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Simultaneous Observation of Order and Dynamics at Several Defined Positions in a Single Acyl Chain Using ^{2}H NMR of Single Acyl Chain Perdeuterated Phosphatidylcholines†

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ABSTRACT: Deuterium nuclear magnetic resonance (^{2}H NMR) spectra from aqueous dispersions of phosphatidylcholines in which perdeuterated palmitic acid is esterified at the *sn*-1 position have several very useful features. The powder spectra show six well-resolved 90° edges which correspond to the six positions closest to the methyl end of the acyl chain. The spectral overlap inherent in the multiple powder pattern line shape of these dispersions can be removed by using a "dePaking" procedure [Bloom, M., Davis, J. H., & Mackay, A. (1981) *Chem. Phys. Lett. 80*, 198–202] which calculates the spectra that would result if the lipid bilayers were oriented in the magnetic field. This procedure produces six well-resolved doublets whose NMR properties can be observed without interference from the resonances of other labeled positions. The presence of a single double bond in the *sn*-2 chain increases the order of the saturated 16:0 *sn*-1 chain at every position in the bilayer compared with a saturated *sn*-2 chain at the same reduced temperature. Surprisingly, addition of five more double bonds to the *sn*-2 chain only slightly reduces the order of the 16:0 *sn*-1 chain at many positions in the bilayer compared with the single double bond. Calculating oriented spectra from a spin–lattice (T_1) relaxation series of powder spectra allows one to obtain the T_1 relaxation times of six positions on the acyl chain simultaneously. As an example of the utility of these molecules, we demonstrate that the dependence of the spin–lattice (T_1) relaxation rate as a function of orientational order for two unsaturated phospholipids differs significantly from the corresponding fully saturated analogue. Interpreting this difference using current models of acyl chain dynamics suggests that the bilayers containing either of the two unsaturated phospholipids are significantly more deformable than bilayers made from the fully saturated phospholipid.

Over the last several years, deuterium nuclear magnetic resonance (^{2}H NMR)[1] methods have become widely recognized as particularly effective probes of molecular motion in anisotropic biological systems. Determination of the orientational order parameter from ^{2}H NMR spectra can directly yield the average amplitude of motions which occur on a time scale of $\sim 10^{-6}$ s or faster. Determinations of ^{2}H NMR relaxation times reflect the rates of these motions and may allow calculation of the reorientational correlation times. In principal, then, it is possible to measure the amplitudes and rates of the molecular motion separately. Furthermore, since the

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[1] Abbreviations: ^{2}H NMR, deuterium nuclear magnetic resonance; T_1, spin–lattice relaxation time; (16:0)(16:0)PC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; (16:0)(16:1)PC, 1-palmitoyl-2-palmitoleoyl-*sn*-glycero-3-phosphocholine; (16:0)(22:6)PC, 1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine; (16:0)(18:1)PC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; $1/T_{2e}$, rate of decay of the quadrupolar echo; θ, reduced temperature; BHT, butylated hydroxytoluene; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid.

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^{2}H NMR "label" is an isotopic substitution for ubiquitous hydrogen, the order and motional information can come from any one of several very specific locations on a labeled molecule whose motional properties are essentially indistinguishable from those of its unlabeled analogue [for reviews, see Seelig (1977) and Davis (1983)].

These characteristics combine to make ^{2}H NMR methods uniquely well suited for detailed studies of anisotropic molecular motion in biological systems. Applications of ^{2}H NMR methods to the study of acyl chain dynamics in phospholipid bilayers have been especially fruitful. Seelig's pioneering work on the position dependence of amplitude fluctuations along the acyl chain greatly refined the notion of a "fluidity gradient" or "order parameter profile" through the bilayer (Seelig & Seelig, 1974). More recent work has shown this order parameter profile to be a general feature of all bilayer-forming pure phospholipids above their gel-phase to liquid-crystalline-phase transitions [for a review, see Seelig & Browning (1978)]. Studies of ^{2}H NMR spin–lattice (T_1) relaxation times have begun to yield information on reorientational rates in the bilayer as a function of acyl chain position (Brown, 1979, 1982, 1984a,b; Brown et al., 1979, 1983).

All of the above studies have used phospholipids labeled at a single acyl chain position. While this type of labeling provides definitive information at the single, labeled position, it represents a significant investment in time and materials. A separate sample must be prepared for each labeled position, and each sample must have its own allocation of ^{2}H NMR instrument time. In those frequent cases where biochemical isolations, unusual synthetic lipids, or instrument time is limited, a single site labeling approach may be prohibitive.

Largely through the work of Davis, Bloom, and co-workers, double acyl chain perdeuterated phospholipids have been extensively utilized in ^{2}H NMR studies of bilayer systems (Davis, 1979; Bienvenue et al., 1982; Davis et al., 1982; Pauls et al., 1983). Though in a few limiting cases it has proved possible to extract position-dependent information (Bloom et al., 1978b; Davis et al., 1980), studies using double acyl chain perdeuterated molecules generally sacrifice detailed, position-dependent information for a large increase in the signal to noise ratio.

In this paper, we indicate how the use of single acyl chain perdeuterated phospholipids offers a valuable compromise approach to obtaining detailed, position-dependent information about motional rates and amplitudes while requiring the preparation of a minimum number of samples. For our ^{2}H NMR studies using unsaturated phospholipids [cf. Deese et al. (1981)], we have synthesized a variety of single acyl chain perdeuterated phospholipids. The ^{2}H NMR spectra from aqueous dispersions of these molecules have several very useful features. The spectra show six well-resolved powder pattern 90° edges (Pake doublets) corresponding to the last six positions at the methyl terminus of the acyl chain. The splittings of these powder patterns are quite sensitive to relatively small changes in the acyl chain motions of the phospholipid, such as those that occur when different fatty acyl chains are esterified at the *sn*-2 position. Oriented spectra calculated by using a numerical "dePaking" procedure (Bloom et al., 1981)—which removes the orientation dependence of the powder pattern and calculates spectra corresponding to a single orientation—show six well-resolved doublets without interference from the other labeled positions in the chain as well as a composite doublet from the plateau region of the bilayer. When oriented spectra are computed from a series of powder spectra produced by a ^{2}H NMR spin–lattice relaxation time (T_1) experiment, one can determine the orientational order and relaxation times for these six known positions along the acyl chain in a single experiment. The ability to simultaneously observe the ^{2}H NMR properties of several defined positions along a single, easily synthesized acyl chain makes single acyl chain perdeuterated phospholipids attractive for detailed studies of acyl chain dynamics in bilayers.

As an example of the potential uses for these molecules, we have measured the orientational order dependence of the spin–lattice relaxation rate (T_1) for one saturated and two unsaturated phospholipids. The two unsaturated phospholipids show significant differences from the saturated phospholipid which we interpret using current models of acyl chain dynamics.

Experimental Procedures

Synthesis of Perdeuterated Hexadecanoic Acid. Perdeuterated hexadecanoic acid was either purchased from KOR or produced by deuterium exchange using a melt of 10% palladium on activated carbon (Aldrich) with hexadecanoic acid (Sigma) under deuterium gas following the general method of Hsiao et al. (1974). At the end of the exchange reaction, the cooled, crude reaction mixture was dissolved in methanol, filtered to remove the catalyst, and rotary evaporated. The dry material was then either recrystallized directly from distilled hexanes (10 mL of hexane/g of hexadecanoic acid) or loaded on a silica gel column in hexanes, washed with hexanes until no more of an uncharacterized alkane eluted, and then eluted off the column with ethyl acetate before subsequent recrystallization from hexanes. The resulting perdeuterated hexadecanoic acid was characterized by mass spectroscopy, high-resolution ^{2}H NMR, melting point, and gas chromatography of its methyl ester. Deuteration levels were at least 95%.

Synthesis of Palmitic-2,2-d_2 Acid, Palmitic-14,14-d_2 Acid, and Palmitic-6,7,8,9,10,11,12,13,14,15,16-d_{23} Acid. Palmitic-*2,2-d_2* acid, palmitic-*14,14-d_2* acid, and palmitic-*6,7,8,9,10,11,12,13,14,15,16-d_{23}* acid were synthesized by using the standard methods of α exchange and Kolbe electrolysis. Synthetic details will be published elsewhere (M. R. Paddy and F. W. Dahlquist, submitted for publication).

Synthesis of Phosphatidylcholines. 1,2-Bis(perdeuteriopalmitoyl)-*sn*-glycero-3-phosphocholine was prepared according to the method of Patel et al. (1979). 1-Perdeuteriopalmitoyl-2-lyso-*sn*-glycero-3-phosphocholine was prepared by phospholipase A_2 treatment of 1,2-bis(perdeuteriopalmitoyl)-*sn*-glycero-3-phosphocholine following the method of Chakrabarti & Khorana (1975) using crude rattlesnake venom (Ross Allen Reptile Farm, Miami, FL) as the source of phospholipase A_2. 1-Perdeuteriopalmitoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine [(per-^{2}H-16:0)(16:0)PC], 1-perdeuteriopalmitoyl-2-palmitoleoyl-*sn*-glycero-3-phosphocholine [(per-^{2}H-16:0)(16:1)PC], and 1-perdeuteriopalmitoyl-2-docosahexeneoyl-*sn*-glycero-3-phosphocholine [(per-^{2}H-16:0)-(22:6)PC] were synthesized essentially as described by Mason et al. (1981). Butylated hydroxytoluene (BHT) was used as an antioxidant in the solvents used for purifying the unsaturated lipids. The solvents were purged with argon prior to use, and silica gel chromatography was carried out under an inert atmosphere as described elsewhere (Brown et al., 1982). Lipid purity was measured by using thin-layer chromatography (TLC), gas–liquid chromatography (GLC), and NMR.

Sample Preparation for ^{2}H NMR. Fifty to eighty milligrams of phosphatidylcholine, dried in vacuo from organic solvent, was hand dispersed in a few milliliters of 20 mM HEPES, pH 7.0, in ^{2}H-depleted water. The dispersion was

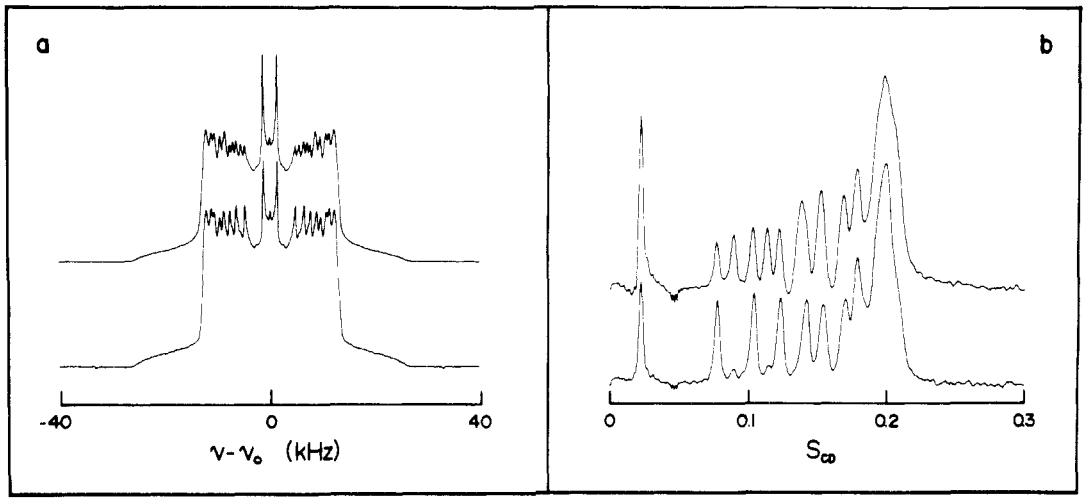

FIGURE 1: ^{2}H NMR powder (a) and oriented (dePaked) spectra calculated from half of the symmetrical powder spectra (b) for (per-^{2}H-16:0)(per-^{2}H-16:0)PC (top) and (per-^{2}H-16:0)(16:0)PC (bottom) at the same reduced temperature, $\theta = 36.1 \times 10^{-3}$ (48 and 50 °C, respectively).

pelleted in a low-speed centrifuge and transferred to 8 mm in diameter × 15 mm flat-bottomed NMR tubes. Total sample volume typically was 500 μL.

^{2}H NMR Methods. ^{2}H NMR spectra and relaxation times were obtained at 23.0 MHz on a Nicolet NT-150 spectrometer equipped with an Oxford Instruments superconducting solenoid and modified to including a home-built, high-power amplifier in the transmitter and a Nicolet Explorer IIIA digital oscilloscope for signal digitization in the receiver. All spectra were acquired by using the Fourier transform quadrupolar echo technique (Davis et al., 1976) in which 4.5-μs 90° pulses were separated by 40-μs delays and the phase of the second pulse was alternatively shifted by 180° (i.e., phase shift of 90° or 270°) to minimize distortions produced by errors in the 90° pulse length. This pulse sequence was repeated at a rate of 4 s^{-1} unless otherwise noted. Spectral widths were ±250 kHz and centered on the symmetry point of the ^{2}H powder pattern using quadrature detection. Typically, one channel of the two quadrature-detected signals was set at zero before Fourier transformation to give spectra symmetric about the center frequency.

Spectra used to determine the deuterium spin–lattice relaxation rate, $1/T_1$, were transformed from the echo signal obtained as a function of the time between the application of an inverting (180°) radio-frequency pulse and the quadrupolar echo pulse sequence. All such spectra were subtracted from that obtained with a very long delay between the inverting and observed pulses. The resulting difference spectra were used for data analysis.

dePaking Methods. The theoretical background for calculating the oriented spectrum for a sample at some arbitrary angle with respect to the applied field from its randomly oriented "powder" spectrum has been described in detail by Bloom and co-workers (Bloom et al., 1982), and applications of these calculations exist in the literature (Davis et al., 1982; Bienvenue et al., 1982; Jarrell et al., 1983; Pauls et al., 1983). Our dePaking calculatons were performed on the Nicolet 1180 minicomputer interfaced with the spectrometer using compiled BASIC software translated from a FORTRAN program kindly provided by James Davis, Department of Physics, University of Guelph. Our dePaking procedure operated on half of symmetrical ^{2}H powder pattern which was contained in a 1024-word data file. The appearance of the resulting dePaked spectrum was improved by interpolating data points in the powder spectrum by zero filling the quadrupolar echo data table to double its length before the Fourier transform. For convenience and maximum resolution, we show the dePaked-oriented spectra that would result if the bilayer normal were oriented 90° to the applied magnetic field.

Results and Discussion

Well-Resolved Powder Patterns Are Observed in Spectra from Single Acyl Chain Perdeuterated Phospholipids Which Are Obscured in Double Chain Perdeuterated Analogues. Figure 1a shows the ^{2}H NMR powder spectra for (per-^{2}H-16:0)(per-^{2}H-16:0)PC (top) and (per-^{2}H-16:0)(16:0)PC (bottom) at approximately the same reduced temperature[2] ($\theta = 36.1 \times 10^{-3}$; 48 and 50 °C, respectively). The spectrum from (per-^{2}H-16:0)(per-^{2}H-16:0)PC has a large number of overlapping powder patterns which make it difficult to identify the spectrum arising from any one labeled position. A much simpler spectrum is obtained from (per-^{2}H-16:0)(16:0)PC. Here, the sharp peaks or 90° edges (resulting from lipids oriented at 90° to the magnetic field) of six powder patterns are readily observed. These are much more poorly resolved in the spectrum from the double acyl chain perdeuterated sample.

The differences in the single and double acyl chain perdeuterated spectra are displayed in another way in Figure 1b, where the half-spectra calculated from the powders using a dePaking procedure are shown. These dePaked spectra represent, to a good approximation,[3] the spectra which would result if all the bilayer normals in the dispersion were oriented perpendicular to the magnetic field. The (per-^{2}H-16:0)-(16:0)PC sample shows six well-resolved lines and a grouping of overlapping, unresolved intensity at higher frequency (larger orientational order parameter).[4] The peak with the largest order parameter arises from about seven methylenes in the

[2] The reduced temperature, θ, is defined as $(T - T_M)/T_M$ where T_M is the gel- to liquid-phase transition temperature [cf. Seelig & Browning (1978)].

[3] The dePaking procedure assumes a line width at each orientation that scales as $P_2(\cos\theta') = {}^1/_2(3\cos^2\theta' - 1)$ where θ' is the angle between the bilayer normal and the applied magnetic field. This assumption produces a small distortion in the line width of each line in the dePaked spectrum which would not be observed in a macroscopically oriented sample (Sternin et al., 1983).

[4] The dePaked spectra of (14,14-d_2-16:0)(16:1)PC and (2,2-d_2-16:0)(16:1)PC show small peaks at other frequencies in the spectrum than that of the major lines. These peaks almost certainly arise from the partial reversal of the labeled *sn*-1 acyl chain into the *sn*-2 position caused by overexposure to phospholipase A_2 [cf. Keough & Davis (1979)].

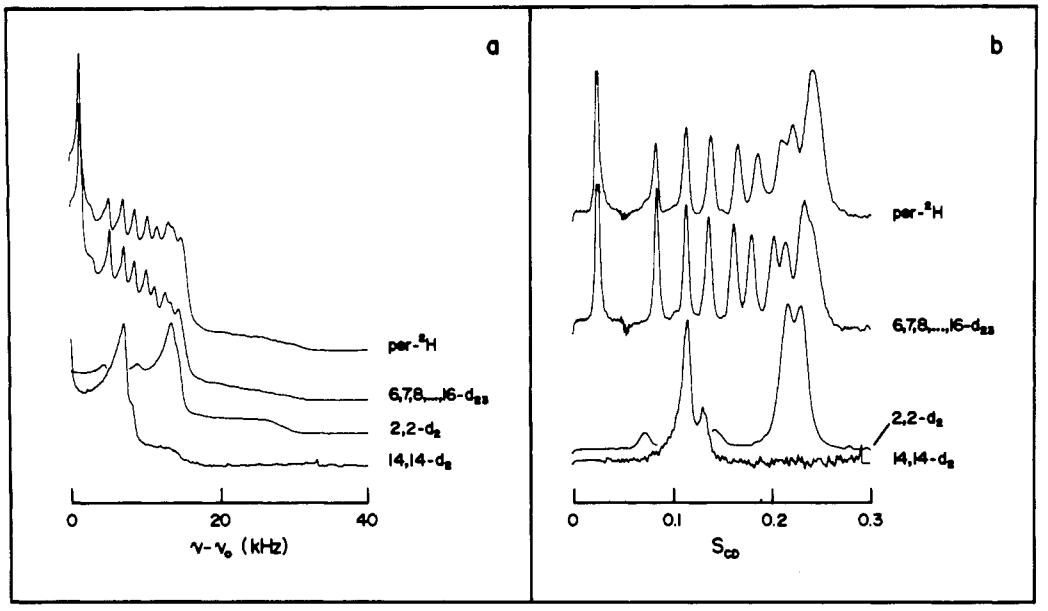

FIGURE 2: ^{2}H NMR powder (a) and oriented (dePaked) spectra (b) at 5 °C for (16:0)(16:1)PC in which the *sn*-1 chain has been deuterium labeled in four different ways, as indicated in the figure.

plateau region of the bilayer near the carboxyl end of the chain that has nearly uniform order. The double acyl chain perdeuterated sample shows an even larger number of lines, although peak areas indicate that only six lines correspond to single sites. In addition, the large number of labeled positions will, in general, make spectra of double acyl chain deuterated samples much more difficult to interpret than the spectra of single chain labeled lipids.

Assignments of Lines in the dePaked Spectrum to Acyl Chain Positions. Figure 2 shows the half-spectrum and the resulting half-dePaked spectrum for four phosphatidylcholines which are identical except for the location of the ^{2}H label on the *sn*-1 acyl chain. These lipids have palmitoleic acid (16:1) esterified at the *sn*-2 position, and the palmitic acid at the *sn*-1 position has been deuterium labeled in four different ways: perdeuterated, deuterated at positions 6–16, and labeled at the single positions 2 and 14. In Figure 2, the line in the dePaked spectrum with the smallest order parameter is clearly that of the methyl terminus because of its very low order parameter, which matches the order parameter of (16,16,16-d_3-16:0)(16:1)PC (Paddy et al., 1981; Deese et al., 1981), because of its relatively long T_1 (data not shown), and because of its significantly greater intensity relative to the other resolved lines. The major line in (14,14-d_2-16:0)(16:1)PC[4] coincides with the third line in the dePaked spectra both of the perdeuterated and of the lower half of the acyl chain labeled (i.e., 6,7,8,9,10,11,12,13,14,15,16-d_{23}) lipids and clearly assigns the third line in the perdeuterated chain to the 14-position. This assignment, when combined with the general order parameter profile obtained by Seelig & Browning (1978), strongly indicates that successive lines with increasing orientational order in the dePaked spectrum represent successive positions along the acyl chain until about position 9. At positions of greater orientational order (S_{CD}) in the dePaked spectrum, one enters the "plateau region" where the different positions have much smaller differences in orientational order, and this simple correlation of position with order parameter breaks down. For example, by comparing the dePaked spectrum of the 2,2-d_2-labeled sample with either the perdeuterated or the 6,7,8,9,10,11,12,13,14,15,16-d_{23}-labeled sample, it is quite clear that the 2-position does not have the greatest orientational order parameter and that the 6-position is as ordered as any position in the chain in these bilayers. Figure 3 in Seelig & Browning (1978) shows that a similar detailed variation within the general order parameter profile has also been observed for (16:0)(18:1)PC using single position labeled phospholipids.

The dePaked spectrum from a single labeled position in the *sn*-1 chain, the 2-position, is clearly split into two lines (see Figure 2b). This splitting is much larger than that observed for (2,2-d_2-16:0)(16:0)PC (M. R. Paddy and F. W. Dahlquist, submitted for publication) and almost certainly represents the motional inequivalence of the two deuterons at this position on the acyl chain (Haberkorn et al., 1977). Presumably, the much larger inequivalence of the two deuterons at the 2-position in (2,2-d_2-16:0)(16:1)PC relative to (2,2-d_2-16:0)-(16:0)PC represents an ordering effect of the cis double bond on the time-averaged structure of the bilayer.

Spectra from Single Acyl Chain Perdeuterated Phosphatidylcholines Are Very Sensitive to the Acyl Chain Esterified at the sn-2 Position. Figure 3 shows the half-powder and dePaked spectra for phosphatidylcholines which all have perdeuterated palmitic acid esterified at the *sn*-1 position but which have three different fatty acids esterified at the *sn*-2 position: palmitic acid (16:0), palmitoleic acid (16:1), andd docosahexaenoic acid (22:6). All spectra are at the same reduced temperature ($\theta = 61.1 \times 10^{-3}$; 58, 5, and 7 °C for the 16:0-, 16:1-, and 22:6-containing phospholipids, respectively).

A striking feature of the three spectra in Figure 3 is the large difference in the width of the powder patterns despite the fact that these lipids are at the same reduced temperature. A rigorous, quantitative measure of the width of these powder patterns is the first moment, M_1 [cf. Davis (1979)]. $M_1 = 5.32 \times 10^4$, 5.07×10^4, and 4.42×10^4 s^{-1} for the 16:1-, 22:6-, and 16:0-containing lipids, respectively (a ratio of 1.20/1.15/1.00). The same qualitative difference in M_1 is observed at all reduced temperatures (data not shown). There are several surprising consequences of these differences in the width of the spectra. For example, the most ordered portions of the bilayer in the plateau region in (per-^{2}H-16:0)(16:0)PC (which appear as a complex, unresolved peak on the right-hand side of the dePaked spectrum) are no more ordered than the 11-position of (per-^{2}H-16:0)(16:1)PC. A qualitatively similar difference between (16:0)(16:0)PC and (16:0)(18:1)PC is

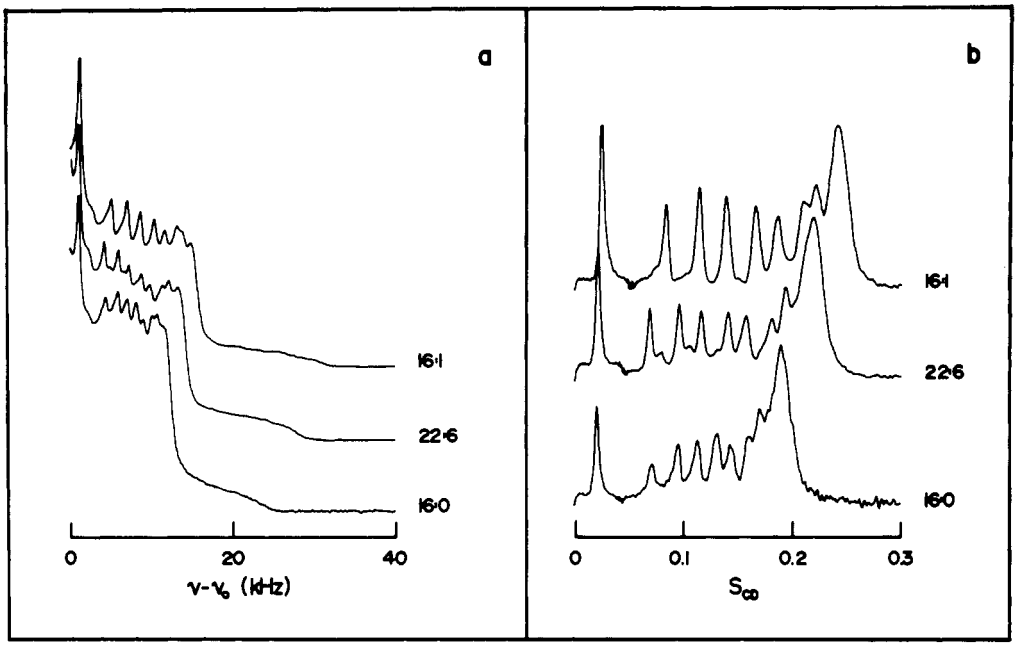

FIGURE 3: ^{2}H NMR powder (a) and oriented (dePaked) spectra (b) for (per-^{2}H-16:0)(16:1)PC, (per-^{2}H-16:0)(22:6)PC, and (per-^{2}H-16:0)(16:0)PC at the same reduced temperature, $\theta = 61.1 \times 10^{-3}$ (5.0, 7.1, and 58.0 °C, respectively).

evident in the data of Figure 3 in Seelig & Browning (1978) obtained from single position deuterium labels.

Comparison of the dePaked spectra of (per-^{2}H-16:0)-(16:0)PC and (per-^{2}H-16:0)(16:1)PC at the same reduced temperature in Figure 3 shows that the addition of the single double bond to the *sn*-2 chain *increases* the order at every position in the 16:0 *sn*-1 chain. Surprisingly, addition of five more double bonds to the *sn*-2 chain (i.e., in 22:6) slightly *reduces* the order of the 16:0 *sn*-1 chain at many positions in the bilayer.

Use of Single Chain Perdeuterated Phospholipids To Measure Spin–Lattice (T_1) Relaxation Times for Six Positions along the Acyl Chain Simultaneously. Figure 4 shows three representations of an inversion–recovery ^{2}H NMR data set used to determine spin–lattice (T_1) relaxation times in (per-^{2}H-16:0)(16:0)PC. Figure 4a shows the inversion–recovery powder spectra which are obtained from the Fourier transform of the solid echoes. Figure 4b shows the fully positive powder spectra which result when the spectrum at any one delay time is subtracted from the spectrum at infinite delay time. These fully positive spectra are more convenient to use in the dePaking procedure. Figure 4c shows the dePaked spectra which are computed from the powder spectra in Figure 4b. Since there is no orientation dependence to the T_1 relaxation of the ^{2}H NMR powder spectrum of fluid-phase phospholipids (Brown & Davis, 1981), one expects the intensities of the lines in the dePaked spectrum to faithfully reflect the integrated intensities of the powder spectra from each labeled position.[5] Figure 4d shows fits of the intensities of the six resolved lines as a function of delay time to single exponentials. The decay rate constants of 23.8, 13.2, and 9.1 s^{-1} measured for the 12-, 14-, and 15-positions) respectively, agree moderately well with the values of 18.0, 10.2, and 7.60 s^{-1} for the 12-, 14-, and 15-positions interpolated from the Arrhenius plot of Brown et al. (1979) measured by using single position labels at 54.4 MHz.

[5] We have checked this result using single position labeled phospholipids and find that T_1 values calculated from the single position labeled powder and dePaked spectra agree well within experimental error (M. R. Paddy and F. W. Dahlquist, submitted for publication).

Spin–Lattice (T_1) Relaxation Time as a Function of Orientational Order along the Same Acyl Chain. Figure 5 shows the spin–lattice (T_1) relaxation time for nine positions in the same acyl chain plotted as a function of the square of the orientational order parameter (S_{CD}) at these positions for each of the 16:1-, 22:6-, and 16:0-containing phospholipids. This plot has been suggested by Brown (1982) as an empirical linear form to fit the varation of T_1 relaxation with orientational order in bilayers. As is clear from Figure 5, this functional form fits the variation of T_1 relaxation along the acyl chain for all three phospholipids very well. There are, however, significant differences between the three phospholipids. The slopes of the 16:1- 22:6-, and 16:0-containing lipid lines have values of 1.81×10^3, 1.34×10^3, and 0.832×10^3 S_{CD}^2 s^{-1}, respectively, which represent ratios of 2.2/1.6/1. The intercept values of 13.7, 11.6, and 2.84 s^{-1} have ratios of 4.82/4.08/1 for the 16.1-, 22.6-, and 16.0-containing phospholipids. It is noteworthy that the slope and intercept of the plot for (per-^{2}H-16:0)(16:0)PC agree well with the values obtained by Brown (1982) (a difference of 25–30% in slope and essentially no difference in intercept), particularly since we have not made any magnetic field dependent corrections to the slope. Finally, though there are distinct differences in slope between all three phospholipids, it is interesting that in broad, qualitative terms the plots from the two unsaturated phospholipids are much more similar to each other than to the fully saturated lipid.

A molecular interpretation of the observed variation in relaxation rate with orientational order is not possible solely on the basis of the available ^{2}H relaxation and orientational order data. However, two recent theories of the relaxation produced by acyl chain motions (Brown, 1982; Marqusee et al., 1984) offer some useful though model-dependent insights. These theories assume two classes of motion: a class fast relative to the resonant frequency which produces relaxation independent of orientational order, and a slow class which is responsible for the orientational order dependent relaxation. The ordinate intercept of the $1/T_1$ vs. S_{CD}^2 plot is determined by the correlation time of the fast class of motions, while the slow class of motions produces the additional relaxation observed at nonzero values of S_{CD}^2. Possible molecular modes suggested for the fast class include local gauche–trans isomerizations

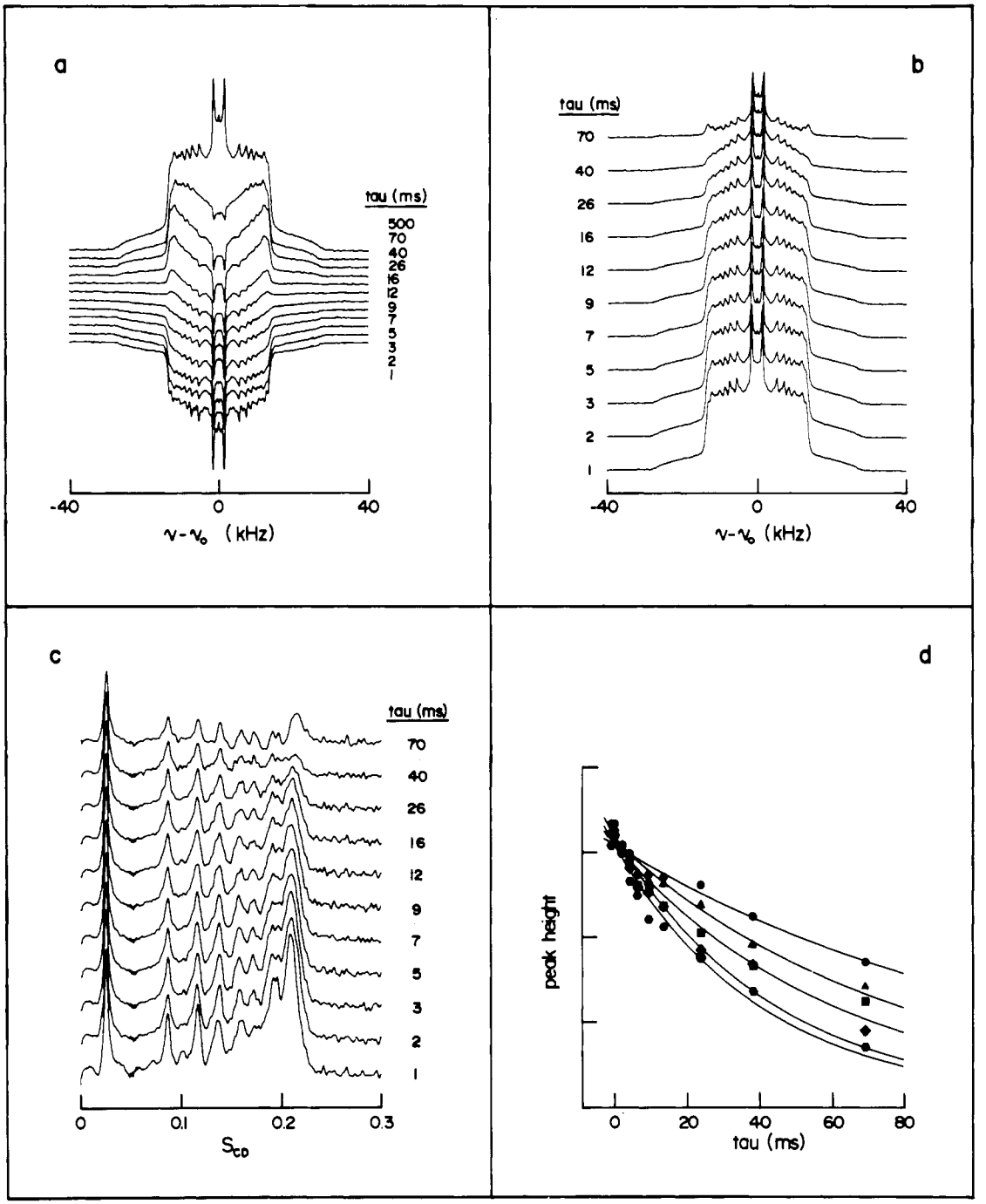

FIGURE 4: Simultaneous determination of the spin–lattice (T_1) relaxation time for each peak in the dePaked spectrum for (per-^{2}H-16:0)(16:0)PC at 46 °C ($\theta = 22.9 \times 10^{-3}$): (a) typical inversion–recovery powder spectrum data set; (b) powder spectra of (a) converted to a format showing only positive intensity by subtracting the spectrum at any one delay time from the spectrum at infinite delay time; (c) oriented (dePaked) spectra calculated from half of the symmetrical powder spectra in (b); (d) intensity-weighted fits of the peak heights of each of the resolved lines in (c) to a single exponential. Best-fit $1/T_1$ values as a function of position are as follows: 11, 25.6 s^{-1}; 12, 23.8 s^{-1}; 13, 17.0 s^{-1}; 14, 8.2 s^{-1}; 15, 9.07 s^{-1}.

along the acyl chain, while the slow class represents collective modes of motion involving the entire chain, which could include random fluctuations in the director axis or twists and splays of the entire bilayer.

Interpreting the data in Figure 5 in light of the Brown or Marqusee et al. models suggests two conclusions. First, the ordinate intercepts of the 16:1- and 22:6-containing lipids are 4–5-fold larger than that of the 16:0-containing lipid. A possible interpretation is that the fast class of motions in the unsaturated lipids is responsible for considerably more spectral density at the resonant frequency as compared with the saturated lipids. Such a conclusion appears not unreasonable, since the geometry of the double bonds might present steric constraints which could hinder intrachain motions on the neighboring *sn*-1 chain. Alternative interpretations are possible.

Second, and more significantly, Marqusee et al. (1984) interpret the slope of the $1/T_1$ vs. S_{CD2} plot as being inversely proportional to the "elastic modulus". The elastic modulus represents the ability of the bilayer to resist deformations by whole-chain fluctuations. Since in this model larger slopes in the $1/T_1$ vs. S_{CD}^2 plot represent larger angular fluctuations of the entire acyl chain, Marqusee et al. (1984) point out that a larger slope can be interpreted as a weaker restoring force in the bilayer. In this interpretion, then, the (per-^{2}H-16:0)(16:1)PC bilayer is least able to resist the deformations

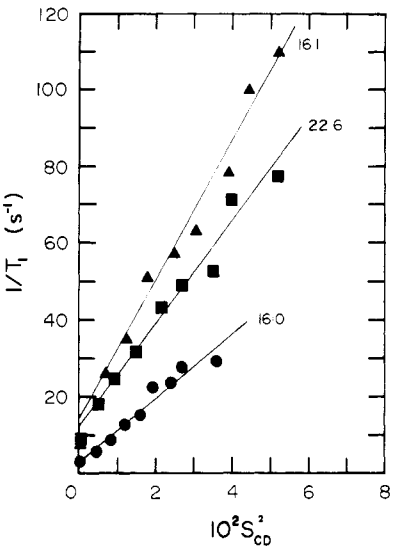

FIGURE 5: Spin–lattice relaxation rate ($1/T_1$) vs. the square of the orientational order parameter for (per-^{2}H-16:0)(16:1)PC (triangles), (per-^{2}H-16:0)(22:6)PC (squares), and (per-^{2}H-16:0)(16:0)PC (circles) at the same approximate reduced temperature (5, 5, and 58 °C, respectively). The lines represent the least-squares best fit to the data excluding the methyl terminus. The positions in the calculated oriented spectrum used to measure $1/T_1$ relaxation rates are the six resolved lines corresponding to positions 11–16, the two less resolved peaks at orientational order immediately above the 11-position, and the maximum of the large, unresolved peak of intensity at highest orientational order due to the plateau region (see Figure 3 for the actual positions of these lines for each of the three lipids).

produced by whole-chain fluctuations, followed by the (per-^{2}H-16:0)(22:6)PC bilayer. Both of these unsaturated bilayers have much weaker restoring potentials than the fully saturated bilayer. This interpretation also appears quite reasonable in molecular terms, for it seems likely that the unsaturated bonds in the 16:1- and 22:6-containing lipids make it more difficult to pack these acyl chains in the bilayer relative to what is possible with the fully saturated (per-^{2}H-16:0)(16:0)PC. Consequently, a bilayer comprised of the unsaturated chains may not be able to offer the resistance/restoring force to while acyl chain fluctuations that a fully saturated bilayer could. In light of this interpretation, what is surprising in Figure 5 is the fact that the 22:6-containing lipid, with six unsaturated double bonds, has a smaller slope than the 16:1-containing lipid, which has only a single double bond. Apparently, the 22:6 chain attains conformations that pack in the bilayer more favorably than the 16:1 chains.

The interpretation of the slope of the $1/T_1$ vs. S_{CD}^2 plot using Brown's theory is less easily related to a single physical parameter. In this theory, the slope is related to both the microviscosity and the elastic modulus.

CONCLUSIONS

The results presented here indicate that single acyl chain perdeuterated phosphatidylcholines are valuable probes for detailed ^{2}H NMR studies of acyl chain dynamics in phospholipid bilayers. The single perdeuterated acyl chains allow the simultaneous observation of six defined positions along a single, easily synthesized acyl chain. As shown in Figures 1 and 3, the ^{2}H NMR spectra of dispersions of these lipids contain six powder patterns with sharp doublets (90° edges) from molecules oriented at 90° to the applied magnetic field. These 90° edges almost certainly represent the lower six positions on the acyl chain, as judged both from the spectra of the specifically deuterated phosphatidylcholines in Figure 2 and from the general order parameter profile that has always been observed with single position labels for bilayer-forming phospholipids [for a review, see Seelig & Browning (1978)]. As one might expect, the quadrupolar splittings of these six positions are quite sensitive to small changes in bilayer structure, such as those caused by changes in the acyl chain esterified at the *sn*-2 position (see Figure 3).

A relatively new method for analyzing powder spectra, dePaking (Bloom et al., 1981), calculates the (approximate[3]) spectrum that would be obtained if the lipids were macroscopically oriented. In the single acyl chain perdeuterated samples described here, dePaking yields half-spectra which contain six well-resolved lines, two less well-resolved lines, and a composite peak from about six to seven methylenes in the plateau region of highly uniform order (see Figures 1 and 3). These dePaked spectra eliminate the spectral overlap inherent in the powder spectra and open additional ^{2}H NMR applications for these molecules in which it is necessary to observe the signal from a single acyl chain position without spectral overlap from other labeled positions. For instance, the dePaking procedure allows the measurement of spin–lattice (T_1) relaxation times at the six positions simultaneously. This is demonstrated for dispersions of (per-^{2}H-16:0)(16:0)PC in Figure 4. These measurements are possible because the shapes of the individual powder spectra (from which the dePaked line shape is calculated) do not change over the T_1 relaxation interval (i.e., there is no orientation dependence to T_1; Brown & davis, 1981).[5] We have recently investigated the orientation dependence of T_2 and have determined that modified dePaking methodologies can be extended to T_{2e} relaxation times as well (M. R. Paddy and F. W. Dahlquist, submitted for publication).

Given that the spectra of phosphatidylcholines containing a single perdeuterated chain give rise to spectra with six well-resolved positions, one might wonder whether acyl chains with fewer deuterated positions would yield spectra with even more resolved lines reaching into the plateau region in the order parameter profile. The probable limiting case of such an approach is shown in Figure 2. Here, a fluid-phase spectrum of (6,7,8,9,10,11,12,13,14,15,16-d_{23}-16:0)(16:1)PC is shown. In the dePaked spectrum, two more lines (presumably positions 10 and 9) are more clearly resolved than in the perdeuterated chain. The three remaining labeled positions (8,7, and 6) are not resolved because the line width of each dePaked line approaches the difference in the splitting between lines. The number of lines resolved in this spectrum (eight) thus is likely to be the maximum that can generally be expected in lipid bilayer systems.

Clearly, the approach outlined here should be very valuable for detailed studies of the position dependence of orientational order and reorientational rates along the acyl chain, as has been done by Seelig, Brown, and co-workers using single position labels [cf. Seelig & Browning (1979), Brown et al. (1979, 1983), brown and (1982, 1984a,b)]. The simultaneous observation of several positions in the same acyl chain should allow considerable savings in time and materials and may, in fact, make some experiments approachable for the first time. In addition, it is easier to synthesize perdeuterated fatty acids than any single position label [except for α to the carboxyl; cf. Oldfield et al. (1978)]. There is not a large loss of information in the perdeuterated spectrum compared with single site labeling, particularly since six positions have well-resolved intensities and the average order in the plateau region is obtained. Further, one has the ability to observe position-dependent effects on the ^{2}H NMR spectrum which might not be anticipated or which might have been obscured by small variations in sample preparations, such as in reconstitutions

with integral membrane proteins (A. J. Deese, F. W. Dahlquist, E. A. Dratz, and M. R. Paddy, unpublished results).

In this paper, we have used this approach to measure the orientational order and spin–lattice (T_1) relaxation rates along the *sn*-1 chain of two unsaturated and one fully saturated phospholipid, as shown in Figure 5. While the detailed interpretation of such data is still controversial, the order-independent and order-dependent parts of the relaxation appear to give insights into the acyl chain dynamics on time scales which are rapid and not rapid compared with the resonant frequency. Furthermore, there appear to be significant differences in these dynamics between phospholipids containing saturated and unsaturated acyl chains. As the composition of most biological membranes is dominated by unsaturated phospholipids, this result appears worthy of further study.

In summary, then, it appears that the possibilities for the simultaneous observation of several defined positions along a single, easily synthesized acyl chain will make single acyl chain perdeuterated phospholipids attractive molecules for a wide variety of studies of acyl chain dynamics in bilayers.

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Registry No. (Per-^{2}H-16:0)(per-^{2}H-16:0)PC, 25582-63-2; (per-^{2}H-16:0)(16:0)PC, 69285-95-6; (per-^{2}H-16:0)(16:1)PC, 97825-79-1; (per-^{2}H-16:0)(22:6)PC, 97825-78-0; (6,7,8,...,16-^{2}H-16:0)(16:1)PC, 97807-57-3; (2,2-^{2}H-16:0)(16:1)PC, 97807-58-4; (14,14-^{2}H-16:0)-(16:1)PC, 97807-59-5; palmitic acid, 57-10-3.

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