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Influence of Ether Linkage on the Lamellar to Hexagonal Phase Transition of Ethanolamine Phospholipids†

J. M. Boggs,* D. Stamp, D. W. Hughes, and C. M. Deber

ABSTRACT: Phosphatidylethanolamine isolated from several sources has been reported to go into the hexagonal phase below physiological temperature, suggesting that a transition to a nonlamellar phase may play a role in membrane function. In the present study, we determined the temperature of the lamellar to hexagonal (L → H) phase transition of bovine white matter ethanolamine phospholipid to be 18 °C. Comparison of this temperature and the hydrocarbon chain composition of bovine white matter ethanolamine phospholipid (77% 1-alk-1-enyl-2-acyl- and 7% 1-alkyl-2-acylglycerophosphoethanolamine) with values reported for phosphatidylethanolamine from other tissues suggested that the alk-1-enyl and alkyl chains on the 1 position of glycerophosphoethanolamine destabilize the lamellar phase. Since this effect could be due either to the double bond between the first and second carbons of the alk-1-enyl chain and/or to the ether linkage, we examined these possibilities using synthetic saturated alkyl and acyl analogues of phosphatidylethanolamine. The temperature of the L → H phase transition was found to increase in the order dialkyl < 1-alkyl-2-acyl ≪ diacyl, while the temperature of the gel to liquid-crystalline (G → L) phase transition increased in the reverse order. Although the ether linkage results in a significant lowering of the temperature of the L → H phase transition, it has little effect on the temperature, enthalpy, and entropy of the G → L phase transition and on the amplitude of motion of fatty acid spin-labels in the gel or liquid-crystalline phases. This indicates that the ether linkage causes only a small increase in the strength of intermolecular hydrogen-bonding interactions between the lipid head groups. This small increase is sufficient to promote the transition to the hexagonal phase, however, which suggests that these interactions for phosphatidylethanolamine have a greater effect on the stability of the hexagonal phase than on the stability of the lamellar gel phase. The presence in myelin of a lipid with a structural modification which destabilizes the lamellar phase suggests that the hexagonal phase may play a role in myelin structure and/or function.

The myelin membrane contains a high content of plasmalogen; 30% of the total phospholipid, mostly the ethanolamine phospholipid fraction (EP)[1] is in this form. The ethanolamine fraction has been found to be 77% 1-alkyl-1-enyl-2-acylglycerophosphoethanolamine (GPE), 7% 1-alkyl-2-acyl-GPE, and 16% diacylphosphatidylethanolamine (PE) (O'Brien & Sampson, 1965; Sun & Horrocks, 1970; Frosolono & Marsh, 1973). A major portion of the 1-alk-1-enyl-2-acyl-GPE of myelin is unusual in that 18:1 is the principal hydrocarbon chain in both the 1 and 2 positions; this species has been reported to be specific for nervous tissue (Sun & Horrocks, 1970; Hack & Helmy, 1977). A high content of plasmalogen lipids is also found in erythrocytes (35% of EP) (Ways & Hanahan, 1964), heart mitochondria (40–60% of EP) (Fleischer et al., 1967; Getz et al., 1968), mouse brain mitochondria (36% of EP) (Sun & Horrocks, 1970), sheep kidney and adrenal cortex (40–60% of EP) Getz et al., 1968), and spermatozoa (64% of total phospholipid and 89% of EP) (Evans et al., 1980).

Plasmalogens have been reported to be more resistant to hydrolysis by phospholipase A_2 than diacyl lipids (Paltauf et al., 1971), but they have other unique properties as well. The ether linkage in analogues of phosphatidylcholine (PC) has been reported to have no effect on lipid fluidity (Schwartz & Paltauf, 1977). However, the phase transition temperature is 2–5 °C higher for the dialkyl analogues of PE, PC (Vaughan & Keough, 1974), and phosphatidic acid (PA) (Blume & Eibl, 1979; Harlos et al., 1979) than for the diacyl analogues while the 1-alkyl-2-acyl analogue of PC has been reported to have a lower phase transition temperature than that of the diester form (Lee & Fitzgerald, 1980).

It has been shown that PE isolated from a number of biological membranes can undergo a lamellar to hexagonal phase transition at temperatures below the physiological temperature

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[1] Abbreviations used: EP, ethanolamine phospholipid fraction; PE, phosphatidylethanolamine; PA, phosphatidic acid; PC, phosphatidylcholine; GPE, 1,2-dialkyl or 1-alkyl-2-acyl derivative of glycerophosphoethanolamine; PE(PC), phosphatidylethanolamine prepared from egg phosphatidylcholine; DMPE, 1,2-dimyristoylphosphatidylethanolamine; DPPE, 1,2-dipalmitoylphosphatidylethanolamine; DSPE, 1,2-distearoylphosphatidylethanolamine; DHPE, 1,2-dihexadecylglycerophosphoethanolamine; HPPE, 1-hexadecyl-2-palmitoylglycerophosphoethanolamine; DOPE, 1,2-dioleoylphosphatidylethanolamine; DEPE, 1,2-dielaidoylphosphatidylethanolamine; PG, phosphatidylglycerol; DTPG, 1,2-ditetradecylphosphatidylglycerol; DMPG, 1,2-dimyristoylphosphatidylglycerol; DMPC, 1,2-dimyristoylphosphatidylcholine; DPPC, 1,2-dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; ESR, electron spin resonance; NMR, nuclear magnetic resonance; Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid.

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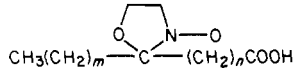
(Reiss-Husson, 1967; Rand et al., 1971; Cullis & de Kruijff, 1978a). Cullis & de Kruijff (1978a) also showed that the lamellar to hexagonal phase transition of PE, which is detectable by ^{31}P NMR, is an endothermic transition which can be detected by differential scanning calorimetry (DSC). Since the hexagonal phase may be involved in certain membrane functions such as fusion and exo- and endocytosis (Rand & Sengupta, 1972; Cullis & de Kruijff, 1978a,b, 1979), it is necessary to determine the phase behavior of ethanolamine plasmalogen in order to understand the role of this lipid in myelin and other membranes.

In the present study, we investigate the effect of the ether linkage on the lamellar to hexagonal phase transition temperature of several natural and synthetic types of ethanolamine phospholipids using DSC and ^{31}P NMR. In addition, we investigate changes in the lipid packing during this transition using fatty acid spin-labels, as studied earlier in the lamellar and hexagonal phases of cardiolipin (Boggs & Hsia, 1973).

Materials and Methods

The ethanolamine phospholipid fraction (EP) from bovine white matter (77% 1-alk-1-enyl-2-acyl, 7% 1-alkyl-2-acyl, and 16% diacyl), egg PE, and PE prepared by transphosphatidylation of egg phosphatidylcholine [PE(PC)] were purchased from Avanti Biochemicals, Inc., Birmingham, AL. 1,2-Dipalmitoylphosphatidylethanolamine (DPPE) and 1,2-dihexadecylglycerophosphoethanolamine (DHPE) were purchased from Calbiochem. 1-Hexadecyl-2-palmitoylglycerophosphoethanolamine (HPPE) was purchased from Berchtold Biochemisches Labor, Switzerland. 1,2-dimyristoylphosphatidylethanolamine (DMPE) was purchased from Fluka (Switzerland). 1,2-Dimyristoylphosphatidylcholine (DMPC) and 1,2-dipalmitoylphosphatidylcholine (DPPC) were purchased from Sigma. All lipids were chromatographically pure and were stored at −20 °C. Fatty acid spin-labels, 5-S-SL [I (12,3)] and 16-S-SL [I (1,14)], were purchased from Syva (Palo Alto, CA).



For ESR and DSC studies, the lipids were dispersed (2–4 mg/0.5 mL) by vortex mixing at a temperature above the T_c. For pH 7.4, Hepes buffer (2 mM) containing 10 mM NaCl and 0.1 mM EDTA was used. For pH 1 and 12.3, 0.5 N HCl and 0.25 N NaOH were used respectively. The pH was checked after dispersion of the lipids.

In order to spin-label the lipid, the lipid and spin-label were dissolved together in chloroform at a molar ratio of lipid to spin-label of 200 to 1. The solvent was evaporated under nitrogen, and the lipid was dispersed as before. The samples was measured in 50-μL disposable micropipets. For DSC studies, the lipid suspension was flushed with nitrogen and centrifuged in an Eppendorf microcentrifuge in the cold at 12000 rpm. Most of the supernatant was removed, and the wet pellet was transferred to an aluminum Perkin-Elmer DSC pan.

Differential Scanning Calorimetry. The temperature and heat content of the thermotropic transitions of the lipids were determined with a Perkin-Elmer DSC-2 differential scanning calorimeter using scanning rates of 2.5-10 °C/min. Samples were heated, cooled, and reheated several times, and the scans were completely reproducible upon scanning at least 3 times. The midpoint of each peak was defined as T_c for the phase transition temperature of the gel to liquid-crystalline transition and as T_h for the phase transition temperature of the lamellar to hexagonal transition. Enthalpy determinations were made by heating the samples at a rate of 10 °C/min and using a fast chart speed of 160 mm/min. Four pans were filled and measured for each sample, and peak areas were determined by weighing as described earlier (Boggs & Moscarello, 1978b). The pans were squeezed open with forceps and dropped into 1 mL of chloroform:methanol, 2:1, to dissolve the lipid. Aliquots were taken for phosphorus analysis by the method of Bartlett (1959). The enthalpy determination was repeated at least twice with identical results being obtained.

Electron Spin Resonance Measurement. Spectra were obtained on a Varian E-104 spectrometer with a Varian temperature control accessory. The microwave power used was 10 mW. An empirical motion parameter, τ_0 (Eletr & Keith, 1972), was derived from the spectral parameters of 16-S-SL as an estimate of the relative rotational correlation time from the expression

$$\tau_0 = (6.5 \times 10^{-10}) W_0 [(h_0/h_{-1})^{1/2} - 1]$$

where W_0 is the width of the center line and h_0 and h_{-1} are the heights of the center and high field lines, respectively.

The order parameter S, a measure of the amplitude of the motion of the molecular long axis about the average orientation of the fatty acid chains in the bilayer, was obtained from the expression (Seelig, 1970; Hubbell & McConnell, 1971)

$$S = \frac{T_\parallel - T_\perp}{T_{zz} - T_{xx}}$$

where $T_\parallel$ and $T_\perp$ are the experimentally determined anisotropic hyperfine splittings measured as described previously (Boggs & Moscarello, 1978a) and T_{zz} and T_{xx} are the rigid lattice principal hyperfine values obtained from single crystal spectra (Seelig, 1970). Where two components were present in the spectra, the $T_\parallel$ values of each were measured individually. The two components could be resolved except at low and high temperature extremes. However, the $T_\perp$ values due to each component could not be resolved. $T_\parallel$ was also used as a measure of the amplitude of motion of the spin-label.

^{31}P NMR. ^{31}P NMR spectra were obtained on a Bruker WH-90 spectrometer operating at 36.4 MHz. All broad-band proton-decoupled spectra were recorded over a 10000-Hz sweep width in 4K data points (0.208-s acquisition time). Spectra were accumulated in 10000 scans using a pulse width of 5 μs (~45° plus angle). External H_3PO_4 was used as a reference. Probe temperatures were maintained to within ±1 °C by a Bruker B-ST 100/700 variable temperature unit and were calibrated by thermocouple measurements. The sample concentration was 50 mg/mL in D_2O which contained 2 mM Hepes buffer, 10 mM NaCl, and 0.1 mM EDTA. The field/frequency lock was provided by the deuterium signal of D_2O.

Results

Phase Transition Temperatures. PE prepared by transphosphatidylation of PC [PE(PC)], bovine white matter EP (84% ether linked), and egg PE all have a main endothermic gel to liquid-crystalline (G → L) phase transition and a secondary endothermic transition at a higher temperature as shown in DSC heating and cooling thermograms in curves a, b, and d, respectively, of Figure 1. The DSC scan of a more oxidized sample of egg PE is also shown in Figure 1c, where the second transition occurs at a lower temperature than that in Figure 1d. The ^{31}P NMR spectrum of PE(PC) was measured at temperatures below and above the temperature of the second transition (63 °C) in order to determine if it is due to the lamellar to hexagonal (L → H) phase transition as shown

Table I: Temperatures and Enthalpies of Gel to Liquid-Crystalline and Lamellar to Hexagonal Phase Transitions of PE from Different Sources[a]

	G → L			L → H		
	T_c (°C)	ΔH (kcal/mol)	ΔS [cal/(mol K)]	T_h (°C)	ΔH (kcal/mol)	ΔT (T_h-T_c)
egg PE	11.3			32–45[b]		20–34
PE(PC)	19.8	6.65 ± 0.20		63	0.70 ± 0.03	43.2
plasmalogen	3			18		15.0
erythrocyte PE	~10[c]			~10[c]		0
rat liver microsomal PE	ND[h]			5[d]		
soy PE	ND			~−30[f]		
E. coli PE	62[c]			62[c]		0
rat liver inner mitochondrial membrane PE	ND			10[g]		
DOPE	−10[e]			~12[c]		22
DEPE	38[e]			~55[c]		17
DPPE	63.7	6.93 ± 0.45	20.6			
DHPE	69.1	7.49 ± 0.35	21.9	88	1.22 ± 0.11	18.9
HPPE	66	7.58 ± 0.45	22.4	102	0.75 ± 0.10	36
DMPC	25	5.6 ± 0.15	18.8			
DPPC	41.5	8.65 ± 0.20	27.5			
DHPE (pH 1)	71.5	7.67 ± 0.75	22.3			
DHPE (pH 12.3)	45.6	9.14 ± 0.35	28.7			

[a] All at pH 7.4 except where noted. [b] We have found that this temperature decreases with the degree of oxidation of the PE. [c] Cullis & de Kruijff (1978a), DSC and ^{31}P NMR. [d] de Kruijff et al. (1980), ^{31}P NMR. [e] van Dijck et al. (1976), DSC. [f] Cullis & de Kruijff (1978b), ^{31}P NMR. [g] Cullis et al. (1980), ^{31}P NMR. [h] ND = not determined.

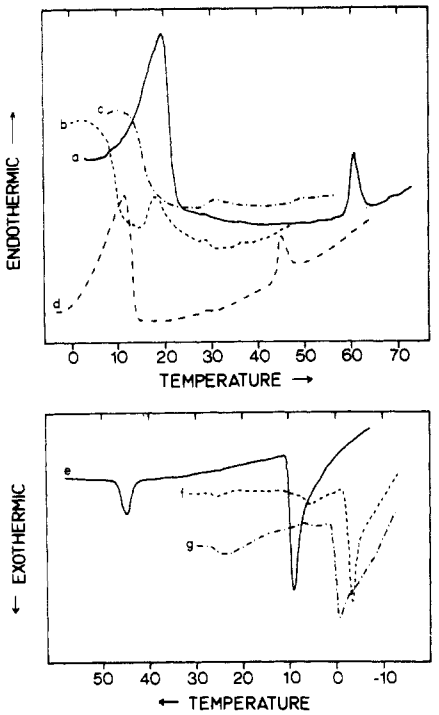


FIGURE 1: Differential scanning calorimetry thermograms on heating (upper panel) and cooling (lower panel) of (a and e) PE prepared from egg PC, (b and f) bovine white matter EP (84% ether linked), (c and g) egg PE which had a trace of yellow color, and (d) egg PE which was completely colorless. Heating and cooling rates were 10 °C min.

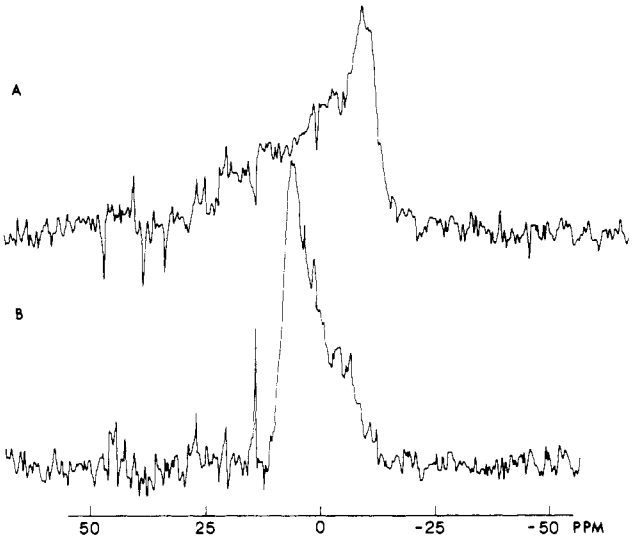


FIGURE 2: ^{31}P NMR spectra at 36.4 MHz of aqueous dispersions of PE prepared from egg PC at (A) 29.3 and (B) 64.9 °C. See Materials and Methods for experimental parameters.

for egg PE (Cullis & de Kruijff, 1978a). The spectrum at 29.3 °C (Figure 2A) is characteristic of the lamellar phase while that at 64.9 °C (Figure 2B), with reversed sign and reduced magnitude of the chemical shielding anisotropy by a factor of two, is characteristic of the hexagonal phase (Cullis & de Kruijff, 1978a). By analogy, it can be concluded that the second transition for ethanolamine plasmalogen is probably also due to the L → H transition (Figure 1b). The variations in temperatures of the G → L and L → H transitions and the temperature intervals between them for the three lipids and for other types of PE, which have been reported elsewhere, are given in Table I.

In order to understand the contribution of fatty acid length and unsaturation on the temperature of the L → H transition, the fatty acid compositions which have been reported in the literature for these types of PE were compiled and are shown in Table II. There is an inverse correlation between the temperature of the L → H transition and the ratio of polyunsaturated to saturated plus monounsaturated fatty acids in PE. An exception is ethanolamine plasmalogen, which has a relatively low content of polyunsaturated fatty acids but goes into the hexagonal phase at a temperature characteristic of a lipid with twice as much polyunsaturated fatty acid. This suggested that either the double bond between the first and second carbons of the alk-1-enyl chain or the ether linkage of this chain to the 1 position of glycerophosphoethanolamine destabilizes the lamellar phase. [The high temperatures of the G → L and L → H transitions of *E. coli* PE are due to its high content of cyclopropane fatty acids (Cullis & de Kruijff, 1978a).]

The contribution of the ether linkage to the low temperature of the L → H transition of ethanolamine plasmalogen was

Table II: Fatty Acid Content of PE from Different Sources

	% of total													polyunsaturated/ saturated + monounsaturated
	16:0	16:1	18:0	18:1	18:2	20:1	20:3	20:4	22:2	22:4	22:5	22:6	other	
egg PE[a]	18		30	18	9.6			15.9		1.9		6.9		0.52
PE(PC)[b]	35		13.5	34	17.5									0.21
bovine white matter EP[c]	14.2		14.9	48.8	0.3	6.6	0.8	3.7	1.3	6.2	0.4	1.3		0.27
human erythrocyte PE[a]	19		13	22	7		1.2	19		5	2.7	3.5	8.7	0.71
rat liver microsomal PE[d]	22.6	2.3	23.4	9.8	10.3			23.1				7.2		0.70
soy PE[e]	23.5		2.8	9.9	59								5.0	1.63
rat liver inner mitochondrial membrane PE[d]	24.8	1.8	27.7	8.8	4.7		0.4	23.8				7.5	0.5	0.57

[a] Cullis & de Kruijff (1978a). [b] Vogel & Bierman (1967). [c] This lipid has 77% plasmalogen; Sun & Horrocks (1970), total of acyl and alk-1-enyl. [d] Colbeau et al. (1971). This lipid has only 3.4% plasmalogen PE in contrast to heart mitochondria (Getz et al., 1968). [e] Cullis & de Kruijff (1978b).

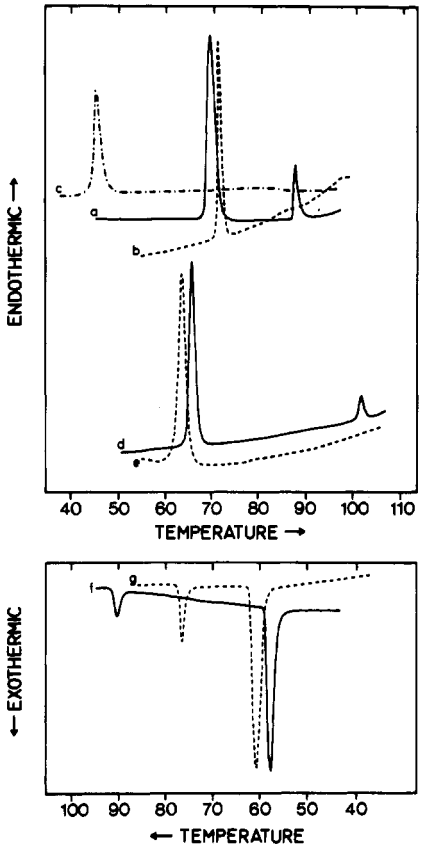


FIGURE 3: Differential scanning calorimetry thermograms on heating (upper panel) and cooling (lower panel) of (a and g) DHPE at pH 7.4, (b) DHPE at pH 1, (c) DHPE at pH 12.3, (d and f) HPPE at pH 7.4, and (e) DPPE at pH 7.4. Heating and cooling rates were 10 °C/min.

investigated by using the synthetic lipids DHPE (dialkyl-GPE), DPPE (diester-PE), and HPPE (1-alkyl-2-acyl-GPE). DSC thermograms of these lipids are shown in Figure 3. Two transitions were observed at 69.1 and 88 °C for DHPE (Figure 3a) and at 66 and 102 °C for HPPE (Figure 3d) while DPPE (Figure 3e) gave only a single transition up to 111 °C, at which point the sample pans ruptured due to water vaporization. The two transitions were also observed on cooling for DHPE and HPPE (Figure 3g,f). When DHPE was studied at pH 1 and 12.3, only single transitions were observed at 71.5 and 45.6 °C, respectively (Figure 3b,c). This result suggests that the higher temperature transition at pH 7.4 is the L → H transition, since PE does not go into the hexagonal phase at high and low pHs (Cullis & de Kruijff, 1978a). The effects of pH on the main transition are in agreement with published results on other types of PE (Eibl, 1977). The ^{31}P NMR spectra of

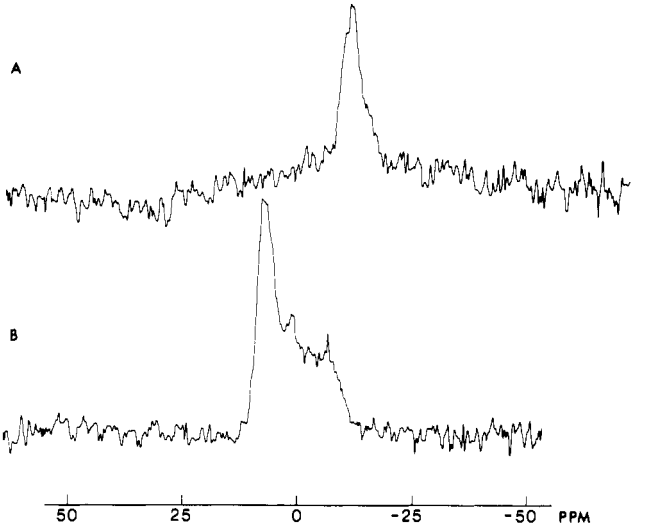


FIGURE 4: ^{31}P NMR spectra at 36.4 MHz of aqueous dispersions of DHPE at pH 7.4 at (A) 77 and (B) 90 °C. See Materials and Methods for experimental parameters.

DHPE at 77 and 90 °C at pH 7.4 (Figure 4A,B) confirmed confirmed that at 90 °C it is in the hexagonal phase. It has also been reported recently that small-angle X-ray diffraction lines of DHPE at 90 °C indicate the existence of a hexagonal phase (Harlos & Eibl, 1980b). By analogy, the second transition at 102 °C for HPPE is probably also due to the L → H transition. Scans of other lipids and buffer blanks had no transition or absorption of energy at this temperature, indicating that this transition is not due to vaporization of water.

The temperatures of the transitions for these three lipids are given in Table I. The T_c of the main transition increases in the order DPPE < HPPE < DHPE in contrast to reported results for analogues of PC where the 1-alkyl-2-acyl form had the lowest T_c (Lee & Fitzgerald, 1980). Although the ether linkage increases the T_c of the G → L transition, it lowers the T_h of the L → H transition. The T_h of the L → H transition increased in the order DHPE < HPPE ≪ DPPE (greater than 111 °C). Thus, the ether linkage destabilizes the lamellar phase.

The enthalpy, ΔH, of the G → L transition was similar for all three analogues of PE and is also similar to ΔH of this transition for DPPC (Table I).[2] ΔH of DHPE also did not

[2] The ΔH values reported here for DMPC, DPPC, and DHPE are similar (within experimental error) to those reported elsewhere (Wilkinson & Nagle, 1981; Vaughan & Keough, 1974) although our value for DPPE is less than the value of 8.8 kcal/mol reported by these two groups.

Table III: Thermodynamic Parameters of EP Transitions Obtained from Both Heating and Cooling Scans

	heating				cooling					
	G → L		L → H		H → L			L → G		
	T_c (°C)	ΔH (kcal/mol)	T_h (°C)	ΔH (kcal/mol)	T_h (°C)	T_h[a] cor (°C)	ΔH (kcal/mol)	T_c (°C)	T_c[a] cor (°C)	ΔH (kcal/mol)
PE(PC)	19.8	6.64 ± 0.20	63	0.70 ± 0.03	47.2	57	0.85 ± 0.2	10	19.8	5.80 ± 0.15
DHPE	69.1	7.70[b] ± 0.14	88	1.14[b] ± 0.07	76.8	84.4	1.32 ± 0.08	61.5	69.1	7.81 ± 0.14

[a] At the heating and cooling rate used (10 °C/min), there is normally 7–10 °C hysteresis in the T_c. The difference between the T_c of the G → L and L → G transitions was assumed to represent instrumental hysteresis and was added on to the T_h of the H → L transitions to obtain the corrected T_c and T_h values. The difference between the T_h of the L → H transition and the corrected T_h of the H → L transition should indicate true hysteresis in phase behavior. [b] ΔH values are different from those in Table I because only those samples for which ΔH was obtained on cooling are averaged in this table while the average in Table I is for data obtained in three sets of measurements.

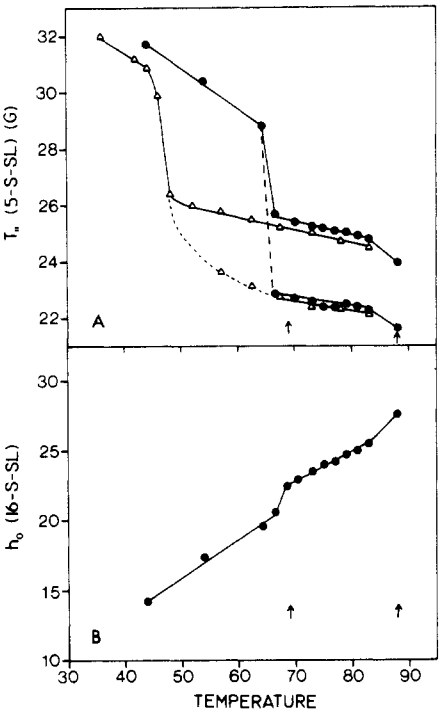


FIGURE 5: ESR spectral parameters of (A) 5-S-SL and (B) 16-S-SL in DHPE (●) and DMPE (△). $T_{\parallel}$ values of both components in the spectra of 5-S-SL above the T_c of DMPE and DHPE are plotted. Arrows indicate the T_c and T_h of the G → L and L → H transitions of DHPE determined by DSC (Table I).

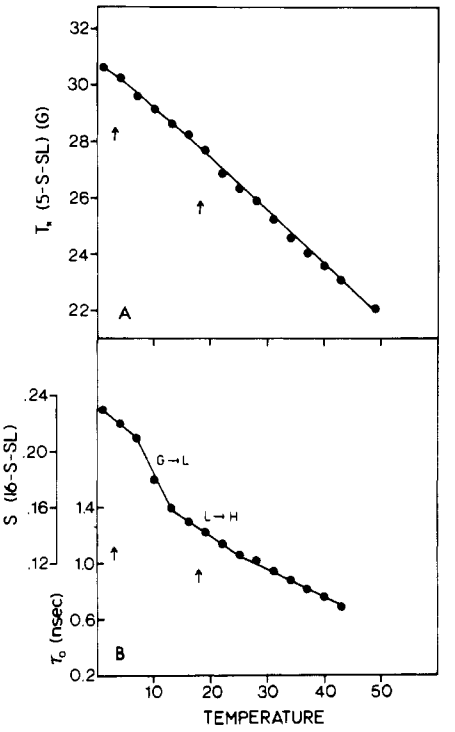


FIGURE 6: ESR spectral parameters of (A) 5-S-SL and (B) 16-S-SL in ethanolamine plasmalogen. Arrows indicate the T_c and T_h of the G → L and L → H transitions of DHPE determined by DSC (Table I). These transitions appear to occur at the increases in slope of the plot of τ_0 against temperature. The overlap of the scales for S and τ_0 was chosen so that samples with a particular S value at intermediate temperatures have a corresponding τ_0 value as indicated by the scale.

vary much with pH. An increase in the entropy, ΔS, is responsible for the lower T_c of DPPC and for the lower T_c of DHPE at pH 12.3 relative to that of PE at neutral pH. However, the data cannot distinguish if it is small differences in ΔH or ΔS which determine the small differences in T_c's among DHPE, DPPE, and HPPE (Table I).

The ΔH of the L → H transition is 10–15% of that for the G → L transition and is greater for DHPE than for HPPE (Table I). There was a hysteresis in the ΔH and T_h of this transition obtained from heating and cooling scans. The ΔH of the reverse H → L transition, obtained from DSC cooling scans, was greater than that for the L → H transition, obtained from heating scans, for both DHPE and PE(PC) (Table III). [The G → L transition had a similar ΔH to the reverse L → G transition for DHPE but not for PE(PC).]

The temperature difference between the G → L and L → H transitions was greater on heating than on cooling. If it is assumed that the difference in T_c of the G → L and L → G transitions is the instrumental hysteresis normally observed at this heating rate (7–10 °C at a heating rate of 10 °C/min), then the T_h of the H → L transition can be corrected for instrumental hysteresis and compared to the T_h of the L → H transition to determine if there is a sample hysteresis. As shown in Table III, the corrected T_h values indicate that the lipid undergoes the reverse transition back into the lamellar phase at a lower temperature than that at which it goes into the hexagonal phase. Similar hysteresis has been reported recently for the L → H and H → L transitions of DLPE[3] (Wilkinson & Nagle, 1981).

Fatty Acid Spin-Label Motion. Changes in fatty acid spin-label motion during the G → L and L → H transitions of DHPE, DMPE, ethanolamine plasmalogen, and PE(PC) were monitored by using 5-S-SL and 16-S-SL as shown in Figures 5–7, respectively. The transition temperatures found by DSC are indicated by arrows. The spin-label signal was rapidly reduced at the temperature of the L → H transition in egg PE probably due to increased oxidation of the lipid in the hexagonal phase. Two components were present in the

[3] It was recently shown that PE containing saturated fatty acids of only 12 carbons in length gives a second transition at 43 °C by DSC at slow heating rates which is probably due to the L → H transition (Wilkinson & Nagle, 1981).

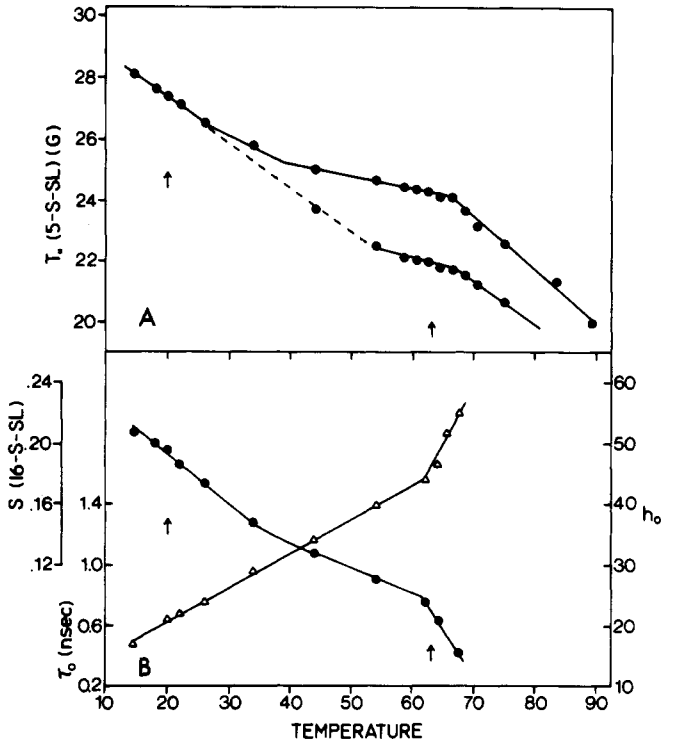


FIGURE 7: ESR spectral parameters of (A) 5-S-SL and (B) 16-S-SL in PE prepared from PC. For 16-S-SL, both S and τ_0 (●) and h_0 (△) are plotted. Other details are as described for Figure 6.

spectra of 5-S-SL above the G → L transition temperature for all types of EP except ethanolamine plasmalogen. $T_{\parallel}$ values but not $T_{\perp}$ of both components could be resolved and are shown in Figures 5–7. The values of $T_{\parallel}$ for both components had parallel behavior with temperature. Their origin is not known, but since they occur in DMPE (Figure 5A) and in phosphatidylcholine and sphingomyelin (Stuhne-Sekalec & Stanacev, 1977), they are not related to the occurrence of the hexagonal phase. The order parameter S and motional parameter τ_0 of 16-S-SL were measured at low and high temperatures, respectively, except for DHPE where another more isotropic component (due to spin-label in water) rendered these measurements inaccurate. In this case, the height of the center line, h_0, was measured and was sensitive to the increase in lipid motion during both phase transitions (Figure 5).

The G → L transition can be observed as a sharp increase in motion of both 5-S-SL and 16-S-SL as expected for all lipids except ethanolamine plasmalogen where the transition could not be detected with 5-S-SL due to the width of this transition and the small temperature difference between it and the L → H transition (Figures 5–7). A second smaller increase in motion of both spin-labels could also be observed at the L → H transition of all the lipids except ethanolamine plasmalogen.

The order and motional parameters of the fatty acid spin-labels in DPPE and DHPE were similar at temperatures where both are in the liquid-crystalline lamellar phase, as shown in Figure 8 at a temperature of 78 °C. However, 12-S-SL could be incorporated into DPPE in the liquid-crystalline phase but not into DHPE, suggesting closer packing in DHPE. These spectral parameters were also similar in both lipids in the gel phase (not shown). At temperatures where DHPE is in the hexagonal phase and DPPE is in the lamellar phase, the order and motional parameters are much lower for DHPE (indicated in Figure 8 at a temperature of 93 °C) due to the greater fluidity of the hexagonal phase as discussed above.

The fluidity gradient [change in fluidity with increasing position (n) from the carboxyl group] is compared in Figure

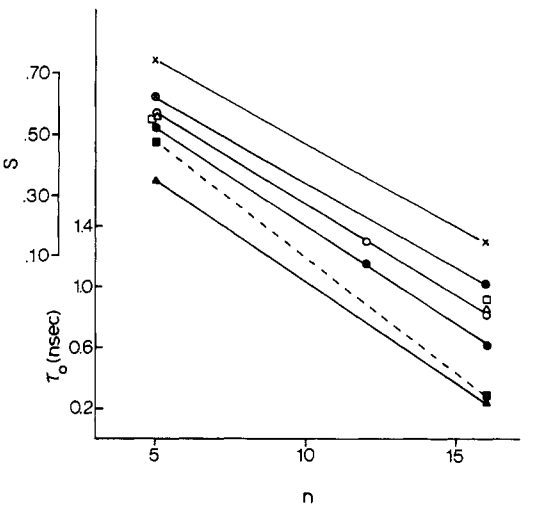


FIGURE 8: Fluidity gradient of fatty acid spin-labels in different types of ethanolamine phospholipids at different temperatures. S and τ_0 are plotted against n, the carbon to which the spin-label is bound, numbered from the carboxyl end. DPPE at 78 (○) and 93 °C (●); DHPE at 78 (△) and 93 °C (▲); PE prepared from PC at 54 (□) and 70 °C (■); ethanolamine plasmalogen at 16 (×) and 28 °C (⊗) (second curve from top).

8 for the liquid-crystalline lamellar phase and the hexagonal phase of DPPE, DHPE, PE(PC), and ethanolamine plasmalogen. This gradient is not necessarily linear as shown, but presentation of the data in this manner serves to compare the change in fluidity in the interior of the bilayer relative to that near the polar head group for the different lipids. The fluidity gradients for the lamellar liquid-crystalline phases of DPPE (78 °C), DHPE (78 °C), PE(PC) (54 °C), and ethanolamine plasmalogen (16 °C) are nearly parallel. The exact values of the order and motional parameters depend on temperature and cannot be compared for the different lipids. The fluidity gradients in the hexagonal phases of DHPE (93 °C) and ethanolamine plasmalogen (28 °C) are also nearly parallel to the fluidity gradients of the lamellar phases. In contrast, the fluidity gradient of the hexagonal phase of PE(PC) is much steeper than that of the lamellar phases and the hexagonal phases of the ether-containing DHPE and ethanolamine plasmalogen.

Discussion

A comparison of fatty acid composition and temperature of the L → H transition for the ethanolamine phospholipid fraction isolated from a number of different tissues suggested that an increase in the percentage of polyunsaturated fatty acids destabilizes the lamellar phase. However, T_h for the EP fraction from bovine white matter, which is 84% ether linked, was lower than might be expected from its low content of polyunsaturated fatty acids. Although a major fraction of white matter plasmalogen contains 18:1 chains in the 1 and 2 positions, its phase transition temperatures are different from those of DOPE; both T_c and T_h are higher while the temperature difference between T_c and T_h is less than that in DOPE (Table I), suggesting that it is not only the high 18:1 content which lowers T_h relative to T_c of white matter EP. This suggested that either the double bond between the first and second carbons of the alk-1-enyl chain or the ether linkage of this chain to the 1 position of glycerophosphoethanolamine also contribute to destabilization of the lamellar phase.

The latter possibility was tested by using synthetic analogues of PE of the diacyl, dialkyl, and 1-alkyl-2-acyl types. The ether linkage resulted in an increase in the G → L transition tem-

perature as reported earlier for analogues of PE, PC (Vaughan & Keough, 1974; Lee & Fitzgerald, 1980), and PA (Blume & Eibl, 1979; Harlos et al., 1979) but a decrease in the L → H transition temperature of analogues of PE. PA and PE can interact intermolecularly by hydrogen bonding, which probably accounts for their relatively high phase transition temperatures compared to that of PC (Jacobson & Papahadjopoulos, 1975; Eibl, 1977; Eibl & Blume, 1979; Eibl & Woolley, 1979; Boggs, 1980). The increase in the G → L transition temperature T_c caused by the ether linkage in these lipids suggests that the ether linkage promotes closer packing of the lipids as well as an increase in the strength of the intermolecular hydrogen bonding for analogues of PE as has been shown for PA (Blume & Eibl, 1979; Harlos et al., 1979).

The lamellar phase is destabilized by a large molecular volume of the hydrocarbon chains caused by unsaturated fatty acids combined with a closely packed head-group structure. Cullis & de Kruijff (1978a,b, 1979) showed that in the case of diacyl-PE, unsaturated fatty acids are required[3] for a hexagonal transition to occur at temperatures below 100 °C. An increase in the content of polyunsaturated fatty acids lowers the temperature of the L → H transition. The intermolecular hydrogen-bonding interaction must also be involved since a pH at which both the phosphate and amine of the head group are ionized is also required. As the temperature is raised, the increased volume of the hydrocarbon region and the lack of expansion at the head-group region can only be accommodated in the hexagonal phase. The fact that DEPE, with a single trans double bond can go into the hexagonal phase at 55 °C while DSPE and even DMPE cannot do so below 100 °C suggests that only a small increase in the volume of the hydrocarbon region is sufficient to destabilize the lamellar phase. Similarly, the results presented here indicate that a small increase in packing at the polar head-group region and in the strength of the intermolecular hydrogen bonding due to the ether linkage can also destabilize the liquid-crystalline lamellar phase even when the hydrocarbon chains are saturated.

The enthalpy of the L → H transition is low, 10–15% of the enthalpy of the G → L transition, indicating that the L → H transition could occur readily upon a slight perturbation as suggested earlier (Boggs & Hsia, 1973; Cullis & de Kruijff, 1978a). The lower T_c found for the reverse H → L transition suggests that the hexagonal phase can undergo the transition back to the lamellar phase less easily, indicating a kinetic barrier to the H → L transition.

A comparison of fatty acid spin-label motion in the lamellar phase of Na^+-cardiolipin with that in the hexagonal phase of Ca^{2+}-cardiolipin showed that the hexagonal phase was more ordered near the polar head group and less ordered further toward the interior of the bilayer than the lamellar phase (Boggs & Hsia, 1973). The present results with PE indicate an increase in amplitude of motion near the polar head group and in the interior of the bilayer upon the transition to the hexagonal phase. However, in the case of PE(PC), the increase in motion and disorder is greater in the interior of the bilayer than near the polar head group so that the fluidity gradient is steeper in the hexagonal phase than in the lamellar phase, as in Ca^{2+}-cardiolipin. An increase in disorder of the hexagonal phase compared to the lamellar phase of DEPE deuterated at the 9 and 10 positions has also been reported in a ^2H NMR study (Gally et al., 1980).

The fluidity gradients in the hexagonal phase of DHPE and ethanolamine plasmalogen are similar to those of the lamellar phase however, suggesting that the ether linkage does not result in greater expansion of the hydrocarbon region (relative to DPPE) at higher temperatures. This supports the suggestion that the destabilizing effect of the ether linkage on the lamellar phase is due instead to a small increase in the strength of intermolecular interactions at the head-group region.[4] Indeed, the ether linkage has a much greater effect on the L → H transition than on the G → L transition, indicating that intermolecular hydrogen-bonding interactions have an even greater effect on the stability of the hexagonal phase than on the stability of the lamellar gel phase.

The ether linkage of the alk-1-enyl and alkyl chains in the 1 position of ethanolamine plasmalogens may therefore contribute to the low T_h of the L → H transition of this lipid relative to the T_c of its G → L transition and its low content of polyunsaturated fatty acids. The double bond between the first and second carbons of the alk-1-enyl chain may of course also contribute to destabilization of the lamellar phase. This cis double bond would be expected to increase the molecular volume of the hydrocarbon region, which would be additive with the destabilizing effect of closer packing in the head-group region due to the ether linkage.

The presence of a high content of EP with the ether linkage in myelin suggests that the hexagonal phase might have a physiological role in this membrane as has been suggested for other membranes. The ethanolamine plasmalogen content may be decreased in the demyelinating disease multiple sclerosis (Yanagihara & Cummings, 1969), although this was not substantiated in a recent study (Gopfert et al., 1980). This latter study reported a reduced content of polyunsaturated fatty acids in EP of multiple sclerosis myelin however. Myelin ethanolamine plasmalogen content has also been reported to be less than normal in the myelin-deficient Jimpy (Nussbaum et al., 1969) and Quaking mice (Dawson & Clarke, 1971; Gregson & Oxberry, 1972), suggesting a role of this lipid in myelination.

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[4] It has been reported recently that the Ca^{2+} complex of diether analogue of phosphatidylglycerol (DTPG) is hexagonal at 90 °C (Harlos & Eibl, 1980a) while the Ca^{2+} complex of the diester analogue of equal hydrocarbon chain length (DMPG) is lamellar up to 100 °C (Farren & Cullis, 1980). This suggests that the ether linkage may allow closer packing in the head-group region of Ca^{2+}-linked dimers of PG as in PE.

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Polynucleotide Melting in Heavy and Light Water†

R. D. Blake

ABSTRACT: The effect on the melting temperature, t_m, of transferring the helix–coil (h → c) transition from H_2O to D_2O has been examined for a wide variety of nucleic acid helixes consisting of either two or three strands twisted into either the A- or B-like secondary structure. Very high precision in measuring the t_m was achieved by monitoring the transition by the absorbance difference approximation method with as many as six different helical species mixed together in the same solution. The average standard deviation in the t_m is ±0.08 °C and ±0.11 °C for the δt_m between solvents. In all cases the melting temperature in D_2O is higher than in H_2O. The δt_m decreases with increasing Na^+ counterion concentration in approximate proportion to the increase in unit transition enthalpy; therefore, most studies were concentrated on the effect of transfer in the moderately low ionic condition of 0.018 M Na^+. At this $[Na^+]$, δt_m for transfer ranged from −0.51 °C for the G·C base pair in DNA to −2.05 °C for the melting of the three-stranded dA·2rU helix and showed a strong dependence on the conformation of the helix. The δt_m for helixes with A-like structures is twice that for B structures. We find no evidence for lattice support of the helix by "ice-like" water nor any effect on δt_m by the chemical nature of the base or pentose. It is proposed that the solvent effect is due to differences in dielectric saturation of Na^+ between the two solvents resulting in a significantly higher dielectric constant in D_2O than in H_2O in the neighborhood of the nucleic acid, where the concentration of Na^+ may exceed 3 M. The difference in free energy for transferring the h → c transition from H_2O to D_2O increases linearly with the difference in $[Na^+]$ condensed to the helix and coil for the different helixes examined in this study. This variation is 40% greater for the A family than for the B family of helixes, reflecting the difference in charge density between these two conformations.

The nature of the interaction of deoxyribonucleic acid (DNA)[1] with water is important to the study of the structure of DNA, since water is necessary in preserving the integrity of the helix. The problem is complex, however, and there still is not a good description of interactions at the molecular level. Certainly some water is firmly bound through hydration–solvation (Tunis & Hearst, 1968). It is less certain how water

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[1] Abbreviations used: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; bp, base pair; EDTA, ethylenediaminetetraacetic acid.

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