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Deuterium NMR study of the effect of *n*-alkanol anesthetics on a model membrane system

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The effects of 25 mol% incorporation of two anesthetics, 1-octanol and 1-decanol, on a deuterated, saturated phospholipid in 50 wt% aqueous multilamellar dispersions have been studied by ^{2}H-NMR spectroscopy and differential scanning calorimetry (DSC). The phospholipid used is *sn*-2 substituted '[$^2H_{31}$]-palmitoylphosphatidylcholine' (PC-d_{31}). DSC thermograms demonstrate that PC-d_{31} has phase behavior qualitatively similar to that of dipalmitoylphosphatidylcholine, with a pretransition at 31°C and a main gel to liquid crystalline transition at 40°C. Analysis of the temperature-dependent ^{2}H-NMR spectra in terms of the first moment, which is extremely sensitive to the phospholipid phase, shows that 1-octanol and 1-decanol depress and broaden the main transition. This is confirmed by DSC, which shows that the pretransition is eliminated by the 1-alkanols. The carbon-deuterium bond order of the phospholipid deuterated acyl chains, in the presence and absence of 1-alkanols, was determined from deuterium quadrupolar splittings. Spectra were analyzed using the depaking technique. A 1-alkanol concentration of 25 mol% had no significant effect on the profile of the carbon-deuterium bond order parameter S_{CD} along the phospholipid acyl chain at 50°C. Thus, it appears that the liquid crystalline phase is able to accommodate large amounts of linear anesthetic molecules without substantial effect on molecular ordering within the membrane bilayer. Preliminary results show that the transverse relaxation rates of the acyl chain segments are significantly decreased by the presence of 1-octanol or 1-decanol.

Introduction

The mode of action of general anesthetics has been a subject of discussion for decades and the question remains unresolved [1,2]. The 'lipid' theories of general anesthesia postulate that anesthetics perturb the lipid bilayer, leading to changes in function of excitable membrane proteins. For a review of lipid theories see Ref. 3. These lipid perturbations may be classified as follows: (i) altered phase behavior of the lipids [4–6]; (ii) changes in the organizational order and/or the rate of motion of the lipid acyl chains [7,8]; (iii) changes in bilayer thickness and/or surface tension [9,10].

Investigation of the effects of long-chain alcohols on membranes provides a particularly useful approach for the study of the mechanism of

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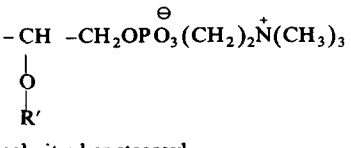
anesthesia [11]. The anesthetic potency (determined in tadpoles) of saturated 1-alkanols increases with increasing chain length up to 1-dodecanol, after which it decreases sharply [12]. The changes induced by members of the *n*-alkanol family in lipid bilayer phase transitions and acyl chain order and mobility have been studied using fluorescence [5,8,13], ESR [7,14–17], DSC [4,18–20] and turbidity measurements [21]. Long-chain alcohols can either raise or lower the main gel to liquid crystalline transition temperature of the phospholipid depending on the length of the alcohol and on the particular lipid used. 1-Alkanols are found to decrease the ESR order parameter of spin-labelled phosphatidylcholine in dipalmitoylphosphatidylcholine/ cholesterol and egg yolk phosphatidylcholine/cholesterol multilamellar liposomes and of spin-labelled stearic acid in synaptosomal plasma membrane preparations. The degree of disordering is proportional to the concentration of anesthetic in the membrane and the effect is small with an ESR order parameter change < 0.1. The degree of disordering also depends upon the lipid composition [22]. 1-Alkanols decrease the steady-state depolarization of the fluorescent probes 1,6-diphenylhexatriene and perylene in microsomal and mitochondrial membranes [8,13]. The depolarization change is attributed to increased motion of the probe and, hence, of the lipid acyl chains, due to the presence of 1-alkanol.

The fluorescence and ESR experiments utilize highly sterically perturbing chemical reporter molecules. These molecules have much lower solubility in gel than liquid crystalline phase, hence, will tend to partition in systems where the two phases coexist. We, on the other hand, report ^{2}H-NMR data on the effect of two of the more potent alcohol anesthetics, 1-octanol and 1-decanol, on aqueous multilamellar dispersions of *sn*-2 substituted '[$^2H_{31}$]palmitoylphosphatidylcholine' (PC-d_{31}).

PC-d_{31}:



R = palmitoyl or stearoyl

R′ = [$^2H_{31}$]palmitoyl

Deuterium is a sterically non-perturbing probe and allows chain behavior within the bilayer to be more accurately monitored in both the gel and liquid crystalline phases. Moreover, in the liquid crystalline phase there is a straightforward relationship between the ^{2}H-NMR powder pattern spectrum and the order parameter of the C–^{2}H bond, S_{CD}. Moments of the ^{2}H spectra were calculated since moments are particularly useful in following the phase transition behavior of acyl chain perdeuterated lipids in the bilayer [23]. Finally, DSC measurements were performed on the PC-d_{31}/1-alkanol mixtures. DSC confirms that the deuterated phospholipid shows qualitatively the same thermotropic profile as its non-deuterated analog and allows correlations to be made between ^{2}H-NMR spectral changes and bulk lipid phase transitions.

Materials and Methods

[$^2H_{31}$]Palmitic acid was a gift from W. Dale Treleaven. Egg yolk lysophosphatidylcholine and deuterium depleted water were purchased from Sigma Chemical Co. Sigma lysophosphatidylcholine contains 66–68% palmitic acid, 24–26% stearic acid, and 6–10% other saturated acids. *n*-Octanol was obtained from Matheson, Coleman and Bell and *n*-decanol from Aldrich Chemical Co.

Synthesis of sn-2 [$^2H_{31}$]palmitoylphosphatidylcholine (PC-d_{31})

Egg yolk lysophosphatidylcholine (3 g) and 1.67 g of [$^2H_{31}$]palmitic acid were condensed with 1,1′-carbonyldiimidazole [24,25] with the following modifications: the reaction was heated by an oil bath at 75–80°C for 2.5 h; the crude product was dissolved in petroleum ether/ethanol (28 ml/4 ml) and precipitated by addition to cold acetone (100 ml); the precipitation step was repeated once; the product was purified on a 60×4 cm Baker Analyzed silica gel column, 60–200 mesh, and eluted with chloroform/ methanol/ water (70 : 26 : 4, v/v).

The mass spectrum of PC-d_{31} is characteristic of the proposed structure, with two sets of lines centered at $M - 183$ (M minus $N(CH_3)_3$-$(CH_2)_2$-OPO_3 : phosphocholine) = 580.1 and 608.1: *m/e*: 578 (4.5%), 579 (12.8%), 580 (14.7%), 581 (9.3%),

582 (4.0%) corresponding to phospholipid with palmitoyl *sn*-1 chains and *m/e*: 606 (1.2%), 607 (2.9%), 608 (3.5%), 609 (2.9%), 610 (1.3%) corresponding to phospholipid with stearoyl *sn*-1 chains.

Multilamellar liposomes

Multilamellar dispersions of PC-d_{31} were prepared by adding approx. 200 mg of PC-d_{31} to approx. 0.20 ml of deuterium-depleted water and thoroughly mixing using a spatula at about 50°C. The PC-d_{31}/1-octanol samples were prepared by dissolving approx. 200 mg of PC-d_{31} in approx. 4 ml of chloroform, evaporating to near dryness with dry N_2, and further drying under high vacuum overnight. Deuterium depleted water ($\approx$ 0.20 ml) was added at about 50°C. Vigorous mixing using a spatula and Vortex mixer ($\approx$ 45 min) produced a homogeneous sample. Sufficient 1-octanol was added at the hydration step to bring the PC-d_{31}/1-octanol molar ratio to 3:1. For the PC-d_{31}/1-decanol sample, the 1-decanol was codissolved with the lipid in $CHCl_3$ prior to vacuum pumping [5]. Samples were stored at -18°C until NMR spectra were determined.

NMR spectroscopy

^{2}H-NMR spectra were recorded at 38.8 MHz using a home-built spectrometer and a 5.9 T superconducting magnet. Sample temperature in the home-built probes was controlled to an accuracy of $\pm$0.5 deg. C. The samples were allowed to equilibrate for a minimum of 20 min at a given temperature and spectra were taken only as a function of increasing temperature to avoid hysteresis effects. Spectra were measured using the quadrupolar echo pulse sequence 90° | 0°-τ-90° | 90°-T. Unless otherwise indicated in figure legends the spectral parameters were: pulse width = 6.5 μs (90° flip angle); sweep width = $\pm$250 kHz; line broadening = 50 Hz; data set = 2 K; τ = 75 μs; T = 1.0 or 1.5 s. Data collection was accomplished with an Explorer III digital oscilloscope while Fourier transformation and moment calculations (see below) were performed using a Nicolet BNC-12 computer.

The spectra were symmetrized by zeroing the out-of-phase quadrature channel and reflecting the spectrum about the central (carrier) frequency, resulting in an increase in signal to noise ratio by $\sqrt{2}$.

The calculation of spectral moments is straightforward for ^{2}H-NMR [26]. First moments M_1 are used in this study to minimize systematic errors introduced by loss of intensity in the wings of the spectrum due to the finite pulse width. The first moment is given by

$$M_1 = \frac{\int_0^\infty \omega f(\omega)\, d\omega}{\int_0^\infty f(\omega)\, d\omega} \tag{1}$$

where the integral becomes a sum in practise, and summation commences at the center of the symmetric spectrum. The intensity at a given frequency displacement ω from the Larmor frequency is $f(\omega)$.

Depaking, the calculation of the 'aligned' ^{2}H-NMR spectrum from the 'random' powder pattern spectrum [27–29], was accomplished on an IBM 4341 computer using the program contained in Ref. 27. Order parameters S_{CD} were calculated from the depaked spectra which correspond to spectra that would be obtained for a planar membrane whose surface is perpendicular to the applied magnetic field using the relationship [30]

$$\Delta\nu_Q = \frac{3}{2}\left(\frac{e^2qQ}{h}\right) S_{CD} \tag{2}$$

where $\Delta\nu_Q$ is the separation of the quadrupole doublet and e^2qQ/h is the static quadrupolar coupling constant (approx. 168 kHz for a C-^{2}H bond in an alkane [31]).

The C-^{2}H order parameter S_{CD} can be represented as

$$S_{CD} = \tfrac{1}{2}\langle 3\cos^2\theta - 1\rangle \tag{3}$$

where θ is the angle between the C-^{2}H bond and the axis of symmetry for acyl chain reorientation (taken to be the normal to the surface of bilayer membranes), and the angular brackets represent a time average.

Differential scanning calorimetry

DSC traces were measured using a DuPont Instruments Series 99 thermal analyzer fitted with a 910 DSC accessory, with approx. 1–2 mg samples contained in sealed sample pans over the

range −30°C to +70°C with a heating rate of 10 deg. C/min. Measurements of the phase transition temperatures follow the convention in Ref. 18. The phase transition temperatures T_m are accurate to ±0.5 deg. C unless otherwise indicated.

Results

A phospholipid, PC-d_{31}, has been prepared from egg yolk lysophosphatidylcholine by attaching [$^2H_{31}$]palmitic acid as the *sn*-2 chain. PC-d_{31}, then, has the usual egg phosphatidylcholine distribution of acyl chains on the *sn*-1 position: 66–68% palmitoyl (16:0); 24–26% stearoyl (18:0); 6–10% others.

Phospholipid multilamellar dispersions (50 wt% deuterium depleted water) of PC-d_{31} and approx. 75 mol% PC-d_{31}/approx. 25 mol% 1-octanol or 1-decanol have been prepared at about 50°C. Both 1-octanol and 1-decanol have high lipid:water partition coefficients so that in a dispersion of equal weights of lipid and water the amount of alcohol in the aqueous phase is negligible [32].

The DSC trace of a PC-d_{31}/water dispersion is given in Fig. 1a and shows a pretransition at 31°C and a main gel to liquid crystalline transition at 40°C (half-height width approx. 2 deg. C). We have also obtained a DSC trace for 50:50 w/w dipalmitoylphosphatidylcholine/water dispersions (trace not shown) which exhibits a pretransition at 35°C and a main transition at 42°C (half width approx. 2 deg. C) in agreement with literature values [18,33].

Upon the addition of 1-decanol at a concentration in the bilayer of 25 mol% the DSC trace (Fig. 1b) shows that the PC-d_{31} pretransition has vanished and the main transition has been lowered (to 37°C) and broadened (half-height width approx. 4 deg. C). 1-Octanol has a greater effect than 1-decanol on the DSC profile of PC-d_{31} as Fig. 1c shows that the main transition occurs at 29°C with a half-height width of 7.5 deg. C. The pretransition, as seen for the 1-decanol/PC-d_{31}/water system, has been eliminated. These changes in transition temperature upon alcohol incorporation are similar to the results of Eliasz et al. [18] who report a decrease in temperature of 4 deg. C for 25 mol% 1-decanol in dipalmitoylphosphatidylcholine and 15 deg. C for 25% 1-octanol in dipalmitoylphosphatidylcholine.

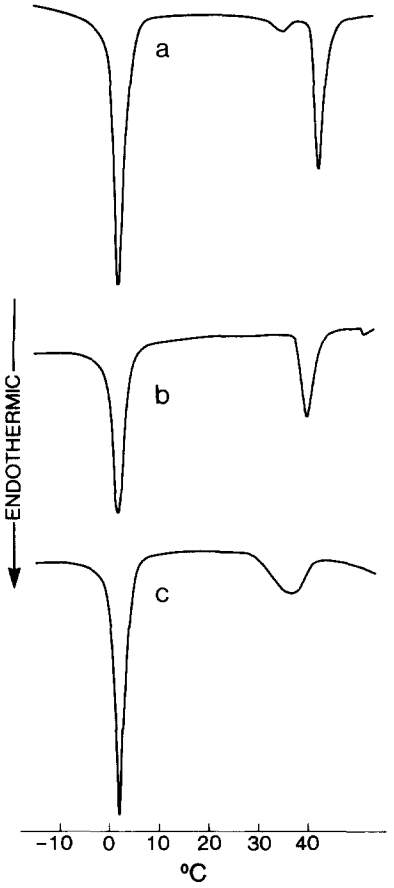


Fig. 1. Differential scanning calorimetry traces: multilamellar dispersions of: (a) 50 wt% PC-d_{31}; (b) PC-d_{31} + 25 mol% 1-decanol; (c) PC-d_{31} + 25 mol% 1-octanol. Scanning rate was 10 deg. C/min over the range −30°C to +70°C.

^{2}H-NMR spectra were measured for the temperature range −15°C to 60°C for 50:50 w/w PC-d_{31}/water and 50:50 w/w PC-d_{31}/water containing 25 mol% 1-octanol. Fig. 2 shows representative spectra for dispersions of PC-d_{31} with and without incorporated 1-octanol. The phase behavior of the lipid samples is such that four distinct spectral shapes are seen. At −15°C (Figs. 2a and 2e) a spectrum containing considerable intensity at ±63 kHz is observed for both samples indicating that a large proportion of the acyl chains are in a rigid state. Figs. 2a and 2e are probably spectra of a metastable lipid state [33–35] and were not studied further. Subsequent experiments

were confined to the temperature range 15°C to 50°C. At 15°C (Figs. 2b and 2f), both samples produce a typical gel phase spectrum with an absence of distinct shoulders and a broad, featureless central region. Such spectra are not simple powder patterns and cannot be analyzed in terms of either a zero or nonzero asymmetry parameter. At 30°C the PC-d_{31} sample (Fig. 2g) is still in the gel phase but the 1-octanol-containing sample (Fig. 2c) is in an intermediate phase between gel and liquid crystal: the broad gel phase component has almost disappeared and sharp edges are beginning to form at ± 24 kHz. When the temperature is raised to 50°C (Figs. 2d and 2h), both samples are in the liquid crystalline phase. This phase is characterized by an axially symmetric powder pattern

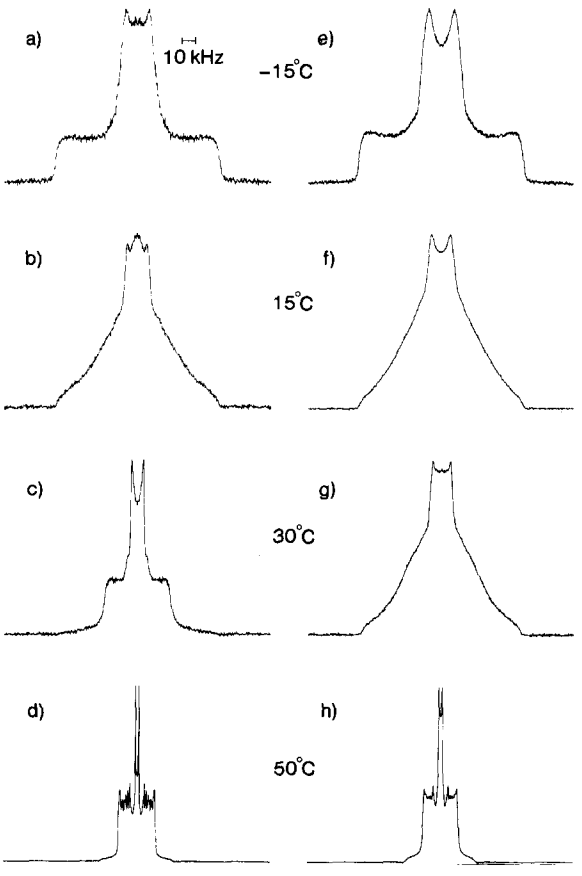


Fig. 2. Temperature dependence of the ^{2}H-NMR spectra of multilamellar dispersions of: (a–d) PC-d_{31} with 25 mol% 1-octanol incorporated; (e–h) PC-d_{31} alone; (d) and (h) sweep width was ± 100 kHz.

^{2}H-NMR spectrum possessing well defined, sharp edges (at ± 13–14 kHz) associated with a plateau in the variation of the C-^{2}H bond order parameter S_{CD} with position along the phospholipid acyl chain. The 1-octanol/PC-d_{31} sample, Fig. 2d, appears to have sharper and more numerous component peaks than Fig. 2h, a feature that is discussed later. The temperature variation of ^{2}H-NMR spectra for the PC-d_{31} sample containing 1-decanol is qualitatively the same as the one containing 1-octanol except the temperature of the gel to liquid crystalline transition occurs at a higher temperature with 1-decanol. Thus, 1-decanol/PC-d_{31}/water dispersions gave a 50°C spectrum virtually identical to Fig. 2d, and a 15°C gel phase spectrum very similar to Fig. 2b. Differences in the ^{2}H-NMR spectra of lipid plus 1-octanol and lipid plus 1-decanol occurred only at temperatures near their respective gel to liquid crystalline phase transitions.

The behavior of 1-octanol/PC-d_{31}/water and PC-d_{31}/water dispersions throughout the gel to liquid crystalline phase transition region, as shown by ^{2}H-NMR, is depicted in Fig. 3. Figs. 3a–3d show the ^{2}H-NMR spectra at 2-deg. C intervals throughout the main transition region of the 1-octanol/PC-d_{31}/water dispersion. The spectral changes are clearly much more gradual than those shown in Figs. 3e and 3f, which occur in the absence of 1-octanol. The phase transition region of a multilamellar dispersion of 1-decanol/PC-d_{31}/water is illustrated in Fig. 4. ^{2}H-NMR spectra at 35, 36 and 37°C indicate that the gel to liquid crystalline transition occurs over a narrower temperature range than in the 1-octanol/PC-d_{31} water dispersion (Fig. 3).

Fig. 5 shows the variation of the first moment M_1 with temperature for PC-d_{31}/water, 1-octanol/PC-d_{31}/water and 1-decanol/PC-d_{31}/water. The uncertainty in the M_1 measurements is small except at the phase transition. Here the first moment depends on the delay time τ between the two 90° pulses of the quadrupolar echo pulse sequence. The values plotted were obtained by extrapolation back to zero delay τ of M_1 recorded as a function of τ. Five τ values in the range 40–125 μs were employed and an uncertainty of not more than 5% was introduced. The dependence of M_1 on τ in the region of the phase

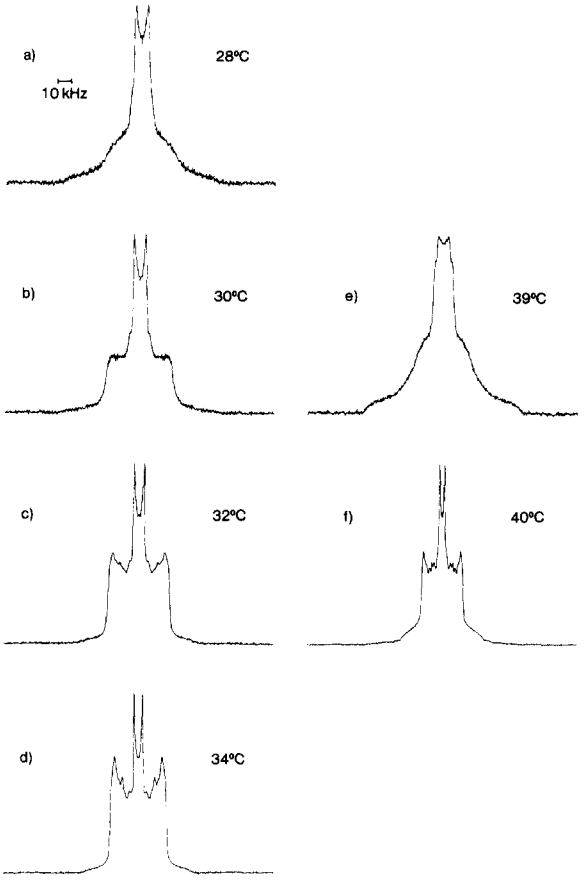


Fig. 3. Changes in ^{2}H-NMR spectra at the main gel to liquid crystalline phase transition: (a–d) PC-d_{31} + 25 mol% 1-octanol; (e–f) PC-d_{31} alone.

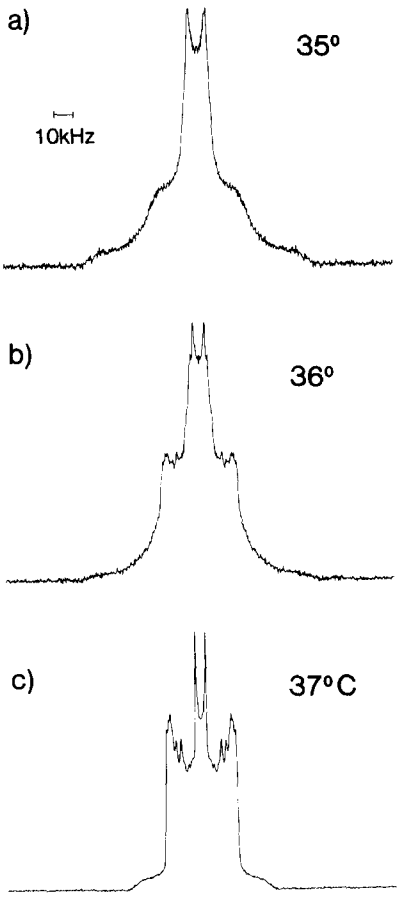


Fig. 4. Changes in ^{2}H-NMR spectra at the main gel to liquid crystalline phase transition for PC-d_{31} + 25 mol% 1-decanol.

transition coincides with a maximum in the relaxation rate $1/T_{2e}$ versus temperature curve. The spectral shape in this region is distorted, with the amount of distortion increasing with increasing τ. The distortion is maximal when $\tau \approx \tau_c$, the ^{2}H motional correlation time [36]. In addition, repeating the measurements with samples which had been stored at −18°C for about 20 weeks gave values of M_1 which agreed to within ±5% of those recorded immediately upon sample preparation.

From Fig. 5 it can be seen that the first moment decreases sharply as the temperature increases through the main gel to liquid crystalline phase transition. For the PC-d_{31}/water this melt occurs abruptly at 40°C, immediately preceded by a region, centered at 31°C, of more gradual decrease which we associate with the pretransition. For 1-octanol/ PC-d_{31}/ water a broad transition centered at 33°C on the M_1 vs. temperature plot is observed. The onset of the transition occurs at about 28°C and it spans about 10 deg. C. The 1-decanol-containing lipid dispersion has a narrower transition (Fig. 5) from approx. 34°C to about 39°C.

The liquid-crystalline spectra observed at 50°C (Figs. 2d and 2h) can be analyzed to give information about the molecular order along the entire lipid acyl chain. Comparing Figs. 2d and 2h, it appears that the spectrum for the sample PC-d_{31}/ 1-octanol possesses a greater number of resolved peaks than does PC-d_{31} alone. The same behavior is observed with the PC-d_{31}/1-decanol sample. Using the depaking technique [29], oriented spectra can be derived from powder pattern spectra so

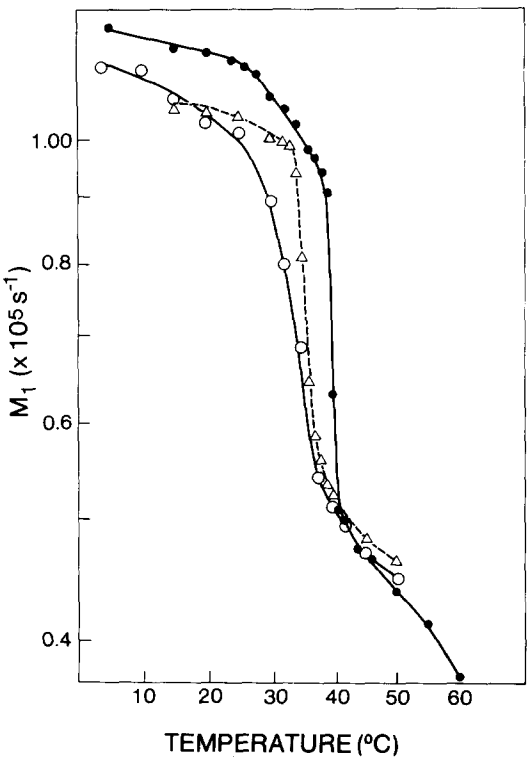


Fig. 5. Variation of the first moment (M_1) with temperature of 50 wt% multilamellar dispersions of: (●) PC-d_{31}; (○) PC-d_{31} + 25 mol% 1-octanol; (△) PC-d_{31} + 25 mol% 1-decanol.

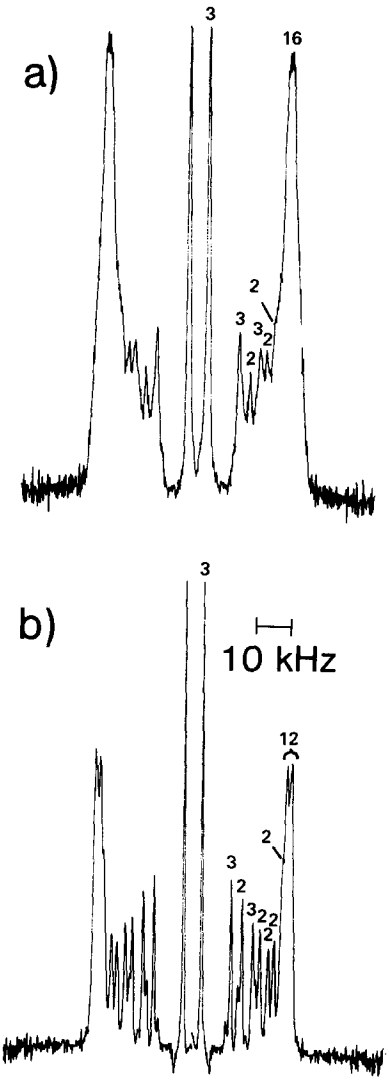


Fig. 6. The depaked ^{2}H-NMR spectra at 50°C of multilamellar dispersions of: (a) PC-d_{31}; (b) PC-d_{31} + 25 mol% 1-octanol. Digits on peaks indicate the number of deuterons assigned to each. Six iterations were performed on the powder pattern data for both (a) and (b).

that resolution is enhanced. Figs. 6a and 6b contain the depaked spectra of 50 : 50 w/w PC-d_{31}/deuterium depleted water, and approx. 75 mol% PC-d_{31}/25 mol% 1-octanol 50 : 50 w/w in deuterium-depleted water, at 50°C. In Fig. 6a five well-defined quadrupole doublets, plus one pair of broad, composite outer peaks can be identified. In Fig. 6b seven pairs of well resolved quadrupole peaks, plus one pair of composite peaks with two distinguishable splittings are visible. Table I lists the peak assignments and quadrupolar splittings ($\Delta\nu_Q$) associated with Fig. 1. Assignment of the various positions of the *sn*-2 [$^2H_{31}$]palmitoyl segments to the spectra in Fig. 6 is based on the work of Pauls et al. [23] wherein it was assumed that, with the exception of the 2-position deuterons, the quadrupolar splittings decrease monotonically towards the C^{2}H$_3$ segment. Assignments of the resonances from the C2 deuterons were based on results with approx. 5 mol% *sn*-2 '[2,2-$^2H_2$]-palmitoylphosphatidylcholine' (PC-d_2) in dispersions of either egg phosphatidylcholine (25°C) or dipalmitoylphosphatidylcholine (48°C) (Parmar, Y.I., Wassall, S.R. and Cushley, R.J., unpublished results). In the former case, the quadrupolar splittings were 11.5 and 17.8 kHz while in the latter case the splittings were 12.2 and 16.9 kHz. In addition, quadrupolar splittings of deuterons on positions 4-, 7-, 11- and 12- reported in Table I are

TABLE I

QUADRUPOLAR SPLITTINGS ($\Delta\nu_Q$) FOR SEGMENTS OF AQUEOUS DISPERSIONS OF PC-d_{31} AND PC-d_{31} + 1-ALKANOL ANESTHETICS AT 50°C

Acyl chain position	$\Delta\nu_Q$ (kHz) [a]			
	PC-d_{31}	PC-d_{31}/ 1-octanol	PC-d_{31}/ 1-decanol	
			Run I	Run II
2	11.75, 17.84	10.95, 17.21	11.11, 17.43	11.62, 18.20
3–8	26.03 [b]	27.97 [c] / 26.63	26.83 [c] / 25.68	27.98 [c] / 26.73
9	26.03 [b]	25.52	24.92	26.29
10	26.03 [b]	23.25	22.94	24.05
11	22.03	21.59	21.59	22.49
12	19.59	19.24	19.43	20.28
13	17.84	17.21	17.43	18.20
14	14.92	14.13	14.30	15.03
15	11.75	10.95	11.11	11.62
16	2.73	2.54	2.54	2.77

[a] Quadrupole powder pattern splittings are reported; i.e., one-half of the depaked spectrum splittings to conform to common usage.

[b] These values are accurate to ±9%.

[c] These values are accurate to ±6%.

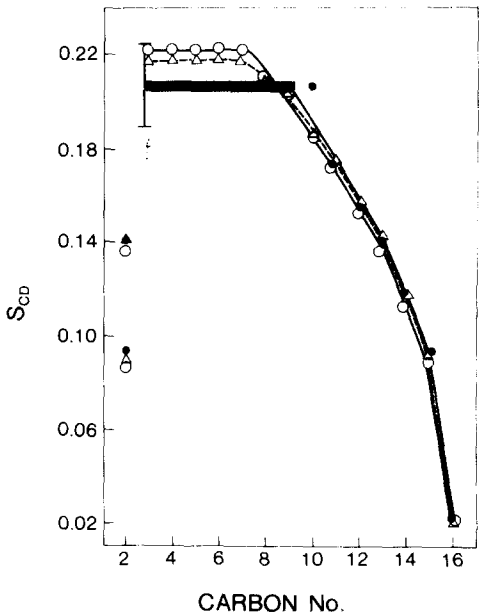


Fig. 7. Order parameter profiles at 50°C: S_{CD} vs. position along the phospholipid *sn*-2 chain. (●) PC-d_{31}; (○) PC-d_{31} + 25 mol% 1-octanol; (△) PC-d_{31} + 25 mol% 1-decanol (average of two determinations). The order parameters for C2 to C9 of PC-d_{31} are indicated by an average value (bar).

within 10% of those found for selectively deuterated *sn*-2 substituted '[$^2H_2$]palmitoylphosphatidylcholines' in the above two systems.

Included in Table I are the results of experiments run with two different PC-d_{31}/1-decanol/water samples. The differences in the $\Delta\nu_Q$ values for the two PC-d_{31}/1-decanol samples indicate the reproducibility of the technique. In all of the depaked spectra of alcohol-containing dispersions of *sn*-2 substituted '[$^2H_{31}$]palmitoylphosphatidylcholine' the C15 and the C14 doublets have shoulders of smaller splittings, $\Delta\nu_Q \approx 9.7 \pm 0.3$ and $\approx 12.7 \pm 0.15$ kHz, respectively (see Fig. 6b). These peaks result from a small amount of acyl chain migration during preparation of the PC-d_{31}. The splittings of the shoulders correspond very closely to values reported by Paddy et al. [37] for deuterons at C15 ($\Delta\nu_Q \approx 9.7$ kHz) and C14 ($\Delta\nu_Q \approx 13.2$ kHz) of *sn*-1-[$^2H_{31}$]palmitoyl-*sn*-2-palmitoylphosphatidylcholine.

The profile of order parameter S_{CD} vs. acyl chain position for PC-d_{31}/water, PC-d_{31}/water/1-octanol and PC-d_{31}/water/1-decanol are shown in Fig. 7. The order parameter for C10 of PC-d_{31}/water does not appear to fit the profile very well. From Fig. 6a one can see that, even in the depaked spectrum, the C10 resonance is not resolved from the broad 'plateau' peak. The splittings in the plateau region do not necessarily correspond to the mean splitting (Table I) but lie within the error bar in Fig. 7 which is determined by the width of the composite peak in Fig. 6a. Upon addition of 1-octanol resolution is sufficiently enhanced in the depaked ^{2}H-NMR spectrum that the C10 position becomes resolved and a slightly narrower splitting than the average 'plateau' value, leading to a smaller S_{CD} value for C10, can be assigned.

Discussion

The lipid used in this study, PC-d_{31}, is deuterated in the *sn*-2 chain only. A saturated phospholipid, it is predicted to behave like di-

palmitoylphosphatidylcholine, rather than the more heterogeneous, unsaturated egg yolk phosphatidylcholine from which it was derived. The DSC profile of PC-d_{31} (Fig. 1) confirms this prediction. Dipalmitoylphosphatidylcholine has a main phase transition at 42°C and a pre-transition at 35°C. [$^2H_{62}$]Dipalmitoylphosphatidylcholine has a main transition at 37°C and a pre-transition at 30°C [38], due to isotope effects caused by the substitution of deuterium for hydrogen. The observed transition temperatures of 40°C and 31°C for PC-d_{31} are consistent with its degree of deuteration and with the presence of about 15% stearoyl chains. The shape of the DSC trace (Fig. 1a) for PC-d_{31} is very similar to that found for dipalmitoylphosphatidylcholine; both display a main transition peak with a half-height width of 2 deg. C. Thus, the heterogeneity of the *sn*-1 chain of PC-d_{31} does not appear to influence its gel to liquid crystalline transition to any appreciable extent. The temperature range of the intermediate (P'_β) phase of PC-d_{31} is approx. 9 deg. C, which is larger than the 7 deg. C range found for dipalmitoylphosphatidylcholine. This is consistent with the presence of *sn*-1 stearoyl chains since there is a 13°C difference between the pre-transition and main transition for 1-stearoyl-2-palmitoyl-*sn*-3-phosphatidylcholine [39].

It is interesting to compare the effects of 1-octanol and 1-decanol on the thermotropic behavior of PC-d_{31} with the results of Lee [5] and Eliasz et al. [18]. Lee examined the change in chlorophyll fluorescence intensity in dipalmitoylphosphatidylcholine liposomes as a function of temperature and alcohol concentration. At a 1-octanol concentration of 25 mol% (2.3 mM) Lee observes a phase transition temperature of 31°C compared with 41°C for the dipalmitoylphosphatidylcholine with no added 1-octanol. This compares well with our results of $T_m = 29$°C (for 25% 1-octanol) and 40°C (for 0% 1-octanol). For 22 mol% 1-decanol (0.13 mM) Lee observes a reduction of 3 deg. C in T_m. We similarly see a reduction in T_m of 3 deg. C upon incorporation of 25 mol% 1-decanol. Eliasz et al. [18] used DSC to determine the change in T_m of dipalmitoylphosphatidylcholine upon incorporation of 1-octanol and 1-decanol. A 25 mol% 1-decanol concentration caused a drop of 4 deg. C in T_m, while 25 mol% 1-octanol produced a drop of 15 deg. C in T_m. These are slightly larger T_m depressions than we observed.

The transition behavior for the gel to liquid crystalline phase change can be examined more clearly using ^{2}H-NMR spectral moments (Fig. 5). The onset temperatures are generally 1–3 degrees lower than the melting points determined by DSC. The onset temperatures determined by NMR correspond more closely with the temperature at which the DSC signal deviates from the baseline, as would be expected. The differential effects of 1-octanol and 1-decanol are due to their respective chain lengths. 1-Octanol disrupts the gel-phase lipid's packing to a greater extent than 1-decanol due to the greater difference in acyl chain length between 1-octanol and PC-d_{31} (≈ 8 carbons) than between 1-decanol and PC-d_{31} (≈ 6 carbons). This has been discussed in the literature [12].

The order parameter profile calculated for PC-d_{31}/water in the liquid crystalline phase (Fig. 7) may be compared with the profile calculated for the *sn*-2 chain of [$^2H_{62}$]dipalmitoylphosphatidylcholine and of selectively deuterated dipalmitoylphosphatidylcholine [23,40,41]. In general our data correspond to within 10% with published results at the same reduced temperature. There is some discrepancy in the assignment of one of the C2 deuteron resonances ($S_{CD} = 0.14$), but those values still agree to within 20%.

The order parameter profile of PC-d_{31}/water is not significantly changed by the presence of 25 mol% 1-octanol or 1-decanol. The first moments (which are directly proportional to the average S_{CD} [26]) for all three systems at 50°C are $0.455 \cdot 10^5\ s^{-1}$ ($\pm 3\%$) (Fig. 5). Apparent differences (Fig. 7) in the plateau regions of the order parameter profiles are the result of the enhanced resolution of peaks in the alcohol-containing samples' spectra. Unresolved shoulders in Fig. 6a are visible in Fig. 6b as a consequence of the narrowing of the spectral lines, which enables a more detailed order parameter profile to be drawn from the latter data. This causes the length of the plateau to be reduced (due to the increased number of resolved peaks) and the apparent order in the plateau region to increase (since splittings may be associated with previously unresolved outer peaks rather than with an average value).

After this work was completed a paper ap-

peared reporting preliminary results of 1-octanol incorporated into a related phospholipid, [$^2H_{54}$]dimyristoylphosphatidylcholine [42]. An apparent small increase in order for the first few segments of the phospholipid acyl chains was also observed.

We have examined the decay of the quadrupolar echo as a function of the pulse spacing delay τ (see Materials and Methods) or PC-d_{31} and PC-d_{31} + 25 mol% 1-octanol dispersions. The intensity of the echo is given by $I(2\tau) = I(0)\exp[-2\tau/T_{2e}]$, yielding the time constant T_{2e}. The significant narrowing of the PC-d_{31} spectral lines in Fig. 6b can be attributed to significantly longer T_{2e} values of PC-d_{31} in the presence of the anesthetic. For example, at 50°C, T_{2e} values for PC-d_{31}, PC-d_{31}/1-octanol and PC-d_{31}/1-decanol are 280 μs, 530 μs and 620 μs, respectively. The enhanced resolution due to T_{2e} illuminates details of the plateau region in a manner not heretofore obtained in a perdeuterated phospholipid bilayer. As slow motions of the phospholipid acyl chains are usually considered to dominate T_{2e} in the liquid crystalline phase [26], the decreased relaxation rate $1/T_{2e}$ for PC-d_{31} in the presence of 1-alkanols suggests that the 1-alkanols decrease τ_c the ^2H correlation time for the deuterated phospholipid chains. A recent paper by Pauls et al. [43] indicates that, above T_m, $1/T_{2e} \propto \tau_c$. A more detailed explanation of the relaxation behavior of PC-d_{31}/1-alkanol systems will be published shortly.

The lack of effect that 1-octanol and 1-decanol (25 mol%) have on the amplitude of lipid acyl chain motions as reflected in S_{CD} is in agreement with the results of a recent paper [44] which concluded that hexane (26 mol%) dissolved in [$^2H_{54}$]dimyristoylphosphatidylcholine did not change the lipid acyl chain order as determined by ^2H-NMR. Incorporation of 20 mol% palmitic acid into [$^2H_{62}$]dipalmitoyl-phosphatidylcholine bilayers has similarly been found to have only a small ($\approx$ 10%) effect upon order within the membrane interior [23].

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