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ORIENTATION AND FLEXIBILITY OF THE CHOLINE HEAD GROUP IN PHOSPHATIDYLCHOLINE BILAYERS

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Summary

The average orientation and flexibility of the phosphorylcholine group are deduced from deuterium and phosphorus-31 nuclear magnetic resonance measurements of unsonicated phosphatidylcholine bilayers in the liquid crystalline state. The experimental data are consistent with a model in which the polar head group exhibits a restricted flexibility characterized by rapid transitions between two enantiomeric conformations. A completely flexible or a completely rigid head group structure can be excluded. The phosphorylcholine residue is found to be bent at the position of the phosphate group, due to a *gauche-gauche* conformation of the phosphodiester linkage. The choline dipole is aligned parallel to the plane of the membrane, which is in agreement with X-ray and neutron diffraction studies. The average orientation of the phosphorylcholine group is therefore the same as that of the phosphorylethanolamine head group.

Introduction

The disposition of the polar groups in lipid bilayers has received a great deal of attention, since the physical and functional properties of bilayer membranes may depend on the nature of the electrostatic interactions [1]. In order to quantitatively understand these electrical interactions, knowledge of the exact orientation and flexibility of the polar groups is of considerable importance. At present two phospholipid head groups, the ethanolamine and the choline group, have been investigated in detail. Using X-ray diffraction techniques [2] and infrared dichroism [3] the ethanolamine dipole has been found to be oriented parallel to the surface of the membrane at temperatures below the phase transition (crystalline or gel state). The same average orientation has been inferred from phosphorus-31 and deuterium NMR measurements for bilayers of

dipalmitoyl-3-*sn*-phosphatidylethanolamine above the phase transition temperature (liquid crystalline state) [4]. The NMR 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. The torsion angles of these conformations are closely related to those characteristic of the phosphorylethanolamine head group in crystalline bilayers [2,4]. With respect to the orientation of the choline head group the situation is less clear, since two different models have been suggested in the literature. By extrapolating the X-ray long spacings of phosphatidylcholine and phosphatidylethanolamine homologues (in the bilayer gel state) to zero hydrocarbon chain length it has been concluded that the preferred orientations of the choline and ethanolamine dipoles are perpendicular and parallel, respectively, to the bilayer surface [5]. Unfortunately, a rather large error is associated with this type of extrapolation. More reliable results are provided by recent high resolution X-ray and neutron diffraction work [6—9]. Although the position of the phosphorylcholine groups can be deduced only indirectly, the latter studies consistently predict an average orientation of the choline dipole parallel to the surface of the bilayer. The objective of this report is to reinvestigate the problem of the phosphorylcholine head group structure using the phosphorus-31 chemical shielding anisotropies and deuterium quadrupole couplings [10,11]. Since the experimental data have been obtained with selectively labeled compounds, they directly reflect the motional behavior of the individual head group segments. In a preliminary and rather qualitative analysis we interpreted the observed differences in the phosphorus and deuterium anisotropies as indicating a progressive increase in the segmental flexibilities from a more rigid glycerol backbone towards a freely moving choline-methyl rotor. The quantitative approach discussed here makes this interpretation rather improbable. Taking into account the torsion angles observed for single crystals of phosphatidylcholine constituents [12—14] the NMR data for dipalmitoyl-3-*sn*-phosphatidylcholine above the phase transition temperature are found to be consistent with the same two-state model as proposed for phosphatidylethanolamine, indicating only a limited head group flexibility. The analysis furthermore yields the average orientation of the choline dipole in the liquid crystalline bilayer membrane.

Parameters of the crystalline state

The structure of the glycerylphosphorylcholine group is depicted in Fig. 1 [14]. The HCH and $^2HC^2H$ bond angles are assumed to be exactly tetrahedral (109.5°C). According to Sundaralingam [14] the torsion angles α_j are measured from the *cis*-planar configuration of the $j+1$ and $j-1$ bonds. The torsion angle about bond j is considered positive (negative) for a right-handed (left-handed) rotation; when looking along the bond j, the far bond $j+1$ rotates clockwise (counterclockwise) relative to the near bond $j-1$.

Crystal structures have been determined for the $CdCl_2 \cdot$ glycerylphosphorylcholine complex [12] and for free glycerylphosphorylcholine [13]. There are two independent conformers, glycerylphosphorylcholine-1 and glycerylphosphorylcholine-2, in the asymmetric part of the unit cell. The corresponding torsion angles, together with those of the single glycerylphosphorylcholine $\cdot$ $CdCl_2$

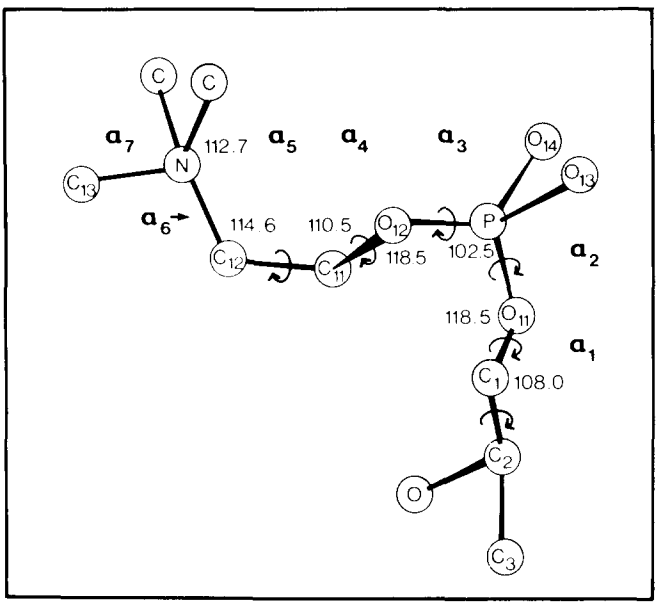

Fig. 1. Structure of glycerylphosphorylcholine according to Sundaralingam [14].

conformation, are listed in Table I [14]. The conformations of the choline part of glycerylphosphorylcholine-1 and glycerylphosphorylcholine · $CdCl_2$ are rather similar, whereas glycerylphosphorylcholine-2 differs from glycerylphosphorylcholine-1 in that all torsion angles α_i in glycerylphosphorylcholine-2 have opposite sense to those in glycerylphosphorylcholine-1. Thus glycerylphosphorylcholine-1 and glycerylphosphorylcholine-2 can be considered as enantiomeric forms with respect to the structure of the choline group. All three structures are characterized by a *gauche-gauche* conformation of the phosphodiester linkage (α_2, α_3) and a *gauche* conformation of the O-C-C-N system (α_5), the *gauche* conformations of glycerylphosphorylcholine-1 and glycerylphosphorylcholine-2 being of opposite sense. The rigid lattice phosphorus-31 chemical shielding tensor of dipalmitoyl-3-*sn*-phosphatidylcholine has been determined by Kohler and Klein [15] and by Griffin [16]. The principal values found by Kohler and Klein (Griffin) are σ_{11} = −81 (−97.6) ppm, σ_{22} = −25 (−34.5) ppm, and σ_{33} = 108 (131.0) ppm. The discrepancy between the two sets of data is presumably caused by the slightly different water contents of the samples used. In Griffin's experiments anhydrous dipalmitoyl-3-*sn*-phosphatidylcholine was prepared by heating the lipid for 4 h at 90°C under vacuum and sealing it without exposure

TABLE I

CONFORMATION OF THE CHOLINE HEAD GROUP IN CRYSTALS OF GLYCERYLPHOSPHATIDYLCHOLINE AND GLYCERYLPHOSPHORYLCHOLINE · $CdCl_2$ [14]

	α_1	α_2	α_3	α_4	α_5
Glycerylphosphorylcholine · $CdCl_2$	169° (t)	−69° (g^+)	−73° (g^+)	178° (t)	73° (g^-)
Glycerylphosphorylcholine - 1	165° (t)	−71° (g^+)	−59° (g^+)	−138°	73° (g^-)
Glycerylphosphorylcholine - 2	−172° (t)	64° (g^-)	65° (g^-)	140°	−75° (g^+)

to atmosphere, while commercially available lipid was used by Kohler and Klein without taking any further precautions. According to Griffin the binding of water to the lipid induces some motion of the lipid head groups so that the rigid lattice limit is no longer maintained. An alternative explanation is that the binding of the first one or two water molecules to the phosphate group changes the components of the chemical shielding tensor but has no effect on the motion of the phosphorus. In the following calculations we have made use of both tensors and found that the calculated torsion angles α_1 and α_2 deviated only by about 5°.

Since the phosphorus NMR experiments were performed with powder-type samples of dipalmitoyl-3-*sn*-phosphatidylcholine no information about the orientation of the chemical shielding tensor relative to the molecular frame could be obtained. Following Kohler and Klein [15] we assume that the orientation of the shielding tensor is the same as that found in single crystals of phosphoryethanolamine, with σ_{11} and σ_{33} parallel to the O(11)—O(12) and O(13)—O(14) connecting vectors, respectively. This is supported by more recent measurements of Griffin (Griffin, R.G., personal communication).

The evaluation of all bilayer quadrupole splittings is based on a static quadrupole coupling constant for the ^{2}H-nucleus in C—^{2}H bond of $(e^2qQ/h) = 170$ kHz. Although the electric field gradient tensor may vary slightly with the position of the deuterium in the lipid molecule, the error introduced is certainly not serious since the static quadrupole couplings in such different compounds as $(^2HOOC)_2C^2H_2$, $N^2H_3C^2H_2COO^-$, and $C_4{}^2H_{10}$ are similar (168, 166.5, and 169.1 kHz, respectively [17—19]), and axially symmetric around the C—^{2}H bond axis (asymmetry parameter $\eta \approx 0$).

Experimental results for the liquid crystalline state

Phosphorus-31 and deuterium NMR studies with oriented multilayers of fully hydrated phosphatidylcholine clearly demonstrate that the choline group as a whole is rotating rapidly around an axis perpendicular to the surface of the bilayer [20,21]. Due to this motion the static phosphorus chemical shielding tensor is averaged to an effective shielding tensor which shows axial symmetry with respect to the rotation axis. The new tensor contains only two components, $\sigma_\parallel$ and $\sigma_\perp$, which are the extreme values of the chemical shielding anisotropy measured parallel and perpendicular, respectively, to the rotation axis. The maximum chemical shielding anisotropy is given by $\Delta\sigma = \sigma_\parallel - \sigma_\perp$. Experimental values for $\Delta\sigma$ measured in mixtures of dipalmitoyl-3-*sn*-phosphatidylcholine and water have been published [10,11,16,20,22]. ^{2}H-labeled dipalmitoyl-3-*sn*-phosphatidylcholines have been synthesized with the deuterium attached at carbon atoms C(1), C(11), C(12), and C(13), respectively [10] *. The residual quadrupole couplings and the phosphorus-31 chemical shielding anisotropy of the corresponding bilayer phases are summarized in the penultimate column of Table II. The quadrupole splitting of dipalmitoyl-3-*sn*-phospha-

* Carbon atom C(1) in Sundaralingam's notation is called C(3) in the stereospecific numbering (*sn*) nomenclature. The lipids abbreviated in ref. 10 as 3-CD_2-DPL, $^+NCH_2CD_2$-DPL, $^+NCD_2CH_2$-DPL, and CD_3N^+-DPL correspond to dipalmitoyl-3-*sn*-phosphatidylcholine with the deuterium label attached at carbon atoms C(1), C(11), C(12), and C(13), respectively, of Fig. 1.

TABLE II

CONFORMATION OF THE CHOLINE HEAD GROUP IN LIPID BILAYERS (NMR)

Torsion angle	NMR	X-ray [a]	Segment	Measured parameter	Experimental result (49°C)	Theoretical prediction
α_1	$\pm 170^\circ$	165° -170°				
α_2	$\mp 60^\circ$	-71° 64°	Phosphate	$\Delta\sigma$	−47 ppm	−47.1 ppm [c]
α_3	$\mp 64^\circ$	-59° 64°				
α_4	$\mp 145^\circ$	-138° 140°	$NCH_2C^2H_2OP\Delta\nu$	$\Delta\nu_\alpha$	5.9 kHz [b]	5.6 kHz [d]
α_5	$\pm 81^\circ$	72° -75°	$NC^2H_2CH_2OP$	$\Delta\nu_\beta$	5.1 kHz [b]	−4.9 kHz [d]
α_6	free rotation					
α_7	free rotation		$C^2H_3(CH_3)_2N$	$\Delta\nu_\gamma$	1.15 kHz [b]	1.25 kHz [d]

[a] Taken from Table I.

[b] Only the absolute value of the quadrupole splitting can be measured. The experimental results refer to unsonicated phosphatidylcholine bilayers (50 wt.% lipid, 50 wt.% H_2O).

c Based on the shielding tensor of Kohler and Klein [15].

d Calculated for powder type spectra with $(e^2 qQ/h) = 170$ kHz.

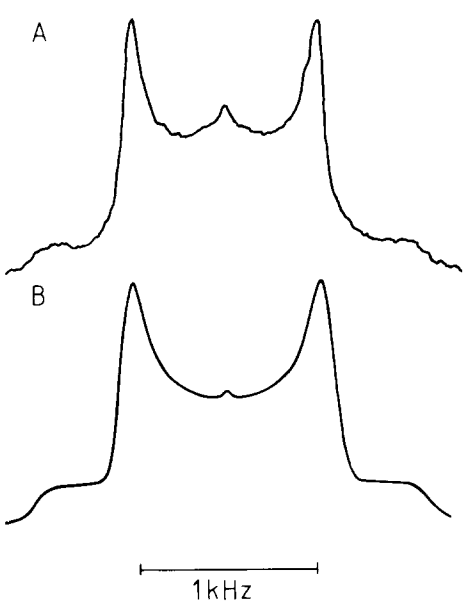

Fig. 2. Deuterium NMR spectrum at 13.8 MHz of unsonicated bilayers of $C^2H_3(CH_3)_2N^+$- dipalmitoyl phosphatidylcholine (50 wt.% lipid, 50 wt.% H_2O; 59°C). A, experimental spectrum; B, computer-simulated spectrum. For the calculation the linewidth formula $\Delta\nu = (\Delta_0 + \Delta_1 \cdot 3\cos^2\Theta - 1)/2$ was assumed [11], with $\Delta_0 = 23$ Hz and $\Delta_1 = 55$ Hz. The calculated residual quadrupole splitting is 1.16 kHz.

tidylcholine deuterated at the methyl rotor (C(13) of Fig. 1) amounts only to about 1 kHz. For such a small splitting the shape of the spectrum is influenced by the width of the resonance lines and the separation of the two most intense peaks yields only an approximate value for the true quadrupole coupling. We have therefore computer simulated this spectrum by a method analogous to that used for the phosphorus-31 NMR spectra [11]. A comparison between the experimental spectrum and its computer simulation is shown in Fig. 2. The computer analysis yields a somewhat larger quadrupole coupling (1.16 kHz at 59°C) than the direct evaluation of the spectrum (1.06 kHz). In Table II only the computed value is listed.

Theoretical analysis

The conformational analysis of the phosphorylcholine head group structure is based on the same model as propounded for the phosphorylethanolamine head group [4]. In this model it is assumed that the C(2)—C(1) axis of the glycerolbackbone constitutes the rotation axis for the motion of the phosphorylcholine moiety. In the crystal structure of dilauroyl-DL-phosphatidylethanolamine the C(2)—C(1) bond vector has been found to be oriented perpendicular to the plane of the bilayer [2] and inspection of molecular models reveals that for this conformation the rotation around the C(2)—C(1) axis is relatively unhindered. This is supported by neutron diffraction data of bilayers of dimyristoyl-3-*sn*-phosphatidylcholine. The Fourier profiles of the neutron scattering densities agree with a model in which the zwitterionic dipole of phosphorylcholine rotates around the C(2)—C(1) axis, so that the choline methyl groups reduce the scattering amplitude density at the bilayer coordinate of the phosphate group [9]. In the liquid crystalline membrane the orientation of the C(2)—C(1) axis is not rigidly fixed perpendicular to the membrane surface. The deuterium resonance spectrum of dipalmitoyl-3-*sn*-phosphatidylcholine deuterated at the glycerol C(1) segment consists of two doublets with frequency spacings of 27 and 29 kHz [10], both couplings being distinctly smaller than anticipated for an immobile C(2)—C(1) axis. The deviation from the rigid lattice limit can be explained by assuming a fast wobbling motion of the C(2)—C(1) axis about the bilayer normal in both cases. In order to characterize the extent of angular excursions of the rotation we introduce the order parameter $S_{C(2)-C(1)}$ of this axis. $S_{C(2)-C(1)}$ can be evaluated from the residual quadrupole coupling of the C(1) segment, but a complication occurs because of the observation of two quadrupole couplings for this segment. Previous studies on the conformation of the fatty acyl chains in the vicinity of the polar group have suggested that the glycerol backbone may assume two different long-lived conformations [23]. The steric restrictions imposed on the C(2)—C(1) axis probably depend on the conformational state of the glycerol and this would explain the two quadrupole splittings observed for C(1). However, since the difference between the two quadrupole splittings is quite small, we shall not discuss the two glycerol conformations separately but shall use the average value of the $S_{C(2)-C(1)}$ order parameters of the two conformations. The average deuterium quadrupole splitting of C(1)-deuterated dipalmitoyl-3-*sn*-phosphatidylcholine thus amounts to $\Delta\nu \approx 28$ kHz at 49°C. The order parameter of the C(1)—^{2}H bond vector is

therefore found to be $|S_{C^2H}| = 0.22$ leading to an order parameter of $S_{C(2)-C(1)} = +0.66$ for the C(1)—C(2) axis (for details of the calculation cf. ref. 4). The general procedure for the analysis of the deuterium and phosphorus NMR data can now be formulated. The static electric field gradient tensor V_P and the phosphorus chemical shielding tensor, $\underset{\sim}{\sigma}_P$ are known in their respective principal coordinate system. For a C—^{2}H bond V_P is axially symmetric around the bond direction (z-axis):

$$V_P = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & V_{zz} \end{bmatrix} \tag{1}$$

The principal coordinate system of the phosphorus shielding tensor $\underset{\sim}{\sigma}_P$ has been defined above. In this coordinate system $\underset{\sim}{\sigma}_P$ takes the form

$$\sigma_P = \begin{bmatrix} \sigma_{11} & 0 & 0 \\ 0 & \sigma_{22} & 0 \\ 0 & 0 & \sigma_{33} \end{bmatrix} \tag{2}$$

By applying cartesian transformation matrices $\underset{\sim}{T}_i\,(\Theta_i, \phi_i)$ these tensors are transformed first into the coordinate system of their respective chain segment and then successively through intervening bonds into the coordinate system of the C(2)—C(1) axis. With $\underset{\sim}{U} = \underset{\sim}{V}_P$ or $\underset{\sim}{\sigma}_P$ this transformation may be written as

$$\boldsymbol{U}_i = \prod_i \boldsymbol{T}(\theta_i,\phi_i)\,\boldsymbol{U}\boldsymbol{T}_i^{-1}(\theta_i,\phi_i) \tag{3}$$

The index i denotes the total number of transformations to be performed, which depends, of course, on the position of the tensor investigated in the choline backbone. For the first transformation, i.e. the transformation from the principal coordinate system into that of the chain segment, Θ_1 and ϕ_1 must be deduced from the chain geometry. For the subsequent steps the Θ_i and ϕ_i are the bond and torsion angles respectively of the i-th transformation [24]. Bond angles Θ_i are taken from the crystal structure of glycerylphosphorylcholine [13]. The parameters to be determined here are thus the torsion angles ϕ_i. The final step in the analysis is to allow for the free rotation around the C(2)—C(1) axis. This eliminates all off-diagonal elements of $\underset{\sim}{U}_i$ and leads to an axially symmetric tensor with $(U_i)_{zz} = U_{\parallel} = -\frac{1}{2}\,U_{\perp}$. The tensor elements are further averaged by the wobbling motion of the rotation axis and must therefore be multiplied by $S_{C(2)-C(1)}$. This result can then be compared with the experiment. In evaluating the ϕ_i three models shall be discussed, namely (i) free rotation around all bonds of the phosphorylcholine backbone, (ii) a rigid structure with all torsion angles fixed at a definite value and (iii) a two-state model with rapid jumps between two enantiomeric conformations.

The free rotation model is certainly not in concord with the experimental results. Consider for example the phosphorus-31 chemical shielding anisotropy. Assuming free rotation around α_2, α_1, and the C(2)—C(1) axis (Fig. 1) yields a calculated shielding anisotropy $|\Delta\sigma| = 5.1$ ppm compared to an experimental value of 47 ppm (cf. ref. 15). Similarly the calculated deuterium quadrupole

couplings are much smaller than the experimental results. A completely flexible head group structure can therefore be excluded.

In the case of the other extreme, i.e. a completely rigid phosphorylcholine group, difficulties are encountered in the interpretation of the deuterium resonance spectra of the C(11) and C(12) segments. In order to explain the magnitude of the quadrupole splittings, a bent configuration of the phosphorylcholine group is required; configurations containing only *trans* and *cis* states do not reproduce the experimental results. In such a bent configuration the two deuterons of a given segment are inclined at different angles with respect to the rotation axis and should produce two separate quadrupole splittings if the choline group were really rigid. This is not borne out by the experiment which clearly shows that the two deuterons are motionally equivalent. We therefore conclude that the choline head group is endowed with a limited flexibility which averages the differences between the two deuterons of the C(11) and the C(12) segment, respectively.

In order to account quantitatively for the observed flexibility a two-state model is suggested here. We assume that a rapid equilibrium exists between two enantiomeric choline conformations, one with all torsion angles α_i positive, the other with all torsion angles α_i negative. If the molecule fluctuates between these two configurations, the corresponding deuterons will exchange their orientation with respect to the rotation axis. For a sufficiently rapid jump rate ($>10^4$ Hz) both deuterons will then experience the same average electric field gradient, leading to the same quadrupole splitting. The experimental support for such a model comes from the very existence of two enantiomeric configurations in crystals of L-α-glycerylphosphorylcholine [13]. It seems likely that these configurations are carried over into the liquid crystalline state and that rapid transitions may then occur between the two states. Based on this model we have calculated the phosphorus-31 chemical shielding anisotropy and the various quadrupole splittings in a configurational space of $\pm 30°$ of the crystallographic torsion angles. The analysis is simplified by the fact that the choline methyl rotor (α_7) and the $N^+(CH_3)_3$ group (α_6) as a whole are characterized by 3-fold symmetry axes. It is safe to assume that these symmetry axes are also rotation axes, so that no torsion angles need to be specified. The computer search then shows that within the configurational space defined above only one solution can be found which provides a consistent explanation of all experimental NMR parameters. This solution is summarized in Table II. The agreement between the experimental results (penultimate column) and the theoretical predictions (last column) can easily be improved by using a finer grid for the torsion angles. Such a precision is, however, meaningless in view of the uncertainties in the phosphorus chemical shielding tensor, the static deuteron quadrupole coupling constants, and also the crystallographic bond angles.

Discussion

Many different models are conceivable for the head group motion as long as the theoretical predictions are compared with just one experimental parameter, e.g. the phosphorus chemical shielding anisotropy [20,22]. The number of possibilities is greatly reduced, however, if several experimental parameters have to

be accounted for. The model presented here is probably the simplest model to provide a consistent explanation of five independent deuterium and phosphorus anisotropies. This is a necessary but not a sufficient condition for the correctness of the model. Additional support comes, however, from recent X-ray and neutron diffraction studies.

The most striking result of Table II is the extremely close agreement of the NMR-determined torsion angles with those obtained from the crystal structure. This is a strong indication that the crystallographic conformations are indeed the predominant ones in the lipid bilayer. Since the C(1)—C(2) rotation axis was assumed to have an average orientation perpendicular to the surface of the membrane, the *gauche-gauche* conformation of the phosphodiester linkage leads to a bending of the phosphorylcholine group at the position of the phosphorus atom, so that the choline part becomes aligned parallel to the plane of the membrane. The average structure and orientation of the phosphorylcholine head group in the liquid crystalline membrane are thus almost the same as observed for the phosphorylethanolamine head group [4]. Some differences between the two head groups do exist, however, with respect to the absolute value of the torsion angles (notably α_4) and with respect to the signs in the *gauche* conformations of the sequence α_2, α_3, α_5 which are g^+, g^+, g^+ in phosphatidylethanolamine but g^+, g^+, g^- in phosphatidylcholine. The theoretical predictions for $\Delta\nu$ and $\Delta\sigma$ (Table II) are quite sensitive to a variation of the torsion angles, e.g. changing α_5 from $\pm 81°$ to $\pm 80°$ decreases the residual quadrupole coupling $\Delta\nu_\beta$ from -4.9 to -5.8 kHz. Only small changes in the torsion angles are therefore required to account for the observed temperature dependence of the NMR parameters [10]. As a qualitative conclusion it follows that no significant changes in the torsion angles are observed in the liquid crystalline temperature range measured (41—70°C). Next let us compare the NMR results with X-ray diffraction and neutron scattering experiments. Lesslauer et al. [6] have performed X-ray diffraction studies of oriented multilayers of dipalmitoyl-3-*sn*-phosphatidylcholine containing various amounts of water. The calculated electron density profiles suggest that the phosphorus and the nitrogen atom of the phosphorylcholine group are lying in a plane parallel to the surface of the membrane at temperatures both below and above the gel-to-liquid crystal transition. The same conclusion has been reached on the basis of neutron diffraction studies involving ^2H_2O-H_2O exchange [9]. More recently, we have employed the selectively deuterated lipids described above in neutron diffraction experiments. Because of the large difference between the coherent scattering amplitudes of hydrogen and deuterium, the deuterated segments can easily be located in the neutron Fourier profiles. The measurements clearly demonstrate that the deuterons attached to the C(13), C(12), and C(11) carbon atoms have almost the same average distance from the center of the bilayer, regardless whether the system is in the gel state (25°C, 5—6 wt. % H_2O, average distance approx. 24.9 ± 0.6 Å) or in the liquid crystalline state (50°C, 25 wt. % H_2O, average distance 21.8 ± 0.6 Å) (Büldt, G., Zaccai, G., Seelig, J. and Gally, H.U., unpublished). This probably constitutes the most direct evidence for a parallel orientation of the choline head group. This orientation seems furthermore to be independent of whether cholesterol is incorporated into the bilayer or whether the lipid region contains unsatu-

rated fatty acyl chains [7,8]. The NMR data presented here are thus in good agreement with the X-ray and neutron diffraction work.

Evidence for an orientation of the choline group perpendicular to the bilayer surface has recently been presented on the basis of T_1-relaxation time measurements using various shift reagents [25]. If correct, these results mean that the presence of trivalent ions changes the choline head group structure. This effect requires further investigations, since the interpretation of T_1-relaxation times is not straightforward and since it is also interesting to know if this finding is relevant for the binding of monovalent or divalent ions (see Note added in Proof).

As a second conclusion it follows from our measurements that the polar head groups in phosphatidylcholine bilayers are endowed with only a limited flexibility. A completely flexible head group with free rotation around all bonds can certainly be excluded as can be the opposite extreme, a rigid head group with completely fixed torsion angles. The NMR data are, however, consistent with a two-state model, in which the phosphorylcholine group jumps between two enantiomeric conformations. These conformations are furthermore in close agreement with those obtained for crystalline model compounds which makes other models involving a large number of different conformations less probable. This is further supported by quantum-mechanical studies of phospholipids and phospholipid constituents. Two well-resolved energy minima have been found by the INDO molecular orbital method for 2,3-diformylglycerol, indicating essentially two energetically favorable conformations in the glycerol part of this model compound [26]. A dynamic equilibrium between two phospholipid conformations characterized by α_2, α_3 = 60°, 60° and −60°, −60° and two possible conformations of the hydrocarbon chains near the glycerol moiety has been postulated by Govil and coworkers [27—29], again based on molecular orbital calculations. With respect to the molecular fluctuations in the phosphatidylcholine head group it thus appears that the molecular constituents oscillate either slowly (the glycerol) or rapidly (the phosphorylcholine) between only a few well-defined minima of the conformational energy. The energetically most favorable states seem to be almost independent of the state of the bilayer and the conformational restrictions persist from the crystalline through to the "more disordered" liquid crystalline state.

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Using shift reagents Barsukov et al. [30] concluded that the phosphorylcholine groups in sonicated bilayer vesicles are oriented parallel to the bilayer surface.

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