Phosphorus-31 Chemical-Shift Tensors in Barium Diethyl Phosphate and Urea–Phosphoric Acid: Model Compounds for Phospholipid Head-Group Studies†

J. Herzfeld, R. G. Griffin,* and R. A. Haberkorn

ABSTRACT: The molecular orientations of the ^{31}P chemical-shift tensors in the phosphodiester barium diethyl phosphate (BDEP) and in the nonesterified urea–H_3PO_4 (UPA) complex were determined from single-crystal studies. Although the tensors possess qualitatively similar orientations, there are differences between the two tensors of the order of 20°. The principal values of the BDEP tensor are within 5% of those found in powder spectra of phospholipids, and, for this and other reasons, which are discussed in detail, we believe BDEP is a good model chemical-shift tensor for lipid phosphate. With the BDEP tensor alone, one can label certain head-group conformations as improbable, and, in conjunction with oriented bilayer spectra, one can determine the conformation of lipid head groups. In addition, we suggest a technique for orienting crystals based on the transformation properties of shift tensors, and we demonstrate this method by applying it to UPA. Finally, our results allow us to assess the effect of shift anisotropy relaxation on ^{31}P liquid spectra at high fields.

Phosphorus-31 nuclear magnetic resonance spectra have been obtained for phospholipids in a variety of forms and have been shown to yield a great deal of interesting information (Berden et al., 1974, 1975; McLaughlin et al., 1975a,b; DeKruijff et al., 1976; Griffin, 1976; Gally et al., 1975; Neiderberger et al., 1976; Seelig et al., 1977). However, since these spectra are dominated by chemical-shift anisotropy effects (except for sonicated vesicles at low fields), the complete interpretation of the ^{31}P spectra of phospholipids depends on a knowledge of the orientation of the chemical-shift tensor in these molecules. In liposomes, where tumbling rates are slow, the partial averaging of the chemical-shift anisotropy seen in the ^{31}P spectra must be due primarily to motion of the lipids within the bilayer. Analysis of this partial averaging in terms of the ^{31}P chemical-shift tensor can provide information about molecular motion and the factors, such as temperature and chemical composition, which govern it. In partially oriented bilayers, the ^{31}P chemical-shift tensor may be used to analyze the angular dependence of ^{31}P chemical shifts, in order to determine the orientation of the phosphate moiety in such a system (Griffin et al., 1978).

With a view toward these applications, we have undertaken two single-crystal studies of ^{31}P chemical-shift tensors. We have chosen barium diethyl phosphate (BDEP[1]) as a model for lipid phosphate because (1) like phospholipids it is a phosphodiester, (2) the principal values of the chemical-shift tensor are within 5% of those found for phospholipids, and (3) the geometry of the phosphate moiety is similar to that found in the one phospholipid and the one phospholipid head-group fragment with known crystal structures. The urea–phosphoric acid complex (UPA) was chosen for the second single-crystal study because it possesses only protonated oxygens and thus provides a contrasting case to the diester BDEP and the monoester phosphorylethanolamine (PEA) which was studied by Kohler and Klein (1976). The pH dependence of PO_4 chemical shifts is well documented (Cohn and Hughes, 1960) and has recently been reported for phospholipids (Seelig and Gally, 1976). In order to interpret these spectra, it is important to know how the chemical-shift tensor is affected by protonation and covalent bonding. Our study of UPA provides some information about the influence of these factors.

The fact that UPA crystallizes in an orthorhombic space group permitted us to employ a new technique in orienting our crystals which relies on the crystal symmetry. Specifically, crystal symmetry requires that chemical-shift tensors transform into one another by rotations about unit cell axes, and we have utilized this constraint in determining the positions of these axes. Although this procedure requires some a priori knowledge of the orientation of shift tensors, it can be very useful in cases such as UPA with orthorhombic symmetry and, to our knowledge, does not seem to have been employed previously.

Experimental Section

Crystals of BDEP were obtained by slow evaporation of aqueous solutions containing stoichiometric quantities of diethyl phosphate and barium hydroxide. The crystals grow as needles elongated in the *c* direction, and specimens chosen for study were mounted in holes drilled in machinable glass cubes. The cubes were then attached to buttons which were transferable between X-ray and NMR probe goniometers. Three orthogonal rotations for the NMR experiments were achieved by attaching the button successively to three different faces of the cube.

The UPA crystals were also grown by slow evaporation from aqueous solutions. A cube-shaped sample was then cut from a large crystal and this was cemented to a square piece of glass. The glass, with the crystal, was remounted for the three or-

† From the Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 (R.G. and R.A.H.), and the Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts 02115 (J.H.). *Received June 3, 1977; revised manuscript received March 24, 1978.* J. H. acknowledges the financial support of the National Institutes of Health (HL 14820 and GM 23316). R.G.G. and R.A.H. acknowledge the support of the National Science Foundation (C-670) and the National Institutes of Health (GM-23289).

[1] Abbreviations used are: BDEP, barium diethyl phosphate, Ba-$[PO_4(C_2H_5)_2]_2$; UPA, urea–phosphoric acid, $(NH_2)_2CO$–H_3PO_4; DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DLPC, dilaurylphosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; DLPE·HOAc, dilaurylphosphatidylethanolamine–acetic acid; PEA, phosphorylethanolamine; L-α-GPC, L-α-glycerophosphorylcholine.

0006-2960/78/0417-2711$01.00/0

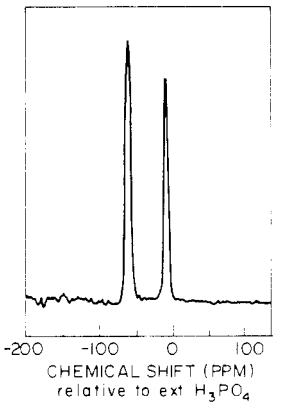

FIGURE 1: BDEP single-crystal spectrum. This spectrum was obtained with the rotation axis approximately along the **c** axis of the crystal. The two lines have widths of ~6 ppm and are due to the two magnetically inequivalent molecules in the unit cell.

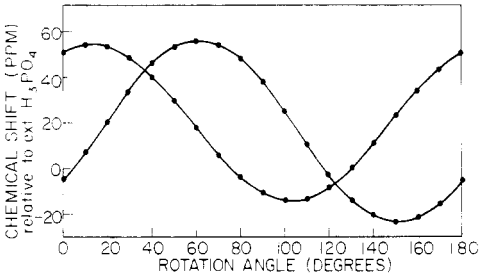

FIGURE 2: BDEP rotation plot obtained by rotating a crystal approximately about the **c** axis of the unit cell.

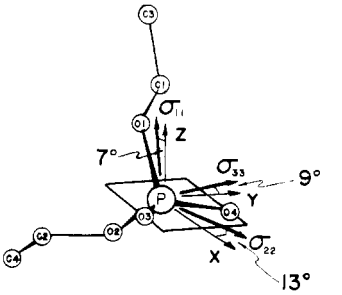

FIGURE 3: Molecular orientation of the ^{31}P chemical-shift tensor in BDEP. Shown is the tensor orientation relative to the *x, y, z* reference frame, chosen as described in the text. The angles between the tensor and the *x, y, z* frame are exaggerated for clarity.

TABLE I: Principal Values, in Parts Per Million Relative to External 85% H_3PO_4, and Direction Cosines, Relative to the **abc*** Unit Cell Axes, for the $^{31}PO_4$ Chemical Shift Tensor in BDEP.[a]

σ_{11}		σ_{22}		σ_{33}	
	−75.9		−17.5		109.8
z	± 1.2	*x*	± 0.2	*y*	± 2.5
−0.7567	−0.6787	0.6345	0.7330	0.1577	0.0443
−0.5560	−0.6090	−0.4976	−0.5281	−0.6658	−0.5919
−0.3440	−0.4105	−0.5915	−0.4287	0.7293	0.8048

[a] Also included are the direction cosines of the *x, y, z* reference frame discussed in the text. Taking the frame and tensor listed below as *x, y, z*, then the other magnetically inequivalent molecule is *x*, $\bar{y}$, *z*. Average errors in the direction cosines are ±1°.

thogonal orientations. We did not determine the orientation of the UPA crystal with X-rays but rather we employed the symmetry operations of the point group to determine the location of the **a, b,** and **c** axes. The details of this method are described in the Appendix.

Anhydrous and monohydrate PCs were prepared by prescriptions in the literature (Chapman et al., 1967). In the case of anhydrous DPPC, this consisted of heating the lipid for ~4 h at 90 °C under high vacuum (10^{-6} Torr) and sealing the sample without exposure to the atmosphere. Chemical analysis of monohydrate samples indicated we were indeed studying DPPC·H_2O. (Anal. Calcd: C, 63.91; H, 10.86; P, 4.13; H_2O, 2.40. Found: C, 64.60; H, 11.14; P, 4.22; H_2O, 2.33.) However, similar analyses of the "anhydrous" samples yielded ~1.3 wt % H_2O. Thus, the literature procedure for the preparation of anhydrous PCs could be producing DPPC·0.5 H_2O; we are currently investigating this point further. Nevertheless, several samples prepared by the above procedure gave reproducible σ_{ii} values which differed by 10–20 ppm from the corresponding monohydrate values. The excess water samples of DPPE were buffered at pH 5.4 and 10.8 with sodium acetate–acetic acid and borax buffers, respectively.

NMR spectra were obtained with a cross-polarization double-resonance experiment (Pines et al., 1973) on a home-built spectrometer operating at a ^{31}P frequency of 118.5 MHz (Griffin and Neuringer, 1973).

Results

The crystal structure of BDEP was determined by Kyogoku and Iitaka (1966), and it was found that the crystals are monoclinic, belonging to the space group $I2/a$, with $\beta = 90°$, and $Z = 8$. This unit cell contains a number of inversion centers and as a consequence the number of magnetically inequivalent PO_4's is reduced to two. A typical spectrum is shown in Figure 1, and Figure 2 is a rotation plot obtained when the crystal is rotated approximately about the *c* axis of the unit cell. These data, together with data from two orthogonal rotations, were employed to obtain the orientation of the tensor in the molecular frame. Table I contains the principal values of the tensor and the direction cosines relating these components to the *a, b,* and *c** axes of the unit cell. Also included in Table I are the direction cosines of an *xyz* system whose orientation was chosen so that *x* bisects the O(3)–P–O(4) angle, *z* is perpendicular to the O(3)–P–O(4) plane, and *y* is orthogonal to these two directions to form a right-handed system. This system is particularly useful for discussing relative tensor orientations and one will note from Table I that the *x, y,* and *z* directions are close to directions of σ_{22}, σ_{33}, and σ_{11}, respectively. Figure 3 is an illustration of the BDEP tensor orientation in the molecular frame. Certain angles are labeled in this figure in order to emphasize the relationship between the tensor orientation and the *x, y, z* axis system.[2]

UPA crystallizes in an orthorhombic lattice *Pcab*, $Z = 8$ (Kostansek and Busing, 1972; Mootz and Albrand, 1972), and in this case the symmetry of the unit cell allows four magnetically inequivalent PO_4 groups. Thus, generally we observe four lines as is shown in Figure 4. Table II contains the principal values and direction cosines relating the PO_4 tensors in UPA to the unit cell axes, as well as the direction cosines of the *x,*

[2] In any magnetic resonance tensor determination, there is an ambiguity in the assignment of the tensors to molecules in the unit cell if there is more than one molecule per unit cell. In the case of BDEP, there are two magnetically inequivalent molecules and thus there are two possible assignments of the tensor to the molecular frame. However, because chemical-shift tensors reflect molecular symmetry, we have chosen the assignment which correlates with the symmetry at the PO_4. This ambiguity has been discussed elsewhere with regard to $^{31}PO_4$ shift tensors (Kohler and Klein, 1976).

TABLE II: Principal Values, in Parts Per Million Relative to External 85% H_3PO_4, and Direction Cosines, Relative to **abc** Unit-Cell Axes, for the $^{31}PO_4$ Chemical Shift Tensors in UPA.[a]

σ_{11}		σ_{22}		σ_{33}	
	−26.6		−2.5		44.6
z	± 1.4	x	± 1.9	y	± 0.4
0.5975	0.7184	−0.3736	−0.4260	0.7095	0.5499
0.4798	0.3783	0.8755	0.9027	0.0570	0.2051
0.6425	0.5838	−0.3063	−0.0607	−0.7024	−0.8097

[a] Also included are the direction cosines of the x, y, z frame discussed in the text. If the frame and tensor listed below are taken as x, y, z, then the three other tensors and frames in the unit cell are x, $\bar{y}$, $\bar{z}$, $\bar{x}$, y, $\bar{z}$, and $\bar{x}$, $\bar{y}$, z. Average errors in the direction cosines are ±1°.

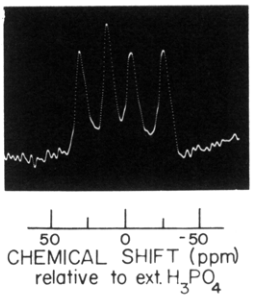

FIGURE 4: UPA single-crystal spectrum showing the four lines obtained from the magnetically inequivalent PO_4s in a general orientation. Due to the presence of ^{14}N and nearby ^{31}Ps, the lines in this spectrum are somewhat broader than found in BDEP crystals.

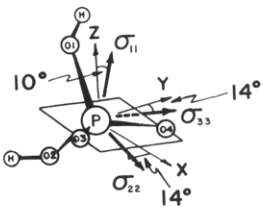

FIGURE 5: Molecular orientation of the ^{31}P chemical-shift tensor in UPA. Shown is the tensor orientation relative to the x, y, z frame discussed in the text. Note the difference in orientations between this tensor and the BDEP tensor.

y, z reference frame, which was chosen in a manner analogous to that for BDEP. As was the case with BDEP, the least-shielded element of this tensor, σ_{11}, is close to the z direction, but it is tilted by 10° toward $+y$ rather than $-z$. As in BDEP, σ_{22} approximately bisects to O–P–O angle, but it is twisted by 14° in the direction of O(3) rather than O(4). Figure 5 illustrates the tensor orientation for UPA, and we note the orientation is clearly different from that found for BDEP, as we might expect on the basis of the principal values of its shift tensor. However, we must point out an ambiguity in the relative orientations of the BDEP and UPA tensors which has to do with the manner in which we have chosen our x, y, z system.

In Table III, we have compiled a list of bond angles and distances obtained from crystallographic data on PO_4 groups pertinent to this study, including DLPE·HAC, (Hitchcock et al., 1974), L-α-GPC (Abrahamsson and Pascher, 1966), and PEA (Kraut, 1961) in addition to BDEP and UPA. From Table III, we see that the P–O distances fall into two groups, and we note that P–O(1) ≈ P–O(2) > P–O(3) ≈ P–O(4). In defining our x, y, z system, we chose x to bisect the O(3)–P–O(4) angle, z perpendicular to the O(3)–P–O(4) plane [i.e., approximately the O(2) to O(1) vector], and y to form a right-handed system [i.e., approximately the O(3) to O(4) vector]. Now, if we reversed the z direction in either BDEP or UPA, by reversing the assignment of O(1) and O(2), and redefined the y direction to maintain a right-handed system, then the two tensors would have very nearly identical orientations. Inspection of Table III reveals that interchanging O(1) and O(2) in BDEP would mean interchanging the P–O(1) and P–O(2) bond lengths, which are the same within the experimental error, interchanging the O(3)–P–O(1) and O(4)–P–O(2) bond angles, which are 104.6° and 103.4°, and interchanging the O(4)–P–O(1) and O(4)–P–O(2) bond angles, which are 111.9° and 110.5°. Thus, the crystallographic data alone are not accurate enough to assign O(1) and O(2) unambiguously. However, if we have reason to believe that the true P–O(1) and P–O(2) bond lengths are significantly different, then the crystallographic data are accurate enough to tell us which is which. We note from Table III that, in all five compounds listed, the P–O(1) bond is longer than the P–O(2) bond. In L-α-GPC, which is remarkably similar to BDEP in its bond angles and distances, this difference is well within the experimental error. We feel that this crystallographic information justifies the choice of assignment which we have made.

As we pointed out, the orientations of the shift tensors in BDEP and UPA are somewhat different in our chosen x, y, z frame and nearly identical in the alternate frame. This outcome raises our confidence in our choice of assignment because we would expect the orientations of the shift tensors to be different in the two compounds. The ^{13}C shift tensors of carboxyl groups, which are the only ones in the literature of which comparative studies have been made (Chang et al., 1974a,b; Griffin et al., 1975; Griffin and Ruben, 1975; Pines and Abramson, 1974), have pronounced variations in orientation, depending on whether the group is ionized, protonated, or esterified. In fact, in carboxyl groups, protonation alone will produce a 20° rotation of the tensor (Griffin and Ruben, 1975), and this is basically the magnitude and the type of difference we observed between UPA and BDEP. However, we do note that there are similarities between the BDEP, PEA, and UPA tensors in that σ_{11} and σ_{22} lie approximately along the z and the x directions, respectively. Since, as shown in Table III, there are similarities among the bond angles and distances in these compounds, these similarities should not be too surprising.

In addition to the single-crystal studies on BDEP and UPA, we have also examined powder spectra of several compounds under various conditions. These results are summarized in Table IV, and a typical powder spectrum is shown in Figure 6. For BDEP, we examined powder spectra at room temperature and at −110 °C and obtained essentially identical results under both sets of circumstances. These data serve as a check on the principal values obtained in our single-crystal study, and the low-temperature experiment ensures us that our single crystal data are rigid-lattice tensor values. The ^{1}H $\mathbf{T}_1$ in UPA is very long at room temperature (~5 min), so we did not think it necessary to perform a low-temperature powder experiment. However, we did study DPPC dispersed in excess water and DPPE at pH 5.4 and 10.8, also in excess water, at −110 °C to explore the possibility that the PO_4 tensor in these systems might be affected by hydrogen bonding with the excess water. Our results indicated that it is not, because the spectra are identical to those obtained for monohydrated DPPC (DPPC·H_2O) and anhydrous DPPE and DLPE at room temperature. This result partially justifies our use of the BDEP tensor in studies of head groups in phospholipids but it is not unequivocal. The remainder of the data in Table IV is for

TABLE III: P-O Bond Distances and O-P-O Bond Angles for Five PO_4 Containing Compounds.[a]

	DLPE · HOAc	L-α-GPC	PEA	UPA	BDEP
P-O(1)	1.63 (2)	1.624 (4)	1.591 (5)	1.560 (3)	1.62 (2)
		1.626 (4)			
P-O(2)	1.62 (2)	1.580 (6)	1.557 (5)	1.553 (3)	1.59 (2)
		1.606 (5)			
P-O(3)	1.47 (2)	1.497 (7)	1.493 (5)	1.519 (3)	1.52 (2)
		1.467 (5)			
P-O(4)	1.43 (2)	1.496(5)	1.503 (5)	1.507 (3)	1.52 (2)
		1.484 (4)			
O(1)-P-O(2)	101 (1)	104.02 (32)			
		102.22 (27)	106.2 (2)	108.2 (2)	103.5 (8)
O(1)-P-O(3)	102 (1)	104.15 (27)			
		104.75 (23)	103.9 (2)	105.2 (2)	104.6 (8)
O(1)-P-O(4)	115 (1)	109.01 (21)			
		109.91 (21)	109.6 (2)	111.9 (2)	111.9 (8)
O(2)-P-O(3)	110 (1)	112.17 (30)			
		110.09 (26)	109.8 (2)	110.8 (2)	110.5 (8)
O(2)-P-O(4)	108 (1)	105.68 (30)			
		105.48 (25)	109.3 (2)	106.9 (2)	103.4 (8)
O(3)-P-O(4)	119 (1)	120.62 (38)			
		122.62 (31)	117.4 (2)	113.7 (2)	121.6 (8)

[a] The labeling of the oxygens has been changed to conform with the labeling employed in the BDEP crystal structure. Errors in the bond lengths and angles are included in parentheses after each entry. The two entries in the L-α-GPC column correspond to the two independent molecules in the asymmetric unit.

TABLE IV: Principal Values, in Parts Per Million Relative to External 85% H_3PO_4, for Various $^{31}PO_4$ Shielding Tensors Obtained from Powder and Single Crystal Experiments.[a]

Compound	Temp (°C)	σ_{11}	σ_{22}	σ_{33}	$\frac{1}{3}\Sigma_i\sigma_{ii}$
BDEP (powder)	20	−79	−19	113	5
BDEP (powder)	−110	−80	−22	108	2
BDEP (crystal)	20	−75.9	−17.5	109.8	5.5
DPPC (anhydr)	20	−98	−34	134	−0.3
DPPC·H_2O	20	−81	−25	110	2.3
DPPC·50 wt % H_2O	20	−40	20	20	0
DPPC·50 wt % H_2O	−110	−81	−21	108	1.3
DMPC (anhydr)	20	−97	−34	133	0.6
DMPC·H_2O	20	−81	−22	110	2
DLPC (anhydr)	20	−96	−32	133	1.7
DLPC·H_2O	20	−79	−19	108	3.3
UPA (powder)	20	−24	−5	45	5.3
UPA (crystal)	20	−26.6 ± 1.4	−2.5 ± 1.9	44.6 ± 0.4	5.2
PEA	20	−63	−8	69	−0.6
DPPE (anhydr)	20	−83	−21	103	2
DPPE·50 wt % H_2O, pH 5.4	−110	−81	−22	104	3
DPPE·50 wt % H_2O, pH 10.8	−110	−80	−21	104	1
DLPE (anhydrous)	20	−84	−23	100	2

[a] Errors for the powder data are ±3 ppm in each component.

various phospholipids and illustrates that, with the exception of the anhydrous lecithins, the principal values for the lipids studied are pretty much identical; that is, they all possess principal values of approximately −80, −20, and +110 ppm; anhydrous lecithins have σ_{ii} which differ by 10–20 ppm from those found for other phospholipids.

We note that some of the σ_{ii} values in Table IV for the various phospholipids are slightly different from those reported previously by Kohler and Klein (1976, 1977); for instance, they obtain −87, −25, and +119 and we find −81, −25, and +110 for DPPC·H_2O.[3] Although the differences are outside the reported experimental errors, they are small and could be due to the fact that the σ_{11} and σ_{33} edges of the powder patterns we obtain are some what sharper than those of Kohler and Klein (1977); i.e., compare Figure 6 with their Figure 1. Because we are at a higher operating frequency, the relative size of the ^{31}P chemical shift and ^{31}P-^{31}P dipolar interaction will be larger, and consequently we expect more well resolved spectra.

[3] Kohler and Klein did not specify the water content of their samples; however, we surmise they were studying something close to DPPC·H_2O.

Discussion

As mentioned above, we believe that BDEP is a good model for lipid phosphate tensors because it is a phosphodiester, and the principal values of its shift tensor (−76, −17, and +110 ppm) are very close to those found in DPPC monohydrate and a number of other phospholipids. In contrast, the other tensor proposed for lipid phosphate is that of the monoester PEA which has principal values of −67, −13, and +69. Thus, the breadth of the PEA spectrum is ~50-ppm smaller than found for phospholipids. These data are summarized in Table IV. In addition, the bond angles and distances in BDEP are close to

those found in DLPE·HAc and in L-α-GPC, as shown in Table III which summarizes the P-O bond distances and angles for five PO_4 moieties pertinent to this study. We note from Table III that the agreement between BDEP and L-α-GPC is very good, while the agreement between BDEP or L-α-GPC and DLPE·HAc is less satisfactory. However, the crystals used for the DLPE·HAc structure were not of high quality, and consequently the differences should perhaps not be taken too seriously.

Assuming then that the BDEP chemical-shift tensor is very similar to that for DPPC·H_2O, the question remains as to whether this tensor may reasonably be employed in the study of phospholipids dispersed in *excess* water. Whereas the ^{2}H quadrupole tensors, employed in chain-dynamics studies, and hyperfine tensors, employed in spin-label studies, would not be directly affected by water, the $^{31}PO_4$ chemical-shift tensors would be perturbed by any changes in protonation or hydrogen bonding resulting from the addition of water. Thus, it is risky to assume, without further examination, that the BDEP chemical-shift tensor accurately represents the chemical-shift tensor of phospholipids in excess water.

We have observed, as shown in Table IV, that the shift anisotropy is smaller in monohydrated lecithins (with tensor principal values −80, −25, 110) than in anhydrous lecithins (with tensor principal values −97, −34, 134). Since the chemical-shift anisotropy reflects departures from cubic symmetry, we conclude that the symmetry at the PO_4 group is reduced by removing the last H_2O molecule. Such a symmetry change would be expected if the H_2O molecule in the monohydrate were hydrogen bonded to the phosphate group. Interestingly enough, we find that in anhydrous DPPE and DLPE the principal values are closer to those of the monohydrate lecithins than the anhydrous lecithins. This could indicate that the NH_3^+ group in PE takes the place of the H_2O in PC. In keeping with this hypothesis, the DLPE·HOAc crystal structure shows that NH_3^+ is hydrogen bonded to the PO_4.

We have reported previously (Griffin, 1976) that the effect of excess water on the ^{31}P spectra of DPPC is to collapse the axially asymmetric spectrum to a symmetric one of 60-ppm breadth at 18 °C. At that time, we pointed out that the trace of the DPPC tensor did not change with addition of water as expected if molecular motion were the source of the narrowing. In contrast, if hydrogen bonding of the water were involved, then we would expect it to perturb the individual tensor elements and thus alter the trace. However, the single-crystal results reported here indicate that this criterion may not be sufficient. We note from Tables I and II that the traces of the BDEP and UPA tensors are +5.5 and +5.2 ppm relative to H_3PO_4, although the tensor orientations and principal values are quite different in the two cases. Consequently, we must conclude that, while this criterion is useful to examine, it by itself is not sufficient to detect the differences in which we are interested. We have attempted to devise other experiments to investigate potential hydrogen bonding. As described above, we have examined the spectrum of DPPE at pH 5.4 and 10.8 and DPPC in excess water at low temperatures. This experiment yielded shift tensors with principal values (see Table IV) essentially identical to that found for DPPC·H_2O and anhydrous PEs. Although these results suggest that there is no hydrogen bonding with the "excess" water, we do not believe that they settle the question, because the hydrogen-bonding structure if it exists could well be temperature dependent.

Taken together, our NMR results indicate that *one* H_2O is probably hydrogen bonded to DPPC in excess water and none to DPPE, and, based on the nearly identical principal values obtained from the BDEP study, we believe the BDEP tensor

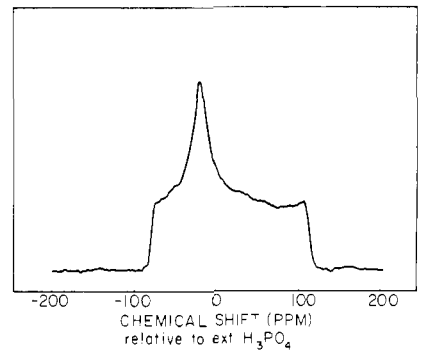

FIGURE 6: Axially asymmetric powder spectrum obtained from a BDEP sample. This spectrum is representative of those found for phosphodiesters such as phospholipids.

provides the best available information on the tensor orientation. However, because of the factors mentioned above as well as the results of our UPA single-crystal study, it is possible that the true $^{31}PO_4$ tensor orientation in phospholipids could be different from that observed in BDEP.

The primary purpose of performing the single-crystal studies reported here was to provide a basis for examining head-group conformation in model and biological membranes. Specifically, we would like to know what PO_4 conformation produces the axially symmetric powder spectrum exhibited by phospholipids in excess water. If the rigid-lattice $^{31}PO_4$ tensors were axially symmetric, then a single spectral measurement would suffice to establish the conformation (or order parameter). Since, as we have shown above, this is not the case, extra information is required to determine the orientation of the PO_4 moiety in the lipid head group. However, one point concerning head-group conformation can be deduced from our $^{31}PO_4$ tensor measurements alone. This point has thus far not been explicitly discussed in the literature and may not be obvious to those not directly involved in this work.

One often finds phospholipids represented with the head group extended parallel to the bilayer normal and the O-P-O plane parallel to the bilayer plane (Lehninger, 1970; Stryer, 1975). If we assume this conformation and let the lipid molecule rotate rapidly about the bilayer normal, then we obtain an axially symmetric powder spectrum with $\sigma_\parallel \simeq -80$ ppm; i.e., the axis of motional averaging is parallel to σ_{11}. Since the trace of the tensor must be invariant to motion, $\sigma_\perp \simeq 40$ ppm and $\Delta\sigma \simeq -120$ ppm. The assumption of rapid rotation about the bilayer normal is based on the observation of the collapse of the ^{2}H powder spectrum in partially deuterated DPPC oriented at the "magic angle" (Stockton, et al., 1974; Seelig and Seelig, 1974) and our own observations of the angular dependence of ^{31}P spectra of oriented bilayers (Griffin, 1977, unpublished results). In order to account for internal motion of the phospholipid, we employ the C(2)-C(3) order parameter of Seelig and Gally (1976) which is 0.66 for DPPC at 48 °C. Thus, the spectrum breadth should be $120 \times 0.66 \simeq 80$ ppm, which is a factor of two larger than is observed at this temperature. Thus, on the basis of our tensor measurements *alone*, we can label any head-group conformation with the nonesterified O-P-O plane parallel to the bilayer plane as quite improbable. This result, as well as the results of Seelig and Gally (1976) and Kohler and Klein (1977), is in disagreement with studies employing paramagnetic ion probes (Hauser et al., 1976). However, we cannot exclude the possibility that the presence of a metal ion alters the head-group conformation.

In order to determine the actual head-group conformation from the spectra, one must either have additional experimental data or substitute educated guesses. We have chosen the former

approach, which consists of examining the angular dependence of oriented bilayer spectra of DPPC·H_2O, and we describe this work in a companion paper (Griffin et al., 1978). Seelig and Gally (1976), Kohler and Klein (1977), and Seelig et al. (1977) have taken the latter approach, using the conformation of DLPE·HOAc, as a starting point for computer simulation of the spectra. In their analyses of phospholipid head-group conformation, these workers employed the chemical-shift tensor reported for PEA where σ_{11}, σ_{22}, and σ_{33} are parallel to our z, x, and y axes, respectively. Our tensor data and oriented bilayer spectra support the qualitative conclusions of these papers, i.e., that the head group is extended parallel to the bilayer plane. However, we believe that our BDEP tensor is a more reasonable model for lipid phosphate than PEA and if we assume this to be the case then the tensor orientation employed by these other workers would be off by a 13° rotation about z and a 7° rotation about y. Thus, use of the BDEP tensor orientation in these calculations could possibly improve the agreement between the torsion angles derived from them and those obtained from the X-ray structure of DLPE·HOAc.

The single-crystal results will also allow us to assess the effect of chemical-shift anisotropy relaxation on $^{31}PO_4$ T_2 values at high magnetic fields. An elegant study of this phenomenon for ^{19}F-labeled fluorotyrosine alkaline phosphatase has recently been published by Hull and Sykes (1975), and they showed that for small proteins (molecular weight $\leq 2 \times 10^4$) shift anisotropy relaxation for ^{19}F nuclei will be important at fields greater than 6.0T, whereas for larger proteins (molecular weight $\approx 10^5$) it can be observed even at ~2.5T fields. Such broadening has been observed for $^{31}PO_4$ lines from phospholipid vesicles (Berden et al., 1974) at ~7.0T and for nucleic acids at 6.4T (Gueron and Shulman, 1975), so it is pertinent to inquire under what circumstances one can expect to observe it in other systems. Since the exact formula for T_2 (and T_1) is cumbersome and depends on the details of the model one chooses for internal motion, we refer the reader to the work of Hull and Sykes for details. However, regardless of the model one chooses,

$$\frac{1}{T_2}, \frac{1}{T_1} \propto \gamma_n^2 H_0^2 \delta_z^2 \sum J_i(\omega_i, \tau_i, \eta, \alpha_i) \qquad (1)$$

where γ_n is the gyromagnetic ratio of the nucleus under study, H_0 is the field strength, and δ_z represents the most shielded component of the shift tensor in its traceless form. The spectral density term, J_i, is a function of the correlation times, the frequency, the asymmetry of the shift tensor, and the Euler angles which relate the diffusion tensor to the shift tensor. Thus, the model for internal motion, which can produce significant line narrowing, is contained in the J_i term.

Using our ^{31}P tensor data, and the monoester data of Kohler and Klein (1976), we have performed some estimates of the size of the line width contribution to ^{31}P solution spectra at high fields (6–12T) for the two hypothetical proteins (mol wt ~ 2×10^4 and 8.6×10^4) considered by Hull and Sykes.[4] For mol wt = 2×10^4, we find significant contributions (~10 Hz) only for phosphodiesters at 12T fields, assuming no internal motion. However, for mol wt = 8.6×10^4 the contributions range from 10 to 100 Hz for diesters, from 10 to 50 Hz for monoesters, and up to 10 Hz for inorganic phosphate. Thus, for small proteins and nucleic acid fragments, shift anisotropy broadening will be of minor importance, while for larger molecular species it will clearly dominate the line widths. We note that these contributions are much smaller than those predicted for ^{19}F spectra and this is primarily due to the γ_n^2 premultiplier in eq 1 which is smaller by a factor of 5.5 for ^{31}P. We also note that the broadening we predict for high molecular weights can be an important source of information. Often there are only a few ^{31}P lines in the spectrum of a biologically interesting molecule and in such cases a field-dependent study could yield correlation times without resort to tedious T_1 experiments.

[4] The line width contributions we discuss here are of course in addition to those arising from dipolar relaxation.

Conclusions

We have demonstrated that ^{31}P shift tensor components and orientations in the two PO_4 groups we have studied possess some common features. Specifically, the σ_{11} element is approximately perpendicular to the O–P–O plane and the σ_{22} element approximately bisects the O–P–O angle. Because BDEP is a phosphodiester and because its principal values are very close to those observed for phospholipids, we believe its tensor currently represents the best data available for lipid phosphate. Nevertheless, we find differences in the orientations of 10–20° between the BDEP and UPA tensors, and thus we conclude that one must use discretion in employing these tensors in lipid head-group conformation studies. However, even with this uncertainty, one can eliminate some head-group conformations and we have shown that any conformation with the nonesterified O–P–O plane parallel to the bilayer plane is rather improbable. Specifically, the common representation of phospholipids with the head group extended parallel to the bilayer normal and the O–P–O plane parallel to the bilayer plane is probably incorrect. In addition, ^{31}P powder spectra of the "anhydrous" and monohydrate forms of lecithins and the anhydrous form of PEs, together with spectra of frozen aqueous dispersions of these lipids, suggest that *one* molecule of H_2O is tightly bound to lecithins and none to PEs when these lipids are dispersed in excess water. Finally, our results allow one to perform a zero-order assessment of the effect of shift anisotropy relaxation in high resolution ^{31}P spectra. Our results indicate that for mono- and diesterified PO_4 at high fields one should observe shift anisotropy line broadening which could be employed to determine molecular correlation times.

Appendix

The chemical-shift tensor for a nucleus is evaluated by measuring the variation of the chemical shielding with orientation. When several symmetry-related molecules are present in the unit cell of the crystal, extra information is obtained for each orientation. This information can be used in two ways. If the symmetry axes have been located by some other means (e.g., by X-ray crystallography), the symmetry relations between the molecules in the unit cell can be used to reduce the number of crystal orientations which must be examined in order to fully evaluate the shift tensors (Weil et al., 1974). If, on the other hand, the shift tensors are evaluated independently of one another, the symmetry relations between them can be used to help locate the symmetry axes. The latter approach is described here. It was used to locate the two fold rotation axis in barium diethyl phosphate, and the result was in close agreement with the X-ray crystallographic determination. It was also used to locate the three twofold unit-cell rotation axes in urea–phosphoric acid, which were then assigned as the **a**, **b**, and **c** axes such that the resulting ^{31}P shift tensor was consistent with that obtained for barium diethyl phosphate.

Consider a set of mutually perpendicular vectors **a**, **b**, and **c**, where we choose arbitrarily, and without loss of generality, to make $\mathbf{b} = \mathbf{a} \times \mathbf{x}$ and $\mathbf{c} = \mathbf{a} \times \mathbf{b}$. Then

$$\hat{\mathbf{a}} = (\mathbf{a}_x, \mathbf{a}_y, \mathbf{a}_z)/\sqrt{\mathbf{a}_x^2 + \mathbf{a}_y^2 + \mathbf{a}_z^2} \quad \text{(A1a)}$$

$$\hat{\mathbf{b}} = (0, \mathbf{a}_z, -\mathbf{a}_y)/\sqrt{\mathbf{a}_y^2 + \mathbf{a}_z^2} \quad \text{(A1b)}$$

and

$$\hat{\mathbf{c}} = \frac{(\mathbf{a}_z^2 + \mathbf{a}_y^2, -\mathbf{a}_x\mathbf{a}_y, -\mathbf{a}_x\mathbf{a}_z)}{\sqrt{\mathbf{a}_y^2 + \mathbf{a}_z^2}\sqrt{\mathbf{a}_x^2 + \mathbf{a}_y^2 + \mathbf{a}_z^2}} \quad \text{(A1c)}$$

A rotation about $\hat{\mathbf{a}}$ by θ produces a new set of vectors

$$\hat{\mathbf{a}}' = \hat{\mathbf{a}} \quad \text{(A2a)}$$

$$\hat{\mathbf{b}}' = \cos\theta\,\hat{\mathbf{b}} + \sin\theta\,\hat{\mathbf{c}} \quad \text{(A2b)}$$

$$\hat{\mathbf{c}}' = \cos\theta\,\hat{\mathbf{c}} + \sin\theta\,\hat{\mathbf{b}} \quad \text{(A2c)}$$

The corresponding transformation matrix, **A**, is given by the equation

$$\begin{aligned}\mathbf{A} &= \hat{\mathbf{a}}'\hat{\mathbf{a}} + \hat{\mathbf{b}}'\hat{\mathbf{b}} + \hat{\mathbf{c}}'\hat{\mathbf{c}} = \hat{\mathbf{a}}\hat{\mathbf{a}} + (\cos\theta\,\hat{\mathbf{b}} + \sin\theta\,\hat{\mathbf{c}})\hat{\mathbf{b}} \\ &\quad + (\cos\theta\,\hat{\mathbf{c}} - \sin\theta\,\hat{\mathbf{b}})\hat{\mathbf{c}} = \hat{\mathbf{a}}\hat{\mathbf{a}} + \cos\theta(\hat{\mathbf{b}}\hat{\mathbf{b}} + \hat{\mathbf{c}}\hat{\mathbf{c}}) \\ &\quad + \sin\theta(\hat{\mathbf{c}}\hat{\mathbf{b}} - \hat{\mathbf{b}}\hat{\mathbf{c}}) = (1 - \cos\theta)\hat{\mathbf{a}}\hat{\mathbf{a}} + \cos\theta(\hat{\mathbf{a}}\hat{\mathbf{a}} + \hat{\mathbf{b}}\hat{\mathbf{b}} + \hat{\mathbf{c}}\hat{\mathbf{c}}) \\ &\quad + \sin\theta(\hat{\mathbf{c}}\hat{\mathbf{b}} - \hat{\mathbf{b}}\hat{\mathbf{c}}) = \mathbf{A}_1 + \mathbf{A}_2 + \mathbf{A}_3 \quad \text{(A3)}\end{aligned}$$

where

$$\mathbf{A}_1 = \begin{bmatrix} \mathbf{a}_x^2 & \mathbf{a}_x\mathbf{a}_y & \mathbf{a}_x\mathbf{a}_z \\ \mathbf{a}_x\mathbf{a}_y & \mathbf{a}_y^2 & \mathbf{a}_y\mathbf{a}_z \\ \mathbf{a}_x\mathbf{a}_z & \mathbf{a}_y\mathbf{a}_z & \mathbf{a}_z^2 \end{bmatrix} \frac{(1 - \cos\theta)}{(\mathbf{a}_x^2 + \mathbf{a}_y^2 + \mathbf{a}_z^2)} \quad \text{(A4a)}$$

$$\mathbf{A}_2 = \cos\theta \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad \text{(A4b)}$$

$$\mathbf{A}_3 = \begin{bmatrix} 0 & \mathbf{a}_z & -\mathbf{a}_y \\ -\mathbf{a}_z & 0 & \mathbf{a}_x \\ \mathbf{a}_y & -\mathbf{a}_x & 0 \end{bmatrix} \frac{\sin\theta}{\sqrt{\mathbf{a}_x^2 + \mathbf{a}_y^2 + \mathbf{a}_z^2}} \quad \text{(A4c)}$$

If two shift tensors, σ and σ', are related by a rotation of θ about the vector axis $\hat{\mathbf{a}}$, then

$$\sigma' = \mathbf{A}^{-1}\sigma\mathbf{A} \quad \text{(A5)}$$

This equation may be rearranged to give

$$0 = \mathbf{A}\sigma' - \sigma\mathbf{A} = \mathbf{T} \quad \text{(A6)}$$

Due to errors in the evaluation of σ and σ', eq A6 will not hold exactly. We therefore choose the values of **a** and θ which minimize the sum of the squares of the nine elements of the test tensor, **T**, i.e. minimize the expression

$$\sum_{i=1}^{3}\sum_{k=1}^{3}\left[\sum_{j=1}^{3}(\mathbf{A}_{ij}\sigma_{jk}' - \sigma_{ij}\mathbf{A}_{jk})\right]^2 \quad \text{(A7)}$$

Since a rotation is independent of the length of the rotation axis $|\mathbf{a}|$, one component of **a** may be fixed at an arbitrary value, such as 1.000, in the least-squares determination. For a twofold rotation axis, θ can be fixed at 180°, leaving only two variable parameters for the least-squares determination. For an nth order rotation axis, θ, can have any one of $n - 1$ values equal to an integral multiple of $360°/n$. In general, therefore, a two-stage least-squares determination is convenient, where in the first stage θ is left freely variable, to determine its approximate value, and in the second stage is fixed at the closest integral multiple of $360°/n$, in order to more accurately determine the two variable components of **a**.

If a crystal has more than one rotation axis, knowledge of the relative orientations of the axes may be used to further improve the least-squares determination. For example, in the case of urea-phosphoric acid there are four shift tensors, σ, σ', σ'', σ''', and three mutually perpendicular twofold rotation axes **a** (relating σ to σ' and σ'' to σ'''), **b** (relating σ to σ'' and σ' to σ'''), and **c** (relating σ to σ''' and σ' to σ''). Constraining **a, b,** and **c** to be mutually perpendicular removes two variables from the least-squares determination. In order to make maximum use of the information available, we then choose the set of mutually perpendicular vectors **a, b,** and **c** such that they minimize the expression

$$\begin{aligned}&\sum_{i=1}^{3}\sum_{k=1}^{3}\left[\left[\sum_{j=1}^{3}(\mathbf{A}_{ij}\sigma_{jk}' - \sigma_{ij}\mathbf{A}_{jk})\right]^2 + \left[\sum_{j=1}^{3}\mathbf{A}_{ij}\sigma_{jk}''' - \sigma_{ij}''\mathbf{A}_{jk}\right]^2 + \left[\sum_{j=1}^{3}(\mathbf{B}_{ij}\sigma_{jk}'' - \sigma_{ij}\mathbf{B}_{jk})\right]^2\right. \\ &\quad + \left[\sum_{j=1}^{3}(\mathbf{B}_{ij}\sigma_{jk}''' - \sigma_{ij}'\mathbf{B}_{jk})\right]^2 + \left[\sum_{j=1}^{3}(\mathbf{C}_{ij}\sigma_{jk}''' - \sigma_{ij}\mathbf{C}_{jk})\right]^2 \\ &\quad \left. + \left[\sum_{j=1}^{3}(\mathbf{C}_{ij}\sigma_{jk}'' - \sigma_{ij}'\mathbf{C}_{jk})\right]^2\right] \quad \text{(A8)}\end{aligned}$$

where the transformation matrices **B** and **C** correspond to the rotation axes **b** and **c** in the same way that **A** corresponds to **a.** It should be noted, however, that in this case the assignment of the symmetry axes is arbitrary and other information is required to identify each of the three axes with a particular crystallographically defined axis.

References

Abrahamsson, S., and Pascher, I. (1966), *Acta Crystallogr. 21,* 79-87.

Berden, J. A., Barker, R. W., and Radda, G. K. (1975), *Biochim. Biophys. Acta. 375,* 186-208.

Berden, J. A., Cullis, P. R., Hoult, D. I., McLaughlin, A. C., Radda, G. K., and Richards, R. E. (1974) *FEBS Lett. 46,* 55-58.

Chang, J., Griffin, R. G., and Pines, A. (1974a), *J. Chem. Phys. 60,* 2561-2562.

Chang, J., Griffin, R. G., and Pines, A. (1974b), *J. Chem. Phys. 61,* 1021-1030.

Chapman, D., Williams, R. M., and Ladbrooke, B. D. (1967), *Chem. Phys. Lipids 1,* 445-475.

Cohn, M., and Hughes, T. R., (1960), *J. Biol. Chem. 235,* 3250-3253.

DeKruijff, B., Cullis, P. R., and Radda, G. K. (1976), *Biochim. Biophys. Acta 436,* 729-740.

Gally, H. Y., Niederberger, W., and Seelig, J. (1975), *Biochemistry 14,* 3647-3652.

Griffin, R. G., (1976), *J. Am. Chem. Soc. 98,* 851-852.

Griffin, R. G., and Neuringer, L. J. (1973), Annual Report of the Francis Bitter National Magnet Laboratory.

Griffin, R. G., Pines, A., Pausak, S., and Waugh, J. S. (1975), *J. Chem. Phys. 63,* 1267-1271.

Griffin, R. G., Powers, L., and Pershan, P. S. (1978), *Biochemistry 17* (following paper in this issue).

Griffin, R. G., and Ruben, D. J. (1975), *J. Chem. Phys. 63,* 1272-1275.

Gueron, M., and Shulman, R. G. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 3482-3485.

Hauser, H., Phillips, M. C., Levine, B. A., and Williams, R. J. P. (1976), *Nature (London) 261,* 390-394.

Hitchcock, P. B., Mason, R., Thomas, K. M., and Shipley, G. G. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 3036-3040.

Hull, W. E., and Sykes, B. D. (1975), *J. Mol. Biol. 98,* 121-

153.
Kohler, S. J., and Klein, M. P. (1976), *Biochemistry 15,* 967-973.
Kohler, S. J., and Klein, M. P. (1977), *Biochemistry 16,* 519-526.
Kostansek, E. C., and Busing, W. R. (1972), *Acta Crystallogr., Sect. B 28,* 2454-2459.
Kraut, J. (1961), *Acta Crystallogr. 14,* 1146-1152.
Kyogoku, Y., and Itaka, Y. (1966), *Acta. Crystallogr. 21,* 49-57.
Lehninger, A. L., (1970), Biochemistry, New York, N.Y., Worth Publishing Co., Chapter 10.
McLaughlin, A. C., Cullis, P. R., Berden, J. A., and Richards, R. E. (1975a), *J. Magn., Reson. 20,* 146-165.
McLaughlin, A. C., Cullis, P. R., Hemminga, M. A., Hoult, D. I., Radda, G. K., Ritchie, G. A., Seeley, P. J., and Richards, R. E. (1975b), *FEBS Lett. 57,* 213-218.
Mootz, D., and Albrand, K. L. (1972), *Acta Crystallogr., Sect. B 28,* 2459-2463.
Neiderberger, W., and Seelig, J. (1976), *J. Am. Chem. Soc. 98,* 3704-3706.
Pines, A., and Abramson, E. (1974), *J. Chem. Phys. 60,* 5130-5131.
Pines, A., Gibby, M. G., and Waugh, J. S. (1973), *J. Chem. Phys. 59,* 564-590.
Seelig, A., and Seelig, J. (1974), *Biochem. Biophys. Res. Commun. 60,* 844-850.
Seelig, J., and Gally, H. U. (1976), *Biochemistry 15,* 5199-5204.
Seelig, J., Gally, H. U., and Wohlgemuth, R. (1977), *Biochim. Biophys. Acta 467,* 109-119.
Stockton, G. W., Polnazek, C. F., Leitch, L. C., Tulloch, A. P., and Smith, I. C. P. (1974), *Biochem. Biophys. Res. Commun. 60,* 844-850.
Stryer, L. (1975), Biochemistry, San Francisco, Calif., W. H. Freeman, Chapter 10.
Weil, J. A., Buch, T., and Clapp, J. E. (1974), *Adv. Magn. Reson. 5,* 183-257.

Head-Group Conformation in Phospholipids: A Phosphorus-31 Nuclear Magnetic Resonance Study of Oriented Monodomain Dipalmitoylphosphatidylcholine Bilayers†

R. G. Griffin,* L. Powers,‡ and P. S. Pershan

ABSTRACT: Angular-dependent ^{31}P NMR spectra of oriented biaxial monodomain DPPC·H_2O multilayers are employed to study head-group conformation in this phospholipid. The results indicate that the O-P-O plane of the phosphate, where the O's are the nonesterified oxygens of the phosphodiester, is tilted at 47 ± 5° with respect to the bilayer normal. This PO_4 orientation could result in the choline moiety being extended parallel to the bilayer plane, and it will explain the breadth of the axially symmetric ^{31}P powder spectrum observed for DPPC in excess water. This work is the first direct observation of this conformation for lecithins and it illustrates the utility of high-resolution solid-state NMR in structural studies of disordered systems.

A knowledge of the molecular structure of biological membranes and their components—lipids, saccharides, and proteins—is central to an understanding of their function, and for this reason a variety of physical techniques have been employed in the elucidation of membrane structure. However, these molecules form disordered liquid-crystalline arrays and consequently classical techniques for determining molecular structure yield only a limited amount of information. For instance, X-ray and neutron diffraction experiments provide data on distances within the bilayer plane and on bilayer thickness (Luzzati and Tardieu, 1974; Janiak et al., 1976; Tardieu et al., 1973; Franks, 1976; Worcester and Franks, 1976; Hitchcock et al., 1975; Zacci et al., 1975), but, because of the presence of disorder, they cannot yield interatomic distances and angles such as are available from single-crystal experiments. Spectroscopic investigations have been helpful in understanding membrane structure and some of the most informative studies have employed magnetic resonance techniques, namely, ESR and NMR.[1] In fact, a reasonably complete picture of acyl chain conformation and dynamics now exists based on ESR spin label and ^{13}C, ^{1}H, and ^{2}H NMR investigations (Lee et al., 1974; Seelig and Seelig, 1974a,b; Stockton et al., 1976; Berliner, 1975). For the most part, this work has been performed on systems in which there is a great deal of molecular motion, and consequently one observes motionally averaged magnetic

† From the Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 (R.G.G.), and the Gordon McKay Laboratory, Division of Engineering and Applied Physics, Harvard University, Cambridge, Massachusetts 02138 (L.P. and P.S.P.). *Received June 3, 1977; revised manuscript received February 24, 1978.* R.G.G. acknowledges the financial support of the National Science Foundation under Grant C-670 to the Francis Bitter National Magnet Laboratory and the National Institutes of Health under Grant 1-R01-GM23280-01. L.P. and P.S.P. acknowledge the support of the Joint Services Electronics Program (U.S. Army, Navy, and Air Force) under contract N00014-75-C-0648, the National Science Foundation under Grants DMR-76-22452 and DMR-76-01111, and the National Institutes of Health Training Grant 5-T01-GM00782-15.

‡ Present address: Bell Telephone Laboratories, Murray Hill, N.J. 07974.

[1] Abbreviations used are: NMR, nuclear magnetic resonance; ESR, electron-spin resonance; DPPC·H_2O, dipalmitoylphosphatidylcholine monohydrate; DMOAP, *N,N*-dimethyl-*N*-octadecyl-3-aminopropyltrimethyloxysily chloride; TBBA, terephthalbis(butylaniline); BDEP, barium diethyl phosphate; DLPE-HOAc, dilaurylphosphatidylethanolamine acetic acid; DSC, differential scanning calorimetry.

0006-2960/78/0417-2718$01.00/0