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Motions and Interactions of Phospholipid Head Groups at the Membrane Surface. 1. Simple Alkyl Head Groups†

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ABSTRACT: As a reference point for comparison with more complex head groups, a set of phospholipids with *simple* alkyl head groups has been studied. These analogues resemble the naturally occurring phospholipids, except they have phosphomethanol, -ethanol, -1-propanol, and -1-butanol as head groups. The gel-to-liquid-crystalline phase transition temperatures were measured with differential scanning calorimetry, and the phase properties of multilamellar dispersions were examined with phosphorus-31 NMR. The effect of the head-group size was found to be rather small. These lipids were synthesized with deuterium labels incorporated into the alcohol portion at all positions in the head groups except butanol, which was labeled only in the C-1 position. Determination of the ^2H residual quadrupole splittings led to an analysis of the head-group ordering properties. Specifically, these data showed that increasing the length of the head group leads to a more perpendicular orientation of the head group relative to the bilayer surface. Phosphorus-31 chemical shift anisotropy data were also compatible with this result. Measurement of surface pressure–area diagrams of monolayers of these compounds revealed that at high pressures (30 dyn/cm) all four lipids occupied similar areas (40–44 Å^2/molecule), yet at lower pressures, the larger the head group, the larger the occupied surface area. This result suggests that in a bilayer the fatty acyl chains occupy similar areas independent of the alkyl head-group size, and the larger head groups cannot pack properly without some conformational adjustment. The addition of phosphatidylcholine with its relatively bulky head group decreases the area available to the alkyl head groups, pushing the alkyl head groups out of the plane of the bilayer surface. Cholesterol, on the other hand, acts as a "spacer", increasing the area available to the head group, and leads to the opposite effect; i.e., the head groups can relax into a conformation more parallel to the bilayer surface. These data illustrate the types of steric effects which can be expected at the membrane surface. Dynamic properties were investigated by measurement of the ^2H spin–lattice (T_1) NMR relaxation times. These relaxation times could be compared with those from other parts of a phospholipid molecule, namely, the glycerol backbone and the fatty acyl chains. The rates of segmental motion in these head groups were similar to the first C-2 to C-8 segments of the fatty acyl chains, indicating considerable head-group flexibility.

The conformation and properties of the polar region of a phospholipid, commonly called the "head group", have been a topic of considerable interest. This attention is well deserved, since it is at the level of the head group that the membrane interacts with its environment. This article is the first of a series of three papers concerned with head-group interactions found at the bilayer surface. The approach is founded basically on measurements of the rates of motion of deuterated segments in the various head groups. By comparison of different head groups, the existence and the molecular nature of the various interactions can be deduced.

This paper describes the properties of simple alkyl head groups. There are no *attractive* inter- or intramolecular interactions such as electrostatic (salt) or hydrogen bonds possible between the alkyl portion of the head groups and neighboring head groups (van der Waals interactions will be ignored in this treatment). All of these analogues bear formally one negative charge under the conditions used here and, other than the size of the head group, are absolutely identical. Therefore, these analogues are suitable for a study of steric effects at the membrane surface and provide a logical reference point for comparison with other head groups. In the second paper, hydroxyl groups will be introduced at various positions, and their effects on the motional and ordering properties will be analyzed. Finally, in the third paper, in what is a more complex case, amine groups will be introduced. Head-group interactions have proven to be extremely difficult to study in the relevant bilayer state. Many of the methods applied to this problem have been reviewed (Hauser & Phillips, 1979; Yeagle, 1978; Büldt & Wohlgemuth, 1981).

In this approach, deuterium and phosphorus NMR methods have been employed. Both nuclei have been successfully used in membrane studies [for representative articles on ^2H NMR, see Seelig (1977), Seelig & Seelig (1980), Büldt & Wohlge-

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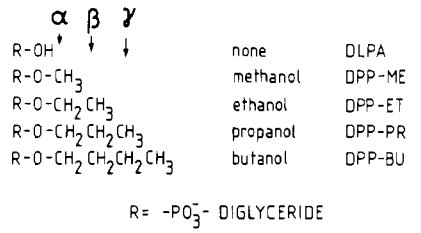

FIGURE 1: Structures of the esterified portions and abbreviations of the head groups employed. "None" refers to dilauroylglycero-3-phosphoric acid; other compounds were the dipalmitoyl derivatives.

muth (1981), Oldfield et al. (1978), and Smith (1978); for ^{31}P NMR studies, see Seelig (1978) and Cullis & de Kruijff (1979)]. These simple analogue phospholipids have been synthesized with deuterium incorporated into specific positions in the head group. Both these deuterons and the naturally occurring ^{31}P have been observed with NMR. With this technique, the *ordering* of the head group was determined from the deuterium quadrupole splittings, and the relative *rates* of *motion* were qualitatively assessed from spin–lattice (T_1) relaxation time data. These data have led to a reasonable evaluation of the role which can be played by steric hindrance at the membrane surface.

Experimental Procedures

Figure 1 gives the names, structures, and abbreviations of the compounds discussed in this paper.

Syntheses. Perdeuterated methanol, ethanol, and 1-propanol as well as (1,1-$^2H_2$)ethanol and (1,1-$^2H_2$)propanol were purchased. 1-(1,1-$^2H_2$)Butanol was synthesized by reduction of *n*-butyric acid with lithium aluminum borodeuteride in diethyl ether by normal techniques (Thomas, 1971).

1,2-Dipalmitoyl-sn-glycero-3-phospho($^2H_3$)methanol. This synthesis will serve to illustrate the technique used for all of the alkyl head-group compounds. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphoric acid [free acid, 1 g, 1.5 mM; synthesized following methods of Baer (1951) or Eibl (1978)] was coupled to ($^2H_3$)methanol (0.5 mL) in 3 mL of pyridine, 3 mL of dry chloroform, and 1.5 g of 2,4,6-triisopropylsulfonyl chloride [following the method of Aneja et al. (1970)] at 50 °C for 2 h, water (0.5 mL) was added, and the entire mixture was concentrated to a syrup followed by drying under vacuum. The resultant solids were dissolved in a small amount of methylene dichloride (approximately 5 mL), and 100 mL of diethyl ether was added. The precipitate was removed by filtration and redissolved in methylene dichloride followed by precipitation with diethyl ether. The ether extracts were pooled and concentrated under reduced pressure. The product was converted to the sodium salt by dissolving it in 50 mL of chloroform and then adding 50 mL of an aqueous saturated sodium chloride solution. The solution was titrated to pH 7.0 with 0.1 M ethylenediaminetetraacetic acid (EDTA),[1] pH 10.0. The organic phase was collected and concentrated. The salt was dissolved in a small amount of methylene dichloride/methanol (50/1 v/v) and applied to a silica gel column. The product was eluted at a ratio of 9/1–4/1 methylene dichloride/methanol. The pure product was treated with Dowex 50 as described by Browning & Seelig (1979), and the sodium salt was re-formed as described above. The product comigrated with the authentic product prepared by an enzymatic procedure (Comfurius & Zwaal, 1977) on silica gel TLC plates; R_f = 0.4–0.5 (range of DPP-ME to DPP-BU) in chloroform/methanol/H_2O (65/25/4) and 0.78 (DPP-ME) in chloroform/methanol/acetic acid/water (45/20/6/1). The methyl, ethyl, and propyl alkyl analogues have been prepared previously by other methods (Träuble et al., 1976; Eibl & Woolley, 1979; Sacré & Tocanne, 1977).

Sample Preparation. Dispersed samples were prepared in a 0.05 M Pipes–Tris buffer, pH 7.0, with 0.1 M NaCl and 1 mM EDTA. The sodium salts were always used, and the pH of the final dispersion was 7.0. Samples were dispersed as previously described (Browning & Seelig, 1980). There can be some difficulty in preparing dispersed DPP-PR and DPP-BU samples which do not have an isotropic signal in the ^{31}P spectra. This signal most likely results from small vesicles. If the samples are dispersed first with only a small amount of buffer (e.g., 100 μL/30 mg of phospholipid), the occurrence of this phase can be minimized. After formation of the bilayers, excess buffer can be added. Mixtures with cholesterol or DMPC were dissolved first in chloroform/methanol (2 h) in 10-mL round-bottom flasks, rotavaped, redissolved in chloroform, and then dried. This dried lipid was dispersed as described above.

Measurements. Differential scanning calorimetry was performed with an apparatus from either Thermonanalyse or Brandt Microcal (MC-1) with a 0.5 °C min^{-1} scanning rate. Samples were typically 1 mg/mL lipid (monosodium salt) in excess buffer or pure water.

NMR spectra were recorded on a Bruker CXP-300 instrument at 121 (^{31}P) and 46 MHz (^2H). ^2H NMR spectra were recorded with a quadrupole echo technique (Davis et al., 1976) with a recycle time of 0.2–1.0 s, depending on whether a $-CD_3$ group was being measured. Spin–lattice relaxation times (T_1) were measured with an inversion–recovery–quadrupole echo combination (180_x–τ_1–90_x–τ_2–90_y–τ_2–echo). τ_2 was 30 μs; the 90° pulse length was 4–6 μs, depending on the amount of sample, and was determined separately for each sample. An interexperiment delay of at least $5T_1$ was used. The T_1 was obtained from a ln ($M_0 - M_z$) vs. τ plot. A weighting factor of $(M_0 - M_z)^2$ was used in the least-squares fitting procedure to account for error in the linearization of an exponential. The error limit is the standard deviation of the unweighted least-squares fit. ^{31}P spectra were obtained with a normal pulse routine with about 1 kW of inverse gated proton decoupling power. Recycle times were 0.4–0.5 s.

Monolayer studies were carried out on a circular trough arrangement after the design of Fromherz (1975). For the recording of the pressure–area diagrams, monolayers were compressed at a rate of 0.5–1.0 Å^2 molecule^{-1} s^{-1}. The plots were recorded directly with an x–y recorder. Lipids were applied to the subphase in a chloroform solution at a concentration of 1.0 mg/mL; the film was allowed to equilibrate at zero pressure for 15–20 min and then scanned. After the first scan to 30 dyn/cm, all following scans were completely reproducible. The apparatus could be preset to apply a specific pressure to the film, thus allowing the determination of the values presented in Table IV.

Results

Phase Behavior. Differential scanning calorimetry of these alkyl analogues in buffer (with 0.1 M NaCl and 1 mM EDTA,

[1] Abbreviations used: DLPA, 1,2-dilauroyl-*sn*-glycero-3-phosphoric acid; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMP-ME, 1,2-dimyristoyl-*sn*-glycero-3-phosphomethanol; DMP-PR, 1,2-dimyristoyl-*sn*-glycero-3-phospho-1-propanol; DPP-ME, 1,2-dipalmitoyl-*sn*-glycero-3-phosphomethanol; DPP-ET, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanol; DPP-PR, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-1-propanol; DPP-BU, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-1-butanol; CSA, chemical shift anisotropy; EDTA, ethylenediaminetetraacetic acid; TLC, thin-layer chromatography; Pipes, 1,4-piperazinediethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

Table I: Gel-to-Liquid-Crystalline Phase Transition Temperatures of Various Alkyl Head-Group Phospholipids[a]

head group	lipid	temp (°C)
dimyristoyl chains (14:0)		
methyl	DMP-ME	31
propyl	DMP-PR	22
dipalmitoyl chains (16:0)		
methyl	DPP-ME	46
ethyl	DPP-ET	41
propyl	DPP-PR	42
butyl	DPP-BU	42

[a] From differential scanning calorimetry of lipids dispersed in 0.05 M Pipes-Tris buffer, 0.1 M NaCl, and 1 mM EDTA, pH 7.0.

pH 7.0) showed sharp transitions at the temperatures given in Table I. Both ^{31}P and ^{2}H NMR measurements were also in agreement with these values. The methyl analogues were found to have consistently a phase transition about 5–10 °C higher than those of the other alkyl compounds. This difference may reflect a better packing of this head group in the bilayer. On the other hand, between the two- and four-carbon head groups, little difference was observed. The data of Träuble et al. (1976) and Blume (1979) for the derivative, DMP-ME, under these conditions are in good agreement with the values presented here. DMP-PR was measured by Eibl & Woolley (1979) with a fluorescence technique, but their value is substantially different. These phase transitions were also measured in pure water (pH 5.4–5.8) where the resultant sodium concentration was low (0.3 mg of DMP-ME monosodium salt/mL of water, i.e., 0.3 mM Na^+). Only 1–3 °C shifts to higher temperatures were observed under this condition.

^{31}P NMR is a very good technique for studying phospholipid phase properties [for a review, see Cullis & de Kruijff (1979)]. A lamellar phase is characterized by phosphorus spectra with a negative chemical shift anisotropy, the shift anisotropy being obtained from the separation of the edges. Alternatively, with a hexagonal phase, a positive shift anisotropy is observed, and spectra of this phase are readily distinguishable from lamellar phases. Typical phosphorus spectra of the alkyl head-group phospholipids are shown in Figure 2. All are characterized by a lamellar structure. Dispersions of the butyl compound DPP-BU and the phosphatidic acid DLPA contained a small amount of an isotropic phase most likely resulting from a small population of small vesicles. Essentially similar results were found by using ^{2}H NMR (Figure 3). Here an isotropic phase gives rise to a sharp signal without a quadrupole splitting. Likewise, a hexagonal phase can be characterized by a smaller quadrupole splitting than the lamellar case (Gally et al., 1980; Wieslander et al., 1978). The small amount of isotropic phase seen in the phosphorus spectrum was also observed in the ^{2}H NMR spectrum as a sharp signal in the center of the spectrum. In most of the spectra, this signal results from the natural abundance of deuterium in the water.

Deuterium NMR Quadrupole Splittings. A α, β, and γ nomenclature for the carbon segments of the head groups will be used here (see Figure 1). ^{2}H NMR spectra of DPP-ME, DPP-ET, DPP-PR, and DPP-BU labeled in the α-CD_2 position of the head group are shown in Figure 3. These spectra have a shape typical of a powder spectrum and are basically similar to those obtained previously for the phosphatidylcholine (Gally et al., 1975), -ethanolamine (Seelig & Gally, 1976), -serine (Browning & Seelig, 1980) and -glycerol head groups (Wohlgemuth et al., 1980). The separation of the peaks is the quadrupole splitting. This splitting is zero for the DPP-PR and DPP-ET/DMPC (1/1, molar ratio) cases. Spectra of

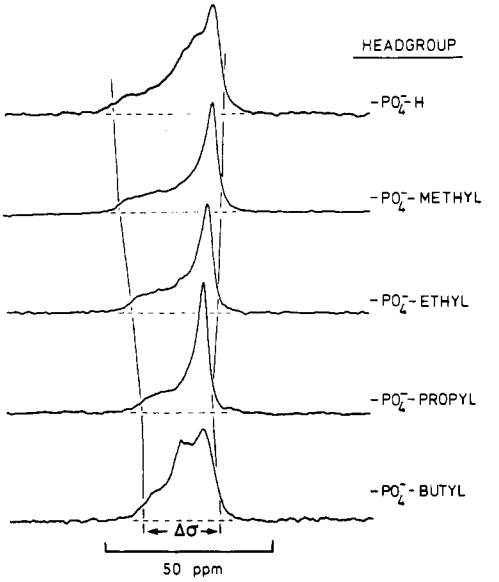

FIGURE 2: Phosphorus-31 NMR spectra (121 MHz) of phospholipid analogues containing short alkyl head groups. Spectra were recorded at 47 °C ($T > T_c$) under gated proton decoupling conditions. The chemical shift anisotropy values were estimated by measuring the signal width at one-half the foot height on the right side of the spectrum to a point extrapolated from the falling edge of the large peak on the left side. Solid lines refer to the positions where the chemical shift anisotropy was measured. Experience has shown that this approximation yields a value for the chemical shift anisotropy close to the value obtained by a computer simulation of the spectrum. All lipids were dipalmitoyl except for $-PO_4^-$–H which was 1,2-dilauroyl-*sn*-glycero-3-phosphoric acid.

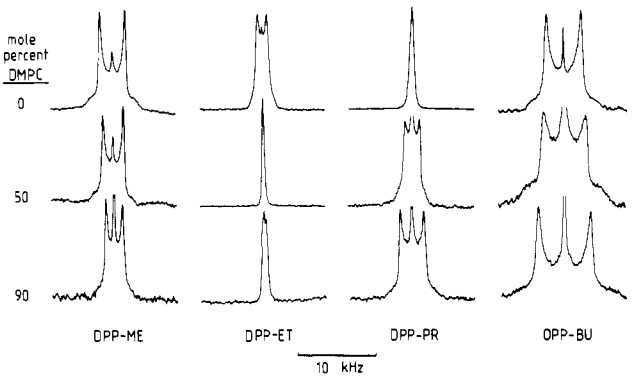

FIGURE 3: ^{2}H NMR powder spectra of alkyl head-group phospholipids deuterated at the α *position* of the head group in the presence and absence of DMPC. Measuring temperature was 47 ± 1 °C, and between 1k and 100k scans were accumulated, depending on the sample.

DPP-ET and DPP-PR perdeuterated in the head group are shown in Figure 4. Assignment of the β-CD_3 signal of DPP-ET was unambiguously made by comparison with the α-CD_2-labeled compound. Additionally, since the ester-linked α deuterons are about 3–4 ppm (at 46 MHz about 150 Hz) downfield from the β-methyl resonance, the two signals, α and β, have different chemical shifts. The difference can be readily seen in Figure 4. In the case of DPP-PR, assignment of the α signal was as above, and a separation of the β-CD_2 and γ-CD_3 signals was based on the differing spin–lattice (T_1) relaxation times. As will be developed, the methyl T_1 is about 5–10 times longer than a methylene T_1. In Figure 4, partially and fully relaxed spectra of perdeuterated DPP-ET and DPP-PR in a DMPC/analogue (9/1) mixture are shown. The relaxation delay has been chosen such that the intensity of the CD_3 signals is about zero [at $\tau = \ln(2T_1)$]. Because of their much shorter relaxation times, the α and β (DPP-PR) deu-

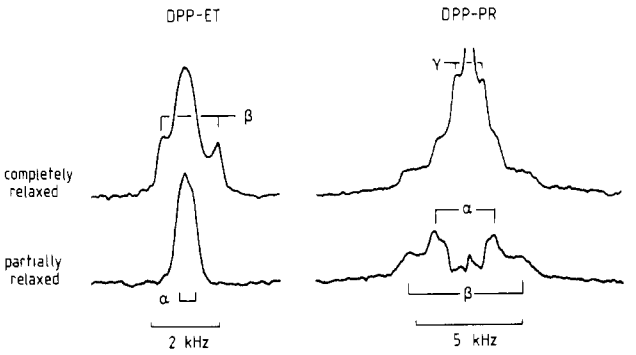

FIGURE 4: Examples of partially relaxed spectra of *perdeuterated* head groups of DPP-ET and DPP-PR in mixtures with DMPC (9/1 molar ratio of DMPC/analogue). Spectra were obtained at 47 ± 1 °C with a $180°-\tau_1-90°-\tau_2-90°-\tau_2$–echo sequence, where τ_2 was 30 μs and the recycle time was 1 s. For DPP-ET (DPP-PR), a completely relaxed spectrum was obtained with a τ_1 = 1 s (3 s), and for the partially relaxed spectrum, τ_1 = 130 ms (80 ms). In both partially relaxed spectra, the terminal $-CD_3$ signal is not observed. When the T_1 data in Table I are used, the expected magnetizations for these $-CD_3$ segments with the above T_1 values are $0.05M_0$ (DPP-ET) and $-0.34M_0$ (DPP-PR). The nonzero value of the calculated magnetization in the DPP-PR case reflects a contribution from the faster relaxing α-CD_2 segment.

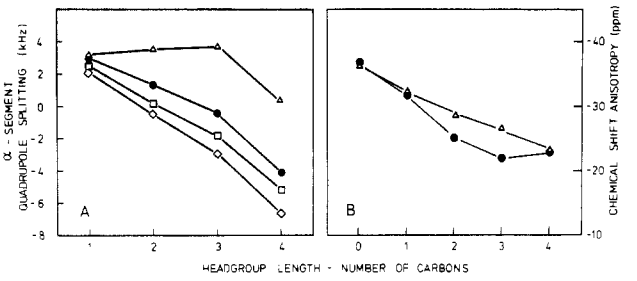

FIGURE 5: (A) ^2H quadrupole splittings of *α-labeled* alkyl head groups as a function of the head-group length. All measurements were made at 47 ± 1 °C. Pure alkyl head-group phospholipid (●), with 50 mol % DMPC (□), 90 mol % DPMC (◇), and 50 mol % cholesterol (△). The sign of the quadrupole splitting has no significance for the measurements but reflects a change in the sign of the segmental order parameter. The assignment of the DPP-ME (1-carbon) splitting as positive is arbitrary. (B) Chemical shift anisotropy of alkyl head groups as a function of the head-group length. Symbols were the same as for (A). Measurements were made at 47 ± 1 °C. The zero-carbon head group refers to dilauroyl-*sn*-glycero-3-phosphoric acid.

terons are almost completely relaxed and appear as normal signals. Thus, with DPP-PR, the smallest quadrupole splitting was assigned to the γ signal, the α signal could already be assigned to the 2.9-kHz peaks, and the remaining 5.5-kHz signal belongs to the β-CD_2 deuterons. In this manner, all the DPP-PR resonances could be assigned.

The size of the quadrupole splittings associated with the α position was found to decrease as the length of the head group became longer. The quadrupole splitting became zero with the propyl head group and then increased with the butyl compound. This trend in the α-position quadrupole splitting is plotted in Figure 5A as a function of alkyl head-group length. The sign of the quadrupole splitting cannot be determined experimentally; nevertheless, it has been shown here to invert. This inversion reflects a continuous change in the orientation of the head group and is a logical trend which has been encountered in other systems (Deloche & Charvolin, 1976). The opposite case, i.e., with DPP-ME negative and the DPP-BU signal positive, is also possible.

The addition of DMPC or cholesterol to these analogues did not abolish this trend but merely shifted the curve in Figure 5A up or down. Two signals for the α-CD_2 segment were seen in the cholesterol mixtures (Figure 6). These two signals are

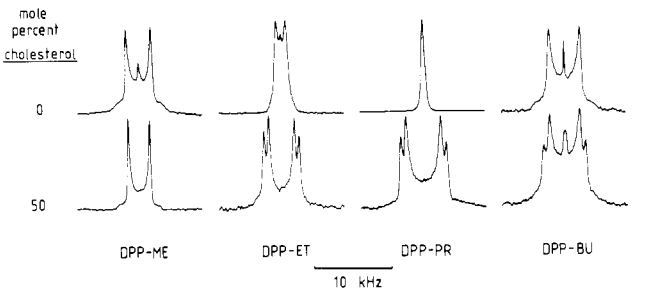

FIGURE 6: ^2H NMR spectra of *α-labeled* alkyl head-group phospholipids in the presence (47 ± 1 °C) and absence (24 ± 1 °C) of 50 mol % cholesterol.

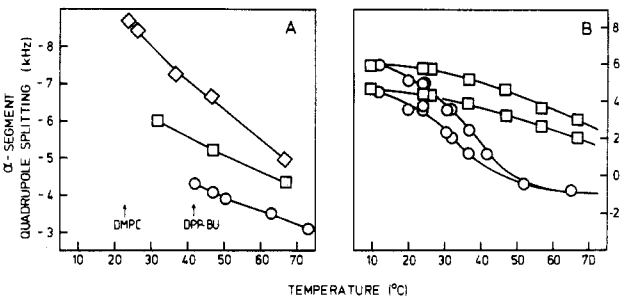

FIGURE 7: Temperature dependence of the ^2H quadrupole splittings of *α-labeled* alkyl head groups. (A) DPP-BU in mixtures with 0 (○), 50 (□), and 90 (◇) mol % DMPC. The sign of the quadrupole splitting conforms with the convention used in Figure 5. (B) DPP-BU (○) and DPP-PR (□) in mixtures with 50 mol % cholesterol.

Table II: ^2H Quadrupole Splittings (kHz) of Dipalmitoyl Alkyl Head-Group Phospholipids (47 °C)

labeled position in head group	head group	DMPC (mol %) 0	50	90	cholesterol (mol %) 50
α	methyl	3.1	2.5	2.1	3.1
	ethyl	1.2	0.3[a]	0.5	3.0, 4.2[b]
	propyl	0.4[c]	1.8	2.9	3.2, 4.6
	butyl	4.0	5.2	6.7	~0.0, 0.5
β	ethyl	1.5[d]	1.5	1.8	0.6
	propyl	4.9	5.4	5.5	~1.4
γ	propyl	1.2	1.3	1.6	1.7

[a] Estimated from the line width at half-height. [b] Values for each deuteron. [c] Resolved in partially relaxed spectra. Data for DMP-PR were identical. [d] Only an approximate value due to overlap from the α-CD_2 segment.

believed to arise from the inequivalence of the two CD_2 deuterons and will be discussed below. The similarity of the spectra shown in Figure 6 suggest that at this temperature the α segments of all the head groups in cholesterol mixtures are in almost identical states. Spectra of a perdeuterated DPP-PR/cholesterol mixture were quite complicated and could only be explained if the β-CD_2 group also gave rise to a pair of quadrupole splittings. Partially relaxed spectra in this case were still rather complex. Generally, the trends in the β and γ signals upon addition of DMPC were similar to those observed at the α position. These data are presented in Table II.

For proper comparison of the data in Figure 5A, a reduced temperature scale [see e.g., Seelig & Browning (1978)] should be used to compensate for the differing transition temperatures of the various mixtures. This was not done in Figure 5A, since there is probably no proper transition in the mixtures with cholesterol. In the case of mixtures with DMPC, a plot like Figure 5A, but on a reduced temperature scale, showed that

Table III: ^2H Spin-Lattice (T_1) Relaxation Times (ms) of Dipalmitoyl Alkyl Head-Group Phospholipids (47 °C)

labeled position in head group	head group	DMPC (mol %)			cholesterol (mol %)
		0	50	90	50
α	methyl	271 ± 8	225 ± 2	194 ± 10	304 ± 7
	ethyl	48 ± 1	44 ± 2	37 ± 3	58 ± 2[a]
	propyl	33 ± 1[e]	27 ± 1	27 ± 1	33 ± 1[a]
	butyl	27 ± 1	23 ± 1	23 ± 1	25 ± 1
β	ethyl	217 ± 6[b]	187 ± 17[c]	173 ± 9[c]	213 ± 4[c]
	propyl	56 ± 10	53 ± 3	44 ± 3	
γ	propyl	195 ± 20[d]	173 ± 20[d]	(160–230)[d]	

[a] Both deuteron signals had the same T_1. [b] Since the α and β signals here exactly overlap, an exact analysis of the β relaxation time was possible by using a T_1 of 48 ms for the α position. [c] Data are from the perdeuterated compound, but the signals could be well separated. [d] Due to extensive overlap with the α signal in the perdeuterated compound, a 10–30% error can be expected. [e] Data for DMP-PR and DPP-PR at the α position were essentially identical.

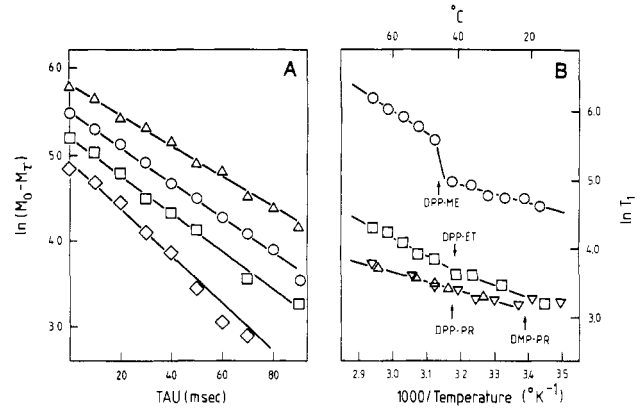

FIGURE 8: ^2H spin–lattice relaxation time data for the alkyl head-group phospholipids. (A) Typical magnetization recovery plots for *α-labeled* DPP-ET in the presence of 0 (○), 50 (□), and 90 (◇) mol % DMPC and (△) 50 mol % cholesterol, measured at 47 ± 1 °C with essentially a 180–τ–90 experiment. The data are expressed as ln ($M_0 - M_\tau$) vs. τ, where M_0 is the magnetization at long pulse intervals and M_τ is the magnetization after a shorter interpulse interval τ. Lines show the fits obtained with the weighted least-squares method described under Experimental Procedures. (B) Temperature dependence of the ^2H spin–lattice relaxation times of the deuterated *methyl* group of DPP-ME (○) and the deuterated *α-methylene* segment of DPP-ET (□), DPP-PR (△), and DMP-PR (▽). Data are expressed in the form of an Arrhenius plot of ln T_1 vs. $1/T$ (K). From the slope of these plots, activation energies for the motions inducing relaxation can be calculated and are presented in Table V. Standard deviations, as determined from the regression analysis, described in (A), were smaller than the symbols. Arrows indicate phase transition temperatures.

the differences between the lines became even more extreme.

The size of the quadrupole splitting was found to increase with decreasing temperature up to the phase transition temperature for all the positions studied. Typical data are presented in Figure 7. At the phase transition temperature, the spectra changed abruptly from the sharp peaked form seen in the above figures to a broadened featureless spectrum typical of gel phase lipids. Even with the methyl compound, no well-defined quadrupole splitting was found in the gel phase. The quadrupole splitting of α-labeled DPP-BU in a mixture with cholesterol varied dramatically with temperature. At temperatures below 20 °C, the measured α-CD_2 quadrupole splitting for DPP-BU was essentially identical with those of the DPP-ME (only one signal), DPP-ET, and DPP-PR. At higher temperatures, the quadrupole splittings rapidly decreased, appearing to go through 0 kHz. This was the only case where such a temperature dependence was observed. Since the quadrupole splitting at higher temperatures approached that of the pure compound, DPP-BU could be separating from the cholesterol under these conditions.

Chemical Shift Anisotropy. The chemical shift anisotropy was determined for each alkyl head-group phospholipid in the presence and absence of cholesterol (Figure 5B). A trend similar to the α-position quadrupole splittings was observed. Increasing the length of the head group decreased the chemical shift anisotropy (absolute value). The addition of cholesterol shifted this curve to larger values. The data for phosphatidic acid have been included since they fell within this pattern.

Spin–Lattice (T_1) Relaxation Times. ^2H T_1 relaxation times of dispersed phospholipids with deuterated alkyl head groups are summarized in Table III. Typical inversion–recovery data are presented in Figure 8A. In all cases, only one decay time was observed as evidenced by the linearity of the plots of ln ($M_0 - M_\tau$) vs. τ. Methylene segments were found to relax 5–10 times faster than a methyl group. This result indicates faster rates of motion for a methyl group, reflecting a smaller energy barrier to rotation about the C–CH_3 bond.

As one progresses from the "anchored point" of a chain (i.e., the glycerol backbone) toward the methyl group, the segments should undergo more and more motion. In the case of DPP-PR, the β-methylene segment relaxation time was always larger than that of the α-methylene segment. This appears to reflect this increasing motion along the chain. In a similar sense, increasing the length of the chain increases the moment of inertia as well as the excluded volume. Both factors lead to a reduction in the overall motion. This is evidenced by the T_1 data. At the α segment, the T_1 decreased from 48 to 27 ms upon going from the ethyl to butyl chains (2 to 4 carbons).

In Figure 8B, some data are presented as an Arrhenius plot of ln T_1 vs. reciprocal temperature. Gel phase relaxation times were determined simply from the height of the broad deuterium spectrum. The plots were linear above and below the phase transition temperature with a discontinuity at the phase transition. From the temperature dependence, it appears that relaxation in both the gel and liquid-crystalline phases is in the short correlation time regime ($\omega_0\tau_c \ll 1$, $\omega_0 = 2.9 \times 10^8$ rad s^{-1}, where ω_0 is the NMR resonance frequency and τ_c is the correlation time). Important in Figure 8B is the comparison of T_1 data from DMP-PR and DPP-PR. These data overlap completely, indicating that an absolute temperature scale is appropriate for comparing data from lipids with differing phase transition temperatures.

Monolayer Properties. Figure 9 contrasts the surface pressure–area diagrams of these four alkyl head-group phospholipids. The compression rate used here was rather high in comparison with that used in earlier work, and there was some hysteresis in the compression–expansion curves. Equilibrium values could be measured (Table IV) and were generally 2–5 $Å^2$/molecule smaller than those found in Figure 9. The diagram for DMP-ME agreed very well with that presented by Blume (1979) for this compound. The limiting areas of the methyl head-group compounds (40–43 $Å^2$/molecule) are typical for phospholipids in a solid film state. The areas under these conditions reflect the area of the fatty acyl chains, i.e., approximately 20 $Å^2$/aliphatic chain (Phillips & Chapman, 1968). The limiting areas were found to be similar for all the head groups except for DMP-PR. Here, the phase transition is close to the measuring temperature, and possibly the lipid was still in the liquid-expanded state. The limiting area of 50 $Å^2$/molecule is closer to the value of 58 $Å^2$/molecule

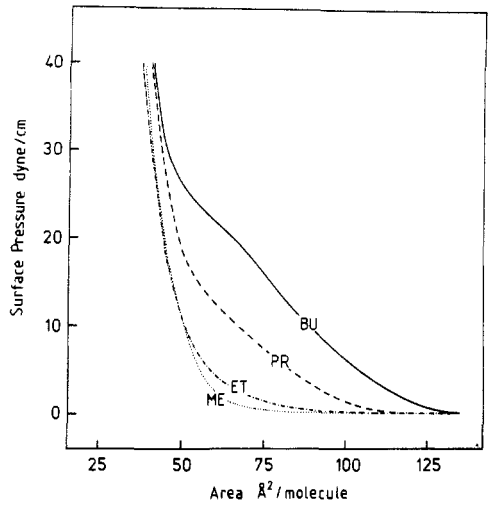

FIGURE 9: Surface pressure–area diagrams for monolayers of alkyl head-group phospholipids. Curves are for the compression of the lipids on a subphase of pure water, pH 5.4–5.8. These data are for a set of lipids with palmitoyl fatty acyl chains.

Table IV: Surface Areas of Various Alkyl Head-Group Phospholipids in Monolayers

		area (Å²/molecule)			
		pure water[a]		buffer, 0.1 M NaCl[b]	
lipid	head group	15 dyn/cm	30 dyn/cm	15 dyn/cm	30 dyn/cm
DPP-ME	methyl	44	40	66	51
DPP-ET	ethyl	43	40	71	53
DPP-PR	propyl	50	44	97	63
DPP-BU	butyl	74	45	109	80
DMP-ME	methyl	46	43		
DMP-PR	propyl	70	50		

[a] The pH was consistently 5.4–5.8 and the temperature 22–24 °C. [b] Pipes-Tris buffer (2 mM), pH 7.0, with 1 mM EDTA.

found for dilauroylglycero-3-phosphopropanol which was definitely in the liquid-expanded state (i.e., correlating with the fluid-bilayer state; Sacrē & Tocanne, 1977).

At lower pressures, films of phospholipids with large head groups were more expanded than those with small head groups. The methyl and ethyl head groups appeared to offer no resistance to compression while lengthening the head group to three- and four-carbon segments resulted in dramatic opposition to compression. With myristoyl (14:0) instead of palmitoyl (16:0) chains, similar effects were observed with these head groups. When a buffer system essentially identical with that used in the NMR measurements was the subphase, larger areas were observed. This effect was previously reported for phosphatidylglycerol and phosphatidylpropanol (Sacrē & Tocanne, 1977) and results at least partially from the higher salt concentration. With this subphase, the transition from a liquid-expanded to a liquid-condensed phase was found to shift to higher pressures and smaller areas as the head group was lengthened. Typically, a distinct break in the curve was observed at about 5 dyn/cm (at 150 Å²/molecule) for DPP-ME and shifted to 40 dyn/cm (at 65 Å²/molecule) for the butyl derivative DPP-BU. The overall effect was similar to that observed with a pure water subphase; the larger head groups produced a more expanded film.

Discussion

This set of relatively simple, noninteracting head groups is useful not only as a set of reference values for comparison with other head groups but also because it provides a good model system for the analysis of steric effects at the membrane surface. In this discussion, it will be shown that these head groups have a distinct average orientation which is sensitive to the packing arrangement at the membrane surface. Agents which decrease or increase the available surface area per lipid head group influence this orientation. Finally, the dynamic properties of these head groups will be dealt with in a section not directly related to the head-group orientation. First, since much of the data presented here involve the use of deuterium NMR, it is necessary to discuss some aspects of this technique as applied to lipid head groups.

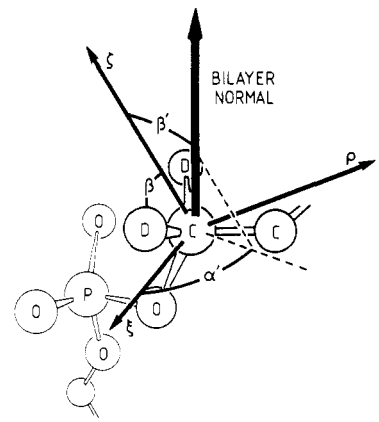

FIGURE 10: Illustration showing the α-CD_2 segment, its segment fixed coordinate system, and angles used in the derivation of eq 2–5. The ζ axis bisects the D–C–D angle, and the ζ–ρ plane includes the O–C_α–C_β atoms.

Evaluation of ^2H NMR Head-Group Spectra. ^2H NMR is a very sensitive tool for the study of phospholipid head groups in liquid-crystalline phase bilayer membranes. For example, diastereomers of both phosphatidylserine (Browning & Seelig, 1980) and phosphatidylglycerol (Wohlgemuth et al., 1980) can be readily distinguished by using this technique. On the other hand, the evaluation of the order parameter, S_{CD}, obtained from the experimental data has been hampered by an inability to separate even qualitatively motional from geometrical contributions. One model has been used to evaluate the data in terms of a preferred head-group conformation by assuming that the head group is rapidly exchanging between two enantiomeric conformations with a superimposed wobbling motion (Seelig et al., 1977; Skarjune & Oldfield, 1979). For use as a quantitative tool, this model suffers from a lack of knowledge of the various internal motions contributing to the segmental order parameters.

The C–D bond order parameter, S_{CD}, can be obtained from the deuterium quadrupole splitting ($\Delta\nu_q$) by

$$S_{CD} = \frac{4}{3}\left(\frac{e^2qQ}{h}\right)^{-1}\Delta\nu_q \qquad (1)$$

where e^2qQ/h is the static quadrupole coupling constant (170 kHz for a C–D bond). The extension from this order parameter to detailed conformational information can be involved [cf. Volino & Dianoux (1979)], and in most cases, the problem is underdetermined. For the (±) deuterons of a CD_2 group, the C–D order parameter can be expressed as (Seelig, 1977)

$$S_{CD}(\pm\beta) = \tfrac{1}{2}((3\cos^2\beta - 1)\langle 3\cos^2\beta' - 1\rangle) \mp \tfrac{3}{4}\sin 2\beta\langle\sin 2\beta'\cos\alpha'\rangle + \tfrac{3}{4}\sin^2\beta\langle\sin^2\beta'\cos 2\alpha'\rangle \qquad (2)$$

where 2β is the D–C–D bond angle and α' and β' are the Eulerian angles for the rotation of a segment fixed coordinate system into that of the bilayer normal, i.e., director axis. The segmental coordinate system is defined in Figure 10. The

brackets signify the time averages of these terms. For a CD_2 group with tetrahedral symmetry, eq 2 reduces to

$$S_{CD}(\pm\beta) = \mp 0.707\langle \sin 2\beta' \cos \alpha' \rangle + 0.5\langle \sin^2 \beta' \cos 2\alpha' \rangle \quad (3)$$

If both deuterons give the same quadrupole splitting, then either the term $\pm\langle \sin 2\beta' \cos \alpha' \rangle$ or $\langle \sin^2 \beta' \cos 2\alpha' \rangle$ must be 0, yielding

$$S_{CD} = +0.5\langle \sin^2 \beta' \cos 2\alpha' \rangle \quad (4)$$

or

$$S_{CD} = \pm 0.707\langle \sin 2\beta' \cos \alpha' \rangle \quad (5)$$

Since the motions involved in the time averaging of these two angles are most likely not independent, it is difficult to further reduce these equations. In the analysis required here, it is necessary to examine the case where $S_{CD} = 0$, i.e., a zero quadrupole splitting. This would occur if the CD_2 segment undergoes sufficient motion (effectively isotropic motion) leading to complete averaging of the quadrupole interactions. This possibility is unlikely in the liquid-crystalline phase. Moreover, in the case of a α-CD_2 group, such motional freedom is not observed at the neighboring segments, C_β and the phosphate. An alternative possibility is that the geometrical contributions, i.e., the angles α' and β', are such that the expressions 4 or 5 for S_{CD} become 0. The important point is not the exact α' and β' combinations which lead to $S_{CD} = 0$ but the fact that when S_{CD} varies from positive to zero and then to negative values (or vice versa) there must be a change in the average orientation of the CD_2 segment. The deuterium data of Deloche & Charvolin (1976) for a liquid-crystal system represent a simplified example of this type of phenomenon.

Head-Group Orientation. It was seen that the α-CD_2 group of DPP-PR had a quadrupole splitting of zero. Equations 4 and 5 show that when $S_{CD} = 0$, only a narrow range of α' and β' angles are possible. The head-group size, where a zero quadrupole splitting was observed, shifted to shorter lengths with increasing head-group lengths and with increasing concentrations of DMPC. The ethyl head-group α-quadrupole splitting decreased to zero and then increased with increasing amounts of DMPC. These effects are interpreted as resulting in a change in the sign of the segmental order parameter, S_{CD}. The physical measurements can determine only the absolute value of this parameter. In Figure 5A, the sign of the quadrupole splitting is shown to change, which is intended to reflect this change in S_{CD}.

This behavior is exemplary of a geometrical change, i.e., a continuous change in the orientation of the α-CD_2 group. At one point, this change passes through the set of α' and β' angles for which $S_{CD} = 0$, and it is this point which corresponds to the orientation of the α-CD_2 segment in DPP-PR [for similar data from a liquid crystal, see Deloche & Charvolin (1976)]. This observed trend upon progressing from DPP-ME to DPP-BU cannot be explained only on the basis of motional considerations. At best, motional effects should be continuous in this series of compounds and cannot result in the zero quadrupole splitting for the α position of DPP-PR.

Thus, the average orientation of the head group is dependent upon the length or the size of the head group. Since these alkyl head groups cannot interact with themselves or with neighboring molecules, steric effects must be responsible for the differing orientations. If models are made of the head groups and arranged in a hexagonal array with an interphosphate distance of 8.2 Å [spacing of DPPC from X-ray data, cf. Shepherd & Büldt (1978)], it is seen that the methyl and ethyl

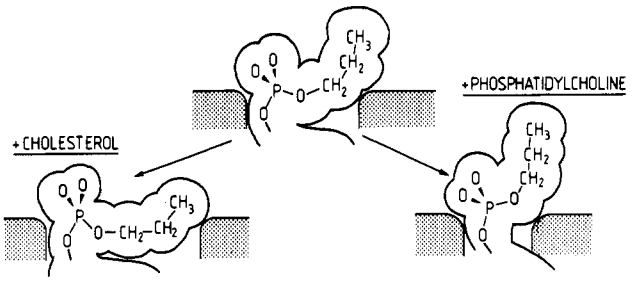

FIGURE 11: Sketch of the effects of phosphatidylcholine and cholesterol on the orientation of the alkyl head groups (in this case a propyl head group).

head groups can easily fit in a parallel conformation in the plane of the membrane surface.[2] With the propyl head group, a parallel conformation can be barely accommodated in this plane. With the longer head groups, i.e., butyl, either a more upright orientation must be assumed or the motions must be very concerted.

If the packing plays an important role, then changes in the packing arrangement should affect the head-group orientation. An increase in the available surface area can be achieved by the addition of cholesterol. Monolayer data on cholesterol/phosphatidylcholine mixtures show that the surface area per phosphatidylcholine head group increases from 60 Å^2 (fluid state without cholesterol) to about 90 Å^2 in a 1/1 molar mixture (Demel & de Kruijff, 1976). This effect is due to the fact that cholesterol does not extend into the head-group region. The hydroxyl group of cholesterol in a phospholipid membrane is known to be located at the level of the glycerol (Worcester & Franks, 1976). Likewise, a decrease in the average area per head group can be obtained in mixtures with phosphatidylcholine. The phosphatidylcholine head group is large and with its parallel conformation [see review by Büldt & Wohlgemuth (1981) and Seelig & Seelig (1980)] occupies at least one-half the available surface area (58–60 Å^2; Büldt et al., 1978).[2]

It is logical that in mixtures with phosphatidylcholine, a reduction in the available space would cause the alkyl head groups to assume a more perpendicular orientation. Increasing the available area, as with the addition of cholesterol, should allow these head groups to retract into a more parallel orientation (Figure 11). On the basis of this model, the data should conform to a number of criteria. The methyl group is small and should not be affected by large amounts of phosphatidylcholine. Likewise in the limit, large head groups should assume a more perpendicular orientation regardless of the presence of phosphatidylcholine. The direction of the change induced by phosphatidylcholine addition should be the same as that caused by increasing the head-group size. That is, decreasing the available surface area by whatever means should affect the quadrupole splittings in a similar way. Finally, increasing the surface area should have the opposite effect. All of these criteria are demonstrated by the experimental data.

This analysis emphasizes changes in the overall orientation of the head group due to steric effects. The above discussion was based on data from the α segment. Are these trends observed at the other neighboring segments? From the data on the β segments of DPP-ET and DPP-PR (Table III), it is

[2] From CPK models arranged in an extended conformation parallel to the bilayer surface, the approximate areas (Å^2) projected into the plane of the bilayer surface for the head groups (including phosphate) are phosphate alone 12, methyl 16, ethyl 20, propyl 25, butyl 29, and choline 32.

seen that similar trends are observed. The quadrupole splittings for this segment in both compounds increased in the following order: 50% cholesterol, 0, 50, and 90% DMPC. Thus, there is at least qualitative agreement with the above analysis. The γ position of DPP-PR was also examined, and here the interpretation is not so clear. At this point, the segment is so flexible that it is becoming questionable whether a preferred orientation of the C_β–C_γ bond exists.

Measurement of the phosphorus chemical shift anisotropy (CSA) shows a trend very similar to that found at the neighboring α segment. An almost linear increase in the CSA accompanied lengthening of the head group. The CSA is a scalar property determined by the trace of the chemical shielding tensor. This tensor is mathematically similar to the electric field gradient tensor which determines the quadrupole splitting [for a review, see Seelig (1978)]. However, two order parameters are needed to describe the CSA, whereas one suffices for the deuterium data. In both cases, the parameters contain both orientational and motional determinants. Here, the observed increase in the CSA is contrary to what would be expected on the basis of motional considerations. The longer head groups undergo less overall motion and should have a larger CSA. Since this is not observed, the orientation must be changing with larger head groups. Addition of cholesterol creates more surface area for the larger head groups. As a consequence, the CSA values approach those associated with a phosphate conformation with a parallel head-group orientation.

The phosphorus CSA data can be qualitatively evaluated in terms of an orientation (i.e., the torsional angles α_1 and α_2 for the bonds C_{Gly}–O–P and C_{Gly}–*O*–*P*, respectively) and an order parameter for the fluctuations of the phosphate about the bilayer normal (Kohler & Klein, 1977; Seelig et al., 1977; Seelig, 1978). In this approach, the CSA value expected for a particular conformation (α_1, α_2) can be calculated. A set of conformations are found which yield the observed CSA [cf. Skarjune & Oldfield (1979)]. In the simplest case, the vector containing the two nonesterified oxygens of the phosphate is parallel to the bilayer surface, and the vector defined by the two esterified oxygens is tilted more vertically as the head groups become more perpendicularly oriented (approximately as in Figure 10). This model is incompatible with the data. In several crystal structures of phospholipids and phospholipid analogues, a trans–gauche conformation is always found for the α_1, α_2 ester linkage [see reviews by Hauser & Phillipis (1979) and Sundaralingam (1972)]. Such a conformation is compatible with the observed CSA data for phosphatidylcholine and phosphatidylethanolamine (Seelig et al., 1977; Seelig & Gally, 1976). If it is assumed that a trans–gauche conformation is present when the head groups are oriented parallel to the bilayer surface, then one can ask the following question: What conformational change would result in a small CSA value? One possibility is a change from a trans–gauche to a roughly gauche–trans (α_1, α_2) conformation. This shift leads to a more perpendicular head-group orientation. Other conformational changes can also account for these data.

The CSA value for phosphatidic acid was contiguous with the data for the alkyl analogues, suggesting a continuous conformational change throughout this set of head groups. This result is noteworthy because phosphatidic acid, as a phosphomonoester, has a distinctly different static chemical shift tensor from that of the similar phosphodiesters [see review by Seelig (1978)]. The expected CSA was calculated as a function of the α_1,α_2 conformation with the phosphatidic acid static chemical shift tensor, and the observed CSA value was found to be still compatible with a trans–gauche conformation for these two bonds.

The monolayer properties of these alkyl head-group phospholipids strongly support the above concepts. The surface area measured in a monolayer reflects a balance between repulsive and cohesive forces. Small changes in either factor can result in differences in the pressure–area diagrams between various phospholipids (Gershfeld, 1970). Nevertheless, in this case a comparison was made with compounds having identical fatty acid chains. Since the transition temperatures are quite similar, the measurements were made at a common reduced temperature. Under such conditions, the state of the hydrocarbon region at least in bilayers is the same in each case (Seelig & Browning, 1978). Therefore, differences in surface pressure–area diagrams can be directly interpreted in terms of differences in head-group properties. The limiting areas (40–44 Å^2/molecule at 30 dyn/cm) of these analogues show that the lipids pack normally in a monolayer with the limit being determined by the area occupied by two fatty acyl chains (Phillips, 1972). However, at lower pressures, the effects of head-group bulk were manifested as an expansion of the surface area. Apparently, the methyl and ethyl head groups can pack well at a limiting surface area of 40 Å^2/molecule, while the propyl and butyl head groups come into contact with neighboring head groups even at low surface pressures. The shape of these surface pressure–area diagrams indicates that as one compresses a phospholipid with a large head group, the head group must undergo a conformational change to accommodate itself to the reduced area. It is envisaged that in a bilayer the forces responsible for the close packing of the chains result in a small surface area with concomitant effects on the head-group packing.

The absolute magnitude of the changes in head-group orientation cannot be determined. The α-segment quadrupole splittings varied over a range of 11 kHz. This is a large variation and is comparable with the magnitude of the effects of divalent cations on the head-group data for phosphatidylcholine (H. Akutsu and J. Seelig, unpublished experiments). The phosphorus CSA changes are rather large, spanning about 15 ppm. Moreover, the effects of varying the head-group size on the monolayer properties also appear to be large. On this basis, it is felt that these orientation changes are also large and on the order of those illustrated in Figure 11.

These points attest to the existence of a preferred average geometry even in these simple head groups. This preferred orientation is modulated by steric forces in the plane of the membrane surface. The use of the term "orientation" does not necessarily imply a particular combination of trans and gauche bonds, but rather an overall picture "averaged" by fast trans–gauche isomerizations. With the ^{2}H NMR technique, motions faster than the reciprocal of the residual quadrupole splitting (10^3–10^5 s^{-1}) lead to averaging. That is, if several conformations are exchanging at a rate slower than this range, then more than one quadrupole splitting will be observed. Since only one quadrupole splitting was observed in this study, the above picture is most likely the average of many conformations. The observation of a distinct orientation is probably not unusual. A "gauche–gauche" conformation is almost always found for the O–P–O diester linkage, whether in a phospholipid, small model compounds, or nucleic acids (Sundaralingam, 1972; Hauser & Phillips, 1979). This conformation introduces a bend at the phosphate, imparting an orientational preference to even these simple alkyl head groups. Steric factors, then, place further limits on the available conformations.

Table V: Comparison of the Motional Properties within a Phospholipid Molecule

labeled position	relaxation time, ^2H T_1 (ms)[a]	app correlation time, $\tau_c \times 10^{11}$ (s)[b]	activation energy[c] (kJ/mol) fluid	activation energy[c] (kJ/mol) gel	ref
alkyl head groups					
α-CD_2 (DPP-ET)	48	4.9	21.3	13.4	*i*
α-CD_2 (DPP-PR)	33	7.1	11.8	<11	*i*
β-CD_2 (DPP-PR)	56	4.2			*i*
CD_3 (DPP-ME)	270	0.9	26.2	10.5	*i*
3-CD_2-glycerol[j]	13	16.8	14.6		*d*
fatty chains[j]					
2-CD_2	21	10.7	14.6		*d*
8-CD_2	33	6.8	14.6		*d*
12-CD_2	56	4.0	14.6		*d*
15-CD_2	130	1.8	14.6		*d*
16-CD_2	275	0.8			*e*
PO_4^--N^+ dipole of DPPC		230	16.7	10.9	*f*
phosphate		100	17.0		*g*
		140			*h*

[a] Data at 47 °C unless otherwise noted. [b] Calculated by using eq 6. [c] Activation energy for relaxation obtained from Arrhenius plots of the relaxation data. For reference, RT is 2.5 kJ/mol or 0.6 kcal/mol at 25 °C. [d] Brown et al. (1979). [e] Davis (1979), 45 °C. [f] Activation enthalpy, Shephard & Büldt (1978), from dielectric relaxation data. [g] Seelig et al. (1981), from the minimum of a ^{31}P T_1 vs. temperature plot (121 mHz), which occurred at about 4 °C. [h] Yeagle et al. (1975), from $^{31}P\{^1H\}$ NOE data. [i] This paper. [j] From DPPC.

Head-Group Motions. The motional properties of these alkyl head groups were studied by means of deuterium spin–lattice (T_1) relaxation time measurements. Deuterium relaxation proceeds by essentially only a quadrupole interaction which in this case is much stronger than the dipolar couplings to adjacent nuclei. With a quadrupolar mechanism, relaxation results from the fluctuation of the molecule fixed electric field gradient with respect to the applied magnetic field. Thus, the ^2H T_1 relaxation time is directly related to the rates of motion of the C–D segment, and there are no intermolecular contributions. With certain assumptions, an apparent correlation time can be calculated as for the fatty acyl chains (Brown et al., 1979; Brown, 1979; Davis, 1979). In the extreme narrowing limit ($\omega_0\tau_c \ll 1$) which is the case for these data, the segmental reorientational correlation time (τ_c) is inversely proportional to the relaxation time and is given by (Brown et al., 1979)

$$\frac{1}{T_1} = \frac{3}{8}\left(\frac{e^2qQ}{\hbar}\right)^2\left(1 + \frac{1}{2}S_{CD} - \frac{3}{2}S_{CD}^2\right)\tau_c \qquad (6)$$

Generally, S_{CD} for these head-group labels was very small, and the term containing this variable can be ignored.

The simplest approach for interpreting the T_1 data is to compare the head-group data with those obtained at other positions in a "typical" phospholipid [cf. Table V of Seelig & Seelig (1980)]. The head-group segmental relaxation times are found to be comparable with the first eight to nine segments (plateau region) of the fatty acyl chains of phosphatidylcholine. Above the phase transition, this region is known to be rather fluid and liquidlike [see Chapman (1975) and Seelig (1977)]. This result indicates that the rates of motion in these alkyl head groups are fast and that these head groups are very flexible.

The deuterium correlation time for an α-methylene segment is calculated to be approximately (5–8) $\times 10^{-11}$ s by using eq 6. This value is 1–2 orders of magnitude faster than the reorientational correlation time of the neighboring phosphorus: 1.4×10^{-9} s (from ^{31}P NOE; Yeagle et al., 1975), 1.0×10^{-9} s (temperature dependence of ^{31}P T_1 in 1,2-dioleoylglycero-3-phosphocholine; Seelig et al., 1981), and 2.3×10^{-9} s (dielectric relaxation; Shephard & Büldt, 1978). The value of 1.0×10^{-9} s from the ^{31}P T_1 data is in even better agreement with the dielectric relaxation data when one considers the differing expressions for the rotational diffusion coefficients as calculated from the correlation time (Abragam, 1961). These data indicate much slower rates of motion for the phosphate when compared with a CD_2 segment only two bonds removed. A likely explanation for this discrepancy is that the phosphorus measurements reflect the overall motion of the head group or at least the relative motion of the phosphate and the choline methyl groups, whereas the ^2H T_1 data are determined primarily by the much faster segmental motions. Alternatively, this difference may reflect the larger size of a PO_4 segment compared to a CD_2 group.

The introduction of the bulky phosphatidylcholine head group into the membrane surface decreased the rates of motion at all segments of all the alkyl head groups. In mixtures with cholesterol, the rates of motion of the shorter head groups were increased. The longer head groups appeared to be unchanged or even less flexible. These effects on the shorter length head groups suggest that even here, where the head groups can pack relatively easily, steric hindrance may be a factor.

The temperature dependence of the T_1 relaxation data shows a change at the phase transition temperature, yet this change is not dramatic. In the fatty acyl chains, ^2H T_1 relaxation times have been observed to shift from the short to the long correlation time regime upon going from the liquid-crystalline to the gel state (L. Tamm and J. Seelig, unpublished data). These data remained in the short correlation time regime in both phases. This result can be expected for the short alkyl head groups. Since the surface area per head group changes by about 10 Å^2 at the phase transition [phosphatidylcholine data of Albrecht et al. (1978), Blume (1979), and Büldt et al. (1979)], the environment of a methyl or a methylene group can be expected to be similar both above and below the phase transition. The activation energies obtained from the ^2H T_1 data above and below the phase transition (Table V) are in good agreement with dielectric relaxation data on phosphatidylcholine (Shepherd & Büldt, 1978).

Inequivalence of the Two Deuterons of a Methylene Segment. The observation of two NMR signals for the two deuterons of a CD_2 group is a common occurrence in the head groups (Wohlgemuth et al., 1980) and provides some insight on the motional properties of the methylene segment. Only recently has sufficient evidence been accumulated to make this assignment, and the conditions producing this inequivalence have not been explicitly formulated. In Figure 12, the con-

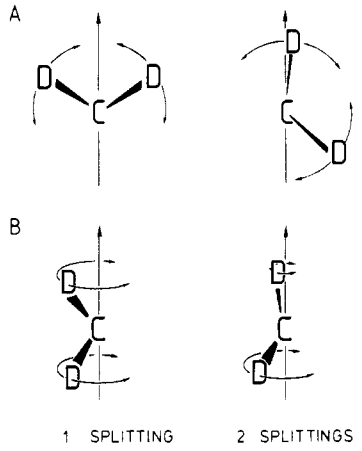

FIGURE 12: Motional conditions resulting in one or two observed quadrupole splittings for a CD_2 segment. The arrow indicates the axis of axial symmetry or the bilayer normal.

ditions leading to the observation of one or two deuterium signals are shown. In general, if the two deuterons have the same average orientation with respect to the director axis, then one "averaged" signal will result. Case A can be easily imagined as resulting from rapid $^{+}$gauche–trans–$^{-}$gauche isomerizations leading to one signal. If this exchange becomes limited to only trans–$^{-}$gauche isomerizations, then two signals will be observed. Rapid exchange between two enantiomers in the phosphaditylcholine head group has been proposed to account for the observation of only one signal at both the α- and β-CD_2 groups (Seelig et al., 1977). This exchange is an example of a symmetrical "averaging" motion. Case B represents an alternate geometry. In both cases, the important determinant is the symmetry of the motion.

Often, the two-signal case is associated with slower rates of motion in the head group. When there is a high degree of flexibility in the head group, the symmetrical motions described in Figure 12 can occur. Reducing the flexibility through hydrogen or electrostatic bonding can lead to a restricted conformational space (i.e., a more limited number of conformational possibilities), where such symmetrical motions cannot take place. An example of this case is seen at the α position of the phosphatidylserine head group where the rates of motion are dramatically slowed [cf. Browning (1981) and Browning & Seelig (1980)] and in the C-1 position of the glycerol backbone (Gally et al., 1981). In both examples, there is limited flexibility, and the CD_2 segment gives rise to two quadrupole splittings of very different size.

On the other hand, the same rates of motion can be present in both the one and two NMR signal cases. This statement is exemplified by the observation of two signals for the α-CD_2 of these alkyl head groups in mixtures with cholesterol. Here, the existence of two slowly exchanging conformers can be excluded since only one signal was observed in the similar DPP-ME/cholesterol mixture. Because of the 3-fold symmetry of a methyl group, only one signal can result. Thus, these two signals must be attributed to the two CD_2 deuterons. In the absence of cholesterol, the head groups are not oriented completely parallel to the bilayer surface. Rapid, symmetrical trans–gauche isomerizations occur, and only one signal per CD_2 group is observed. With cholesterol, a parallel (or more parallel) orientation is obtained, and there may be some steric interaction with the actual surface of the bilayer (e.g., the glycerol backbone region). This could lead to a restriction in the conformational space, resulting in a signal for each deuteron. At the α position of these head groups in the presence of cholesterol, no large reduction in the length of the T_1 was observed. Therefore, the rates of segmental motion at least in this high-frequency range were not altered, yet the two deuterons are inequivalent.

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Motions and Interactions of Phospholipid Head Groups at the Membrane Surface. 2. Head Groups with Hydroxyl Groups†

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ABSTRACT: A set of phospholipids with zero, one, or two hydroxyl groups at various positions in the head group were synthesized to approach the question of hydrogen bonding at the surface of phospholipid membranes. These lipids had as head groups the alcohols propanol, ethanediol, 1,3-propanediol, and glycerol esterified to the phosphate. The four different properties of these lipids that were studied were the following: phase transition temperatures, packing properties in monolayers, the relative rates of head-group motions, and the "ordering" of the head group. The gel-to-liquid-crystalline phase transition temperatures of these derivatives were measured by differential scanning calorimetry, and the effect of hydroxyl group addition was found to be small. The phase properties were examined with phosphorus-31 NMR, and all lipids formed normal bilayer phases in aqueous mixtures. Measurement of the surface pressure–area diagrams for monolayers of these lipids showed that incorporation of a hydroxyl group into the head group had a "condensing" effect. This effect was dramatic and was attributed to the formation of hydrogen bonds within the plane of the lipid surface. These lipids were synthesized with deuterium labels on essentially every carbon segment in the head group. Measuring the ^2H NMR spin–lattice (T_1) relaxation time allowed a determination of the relative rates of head-group segmental motions. The addition of hydroxyl groups to the propyl head-group "skeleton" substantially reduced the rates of motion in both the liquid-crystalline and gel states. The head groups could be ordered in terms of increasing rigidity as propanol (0 -OH) < 1,3-propanediol (1 -OH) < ethanediol (1 -OH) < glycerol (2 -OH). This trend was observed for labels attached to all of the head-group carbon segments of the lipids in this set. The rates of motion of the different segments within the ethanediol or glycerol head groups were almost identical, whereas those of the propyl head group increased as one progressed toward the free end of the head group. The average activation energy for the motions involved in T_1 relaxation increased upon introduction of a hydroxyl group. Measurement of the deuterium residual quadrupole splittings showed that the initial PO_2–O–CD_2– segments of the hydroxyl-containing head groups have very similar ordering properties which differed distinctly from those of the propyl head group. Thus, the introduction of one hydroxyl group appears to alter the head-group conformation to a specific conformation which is shared by all of these hydroxyl group containing head groups.

The properties of the water–lipid interface region of phospholipid membranes are important from the viewpoints of both the physical chemistry of lipid surfaces and the structure and function of biological membranes. In this paper, the question of noncovalent bonds or interactions between phospholipid head groups at this interface will be addressed. The properties of a set of phospholipids with simple, short alkyl chains as head groups were explored in the preceeding paper in this issue (Browning, 1981a). The data obtained from this "reference set" of head groups will be applied to a more complicated set, those head groups containing hydroxyl groups. In this set, hydroxyl groups were placed at various positions on either an ethyl or a propyl "skeleton". By making these slight alterations in these head groups and then determining the effects of the alterations on the properties of the head groups in membranes, one can hope to infer the existence or nonexistence of hydrogen

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