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Conformation and Motion of the Choline Head Group in Bilayers of Dipalmitoyl-3-*sn*-phosphatidylcholine†

Hans-Ulrich Gally, Werner Niederberger, and Joachim Seelig*

ABSTRACT: The conformation and motion of the choline head group in lipid bilayers above and below the gel-to-liquid crystal transition point are studied by means of deuterium and phosphorus magnetic resonance. For this purpose dipalmitoyl-3-*sn*-phosphatidylcholine is selectively deuterated at various positions on the choline and glycerol constituents. The residual deuteron quadrupole couplings and the phosphorus chemical-shift anisotropy of the corresponding lipid–water mixtures yield quantitative information on the segmental motions. The choline methyl group is only slightly hindered in its movement, but the motional freedom becomes increasingly restricted the closer the segment is located to the glycerol backbone. The average value of the OC–CN bond rotation angle changes with temperature. Increasing the temperature rotates the choline methyl group into the vicinity of the phosphorus atom. The choline group as a whole is thus characterized by a flexible, temperature-dependent structure. Its orientation in space is not fixed, either parallel or perpendicular to the bilayer surface. Instead all segments execute angular oscillations with varying degrees of restriction around the normal on the bilayer surface. The gel-to-liquid crystal phase transition at 41° is clearly reflected in the deuterium and phosphorus resonance spectra of the choline moiety, while no change is observed at 34°. The calorimetric pretransition at 34° seems not to be associated with a conformational change in the choline group.

The structure and function of lipid–water systems depend in large part on the nature of the polar groups. The interaction of charged hydrophilic polar groups with each other and with water and the van der Waals attractions between the hydrophobic fatty acyl chains constitute the physical basis for the arrangement of the lipid molecules in many different structures—micelles, cylindrical rods, bilayer leaflets, etc. (Luzzati, 1968; Shipley, 1973; Parsegian, 1973). The role of the polar head groups is of particular interest in relation to the biophysical reactivity of lipid–water systems. Ion-binding or pH-induced charge alterations at the polar groups can lead to a completely different long-range or short-range order (Rand and Sengupta, 1972; Träuble and Eibl, 1974). It is also plausible, though not yet proven experimentally, that biochemical reactions or cell–cell intersurface contacts could be triggered by small changes in the conformation, orientation, or charge of the polar groups.

In looking at the properties of the polar groups with physicochemical methods the most detailed information has so far been provided by single-crystal X-ray studies of phospholipids and their constituents. A precise picture of the molecular conformation, the bond angles, and the bond distances for the choline and ethanolamine head groups in the crystalline state has been derived (Sundaralingam, 1972; Hitchcock et al., 1974; Phillips et al., 1972; Abrahamsson and Pascher, 1966; DeTitta and Craven, 1973). It has been suggested that these conformations most likely will also be exhibited in solution and in the liquid crystalline state characteristic of biological systems. For lipid molecules in solution some of these predictions have been confirmed by means of proton and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR) (Birdsall et al., 1972; Dufourcq and Lussan, 1972; Shaw et al., 1973; Richard et al., 1974; Lichtenberg et al., 1974). The liquid crystalline state, however, has received relatively little attention in this respect since the conditions necessary to obtain high-resolution proton or ^{13}C NMR spectra are generally not met. Here the introduction of deuterium magnetic resonance holds promise of major advances (Oldfield et al., 1971; Charvolin et al., 1973; Seelig and Niederberger, 1974a,b; Seelig and Seelig, 1974a,b; Niederberger and Seelig, 1974; Stockton et al., 1974; Fujiwara et al., 1974). If a chain segment of the polar head is specifically deuterated any anisotropic motion of this segment should give rise to a doublet splitting of the deuterium magnetic resonance signal. The separation of the two deuterium transitions is then connected in a simple way with the degree of order of the C–D bond vector. We have therefore synthesized a series of dipalmitoyl-3-*sn*-phosphatidylcholines specifically deuterated at the three carbon atoms of the choline group and also at the C-3 position of the glycerol moiety, the latter being generally considered the rigid core part of the lipid molecule. The deuterium magnetic resonance spectra of the corresponding lipid–water systems have been measured as a function of temperature. In order to elucidate the motion of the phosphate group, the phosphorus-31 nuclear magnetic resonance spectra of the same systems have also been recorded as a func-

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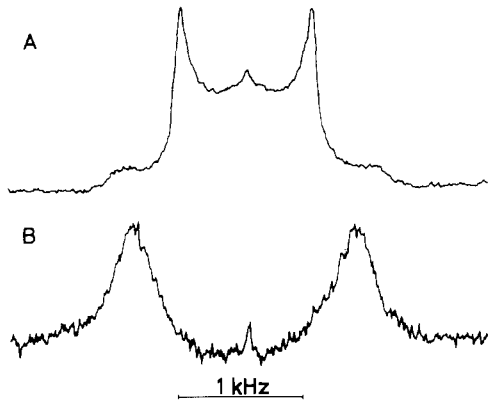


FIGURE 1: Deuterium magnetic resonance spectra (13.8 MHz) of bilayers of CD_3N^+-DPL (DPL 50 wt %; H_2O 50 wt %): (A) above the phase transition (59°); pulse width 18 μsec; dwell time 100 μsec; data memory 1K real; noise modulation width ±1.5 kHz at 5 W decoupling power; accumulation of 29,000 free induction decays; (B) below the phase transition (37°); otherwise the same conditions as A; 5000 free induction decays.

tion of temperature. In this case the information relating to the motional properties can be drawn from the chemical-shift anisotropy of the phosphorus nucleus. In combining the various measurements certain quantitative conclusions about the motion and the conformation of the choline head group can then be made.

Experimental Section

Chemical Synthesis. (2-Hydroxyethyl)-*methyl*-d_3-dimethylammonium iodide (**1**) was synthesized according to Dauben and Gee (1952):

$$(CH_3)_2NCH_2COOEt \xrightarrow{LiAlH_4} (CH_3)_2NCH_2CH_2OH \xrightarrow{CD_3I} [CD_3(CH_2)_2NCH_2CH_2OH]^+I^-$$

1

Choline-*1*-d_2 iodide (**2**) was prepared according to Douglas and Burditt (1955):

$$NaCN + HCHO \rightleftharpoons HOCH_2CN \xrightarrow{C_6H_5COCl} C_6H_5COOCH_2CN \xrightarrow{LiAlD_4} HOCH_2CD_2NH_2 \xrightarrow{CH_3I} [(CH_3)_3NCD_2CH_2OH]^+I^-$$

2

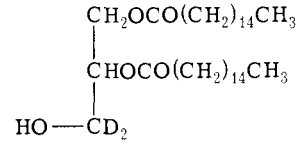
Choline-*2*-d_2 iodide (**3**) was made analogous to **1**:

$$(CH_3)_2NCH_2COOEt \xrightarrow{LiAlD_4} (CH_3)_2NCH_2CD_2OH \xrightarrow{CH_3I} [(CH_3)_3NCH_2CD_2OH]^+I^-$$

3

The selectively deuterated cholines **1**, **2**, and **3** were condensed with dipalmitoyl-3-*sn*-phosphatidic acid dichloride following the procedure by Baer and Kindler (1962). For the latter synthesis commercially available 1,2-dipalmitoyl-*sn*-glycerol (Fluka, Switzerland) was used. The corresponding dipalmitoyl-3-*sn*-phosphatidylcholines are abbreviated as CD_3N^+-DPL, $^+NCD_2CH_2$-DPL, and $^+NCH_2CD_2$-DPL, respectively.[1]

Selectively deuterated 1,2-dipalmitoyl-*sn*-*3*-d_2-glycerol (**4**) was synthesized in several steps starting from *d*-mannitol (Baer and Fischer, 1939; Reichstein et al., 1935; Howe and Malkin, 1951). The deuterated 1,2-dipalmitoyl-*3*-d_2-*sn*-phosphatidylcholine (3-CD_2-DPL) was prepared from **4** following Baer and Kindler (1962).



4

The purity of the selectively deuterated lecithins was examined with thin-layer chromatography. For all compounds a single spot was obtained in chloroform–methanol–water (65:25:4, v/v). Optical rotation measurements indicated that the stereochemistry was retained during synthesis. The optical rotation $[\alpha]^{25}D$ was in the range of 6.2–6.8° compared to $[\alpha]^{25}D$ 6.9° for commercially available DPL (methanol–chloroform solvent 1:1, v/v). The selectively deuterated lecithins and their chemical precursors were further characterized by their proton and deuterium magnetic resonance spectra and their infrared spectra.

Preparation of Dipalmitoyl-3-sn-phosphatidylcholine Bilayers. DPL bilayers were prepared by thoroughly mixing DPL (50 wt %) and water (50 wt %). Approximately 200–500 mg of liquid crystalline phase was used for measuring powder type spectra. For magnetic resonance experiments a sealed ampoule containing the liquid crystalline phase was placed inside a 10-mm NMR tube. Measurements were made above and below the phase transition temperature of 41° (Chapman et al., 1967).

Deuterium and Phosphorus Magnetic Resonance Measurements. The deuterium (13.8 and 41.4 MHz) and phosphorus-31 (36.5 MHz) resonance spectra were obtained with Bruker-Spectrospin HX-90-FT and WH-270-FT spectrometers equipped with a variable temperature unit. The temperature unit was calibrated with a standard thermometer and the temperatures are correct to within ±1°. Proton decoupling experiments were performed with a Bruker-Spectrospin B-FS-100 frequency synthesizer and modulation unit. Deuterium T_1 relaxation time measurements were made by employing the conventional (180°–τ–90°) pulse sequence where τ is the delay between the 180 and 90° pulses. A 90° pulse had a duration of 18 μsec.

Results

Figure 1 shows typical deuterium magnetic resonance spectra of bilayers of CD_3N^+-DPL at two different temperatures. In the liquid crystalline state (Figure 1A) the spectrum is the kind expected for "powder type" samples of nuclear spin $I = 1$ if the average electric field gradient at the deuterium nucleus is axially symmetric (i.e., the asymmetry parameter η is zero) (Cohen and Reif, 1957; Chiba, 1962). The observed separation of the central peaks is:

$$\Delta\nu = (3/4)(e^2qQ/h)S_{CD} \quad (1)$$

where (e^2qQ/h) is the static deuteron coupling constant and S_{CD} is the order parameter of the C–D bond. For paraffinic chains the static deuteron coupling constant (e^2qQ/h) is approximately 170 kHz (Burnett and Muller, 1971). As anticipated from the theory the separation of the outer wings in Figure 1A amounts to approximately twice the separation of the central lines. On lowering the temperature through the phase transition to 37° (Figure 1B) the resonances broaden and the outer wings can no longer be observed.

[1] Abbreviation used is: DPL, dipalmitoyllecithin (= 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine).

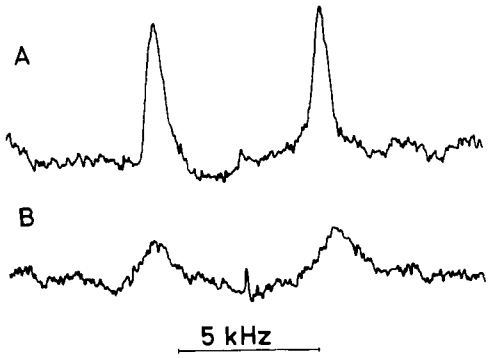


FIGURE 2: Deuterium magnetic resonance spectra (13.8 MHz) of bilayers of $^{+}NCH_2CD_2$-DPL (DPL 50 wt %; H_2O 50 wt %); (A) above the phase transition (50°); pulse width 18 μsec; dwell time 20 μsec; data memory 1K real; 20,000 free induction decays; (B) below the phase transition (39°); otherwise the same conditions as A; 22,000 free induction decays.

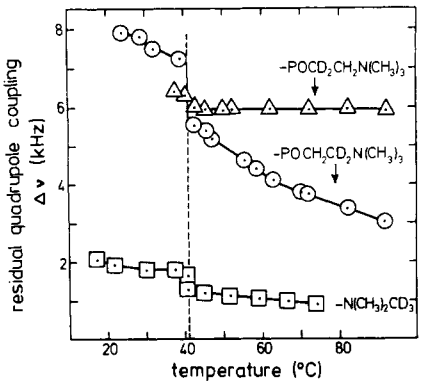


FIGURE 3: Variation of the residual quadrupole splittings of deuterated DPL bilayers with temperature; DPL–H_2O mixtures (50:50, wt %); deuterium labeling of the choline segments: (□) CD_3N^{+}-DPL; (○) $^{+}NCD_2CH_2$-DPL; (△) $^{+}NCH_2CD_2$-DPL.

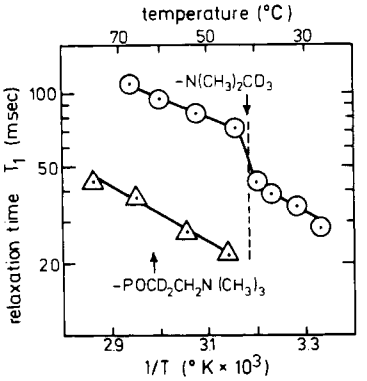


FIGURE 4: Variation of the deuteron T_1 relaxation times with temperature. Semilogarithmic plot of T_1 vs. the reciprocal absolute temperature; unsonicated DPL–H_2O mixtures (50:50 wt %): (○) CD_3N^{+}-DPL; (△) $^{+}NCH_2CD_2$-DPL.

Furthermore, the separation of the central lines increases by approximately a factor of 2, indicating a more restricted motion of the CD_3 group in the gel state.

Analogous measurements for bilayers of $^{+}NCH_2CD_2$-DPL are presented in Figure 2. Here the residual quadrupole splitting is approximately 6 kHz for most temperature measured. Due to the decreased sensitivity only the central peaks can easily be detected. As the temperature falls below the liquid crystalline transition point a marked line broadening occurs which makes signal detection impossible below 37°. It should also be mentioned that the spectra in Figure 2 were recorded without proton decoupling. Under decoupling conditions a sharpening of the lines occurs above the phase transition and two signals with slightly different quadrupole splittings appear (difference between the signals ~ 200 Hz).

The variations of the residual quadrupole splitting, $\Delta\nu$, of the deuterated choline segments with temperature are summarized in Figure 3. Above the phase transition, depending on the segment involved, the temperature dependence shows two different appearances. In one case ($^{+}NCH_2CD_2$-DPL) the quadrupole splitting remains constant from 70 to 41°. In the other case ($^{+}NCD_2CH_2$- and CD_3N^{+}-DPL) it increases with decreasing temperature, the relative change being approximately equal for both lipids. In going through the phase transition all three choline segments show larger quadrupole splitting. The relative change in $\Delta\nu$ is small for $^{+}NCH_2CD_2$-DPL, while a particularly marked increase is found for $^{+}NCD_2CH_2$-DPL. Below the transition point all three choline segments exhibit some temperature dependence. For CD_3N^{+}-DPL at 31° the measured quadrupole splitting is in approximate agreement with Stockton et al. (1974).

The deuteron T_1 relaxation times for *unsonicated* bilayers of CD_3N^{+}- and $^{+}NCH_2CD_2$-DPL are given in Figure 4 as a plot of log T_1 vs. the reciprocal absolute temperature. Above the phase transition the activation energy, E_a, of the relaxation of the CD_3 deuterons is calculated as E_a = 3.7 ± 1 kcal/mol, below the phase transition E_a = 6 ± 2 kcal/mol. For $^{+}NCH_2CD_2$-DPL the activation energy above the critical temperature is found to be E_a = 5 ± 2 kcal/mol; below the phase transition the relaxation time could not be measured due to sensitivity problems.

In addition to the methyl and methylene segments of the choline moiety the motion of the phosphate group was studied by means of phosphorus-31 resonance. Up until now such experiments have mainly been performed with *sonicated* lipid dispersions (Barker et al., 1972; Horwitz and Klein, 1972; Sheetz and Chan, 1972; Michaelson et al., 1974; Bystrov et al., 1972; Davis, 1972; Berden et al., 1974) under which conditions the chemical-shift anisotropy is rotationally averaged and enters only through the relaxation times. Most of the structural information contained in the anisotropy of the phosphorus-31 shielding tensor is thus lost. Experiments with *unsonicated* lipid samples have also been reported (Horwitz and Klein, 1972; Sheetz and Chan, 1972). Here the difficulty lies in decoupling the proton dipolar interactions from the phosphorus nucleus. Incomplete decoupling will obscure the exact evaluation of the chemical-shift anisotropy. In order to minimize this problem an intense decoupling field was employed in our experiments and the proton decoupling frequency was adjusted to the CH_2 resonances of the choline moiety. A comparison of decoupled and nondecoupled phosphorus-31 spectra of homogeneous, *nonsonicated* DPL–water mixtures is given in Figure 5. Also included is a spectrum of a sonicated sample. The decoupled phosphorus spectrum (Figure 5C) has exactly the form anticipated for powder-type spectra with chemical-shift anisotropy. The evaluation of the chemical-shift anisotropy, $\Delta\sigma_{obsd} = \sigma_{\parallel} - \sigma_{\perp}$, is thus straightforward. The results of measurements at various temperatures are summarized in Figure 6. In order to complete the study of the choline head group motion and to obtain a handle on the glycerol backbone of DPL, the C-3 carbon atom of the glycerol moiety was also deuterated. At this position the mo-

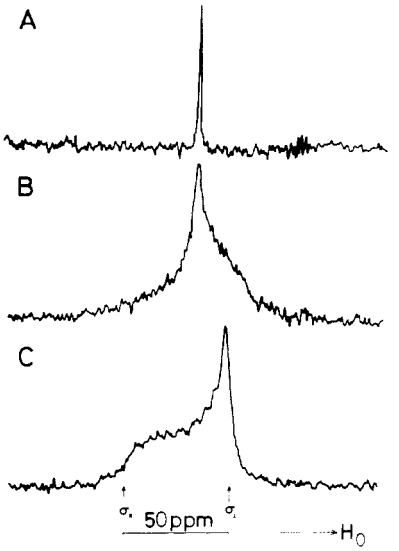


FIGURE 5: Phosphorus-31 resonance spectra (36.5 MHz) of DPL bilayers at 44°: (A) sonicated lipid dispersions (70 mg of DPL/ml of H_2O); chemical shift +1 ppm relative to external 85% H_3PO_4; (B) nonsonicated DPL bilayers (DPL 50 wt %-H_2O 50 wt %); *no* proton decoupling; (C) the same as B but *with* proton decoupling.

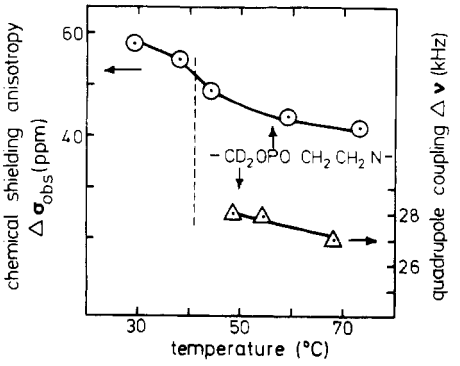


FIGURE 6: (○) Variation of the phosphorus chemical-shift anisotropy (in parts per million, left scale) with temperature. Unsonicated bilayers of DPL-water mixtures (50:50 wt %). $\Delta\sigma_{obsd}$ is determined as shown in Figure 5C. (△) Variation of the residual quadrupole coupling, $\Delta\nu$, of 3-CD_2-DPL bilayers with temperature. Average value of two couplings with a difference of about 2 kHz.

tional freedom is drastically restricted compared to the segments of the choline residue since the deuterium magnetic resonance spectra reveal two quadrupole splittings with separations of about 27 and 29 kHz. Such large splittings have so far been observed only for the fatty acyl chains in the lipid core region (Seelig and Seelig, 1974a,b). The average value of both splittings as a function of temperature is also included in Figure 6.

Discussion

The structure of a part of the choline head group in the extended conformation is depicted in Figure 7. The bond angles are the same as proposed by Sundaralingam (1972). The torsion angle φ indicates the rotation around the C_α-C_β bond. The 0° value corresponds to the planar trans arrangement of the O-C_α and C_β-N bonds.

Given the bond angles and bond lengths the problem is then to find the *average* conformation of the choline group in its bilayer environment, that is to determine the *average* torsion angles of the bonds between the glycerol backbone and the choline C_γ atom and also the average positions of

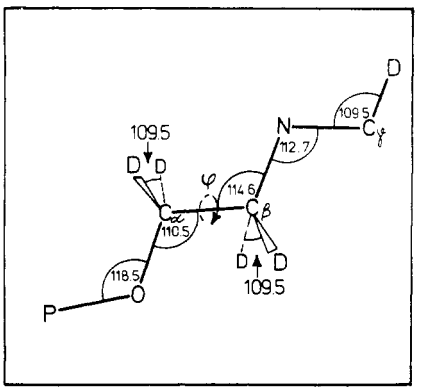


FIGURE 7: Bond angles and notations for carbon atoms of the choline polar group.

the segments with respect to the bilayer surface. For the terminal methyl group and for the $N(CH_3)_3$ group as a whole this problem is easily solved. Both groups possess a C_3-symmetry axis, therefore the average orientation is defined unequivocally by specifying the order parameters of the symmetry axes. For the methyl group the symmetry axis is given by the direction of the N-C_γ bond. From the bond angles in Figure 7, the order parameter $S_{N\text{-}C_\gamma}$ is calculated from the quadrupole splitting of the N-CD_3 group, $\Delta\nu_{CD_3}$, according to:

$$|S_{N\text{-}C_\gamma}| = 3|S_{C\text{-}D}| = (9/4)(h/e^2qQ)\Delta\nu_{CD_3} \qquad (2)$$

The symmetry axis of the $N(CH_3)_3$ group is the C_β-N bond direction. Its order parameter, $S_{C_\beta\text{-}N}$, is given by:

$$|S_{C_\beta\text{-}N}| = 3.6|S_{N\text{-}C_\gamma}| \qquad (3)$$

Using the results of Figure 3, the order parameter $S_{C_\beta\text{-}N}$ is found to range from 0.07 to 0.17 in the temperature interval measured. This demonstrates that the movement of the choline methyl and $N(CH_3)_3$ groups is not significantly restricted. The C_β-N bond vector does not occupy a fixed position with respect to the bilayer surface but executes oscillations of large angular amplitudes. This is in agreement with the interpretation of the proton T_1 relaxation time of the choline methyl group in coarse lecithin dispersions (Feigenson and Chan, 1974). It can also be shown that the central axis of the angular fluctuations, i.e. the axis of motional averaging, is normal to the bilayer surface. This follows directly from the observed collapse of the residual quadrupole splitting if planar oriented DPL bilayers are inclined at the magic angle with respect to the magnetic field (Stockton et al., 1974). By pressing the CD_3N^+-DPL-water phase (50/50 wt %) between planar glass plates we were able to confirm this result of Stockton et al. (1974).

The rate of the molecular reorientation follows from the relaxation time T_1. The dominant relaxation mechanism for deuterons is the quadrupole relaxation. For the case of a rapid isotropic reorientation the relaxation time T_1 is related to the correlation time τ_c according to eq 4 (Abragam, 1961):

$$(1/T_1) = (3\pi^2/2)(e^2Qq/h)^2\tau_c \qquad (4)$$

Since the movement of the choline methyl deuterons is almost isotropic, eq 4 may be used as a good approximation. Assuming a static quadrupole coupling constant of 170 kHz the correlation times of the choline methyl deuterons are determined from Figure 4 as: $\tau_c = 1.9 \times 10^{-11}$-$3.3 \times 10^{-11}$ sec (44-67°). The motion of the choline methyl group has

previously been investigated by measuring the proton relaxation time T_1 (Feigenson and Chan, 1974). Their data are interpreted by using two correlation times: $\tau_\parallel = 1 \times 10^{-10}$–$5 \times 10^{-11}$ sec for the reorientation about the rotor axis and $\tau_\perp \geq 4 \times 10^{-7}$ sec for off-axis excursions. The deuteron relaxation times τ_c thus correspond to the fast reorientation about the rotor axis. The activation energy of the reorientation, $E_a = 3.7$ kcal/mol, is in agreement with previous proton T_1 measurements of sonicated bilayers (Lee et al., 1972; Horwitz et al., 1972). Stockton et al. (1974) have measured the deuteron relaxation time T_1 of *sonicated* bilayers of egg-yolk lecithin, specifically deuterated at all three choline methyl groups. The observed spin–lattice relaxation time was 46 msec which is the same order of magnitude as observed for unsonicated systems.

Let us now proceed to a description of the conformational state of the C_α–C_β bond as revealed by Figures 3 and 6. The experimental parameters, i.e. the residual quadrupole coupling and the chemical-shift anisotropy, are directly connected with the motional restrictions. Then it follows for temperatures above the phase transition that the angular oscillations of the glycerol-C-3 segment, the phosphate group, and the choline-C_α segment show little variation with temperature, while the C_β and C_γ segments exhibit a pronounced temperature dependence, increasing temperature inducing smaller quadrupole splittings. The obvious explanation of this effect is torsional oscillations around the C_α–C_β bond. The *average* torsion angle must change with temperature. A qualitative picture of the direction of rotation is provided by phosphorus-31 measurements of CD_3N^+-DPL bilayers. In these experiments proton decoupling alone is not sufficient to yield spectra characteristic of pure phosphorus chemical-shift anisotropy. Instead, the experimental spectra are still distorted by the remaining deuteron–phosphorus dipolar interactions. Interestingly enough these deviations from the theoretical powder-type spectrum are small at 41°, but increase steadily with increasing temperature (Niederberger, W., Gally, H., and Seelig, J., manuscript in preparation). This is strong evidence that the choline CD_3 group moves closer to the phosphorus with increasing temperature. Inspection of molecular models shows that such an effect is easily explained by assuming a rotation of the C_α–C_β bond toward the gauche conformation, since the separation of the CD_3 group from the phosphorus is clearly shorter in the gauche than in the trans conformation. A more quantitative analysis is in preparation.

The two remaining segments to be discussed are the phosphate and the glycerol-3-CD_2 groups. Figure 6 shows that the phosphorus chemical-shift anisotropy amounts to 40–50 ppm for liquid crystalline DPL bilayers. Unfortunately, the elements of the static chemical-shift tensor of the DPL molecule are not known. From related data on other systems a maximum shift anisotropy of $\Delta\sigma_{max} \simeq 200$ ppm seems most probable (Appleman and Dailey, 1974). This allows a crude estimate of the order parameter, $|S_p|$, of the phosphate principal axis, namely $|S_p| \geq 0.2$. An analogous estimate can be made for the glycerol-3-CD_2 group. Here the experimental quadrupole splitting is about 28 kHz, yielding an order parameter of $|S_{CD}| = 0.22$ for the C–D bond. If the motion of this segment is assumed to be axially symmetric, the order parameter of the segment direction, defined by the normal to the plane spanned by the two C–D bond vectors, is given by $S_{mol} = 2|S_{CD}| = 0.44$. Even though these results are approximate, they nevertheless indicate an increasing restriction of the segmental motion toward the glycerol backbone of the lipid molecules. On the other hand, the observed order parameters are less than expected for a completely anisotropic motion. Even the glycerol-3-CD_2 segment has a remarkable flexibility in the lipid bilayer.

Summary

The orientation of the choline group in a bimolecular leaflet is generally discussed in terms of two extreme models (Hanai et al., 1965). Two orientations with respect to the bilayer surface are thought to be attainable by the zwitterion: (a) the choline group may be oriented with its axis normal to the bilayer surface or (b) it may be extended parallel to the bilayer surface. Analyzing the X-ray spacings of a series of synthetic phosphatidylcholines, Phillips et al. (1972) concluded that in *crystals* of phosphatidylcholine the zwitterionic group is oriented normal to the bilayer surface. The end-group contribution is 11 Å per lecithin and the C_α–C_β bond is postulated to be in the gauche conformation.

Above the gel-to-liquid crystal transition point the situation is less clear. Hydration studies with D_2O as well as proton and phosphorus resonance measurements of sonicated dispersions have yielded qualitative information about the head group motion (Finer and Darke, 1974; Michaelson et al., 1974). By comparing the choline head group with the ethanolamine and serine head groups it was concluded that the choline has the least motional restriction. Our results extend the previous studies in that they provide quantitative estimates for the motional restrictions of the various choline segments. The choline group is found to possess a flexible, temperature-dependent conformation. This effect is especially pronounced for the C_α–C_β bond. The phosphorus experiments suggest a bent structure with the positively charged tetramethylammonium group in the vicinity of the negatively charged phosphate. Increasing temperature stabilizes this structure. This ties in neatly with conformational studies of choline and phosphatidylcholine in *solution* where the C_α–C_β bond was found to be either exclusively (phosphatidylcholine) or predominantly (choline) in the gauche conformation (Birdsall et al., 1972; Dufourcq and Lussan, 1972; Lichtenberg et al., 1974). In comparing the different segments of the choline head group the order parameters are found to be smallest for the methyl group and largest for the glycerol-3-CD_2 segment. The latter order parameter is similar in magnitude to those observed for the fatty acyl chain segments close to the glycerol (Seelig and Seelig, 1974a,b). The choline group as a whole executes rapid angular oscillations around an axis *normal* to the plane of the bilayer. Due to these fluctuations and also due to the segmental flexibilities neither of the above-mentioned *static* pictures provides a suitable description of the conformation of the polar group; instead, a dynamic model using order parameters is preferable.

Below the phase transition one interesting aspect deserves notice. Dipalmitoylphosphatidylcholine has been found to display a calorimetric pretransition at about 34° (Chapman et al., 1967; Hinz and Sturtevant, 1972). It was suggested that this transition is associated in some way with the packing of the choline head group. In our experiments no discontinuities are observed at this temperature either in the phosphorus chemical-shift anisotropy or in the quadrupole splittings of the C_β or C_γ segment, indicating that the pretransition should probably be attributed not so much to the choline moiety but to the DPL molecule as a whole.

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