of the penetration and packing of the *N*-acyl chain in the hydrophobic interior of the lipid membrane. In addition, thermodynamic analysis of the chain-length dependence and end effects in the calorimetric properties yields important insights into the chain structure of the aqueous lipid assemblies of *N*-acyl PEs.

MATERIALS AND METHODS

Materials

DLPE, DMPE, DPPE, and DSPE were obtained from Avanti Polar Lipids (Birmingham, AL). Lauric acid, myristic acid, palmitic acid, and stearic acid were obtained from Fluka (Buchs, Switzerland) or Sigma Chemical Company (St. Louis, MO). Oxalyl chloride was a product of Fluka. All other reagents and solvents were of analytical grade. The 5-PCSL phosphatidylcholine spin label bearing the nitroxide oxazolidine ring on the 5 C atom of the *sn*-2 chain was synthesized as described by Marsh and Watts (1982).

Synthesis of *N*-acyl phosphatidylethanolamines

Lauric acid, myristic acid, palmitic acid, and stearic acid were converted to the corresponding acid chlorides by treating with 4 mol equivalents of oxalyl chloride, under stirring at room temperature in a nitrogen atmosphere. The excess reagent was then removed by adding hexane (2 × 10 ml) and blowing dry nitrogen gas over the sample.

N-12 DLPE, *N*-14 DMPE, *N*-16 DPPE, and *N*-18 DSPE (see Fig. 1) were synthesized by the reaction of the appropriate acid chlorides with the corresponding saturated diacyl PEs, essentially according to the procedure described by Akoka et al. (1988). The PE (~200 mg) was dissolved in ~5 ml of dichloromethane. Approximately 1 mole equivalent of the acid chloride and triethylamine were then added, and the mixture was kept under stirring at room temperature. The progress of the reaction was monitored by TLC on silica gel (solvent system: dichloromethane/methanol/ammonia 65/25/4, v/v/v), and additional amounts of acid chloride and triethylamine were added if necessary. The reaction was stopped when the TLC spot corresponding to PE had completely disappeared. The solution was then shaken in a separation funnel with 1 M sodium acetate (pH 9.0) to convert it to the sodium salt. The organic layer was evaporated to dryness, the residue was then redissolved in a small volume of dichloromethane, and 10 volumes of acetone were added. The solution was kept at −20°C overnight and then filtered. The precipitate was checked by TLC for purity and, if not fully pure, was subjected to further purification by HPLC.

Sample preparation

Samples for differential scanning calorimetry were prepared by weighing ~3–8 mg of the lipid into the DSC sample ampules and adding 0.5 ml of 10 mM HEPES buffer, 1 mM EDTA (pH 7.4), with or without 1 M NaCl. The ampules were then tightly sealed with screw caps and placed in the calorimeter. The reference ampule contained an equal volume of the buffer alone. Hydration of the lipid was achieved by heating the samples above the chain-melting phase transition temperature (to 95°C) in the calorimeter, incubating for 20 min, then cooling to 20°C or lower and incubating for a further 20 min. After this hydration procedure, the samples were subjected to two heating and two cooling scans, with nearly identical results for the repeated heating and repeated cooling scans. Samples for ESR spectroscopy were prepared by codissolving 1 mg of the lipid and 1 mol% of the spin label in dichloromethane. The solvent was evaporated with a gentle stream of dry nitrogen gas, and the residual solvent was removed by placing the sample in a vacuum desiccator overnight. The sample was then hydrated with 100 μl of the required buffer by heating it above the phase transition temperature and vortexing. The sample was then transferred to a 100-μl glass capillary (1 mm ID) and pelleted by centrifuging at 10,000

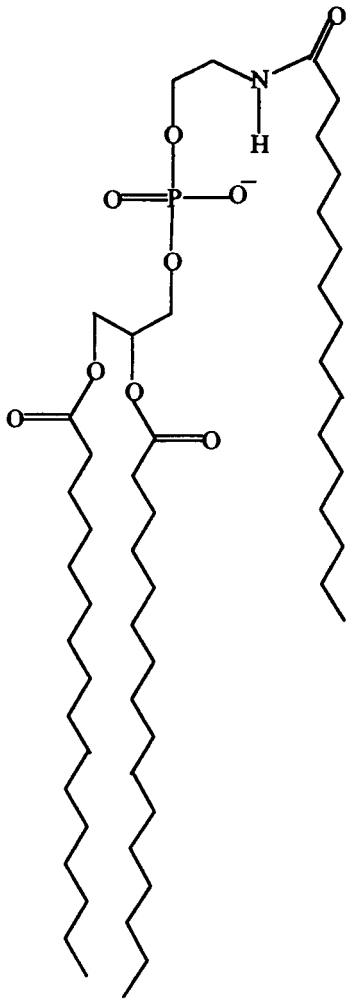

FIGURE 1 Structure of *N*-acylphosphatidylethanolamines. The *sn*-1, *sn*-2, and *N*-acyl chains consist of 12 (*N*-12 DLPE), 14 (*N*-14 DMPE), 16 (*N*-16 DPPE), or 18 (*N*-18 DSPE) C-atoms.

rpm in a Biofuge (Heraeus). Excess supernatant was then removed and the capillary was flame-sealed.

Differential scanning calorimetry

Differential scanning calorimetry was performed with a model 4207 heat-flow calorimeter from Hart Scientific. Samples were prepared as described above, and the scan rate used was 10°/h. Transition enthalpies were estimated by using the peak integration software provided with the instrument. Transition entropies were estimated from the transition enthalpies, assuming a first-order transition according to the expression (Marsh, 1990)

$$\Delta H_t = T_t \Delta S_t \quad (1)$$

where ΔH_t, ΔS_t, and T_t are the transition enthalpy, entropy, and transition temperature, respectively.

Electron spin resonance

ESR spectra were recorded on a Varian E-line 9-GHz ESR spectrometer equipped with nitrogen gas flow temperature regulation. Samples in 1-mm ID glass capillaries were placed in a standard quartz ESR tube containing light silicone oil for thermal stability. Temperature was measured with a fine-wire thermocouple positioned in the silicone oil just above the ESR cavity.

RESULTS AND DISCUSSION

Differential scanning calorimetry of *N*-acyl-PEs

The differential scanning calorigrams of saturated, symmetrical *N*-acylphosphatidylethanolamines with even-numbered and matched *O*-acyl and *N*-acyl chain lengths of C(12:0) to C(18:0), dispersed in 10 mM HEPES, 1 mM EDTA (pH 7.4), with and without 1 M NaCl, are given in Fig. 2, *A* and *B*, respectively. In each case the lipid dispersions show sharp, reversible thermotropic phase transitions in the heating scans. A second heating and a second cooling scan gave results nearly identical to those of the first heating and cooling scans, respectively. The cooling scans gave multiple peaks, indicative of metastability (not shown). The

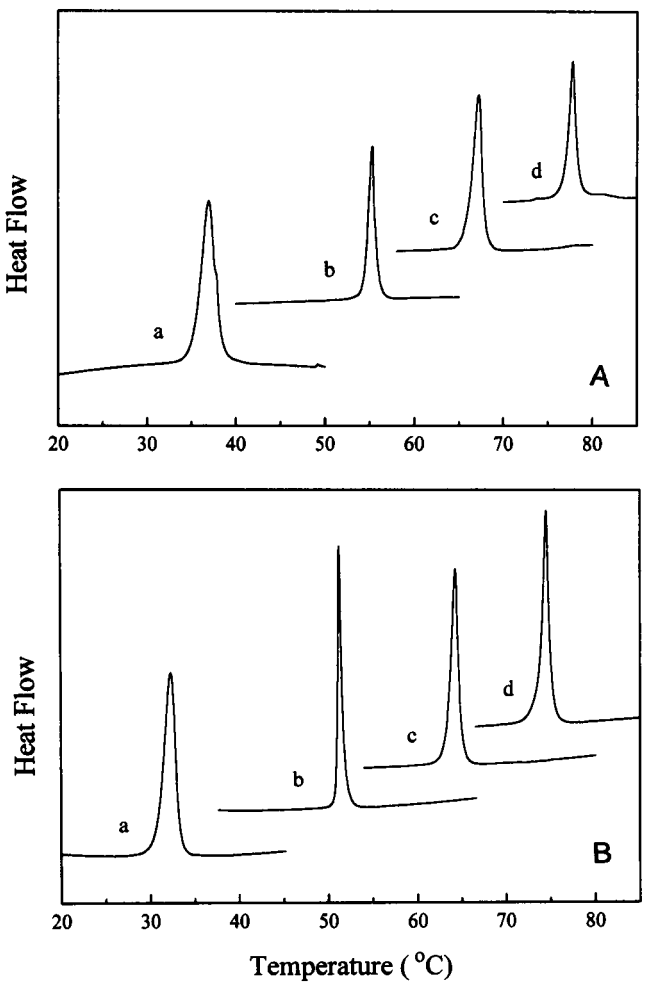

FIGURE 2 Differential scanning calorimetric scans of *N*-acyl PEs with matched *N*-acyl and *O*-acyl chains. The DSC traces shown correspond to (*a*) *N*-12 DLPE, (*b*) *N*-14 DMPE, (*c*) *N*-16 DPPE, and (*d*) *N*-18 DSPE. Samples were dispersed in 10 mM HEPES buffer, 1 mM EDTA (pH 7.4) containing (*A*) 1 M NaCl and (*B*) no salt.

reason for this is not known with certainty, but could be related to the difficulty in effectively packing the *N*-acyl chain in the lipid membrane in the gel phase. The half-widths of transitions observed in the heating scans are all in the range of 0.5–1.0°, with the exception of *N*-12 DLPE, which gave somewhat broader transitions, with half-widths of 1.5 and 1.9°, respectively, for samples in the absence and in the presence of 1.0 M NaCl. Transition widths in the presence of salt were generally similar to those observed in the absence of salt.

The transition enthalpies observed (see later) are not sufficiently large to be attributable to a chain-melting transition from a crystalline phase (cf. Lewis and McElhaney, 1993; Seddon et al., 1983b). Nor are additional transitions of appreciable enthalpy detected at higher temperatures that could be attributed to part of the sample being in a crystalline phase (cf. Seddon et al., 1983b). As summarized in the Introduction, all available structural evidence indicates that *N*-acyl PEs with fully saturated chains form lamellar phases. The negative charge on the headgroup of *N*-acyl PEs will tend to suppress formation of inverted nonlamellar phases (see Seddon et al., 1983c; Heimburg and Marsh, 1990). The only reports of inverted hexagonal (H_{II})-phase formation by *N*-acyl PEs are for those with unsaturated chains, which are known to promote H_{II} formation (e.g., Sankaram et al., 1989). In one case, the dioleoyl species *N*-16 DOPE is still lamellar immediately above the chain-melting transition and only forms H_{II} phases at higher temperatures (Akoka et al., 1988). In the other case, an H_{II} phase is induced with the trioleoyl species *N*-18:1_c DOPE by Ca^{2+} binding (Newman et al., 1986), which, besides neutralizing the lipid charge, also causes interfacial dehydration that is known to favor H_{II}-phase formation (e.g., Seddon et al., 1983a). Therefore it seems most likely that the fully saturated matched-chain *N*-acyl PEs are lamellar in structure, both in the gel phase and in the fluid phase immediately above the chain-melting transition.

The transition temperatures, transition enthalpies, and transition entropies obtained from the heating scans for the *N*-acyl PEs in the absence and in the presence of 1 M NaCl are listed in Table 1. The values of transition entropies were calculated, assuming a first-order transition (Eq. 1) for each of the *N*-acyl PEs. For all four *N*-acyl PEs investigated in the present study, the values of transition enthalpy obtained in the presence of 1 M NaCl are comparable to those obtained in the absence of salt. The transition temperatures obtained in the presence of 1 M NaCl are, however, higher than those obtained in the absence of salt by 3.0–4.5°.

TABLE 1 Transition temperatures (T_t), transition enthalpies (ΔH_t), and transition entropies (ΔS_t) of *N*-acyl phosphatidylethanolamines of matched *N*-acyl and *O*-acyl chain lengths (*n*) dispersed in 10 mM HEPES, 1 mM EDTA (pH 7.4), with and without 1 M NaCl

n (C atoms)	T_t (°C)	ΔH_t (kcal/mol)	ΔS_t (cal/mol/K)
0 M NaCl			
12	32.5	6.4 ± 0.3	21.0 ± 1.0
14	51.2	9.6 ± 0.1	29.6 ± 0.3
16	64.2	12.0 ± 0.1	35.6 ± 0.3
18	74.7	14.3 ± 0.2	41.2 ± 0.6
1 M NaCl			
12	36.9	6.5 ± 0.1	21.0 ± 0.3
14	55.2	8.8 ± 0.6	26.8 ± 1.8
16	67.2	11.6 ± 0.2	34.0 ± 0.6
18	78.2	14.5 ± 0.3	41.3 ± 0.9

Electron spin resonance

The transition enthalpies observed here for the *N*-acyl PEs with matched *O*-acyl and *N*-acyl chains are somewhat larger than those obtained for the gel-to-fluid phase transitions of the aqueous dispersions of the parent diacylphosphatidylethanolamines, as well as for other diacylphospholipids (Marsh, 1990). This suggests that the endothermic transitions observed upon heating the hydrated *N*-acyl PEs are related to the chain-melting transitions from an ordered gel to a fluid liquid-crystalline phase. Further confirmation of this interpretation is drawn from spin-label electron spin resonance studies on the thermally induced changes in the lipid chain dynamics of a spin-labeled phospholipid (5-PCSL) used as a probe in the *N*-acyl PE dispersions. The ESR spectra of the 5-PCSL spin label, bearing the nitroxide radical at the C-5 position of the *sn*-2 chain of phosphatidylcholine, in dispersions of *N*-14 DMPE in 10 mM HEPES, 1 mM EDTA (pH 7.4), recorded at various temperatures below and above the transition of the lipid, are given in Fig. 3. At low temperatures the spectra are all typical immobilized powder patterns, characteristic of a phospholipid gel phase. The spin-labeled lipids are well

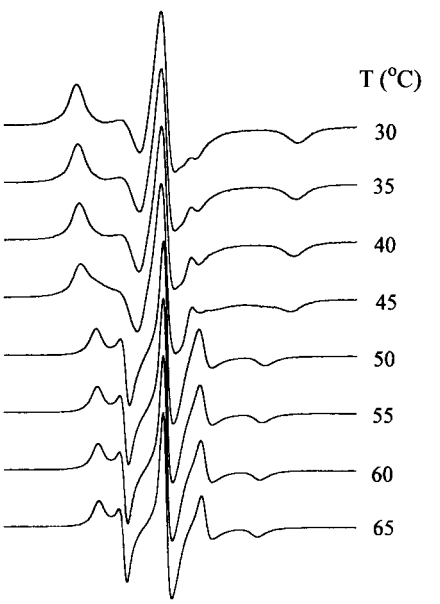

FIGURE 3 ESR spectra of the 5-PCSL spin label in *N*-14 DMPE dispersed in 10 mM HEPES, 1 mM EDTA (pH 7.4). The temperature at which each spectrum was recorded is indicated. Total scan width = 100 G.

dispersed in the gel phase. There is no evidence in the spectra for spin-spin broadening, which takes place strongly when such spin probes are squeezed out (as they usually are) from crystalline phospholipid phases. The ESR spectra change very little with increase in temperature up to 45°C. The outer hyperfine splitting, $2A_{max}$, which is 63.1G at 30°C, decreases only slowly when the temperature is raised to 45°C (59.8G). At 50°C, the ESR spectrum changes to a typical, partially motionally averaged, axial line shape with a considerably smaller hyperfine anisotropy ($2A_{max}$ = 47.9G), which is characteristic of fluid liquid-crystalline phases. This indicates that *N*-14 DMPE, hydrated with buffer containing no salt, undergoes a gel-to-fluid phase transition between 45°C and 50°C, which is in reasonable agreement with the results obtained by the DSC studies (Table 1). The outer hyperfine splitting then decreases gradually with increase in temperature to a value of 45.3G at 65°C, which is also consistent with expectations for a fluid liquid-crystalline phase. Similar results were obtained for the ESR studies on *N*-16 DPPE dispersions using the 5-PCSL spin-label, except that chain-melting took place at a correspondingly higher temperature.

Transition enthalpies and entropies

The chain-length dependences of the enthalpies and entropies of chain melting are given in Fig. 4 for the *N*-acyl PEs in the absence and in the presence of 1 M NaCl. The values for the *N*-acyl PEs in the presence and absence of salt are rather similar but, as expected, are greater than those for the corresponding normal diacyl PEs. However, the latter difference is considerably smaller than that obtained by increasing the number of C atoms in the chains of the diacyl PEs by an amount equal to the number of C atoms in the *N*-acyl chain. For instance, the transition enthalpy and entropy of *N*-12 DLPE correspond to a diacyl PE with chains containing a total of four to six more C atoms than DLPE.

Both the transition enthalpy and transition entropy are approximately linearly dependent on the chain length, *n*, according to

$$\Delta H_t = n\Delta H_{inc} + \Delta H_o \quad (2)$$

$$\Delta S_t = n\Delta S_{inc} + \Delta S_o \quad (3)$$

as is seen from Fig. 4. The incremental values (ΔH_{inc}, ΔS_{inc}) and the effective end contributions (ΔH_o, ΔS_o) obtained from linear regression to the calorimetric data are given in Table 2. The incremental values for the *N*-acyl PEs are greater than those obtained from normal diacyl PEs, but by less than 50%. An increase of 50% would be expected if the incremental change at the transition for each of the three chains of the *N*-acyl PEs was equal to that for each of the chains of the normal diacyl PEs. One possible explanation for the discrepancy could be that the change at the transition for the *N*-acyl chain is smaller than that for the *O*-acyl (i.e., glycerol) chains. This explanation is not unique, but would imply that either the *N*-acyl chain is less well packed in the gel phase or less configurationally disordered in the fluid phase than are the *O*-acyl chains of the *N*-acyl PEs. Further analysis, including data from a homologous series of *N*-acyl DPPEs, is given later to resolve this point.

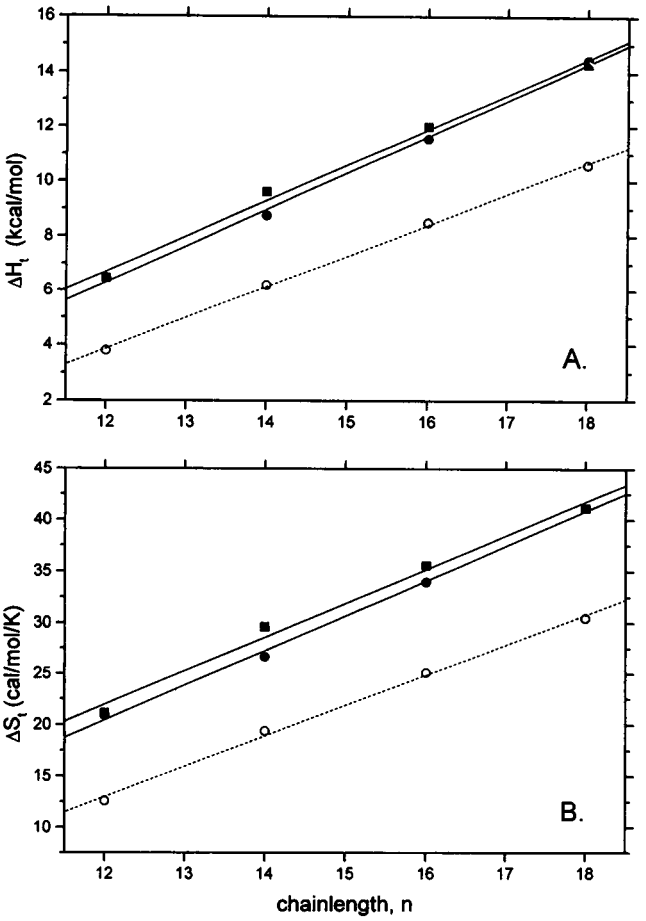

FIGURE 4 Chain-length dependence of (*A*) the transition enthalpy, ΔH_t, and (*B*) the transition entropy, ΔS_t, of *N*-acyl PEs in 1 M NaCl (●) and without salt (■). The *N*-acyl chain length, *n*, is matched to that of the *O*-acyl (glycerol) chains in each case. The full lines are linear regressions, the parameters given in Table 2. Corresponding data for diacyl PEs in H_2O (○, average values from Marsh, 1990) are given for comparison.

The end contributions to the calorimetric quantities are rather similar for the *N*-acyl PEs and the normal diacyl PEs. This result is somewhat surprising, in view of the different structures of the two classes of lipids, with respect to both the polar headgroup acylation and the probable location of the end of the *N*-acyl chain. One possibility could be that there is appreciable cancellation of opposing modified end contributions in the case of the *N*-acyl PEs.

The transition enthalpy for *N*-16 DPPE found here is within the range reported by Domingo et al. (1995) (ΔH_t = 11.6 ± 0.5 kcal/mol), but is considerably larger than that reported by Akoka et al. (1988). If the present value for *N*-16 DPPE is combined with the data for other *N*-acyl DPPEs reported by the latter authors (Akoka et al., 1988), it is found that both the calorimetric enthalpy and entropy are linearly dependent on the *N*-acyl chain length (n^N = 12–18),

TABLE 2 Incremental values (ΔH_{inc}, ΔS_{inc}) of the chain-length dependence and end contributions (ΔH_o, ΔS_o) to the calorimetric enthalpy and entropy, respectively, for *N*-acyl phosphatidylethanolamines of matched *N*- and *O*-acyl chain lengths (n = 12, 14, 16, 18) in 10 mM HEPES, 1 mM EDTA (pH 7.4), with and without 1 M NaCl*

Lipid	ΔH_{inc} (kcal/mol/CH_2)	ΔH_o (kcal/mol)	ΔS_{inc} (cal/mol/K/CH_2)	ΔS_o (cal/mol/K)
N-acyl PE (0 M NaCl)	1.30 ± 0.07	−8.9 ± 1.1	3.30 ± 0.24	−17.7 ± 3.7
N-acyl PE (1 M NaCl)	1.34 ± 0.05	−9.7 ± 0.8	3.40 ± 0.13	−20.4 ± 1.9
PE	1.13 ± 0.02	−9.7 ± 0.4	2.97 ± 0.11	−22.6 ± 1.7

*Obtained from linear regressions given in Figs. 4A and 4B (see Eqs. 2, 3).
Corresponding data for nonderivatized diacyl phosphatidylethanolamines (PEs) in water evaluated over the same range are also included (average values from Marsh, 1990).

for constant *O*-acyl chain length (see Fig. 6, *A* and *B*). The incremental values and end contributions are 0.925 ± 0.041 kcal/mol/CH_2, −2.65 ± 0.64 kcal/mol and 2.43 ± 0.14 cal/mol/K/CH_2, −2.9 ± 2.2 cal/mol/K for the transition enthalpy and entropy, respectively. The "end contributions" (corresponding to $n^N = 0$) are, however, very different from the calorimetric enthalpy and entropy of the parent DPPE (cf. Marsh, 1990). This suggests that there is a contribution to the calorimetric properties that is dependent on the difference in length, $n^O - n^N$, between the *O*-acyl and *N*-acyl chains, as reasoned previously for the contributions from chain mismatch in mixed-chain diacyl phosphatidylcholines of unequal chain length (Marsh, 1992). These contributions arise from deficits in the chain packing in the region of mismatch between the *N*-acyl and *O*-acyl chains. Assuming, as found for the mixed-chain phosphatidylcholines, that the contributions from mismatch are linearly dependent on the chain-length difference, i.e., are of the form $\Delta h(n^O - n^N)$ and $\Delta s(n^O - n^N)$, then allows estimation of the various component values (see Appendix).

For the "end contributions" of the *N*-acyl DPPEs (see Eqs. A7 and A8), subtracting the calorimetric parameters for DPPE itself (Marsh, 1990) yields values of Δh = −0.70 ± 0.09 kcal/mol/CH_2 and Δs = −1.75 ± 0.28 cal/mol/K/CH_2 for the deficit contributions in the region of chain mismatch, taking $n^O = 16$. This then further yields values of $\Delta H^N_{inc} = 0.23 \pm 0.13$ kcal/mol/CH_2 and $\Delta S^N_{inc} = 0.69 \pm 0.42$ cal/mol/K/CH_2 for the contributions of the *N*-acyl chain itself to the incremental calorimetric quantities, in agreement with the suggestion that the *N*-acyl chain makes a less significant contribution than do the *O*-acyl chains (cf. Table 2). For the *N*-acyl PEs with matched chain lengths (see Eqs. A5 and A6), subtracting these values from the total incremental quantities then yields values of $\Delta H^O_{inc} = 1.08 \pm 0.20$ kcal/mol/CH_2 and $\Delta S^O_{inc} = 2.64 \pm 0.66$ cal/mol/K/CH_2 for the contribution of the *O*-acyl chains, which are close to the values for normal diacyl PEs (see Table 2), in agreement with the expectations of the model (see Appendix). As already noted, the end contributions to the calorimetric quantities for the *N*-acyl PEs with matched chains are rather close to those for the normal diacyl PEs (i.e., ΔH^O_o and ΔS^O_o), which is also in agreement with the model as presented in the Appendix. Thus, according to the interpretation of the calorimetric data by the thermodynamic model, the *O*-acyl chains are packed in a manner similar to that of normal diacyl PEs, except in the region of mismatch with the *N*-acyl chains, the packing of which is different from that of the *O*-acyl chains.

Transition temperatures

The temperatures of the chain-melting transition for the different *N*-acyl PEs in which the *N*-acyl chain is of the same length as that of the *O*-acyl (i.e., glycerol) chains are given for lipid dispersions in the presence and absence of 1 M NaCl in Table 1. These values are also plotted as a function of the chain length, n, in Fig. 5. In the absence of salt, the transition temperatures lie close to those of the corresponding normal diacyl PEs in H_2O (taken from Seddon et al., 1983a). This indicates a high degree of entropy-enthalpy compensation between the two systems, because the transition enthalpies and entropies differ considerably between the *N*-acyl PEs and the normal PEs (cf. above). The

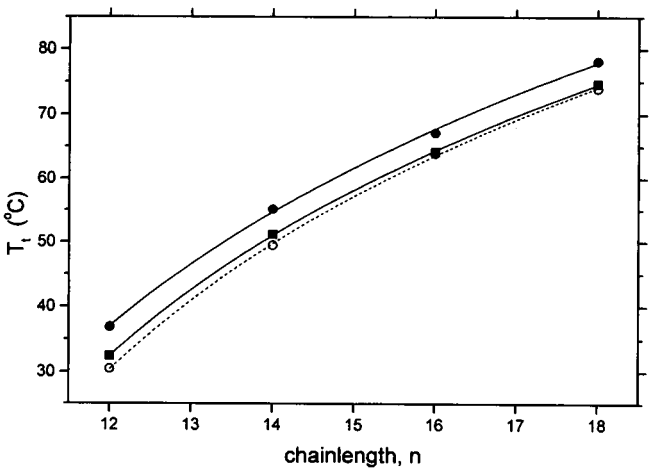

FIGURE 5 Chain-length dependence of the gel-to-fluid phase transition temperatures, T_t, of *N*-acyl PEs in 1 M NaCl (●) and without salt (■). The *N*-acyl chain length, n, is matched to that of the *O*-acyl (glycerol) chains in each case. The continuous lines are nonlinear least-squares fits to Eq. 4, with fitting parameters: T_t^∞ = 422 (429) K, n_o-n'_o = 2.9 (3.2), n'_o = 1.4 (0.6) in the absence (presence) of salt. Corresponding data for diacyl PEs in H_2O (○, Seddon et al., 1983a) are fitted with T_t^∞ = 422 K, n_o-n'_o = 2.9, n'_o = 1.7 (*dashed line*).

chain-length dependences of the transition temperatures, T_t, become less steep with increasing chain length in both systems, and are well fitted by the expression (Marsh, 1991; Seddon et al., 1983a)

$$T_t = T_t^{\infty}[1 - (n_o - n_o')/(n - n_o')] \quad (4)$$

which was proposed on the assumption of a linear dependence of the transition enthalpy and transition entropy on chain length, n. The values of the fitting parameters are given in the legend to Fig. 5. The transition temperature, T_t^{∞}, extrapolated to infinite chain length, is similar for all three systems, as expected.

The transition temperatures in the presence of salt are 3–4.5° higher than those in the absence of salt, with the difference, ΔT_t, decreasing with increasing chain length. The product, $\Delta T_t \cdot \Delta S_t$, which represents the salt-induced change in the free energy difference between the gel and fluid states in the region of the transition (Cevc and Marsh, 1987), is in the range 100–140 cal/mol. If it is assumed that this difference is attributed solely to the electrostatic double layer, then the fractional change in the area per N-acyl PE molecule at the transition, $\Delta A_t/A$, would be in the region of 0.08–0.1 (cf. Swamy et al., 1994). This is considerably smaller than the area expansion normally observed at the chain melting transition of lipid bilayers (see Marsh, 1990). Both this comparison and the rather small size of the salt-induced transition temperature shifts suggest that electrostatic effects are relatively unimportant for the thermodynamics of chain melting of the N-acyl PEs.

For N-acyl DPPEs, by taking the transition temperature for N-16 DPPE together with those for other DPPE derivatives from N-10 to N-18 (Akoka et al., 1988; Lafrance et al., 1990), the dependence on the N-acyl chain length can also be fit by Eq. 4 (see Fig. 6 C). This yields values of T_t^{∞} = 361.3 K, $n_o - n_o'$ = 0.665 and n_o' = 6.003 (χ^2 = 0.02). The difference between these parameters and those for the chain-length dependence of normal diacyl PEs (see Fig. 5 legend) again emphasizes the difference in the contribution of the N-acyl chain from that of the O-acyl chains to the calorimetric properties of chain melting.

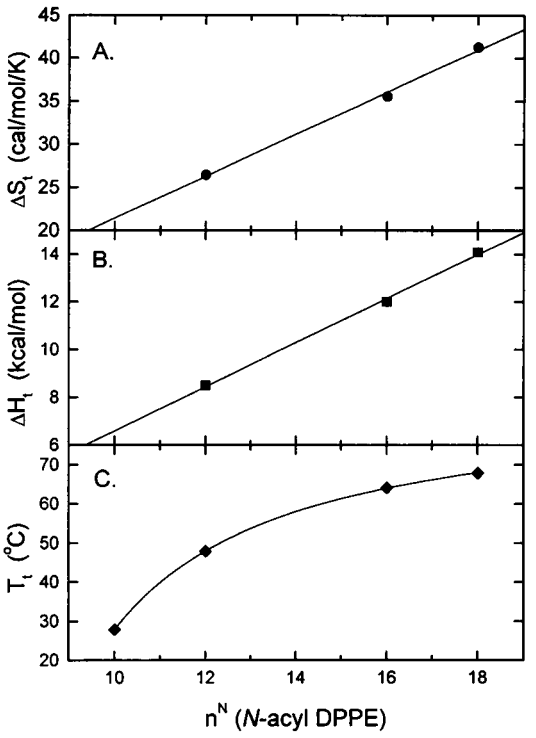

FIGURE 6 Dependence on the N-acyl chainlength, n^N, of (A) the transition entropy, ΔS_t; (B) the transition enthalpy, ΔH_t; and (C) the transition temperature, T_t, for N-acyl DPPEs. The solid lines in A and B are linear regression lines. In C it is a nonlinear least-squares fit to Eq. 4. Fitting parameters and sources of the data are given in the text.

CONCLUSIONS

The chain-melting enthalpies and entropies of N-acyl PEs in which the N-acyl chain length is equal to that of the O-acyl (glycerol) chains depend linearly on the number of C atoms in the matched chains (n = 12–18). The incremental transition enthalpies and entropies per CH_2 group are less, however, on a per-chain basis, than are those for normal diacyl PEs. This implies that the chain packing in the gel and/or fluid states differs between the N-acyl PEs and normal diacyl PEs. Analysis of the calorimetric quantities by using the thermodynamic model given in the Appendix shows that this difference reflects the packing properties of the N-acyl chain itself. According to the model, there is a packing deficit for the O-acyl chains in the region of mismatch with the N-acyl chains, but outside this region the O-acyl chains are packed in a manner similar to that for normal diacyl PEs.

The large degree of entropy-enthalpy compensation that results in the chain-melting transitions of the N-acyl PEs lying very close to those of the corresponding parent PEs is also found in the comparison of phosphatidylcholines with interdigitated and noninterdigitated gel states (Swamy and Marsh, 1995). This may prove to be a unifying thermodynamic principle for the viable organization of lipid bilayer membranes with nonconventional chain-packing motifs.

APPENDIX: CHAIN-LENGTH DEPENDENCE OF THE CALORIMETRIC PROPERTIES FOR *N*-ACYL PEs

The simplest model that is consistent with the experimental data is given here for the dependence of the transition enthalpy and transition entropy on the length of the O-acyl and N-acyl chains, n^O and n^N, respectively (cf. Figs. 4 and 6). It is assumed that the contribution from the O-acyl chains is linearly dependent on n^O with a constant end contribution, as for normal diacyl PEs (see Seddon et al., 1983a). It is further assumed that the contribution from the N-acyl chains is linearly dependent on n^N, but with an end contribution that is linearly dependent on the difference or mismatch, $n^O - n^N$, between the lengths of the O-acyl and N-acyl chains (see Fig. 6). This deficit arising from chain mismatch is similar to that introduced previously for mixed-chain diacyl phospholipids (Marsh, 1992). Direct correlation with molecular structure has been achieved in the latter

case by the extensive work of Huang and co-workers (see, e.g., Huang, 1990). The dependence of the calorimetric properties on n^O and n^N is then given by

$$\Delta H_t = \Delta H^O_{inc}(n^O - n^O_o) + \Delta H^N_{inc} \cdot n^N + \Delta h(n^O - n^N) \quad (A1)$$

$$\Delta S_t = \Delta S^O_{inc}(n^O - n^{O\prime}_o) + \Delta S^N_{inc} \cdot n^N + \Delta s(n^O - n^N) \quad (A2)$$

where ΔH^O_{inc}, ΔS^O_{inc} are the incremental transition enthalpy and entropy, respectively, for the *O*-acyl chains and ΔH^N_{inc}, ΔS^N_{inc} are the corresponding values for the *N*-acyl chain. The end contributions to the transition enthalpy and transition entropy are $\Delta H^O_{inc} n^O_o$ and $\Delta S^O_{inc} n^{O\prime}_o$, respectively, for the *O*-acyl chains and those for the *N*-acyl chains are given by the final terms on the right-hand side of Eqs. A1 and A2, respectively. The latter correspond to the packing deficits in the region of *O*-acyl and *N*-acyl chain mismatch. When we collect terms in Eqs. A1 and A2, the dependences on n^O and n^N are given by

$$\Delta H_t = (\Delta H^O_{inc} + \Delta h)n^O + (\Delta H^N_{inc} - \Delta h)n^N - \Delta H^O_{inc} n^O_o \quad (A3)$$

$$\Delta S_t = (\Delta S^O_{inc} + \Delta s)n^O + (\Delta S^N_{inc} - \Delta s)n^N - \Delta S^O_{inc} n^{O\prime}_o \quad (A4)$$

where n^O_o and $n^{O\prime}_o$ are constant and expected to be approximately equal to the corresponding values for normal diacyl PEs.

For *N*-acyl PEs with matched chains, as studied here, $n^O = n^N = n$, and the dependence of the calorimetric properties on n is given by

$$\Delta H_t = (\Delta H^O_{inc} + \Delta H^N_{inc})n + \Delta H^O_o \quad (A5)$$

$$\Delta S_t = (\Delta S^O_{inc} + \Delta S^N_{inc})n + \Delta S^O_o \quad (A6)$$

where $\Delta H^O_o = -\Delta H^O_{inc} n^O_o$ and $\Delta S^O_o = -\Delta S^O_{inc} n^{O\prime}_o$. Correspondingly, for the situation in which the *O*-acyl chain length, n^O, is constant and only that of the *N*-acyl chain (i.e., n^N) is varied:

$$\Delta H_t = (\Delta H^N_{inc} - \Delta h)n^N + \Delta h \cdot n^O + \Delta H^O_t(n^O) \quad (A7)$$

$$\Delta S_t = (\Delta S^N_{inc} - \Delta s)n^N + \Delta s \cdot n^O + \Delta S^O_t(n^O) \quad (A8)$$

where $\Delta H^O_t(n^O)$ and $\Delta S^O_t(n^O)$ are constant and expected to be equal to the transition enthalpy and entropy, respectively, of the corresponding normal diacyl PE with chain length n^O.

Further refinement could be made by adding a constant end contribution to the final terms in Eqs. A1 and A2 (i.e., effectively a different value of n^N), but this would not be necessary for the currently available data.

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