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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 31P NMR spectra of oriented biaxial monodomain DPPC-H2O 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 PO4 orientation could result in the choline moiety being extended parallel to the bilayer plane, and it will explain the breadth of the axially symmetric 31P 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 one observes motionally averaged magnetic 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; Zaccè 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. In fact, a reasonably complete picture of acyl chain conformation and dynamics now exists based on ESR spin label and 13C, 1H, and 2H 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

† Abbreviations used are: NMR, nuclear magnetic resonance; ESR, electron-spin resonance; DPPC-H2O, dipalmitoylphosphatidylcholine monohydrate; DMOAP, N,N-dimethyl-N-octadecyl-3-aminopropylmethoxyxyl chloride; TBBA, tetraphenylborate (butylamine); BDEP, barium diethyl phosphate; DLPE-HOA C, dilaurylphosphatidylethanolamine acetic acid; DSC, differential scanning calorimetry.

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resonance parameters. Interpretation of these observations is thus complicated by the need to make specific assumptions concerning molecular structure (Lee et al., 1974; Seelig and Seelig, 1974) and for many results there exists a degree of ambiguity. Some of these assumptions can be evaluated by a study of rigid systems for which interpretation is less ambiguous. Information on the structure of rigid systems could then lead to a better understanding of systems in which there is molecular motion. In the last few years, techniques have been developed for obtaining high-resolution NMR spectra of magnetically diluted spins in solid samples (Pines et al., 1973), and these techniques are well suited to the study of semisolid model membranes and biological membranes (Urbina and Waugh, 1974; Kohler and Klein, 1976; Griffin, 1976). With these techniques one can examine the anisotropy of certain magnetic interactions, such as the chemical-shift tensor, in “rigid” as well as motionally averaged samples (Mehring et al., 1971; Griffin et al., 1972), and if one knows the orientations of these tensor interactions relative to molecular bonds then an angular-dependent study of chemical shifts will allow one to determine molecular orientation in different samples. Moreover, with these techniques one can examine disorder in a more incisive manner than has previously been possible.

We have employed this approach in the study of head-group orientation in the phospholipid DPPC·H2O. Specifically, in a companion paper (Herzfeld et al., 1978), we described a determination of the orientation of the 31P chemical-shift tensor in the molecular frame for a phosphodiester. Knowledge of this tensor orientation, together with the observation of angular-dependent chemical shifts in highly oriented DPPC·H2O samples, has allowed us to deduce the orientation of the PO4 part of the head group in this phospholipid. We find from these experiments that the O-P-O plane (where the O's are the nonesterified oxygens of the phosphodiester) is tilted at an angle of 47 ± 5° with respect to the bilayer normal. This PO4 orientation could result in the remaining part of the head group, e.g., the choline moiety, being extended parallel to the bilayer plane. Moreover, this conformation will explain the breadth of the axially symmetric 31P powder spectrum observed for unoriented DPPC dispersed in excess water.

Experimental Section

Samples for the 31P NMR experiments were prepared by the method described by Powers and Clark (1975). Briefly, this consists of coating two glass plates with a silane surfactant, DMOAP, and sandwiching the phospholipid between these plates. The sample is then heated to ~125 °C and allowed to anneal over a period of hours, the alignment during this time being monitored optically. After annealing, the sample is cooled to room temperature, again over a period of hours. The result is a biaxial monodomain sample of oriented lipid bilayers with typical sample dimensions of 4 × 4 × 0.13 mm. This thickness (0.13 mm) amounts to approximately 103 bilayers, which, although very large for an oriented multilamellar sample, corresponds to only 4 μmol of PO4. Thus, our high-field (6.8T) spectrometer (Griffin and Neuringer, 1973) was essential to obtain the 31P spectra.

Following alignment, the sample was transferred to a sample holder which could be inserted into the NMR probe goniometer. The holder consists of a 6-mm diameter delrin rod which had been sliced along its long axis and a slot machined in each half of the rod to accommodate the glass plates and the bilayer. The rod could then be rotated about its long axis which was perpendicular to H0, for angular dependent studies. The doublet structure described below is only observed for near coincidence between the rotation axis of the sample and one of the symmetry directions for the optical biaxial pattern. Although details of this phenomenon were not studied systematically, it is clear that the optical biaxiality is closely linked to the orientational dependence of the NMR spectra.

31P NMR spectra were obtained with a double-resonance experiment described elsewhere (Pines et al., 1973). Spectra were generally taken at 10° intervals, with 0° being the orientation where the bilayer normal was parallel to H0. To date, we have obtained good quality spectra only up to rotation angles of ~60°. At this point the spectra become very wide (~150 ppm), and the signal to noise decreases dramatically.

Results and Discussion

Figure 1 shows 31P spectra of the DPPC·H2O bilayers for T = 20°C at θ = 0° (Figure 1a) and 50° (Figure 1b), where θ denotes the angle between the bilayer normal and H0. Figure 1c is a spectrum of a single crystal of BDEP which we include to illustrate the disorder present in the phospholipid samples. Specifically, the line widths in the single-crystal spectrum are ~6 ppm, whereas in the θ = 0° bilayer sample the full width at half maximum is ~60 ppm. This factor of 10 increase in line width can only be attributed to disorder and we believe illustrates the fact that, while the monodomain samples are indeed highly oriented on a macroscopic level, there remains considerable microscopic disorder. In Figure 1a, the line has a chemical shift of -46 ppm (relative to external 85% H3PO4), and we note that at θ = 50° the singlet has broken up into a
The bilayer sample is such that a significant fraction have a chemical-shift tensor relative to the PO₄ group and on nothing more. We note that this conclusion is predicated on a knowledge of the orientation of the PO₄ is assumed to be uniform about, for instance, the symmetry direction of the optical biaxial pattern, and, therefore, it does not permit the PO₄'s to be disordered around the bilayer normal. We then allow for discrete disorder about and perpendicular to the bilayer normal in these calculations. That is, the in-plane orientation of the PO₄ is assumed to be uniform about, for instance, the symmetry direction of the optical biaxial pattern up to an angle α, where α denotes the angle between the symmetry direction and say the s₁₁, s₂₂ plane of the tensor, and, zero elsewhere. With this model of the disorder and α = ±45°, we obtain simulated spectra which agree quite well with those of Figure 1. Specifically, the line positions and intensities (Figure 1b) are correct and the shoulder at ~100 ppm is present. However, this model of disorder is, for obvious reasons, physically implausible, and, because it does not permit the PO₄'s to assume an orientation with s₃₃ approximately along H₀ at θ = 90°, it will not explain the intensity in the ~100-ppm region observed at this rotation angle. A more realistic model for the disorder might allow for a Gaussian distribution of orientations with a width of ±45° and such a distribution would contain molecules which would have shifts of ca. +100 ppm at θ = 90°. We are currently extending these calculations to allow for this type of disorder and will report on them in a future publication. Nevertheless, these calculations indicate that in the multilayer samples considered here the distribution of PO₄ orientations in the bilayer plane amounts to ca. ±45° with respect to the symmetry axis of the optical biaxial pattern, and, based on the width of the line at θ = 0°, the PO₄'s are disordered by ±15° with respect to an axis lying in the bilayer plane and perpendicular to θ and the optical symmetry axis.

Given the tilted PO₄ conformation which we measure, we now inquire as to the conformation of the remainder of the head group, i.e., the choline moiety, in lecithin molecules. If we assume the tilted PO₄ conformation discussed above, then inspection of molecular models reveals that it is possible for a possible conformation of the choline moiety to be one in which it is extended parallel to the bilayer plane as is illustrated in Figure 3a. However, it is possible that the choline group folds back on itself with the -N(CH₃)₃⁺ electrostatically bonded to the PO₄. Although we do not at present have direct evidence for either of these choline conformations, we prefer the former because of its similarity to the crystal structure of DLPE-HOAc reported by Hitchcock et al. (1974) and because the conformation with the choline folded toward the PO₄ appears sterically
unfavorable. Moreover, there are diffraction and other NMR experiments, which we discuss below, that support this conformation.

In addition, we should inquire if the tilted PO₄ conformation is consistent with the ³¹P spectrum of unoriented DPPC observed in excess water. At −10 °C DPPC in excess water exhibits a motionally averaged powder spectrum which is axially symmetric and has a breadth of 69 ppm with shifts of −46 and +23 ppm. For T < −10 °C, the spectrum begins to assume an axially asymmetric shape, so we take this as the limiting breadth. At higher temperatures this spectrum narrows to ~47 ppm at 48 °C, which is slightly above the chain-melting temperature of 41 °C. We assume that at the low temperature the predominant mode of the head group is rotation around the normal to the lipid bilayers (Stockton et al., 1974; Seelig and Seelig, 1974a), and this motion is fast compared to the size of the PO₄ chemical shift tensor. If this is the case, then the tilted conformation which we measure will produce an axially symmetric powder spectrum with σ₁ = −46 ppm, and, since the trace of the tensor must be invariant to molecular motion, σ₁ = +23 ppm and Δσ = |σ₁| − σ₁ = 69 ppm. As mentioned above, we observe |σ₁| = 69 ppm at −10 °C with σ₁ = −46 ppm and σ₂ = +23 ppm. At higher temperatures additional modes of motion are present and the easiest manner to account for these is to employ the C(2)−C(3) order parameter measured by Gally et al. (1975) in ²H NMR experiments. This order parameter is 0.66 at T = 48 °C (Seelig and Gally, 1976) and results in Δσ = 69 × 0.66 = 45.5 ppm, which agrees well with the measured Δσ of 47 ppm at this temperature. Thus, the PO₄ orientation which we determine from our oriented bilayer experiments will explain the breadth and principal values observed in the ³¹P spectra of DPPC in excess water. In regard to this point, we should also comment that the commonly assumed conformation of lecithins (Lehninger, 1970; Stryer et al., 1975) with the O−P−O plane parallel to the bilayer plane and the head group extended parallel to the bilayer normal (cf. Figure 3b) will yield much too wide a spectrum. Using arguments identical to those used above, we find that at −10 °C this conformation would yield a spectrum of ~120-ppm breadth, and at 48 °C we expect a spectrum of ~80-ppm breadth. Thus, we believe we can label this conformation as highly improbable.

The fact that we can predict the observed ³¹P powder spectra breadth of DPPC in excess water leads to another interesting but somewhat speculative conclusion concerning the molecular changes which occur at the chain-melting temperature Tc. For DPPC in excess water below Tc, it has been shown with X-ray diffraction that the chains are tilted at ~30° with respect to the bilayer normal (Janiak et al., 1976), and this tilt decreases with decreasing water content to a limiting value of ~10° (Tardieu et al., 1973; Stamatoff et al., 1977). In contrast, raising the temperature of such a sample results in the chain tilt decreasing until the main transition temperature where the chains are disordered and parallel to the bilayer normal (Janiak et al., 1976). The fact that we can predict the temperature dependence of the ³¹PO₄ spectrum indicates that, despite the alteration in chain tilt, the PO₄ must maintain an approximately constant orientation. Such an observation is somewhat surprising but supports the hypothesis that the main transition observed in DSC experiments has little or nothing to do with the head group.

Our results also shed some light on the structure of certain liquid-crystalline phases. Recently, Luz and Meiboom (1974) have reported the observation of molecular motion and field alignment in the smectic-B phase of the liquid-crystal TBBA. The oriented bilayers of DPPC·H₂O that we study here share many of the properties of smectic-B liquid crystals, but, in contrast to many of the thermotropic liquid crystals, we believe the DPPC samples are rigid on the time scale of our ³¹P NMR measurements. Specifically, we obtain the same ³¹P NMR chemical-shift powder spectrum at 20 and at −110 °C, so we conclude they are rigid at room temperature. In addition, we have made a few unsuccessful attempts to align DPPC·H₂O with a magnetic field. Thus, we conclude that the presence of molecular rotation and field alignment are not general properties of the lipid smectic-B-like phases. The absence of these two properties in phospholipids is not too surprising, since, for instance, the diamagnetic susceptibility anisotropy for aromatic rings is certainly larger than is found for polymethylene chains.

Finally, we should compare our results on head-group orientation with those obtained from other studies. Recently, a crystal structure of the DLPE·HOAc complex was reported (Hitchcock et al., 1974) and in this particular compound the O−P−O plane is also tilted with respect to the bilayer normal. In fact, if we assume the a unit cell direction to be the bilayer normal, then we find the O−P−O plane is tilted at 45° with the bilayer normal. Thus, our results are very similar to the crystal structure reported by Hitchcock et al. Moreover, recent X-ray and neutron-diffraction results, which are based on differential H₂O/D₂O scattering in egg yolk lecithin (Worcester and Franks, 1976), are consistent with a head-group conformation in which the choline moiety lies in the bilayer plane. In addition, Seelig and Gally (1976), Kohler and Klein (1977), and Seelig et al. (1977) have addressed the problem of head-group conformation in computer analyses of ³¹P and ²H NMR experiments. In their work, these authors assume the crystallographic conformation of DLPE·HOAc as a starting point and then they calculate the expected spectral breadths for various motional models and/or departures of the lipid molecule from the crystallographic torsion angles. Kohler and Klein suggest two models which explain the breadth of the ³¹P spectrum and one of these (their model B) seems to result in a slightly better fit to the temperature dependence of their data. This model consists of a fast rotation about the P−O glycerol bond, followed by an additional rotation about the bilayer normal, and the temperature dependence is introduced by permitting the lipid molecule to "wobble" about the bilayer plane.
normal. Seelig and co-workers assume motion about the C(2)–C(3) glycerol bond, which produces an axially symmetric $^{31}$P tensor and account for "wobbly" with the C(2)–C(3) order parameter which we have employed above. In order to explain the motional equivalence of the deuterons in the choline, Seelig and co-workers invoke a rapid equilibrium between two enantiomeric conformations, such as are observed in crystals of t-α-glycerophosphorylcholine (Abrahamsson and Pascher, 1966). Our results, which are completely independent of the diffraction experiments, suggest that the assumption of the DLPE-HOAc conformation as a starting point for these calculations is not unreasonable. As mentioned above, we find the O–P–O planes in DPPC-H$_2$O and DLPE-HOAc are tilted at very similar angles with respect to the bilayer normal. Furthermore, these workers arrive at conclusions very similar to ours in regard to the head-group conformation. Finally, we should mention that there are nuclear Overhauser experiments (Yeagle et al., 1977) and potential energy calculations (Pullman et al., 1975) which provide supporting evidence for the head-group conformation with the choline moiety parallel to the bilayer plane.

Conclusions

With high-resolution solid state $^{31}$P NMR experiments, we have demonstrated that the PO$_4$ part of the head group in DPPC is oriented so that the O–P–O plane is inclined at an angle of 47 $\pm$ 5° with respect to the bilayer normal. This is the first direct observation of this orientation in lecithin molecules. With this orientation for the PO$_4$ the remainder of the head group probably assumes an orientation approximately parallel to the bilayer plane. Moreover, this tilted orientation of the PO$_4$ will explain the breadth of the axially symmetric powder spectrum observed in excess water, and we believe this provides strong support for the contention that this is the conformation that exists under these conditions. Finally, this work illustrates the manner in which high-resolution solid-state NMR experiments can be employed in structural studies of disordered systems.

References


