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Investigation of Phosphatidylethanolamine Bilayers by Deuterium and Phosphorus-31 Nuclear Magnetic Resonance†

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ABSTRACT: The motion of the ethanolamine head group in unsonicated lipid bilayers above and below the phase transition is studied by means of deuterium and phosphorus magnetic resonance. For this purpose, dipalmitoyl-3-*sn*-phosphatidylethanolamine is selectively deuterated at the two ethanolamine carbon atoms. The deuterium quadrupole splittings of the corresponding bilayer phases are measured at pH 5.5 as a function of temperature. In addition, the phosphorus-31 chemical shift anisotropies of planar-oriented and randomly dispersed samples of dipalmitoyl-3-*sn*-phosphatidylethanolamine are measured at pH 5.5 and 11 by applying a proton-decoupling field. The knowledge of the static chemical shift tensor (Kohler, S. J., and Klein, M. P. (1976), *Biochemistry 15,* 967) provides the basis for a quantitative analysis of the head-group motion. The nuclear magnetic resonance data are consistent with a model in which the ethanolamine group is rotating flat on the surface of the bilayer with rapid transitions occurring between two enantiomeric conformations.

Unlike proton and carbon-13 nuclear magnetic resonance (NMR), ^{2}H NMR has the advantage of measuring segmental fluctuations quantitatively in terms of order parameters (Charvolin et al., 1973; Stockton et al., 1974; Seelig and Niederberger, 1974; Seelig and Seelig, 1974a). The method is particularly useful for the study of lipid head groups where the attachment of a spin-label or fluorescence label would create too large a perturbation of the system. A detailed study on the motion and conformation of the choline head group in lipid bilayers has been published (Gally et al., 1975). In this communication, we report the results of deuterium and ^{31}P NMR investigations of the ethanolamine head group in bilayers of dipalmitoyl-3-*sn*-phosphatidylethanolamine (DPPE[1]).

Experimental Section

2-Aminoethanol-1-d_2 hydrochloride was prepared according to Weissbach and Sprinson (1953). 2-Aminoethanol-2-d_2 hydrochloride was synthesized by the same method but starting from glycine-2-d_2 ethyl ester hydrochloride (Blomquist et al., 1966) and reduction with $LiAlH_4$. Reduction of glycine-2-d_2 ethyl ester hydrochloride with $LiAlD_4$ led to doubly deuterated 2-aminoethanol-1,2-d_4 hydrochloride.

The corresponding lipids were prepared from dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) by means of phospholipase D (Yang et al., 1967). DPPC (400 mg) was suspended in 100 ml of 0.2 M acetate buffer and the suspension was sonicated. Deuterated 2-ethanolamine (3.2 g), $CaCl_2 \cdot 2H_2O$ (360 mg), and phospholipase D (80 mg, Calbiochem) were added. This mixture was covered with 50 ml of ether and vigorously shaken for 3 h. The lipids were extracted with chloroform–methanol and the DPPE (147 mg) was purified by thick-layer chromatography. The deuterated dipalmitoyl-3-*sn*-phosphatidylethanolamines are abbreviated in the following as $^+NCH_2CD_2$-DPPE, $^+NCD_2CH_2$-DPPE, and $^+NCD_2$-CD_2-DPPE. The corresponding quadrupole splittings are called $\Delta\nu_\alpha$ (= $^+NCH_2CD_2$-DPPE) and $\Delta\nu_\beta$ (= $^+NCD_2CH_2$-DPPE) for short. The purity of the lipids was established by thin-layer chromatography, ^{1}H and ^{2}H NMR, infrared spectroscopy, and, in part, by elemental analysis. Nondeuterated DPPE and DPPC were purchased from Fluka, Switzerland.

Random multilayers were prepared by mixing 0.15 g of DPPE with 0.3 ml of sodium acetate–acetic acid buffer (pH 5.5; 0.2 M) or with 0.3 ml of Borax–sodium hydroxide buffer (pH 11; 0.2 M). The samples were thoroughly mixed in a sealed ampule and the latter placed in a 10-mm NMR tube. Oriented lipid bilayers were produced as follows. A homogeneous lipid–water phase of the above composition (pH 5.5) was sandwiched between a stack of 20–25 glass plates (20 × 6.5 × 0.15 mm). The package was compressed gently and kept in a sealed NMR tube fr 12 h at about 70 °C.

All phosphorus-31 measurements were made with multilayers of nondeuterated DPPE.

The deuterium (13.8 and 41.3 MHz) and phosphorus-31

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[1] Abbreviations used are: DPPE, dipalmitoyl-3-*sn*-phosphatidylethanolamine; DPPC, dipalmitoyl-3-*sn*-phosphatidylcholine.

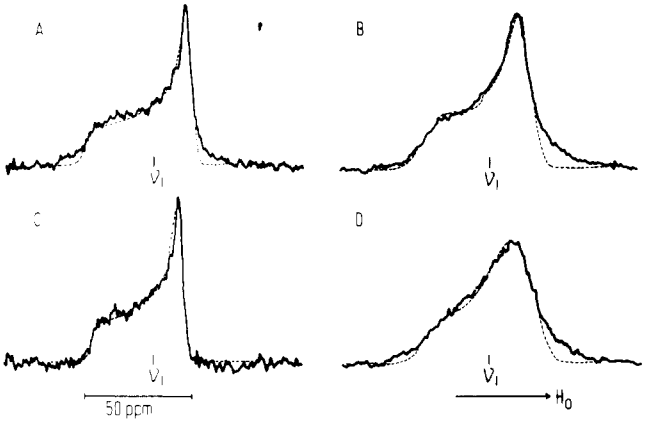


FIGURE 1: Proton-decoupled ^{31}P NMR spectra (36.5 MHz) of nonsonicated DPPE bilayers. Pulse width 4 μs; dwell time 50 μs; 10 000 free induction decays. Solid lines: experimental spectra; dashed lines: computed spectra. ν_i resonance position of bilayer vesicles (isotropic tumbling). (A) pH 5.5, 88 °C, liquid crystalline state; (B) pH 5.5, 57 °C, gel state; (C) pH 11, 61 °C, liquid crystalline phase; (D) pH 11, 52 °C, gel state.

(36.5 MHz) resonance spectra were obtained with Bruker-Spectrospin HX-90-FT and WH-270-FT spectrometers equipped with a variable temperature unit (Gally et al., 1975).

Results

Figure 1 shows ^{31}P NMR spectra of random multilayers of DPPE for two different pH values above and below the gel-to-liquid crystal phase transition. The phosphorus-31 chemical shift anisotropy $\Delta\sigma$ corresponds approximately to the separation of the edges in the powder-type spectra. A more accurate value is obtained by a computer simulation of the spectra (Niederberger and Seelig, 1976) and the theoretical spectra are indicated by the dashed lines in Figure 1. These curves have been computed assuming Gaussian line shapes of *constant* line width. The available proton-decoupling field is, however, too small to produce perfect proton-decoupled ^{31}P spectra at temperatures below the phase transition. The residual dipolar couplings broaden the edges of the spectra and account for the differences between the experimental spectra and those calculated for pure chemical shielding anisotropy (cf. Niederberger and Seelig, 1976).

The chemical shielding anisotropies of DPPE at pH 5.5 and 11 are plotted in Figure 2 as a function of temperature. The following conclusions can be drawn from Figure 2. (1) The motion of the phosphate group in bilayers of DPPE is clearly anisotropic. (2) The freezing or melting of the hydrocarbon chains is reflected in the motional freedom of the head group. Cooling the bilayer below the phase transition increases the chemical shift anisotropy by about 20 ppm. As judged from the steepness of the curves, the phase transition of the hydrocarbon chains has a much stronger effect on the polar group of DPPE than on that of DPPC. (3) A change in the pH from 5.5 to 11 is accompanied by a transition-point depression. This is seen more clearly in Figure 3 where the line width at half-height of nondecoupled ^{31}P spectra (powder-type spectra) is plotted as a function of temperature. The distinct line broadening below the phase transition is caused by a rapid increase in the proton–phosphorus dipolar couplings. Figure 3 shows that a shift in the pH from 5.5 to 11 lowers the transition temperature from 63 to 57 °C. Such a pH effect has been observed in related bilayer systems by Träuble and Eibl (1974) and was rationalized by a change in the electrostatic charge of the polar head. At pH 5.5 DPPE is zwitterionic, while at pH

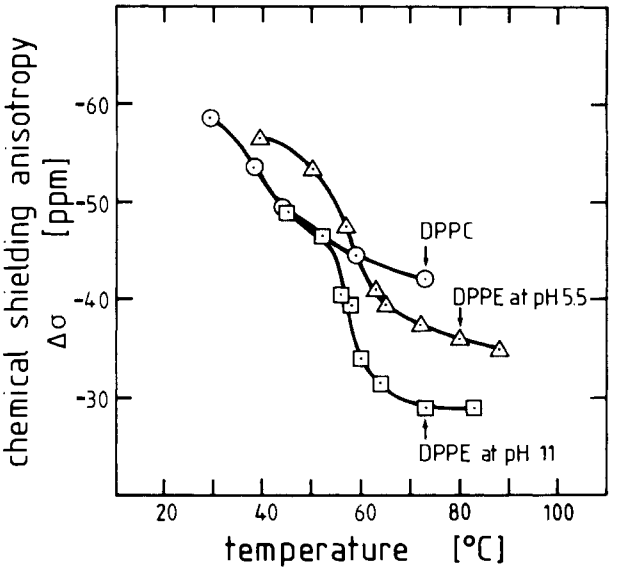


FIGURE 2: Variation of the phosphorus-31 chemical shielding anisotropy (in parts per million) with temperature. Data for DPPC (50% lipid/50% H_2O, no buffer), taken from Gally et al. (1975).

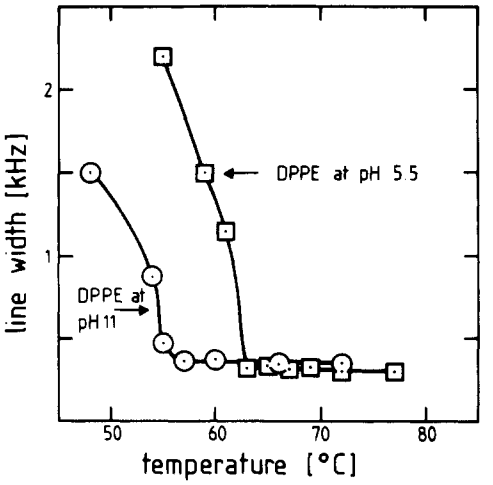


FIGURE 3: Temperature dependence of the line width at half-height for powder-type ^{31}P NMR spectra. No proton-decoupling field applied.

11 the amino group becomes deprotonated and DPPE then carries a net negative charge. The concomitant increase in the electrostatic repulsion between the head groups is believed to cause a melting of the hydrocarbon chains at lower temperatures. Recently, more indirect mechanisms have also been suggested (MacDonald et al., 1976).

The absolute value of the ^{31}P shielding anisotropy of DPPE is smaller at pH 11 than at 5.5. This could be due to one or more different effects. (1) The alteration of the head-group charge could induce a change in the static chemical shielding tensor. It is difficult to estimate the magnitude of this effect, since at least the formal charge on the phosphate group remains constant. (2) The quantitative expression for the shielding anisotropy in liquid crystalline bilayers is given by (Niederberger and Seelig, 1976)

$$\Delta\sigma = (\sigma_{11} - \sigma_{22})S_{11} + (\sigma_{33} - \sigma_{22})S_{33} \qquad (1)$$

Here the S_{ii} are so-called order parameters (cf. Saupe, 1964), which depend on both the average orientation of the ^{31}P shielding tensor with respect to the axis of rotation and also on

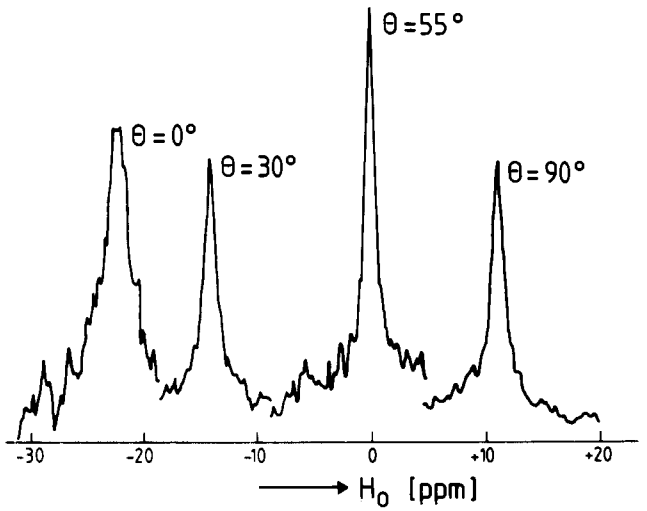


FIGURE 4: Representative examples of proton-decoupled ^{31}P NMR spectra (36.5 MHz) obtained from planar-oriented DPPE multilayers at various orientations with respect to the applied magnetic field. θ is the angle between the magnetic field and the normal to the plane of the membranes. Temperature 77 °C and pH 5.5. The variations in line width could be caused by imperfect ordering of the multilayers.

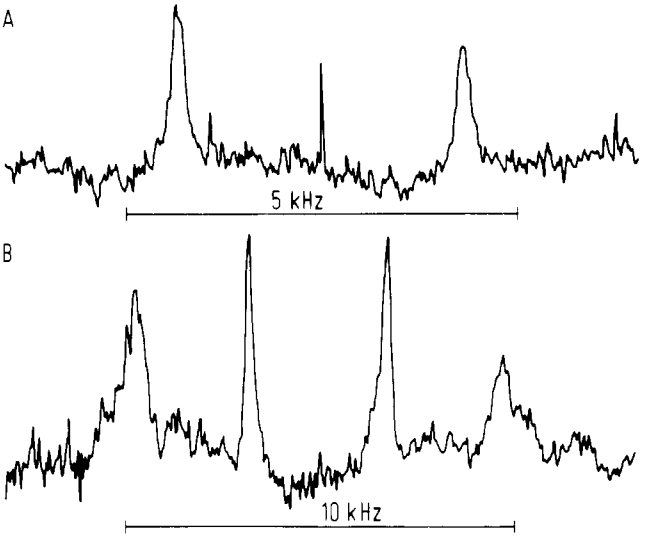


FIGURE 5: Deuterium magnetic resonance spectra (13.8 MHz) of non-sonicated DPPE multilayers. 0.15 g of lipid in 0.3 ml of buffer (pH 5.5). Pulse width 18 μs. (A) $^{+}NCD_2CH_2$-DPPE at 73 °C, dwell time 50 μs, 30 000 free induction decays. (B) $^{+}NCD_2CD_2$-DPPE at 78 °C, dwell time 25 μs, 20 000 free induction decays.

the extent of angular fluctuations around this rotation axis. Thus, a conformational change in the head-group structure as well as larger fluctuations (more disordered motion) could also account for the observed changes in $\Delta\sigma$. On the basis of the available experimental data, no decision can be made between the various alternatives. Proton and phosphorus line width studies of *sonicated* vesicles of bacterial phosphatidylethanolamines are, however, interpreted in the sense that there is a less restricted molecular head group motion when the phosphatidylethanolamine is charged than when it is zwitterionic (Michaelson et al., 1974).

Experiments with planar oriented multilayers allow a determination of the axis of motional averaging. As may be seen from Figure 4, the position of the phosphorus-31 resonance line shifts as the angle θ between the magnetic field and the normal on the glass plates is varied. The shift follows a $(3\cos^2\theta - 1)$ dependence (cf. McLaughlin et al., 1975b) and the separation between the two extreme positions $\theta = 0°$ and $\theta = 90°$ agrees with the chemical shift anisotropy $\Delta\sigma$ of the corresponding random dispersion. At the so-called magic angle ($\theta = 54.7°$), the resonance signal is found at a frequency characteristic of single-walled bilayer vesicles (rapid isotropic tumbling). From these results it can be concluded that the motion of the phosphate group in bilayers of DPPE is rotationally symmetric around the normal to the bilayer surface. Similar studies have been performed with oriented bilayers of DPPC. It was found that also in this system the bilayer normal is the rotation axis for the phosphate of the phosphorylcholine head group (Stockton et al., 1974; McLaughlin et al., 1975b) as well as for the hydrocarbon chains (Seelig and Seelig, 1974b), which suggests this to be a rather general property of lipid bilayers.

Representative ^2H NMR spectra of random multilayers of $^{+}NCD_2CH_2$-DPPE and $^{+}NCD_2CD_2$-DPPE above the gel-to-liquid crystal transition point are shown in Figure 5. Each CD_2 segment gives rise to just one quadrupole splitting, which means that the two deuterons of a given segment are motionally equivalent. The variaton of the quadrupole splittings and the phosphorus-31 chemical shift anisotropy with temperature is shown in Figure 6. As opposed to the phosphorus signal, the deuterium resonances can no longer be observed below 63 °C.

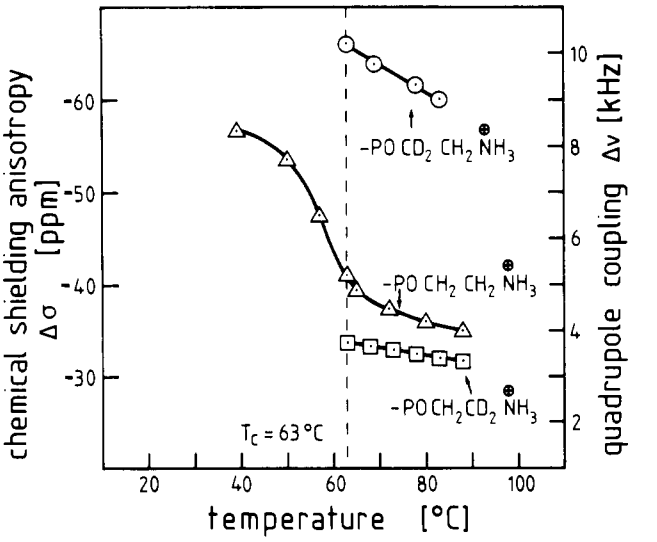


FIGURE 6: Variation of the quadrupole splittings and the phosphorus shielding anisotropy of DPPE multilayers with temperature. Gel-to-liquid-crystal transition temperature T_c = 63 °C; pH 5.5.

The transition from well-resolved spectra to a complete loss of signal is rather sharp and occurs exactly at the gel-to-liquid crystal transition point as determined by differential scanning calorimetry (Vaughan and Keough, 1974). Below this critical temperature the ethanolamine head group is presumably locked into a rigid conformation, which, together with a reduction of the reorientation rate, makes the quadrupole splittings too large and the lines too broad to be detected. This behavior is different from that of bilayers of DPPC where the deuterium quadrupole splittings of the deuterated choline head group can be observed well below the phase transition (Gally et al., 1975). Even though the difference in the mobility of the two head groups is most pronounced in the gel state, studies with vesicles of bacterial phosphatidylethanolamine and phosphatidylcholine above the phase transition have led to a similar conclusion, namely, that the molecular motions of phosphatidylethanolamine are more restricted than are those of phosphatidylcholine (Michaelson et al., 1974). No ^2H NMR

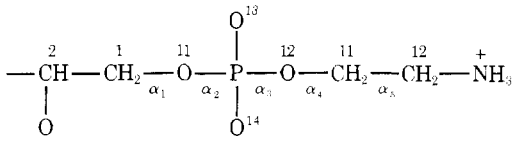
measurements were made at pH 11. The following analysis is therefore restricted to the phosphorus-31 and deuterium spectra obtained at pH 5.5.

Discussion

The angular dependence of the phosphorus-31 resonance line in oriented multilayers of DPPE clearly demonstrates that the ethanolamine head group rotates around an axis perpendicular to the bilayer surface. The questions then remain of how flexible or rigid is the molecular frame of the polar group and of how the polar group is oriented with respect to the rotation axis.

According to Sundaralingam (1972), the phosphatidylethanolamine is represented by the following scheme.

Scheme I



The torsion angles α_i are measured from the cis-planar configuration. Crystal structures have been described for L-α-glycerylphosphorylethanolamine (DeTitta and Craven, 1971, 1973) and for 1,2-dilauroyl-DL-phosphatidylethanolamine (Hitchcock et al., 1974). Common to both structures is the gauche conformation of the O-C-C-N^+ system (torsion angle α_5) and the gauche-gauche rotation of the phosphodiester linkage (α_2, α_3) leading to a boomerang shape of the ethanolamine group in the crystal (DeTitta and Craven, 1971).

The phosphorus-31 chemical shielding tensor of solid dipalmitoylphosphatidylethanolamine (and also of other lipids) has recently been determined by Kohler and Klein (1976). The principal values were found to be $\sigma_{11} = -85$ ppm (relative to H_3PO_4), $\sigma_{22} = -14$ ppm, and $\sigma_{33} = 87$ ppm. Following Kohler and Klein (1976), we assume that the orientation of the chemical shielding tensor in DPPE is the same as found in single crystals of phosphorylethanolamine with σ_{11} and σ_{33} parallel to the O(11)-O(12) and O(13)-O(14) connecting vectors, respectively. This is probably an approximation, since phosphorylethanolamine is a monoester while DPPE contains a phosphodiester linkage. Also, the principal values of the DPPE shielding tensor should be reexamined in view of more recent results on the influence of water on the chemical shielding tensor of DPPC (Griffin, 1976). On the other hand, phospholipids, so far, have eluded all efforts to prepare single crystals of sufficient size and good quality and the results of Kohler and Klein are presumably the best approximation possible under the present circumstances.

In the liquid crystalline bilayer, the static ^{31}P shielding tensor is averaged by the head-group motion and is replaced by an effective tensor which is axially symmetric with respect to the bilayer normal. Previously, when the components of the static tensor were not yet known, the effective chemical shielding anisotropy, $\Delta\sigma$, was interpreted in terms of *one* order parameter (McLaughlin et al., 1975a,b). However, since the asymmetry of one shielding tensor has now been established, this procedure is certainly not valid. The proper interpretation of $\Delta\sigma$ requires *two* order parameters, as shown in eq 1 (Niederberger and Seelig, 1976). Thus, the measurement of $\Delta\sigma$ alone is not sufficient for an unambiguous description of the phosphate group motion and additional assumptions on the nature of the motion must be made.

The simplest motion, namely an unhindered rotation around the O(11)-P axis (torsion angle α_2), has been discussed by Kohler and Klein (1976) in connection with bilayers of DPPC. It was concluded that this motion was not in concord with the observed spectra, since the predicted $\Delta\sigma$ was clearly too large. A similar conclusion follows for bilayers of DPPE. Using the bond angles of DeTitta and Craven (1973) and performing the same calculation as Kohler and Klein (1976), $\Delta\sigma$ for a free rotation around the O(11)-P bond (α_2) is found to be -83 ppm compared to an experimental value of about -35 to -40 ppm ($T > 63$ °C). If free rotation is assumed also for the C(1)-O(11) bond (torsion angle α_2), this anisotropy is further reduced by the factor $(1/2)(3\cos^2 122.3 - 1)$, where $\theta = 122.3°$ is the C(1)-O(11)-P bond angle. The resulting chemical shielding anisotropy then becomes $|\Delta\sigma| = 6.0$ ppm, which is now too small. We, therefore, like to discuss a model in which the flexibility of the head group is limited to a rather narrow configurational space. We start from the basic premise that the motion of the ethanolamine group is axially symmetric around the glycerol C(2)-C(1) axis. Support for this contention may be drawn from the x-ray analysis of dilauroyl-DL-phosphatidylethanolamine, which shows the C(2)-C(1) axis to be oriented perpendicular to the bilayer surface (Hitchcock et al., 1974). If this configuration is carried over into the liquid crystalline state, both a rotation of the whole lipid molecule or a free rotation around the C(2)-C(1) axis (or at least a threefold symmetric jump) will produce an axially symmetric shielding tensor. The order parameter of the C(2)-C(1) axis can be estimated from ^2H NMR spectra of bilayers of DPPC where the C(1) atom of the glycerol constituent has been labeled (Gally et al., 1975). Above the phase transition, the quadrupole splitting of this segment amounts to $\Delta\nu = 28$ kHz. The order parameter, S_{CD}, of the C(1)-D bond vector is related to the quadrupole splitting of the powder-type spectrum according to

$$\Delta\nu = (3/4)(e^2qQ/h)S_{CD} \qquad (2)$$

Only the absolute value of S_{CD} can be determined, since the sign of $\Delta\nu$ is unknown. With a static quadrupole constant of $e^2qQ/h = 170$ kHz (cf. Table I, footnote d) it then follows that $|S_{CD}| = 0.22$. The C(1)-D bond vector makes an angle of 109.5° with the C(2)-C(1) axis. Since an axially symmetric motion around the C(2)-C(1) bond direction is assumed, the order parameter of this axis, $S_{C(2)\text{-}C(1)}$, is given by

$$S_{C(2)\text{-}C(1)} = [(3\cos^2 109.5 - 1)/2]^{-1} S_{CD} = -3S_{CD}, \qquad (3)$$

yielding $S_{C(2)\text{-}C(1)} = \pm 0.66$. Order parameters cannot be smaller than -0.5; therefore, only $S_{C(2)\text{-}C(1)} = +0.66$ is a solution.

We assume that this result, to a first approximation, is also valid for bilayers of DPPE. First, the two phospholipids DPPC and DPPE differ only in the chemical structure of the head group. The packing of the hydrocarbon chains is found to be rather similar when comparing ^2H NMR measurements of DPPC with x-ray diffraction studies of dilauroyl-DL-phosphatidylethanolamine (Seelig and Seelig, 1974b). In both systems, the beginning of one fatty acyl chain is oriented parallel to the surface, while the other is oriented perpendicular. This unique arrangement of the first segments of the chains requires a very similar conformation of the glycerol backbone in both lipids. Second, even though there are distinct numerical differences (of the order of 30%) between the quadrupole splittings of the choline and ethanolamine segments, this does not imply a different head group structure. The quantitative analysis of the DPPC bilayer shows that the differences are caused by relatively small changes in the torsion angles α_i. In fact, the head-group structure of DPPC closely resembles that

TABLE I: Torsion Angles α_i and Comparison of Experimental Parameters with Theoretical Predictions for the Head Group of DPPE in Lipid Bilayers.

Torsion Angle	X-Ray[a]	NMR	Segment	Measured Parameter	Experimental[b] Result (71 °C)	Theoretical Prediction
α_1	211 (t)	±193				
α_2	51 (g^-)	±50	PO_4^-	$\Delta\sigma$	−37.5 ppm	−39 ppm[c]
α_3	64 (g^-)	±64				
α_4	101	±96	$^+NCH_2CD_2OP$	$\Delta\nu_\alpha$	9.7 kHz	9.8 kHz[d]
α_5	77 (g^-)	±87	$^+NCD_2CH_2OP$	$\Delta\nu_\beta$	3.7 kHz	−3.8 kHz[d]

[a] Taken from Hitchcock et al. (1974). [b] Only absolute values of the quadrupole splitting can be measured. [c] Based on the static shielding tensor of Kohler and Klein (1976). [d] Calculated with a static quadrupole splitting of 170 kHz. The quadrupole coupling constant may vary slightly with the position of the deuterium in the lipid molecule. The error introduced by assuming e^2qQ/h = 170 kHz is certainly not serious, since the quadrupole couplings in such different compounds as $(DOOC)_2CD_2$, $^+ND_3CD_2COO^-$, and C_4D_{10} are already rather similar, namely, 168, 166.5, and 169.1 kHz, respectively (Derbyshire et al., 1969; Barnes and Bloom, 1973; Burnett and Muller, 1971).

of DPPE (J. Seelig and H. U. Gally, manuscript in preparation). Third, the quadrupole splitting of the glycerol C(1) segment (~28 kHz) is much larger than the splittings of the choline or ethanolamine segments (~5 kHz), suggesting less conformational freedom in the glycerol part than in either head group.

The next step is to transform the static chemical shift tensor from its principal coordinate system through intervening bonds into the coordinate system of the C(2)–C(1) bond. This can be accomplished using either Cartesian transformation matrices (cf. Carrington and McLachlan, 1967; p. 250) or Wigner rotation matrices (cf. Rose, 1957). The rotational symmetry around the C(2)–C(1) axis is invoked and the resulting axially symmetric tensor can be compared with the experimental result. In this coordinate transformation, the torsion angles α_1 and α_2 are unknown parameters. A computer search shows, however, that only a limited number of combinations of α_1 and α_2 will produce the experimental $\Delta\sigma$. Within a conformational space of ±30° around the crystallographic equilibrium values of α_1 = 211° and α_2 = 51° (Hitchcock et al., 1974), only the torsion angles α_1 = 193° (~trans) and α_2 = 50° yield the correct result. Using the latter values, the chemical shielding anisotropy in the C(1)–C(2) coordinate system is predicted to be $\Delta\sigma$ = −59 ppm, which, after multiplication with the order parameter of the C(1)–C(2) axis (S = 0.66), yields $\Delta\sigma$ = −39 ppm, in agreement with the experiment. (The sensitivity of the method is illustrated by repeating this calculation for slightly different torsion angles. Using α_1 = 190° and α_2 = 45°, one obtains $\Delta\sigma = -70.6 \times 0.66 = -46.7$ ppm.) Leaving α_1 and α_2 unchanged, this procedure can now be extended to determine α_3, α_4, and α_5 from the deuterium quadrupole splittings. Again, several solutions are possible, but for deviations within ±30° from the crystallographic conformation the situation is unambiguous and the results are summarized in Table I. In deriving these results, the motional equivalence of the deuterons attached to the same segment has been invoked. In a completely rigid head group conformation, the individual deuterons of a CD_2 segment would be inclined at different angles with respect to the rotation axis and would produce two separate splittings. Since this is not borne out by the experiment, the simplest explanation is to assume a rapid equilibrium between two enantiomeric conformations, one with all torsion angles α_i positive and the other with all torsion angles negative. A transition from one conformation to the other would exchange the orientations of the corresponding deuterons. For a sufficiently rapid jump rate ($>10^4$ Hz), both deuterons would then experience the same average electric field gradient, leading to the same quadrupole splitting. The existence of two enantiomeric forms has, indeed, been established for crystals of L-α-glycerophosphorylcholine (Abrahamsson and Pascher, 1966) and it is rather probable that these conformations are also among those preferred by the molecule in the liquid-crystalline bilayer. The torsion angles in Table I have therefore been calculated on the basis of a raid equilibrium between these two enantiomers.

Inspection of Table I then leads to two conclusions. First, for a quantitative description of the NMR data, it suffices to consider an equilibrium between just two enantiomeric conformations. Even though the conformational pathway connecting the two enantiomers is not known and other conformations are, therefore, not excluded, this suggests quite an ordered structure of the polar head group. Second, the NMR data are in accord with the head group conformation of crystalline bilayers, as determined by x-ray diffraction (Hitchcock et al., 1974) and infrared spectroscopy (Akutsu et al., 1975). Within a conformational space of ±30° of the crystallographic torsion angles, the calculated conformation is the only solution to the NMR measurements. The importance of this particular conformation is also stressed by a quantum mechanical computation of hydrated phosphatidylethanolamine, which reveals a distinct energetic minimum for the crystallographic torsion angles (Pullman et al., 1975). Finally, studies on phosphatidylethanolamine in solution demonstrate that at least the $O-C-C-NH_3^+$ system must be in a gauche conformation (Richard et al., 1974). Although not providing a conclusive proof, the NMR data presented here are then consistent with a picture in which the polar group rotates flat on the surface of the bilayer and assumes the shape of a right- or left-handed boomerang with rapid transitions occurring between the two enantiomers.

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