

ORIENTATIONAL ORDER AND ROTATIONAL DIFFUSION OF THE HEAD GROUP IN THE BILAYER MEMBRANE

A NUCLEAR MAGNETIC RESONANCE STUDY

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ABSTRACT An order parameter-based interpretation is applied to the temperature dependence of the deuterium magnetic resonance splittings and the anisotropic contribution to the chemical shift for ^{31}P from the head group of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). It is shown that the rotational motion of the molecule about its long axis is not a free rotational motion as normally assumed, but instead a biased one. Changes in the degree of biasing appear to be primarily responsible for the variation of the NMR spectra with temperature. The degree of biasing is described by orientational order parameters. With the use of these order parameters, it is shown that the temperature dependence of the anisotropic contribution to the chemical shift for ^{31}P can be predicted from that of the deuterium quadrupole splittings.

INTRODUCTION

Rotation of the phospholipid molecule about its long molecular axis in the bilayer membrane is a well established fact (1). It is normally assumed that this rotational motion is a free rotational motion in the interpretation of nuclear magnetic resonance (NMR) spectra from membranes (2). This often can be a good approximation and of considerable value in some studies, for it greatly simplifies the interpretation of the spectra. In this paper, we show evidence that this motion is not free but is instead strongly biased. Furthermore, we show that making the free rotation approximation can lead not only to misleading results in the interpretation of the NMR spectra, but also to loss of useful information, particularly in the interpretation of the temperature dependences of the spectral splittings or shifts.

To examine the rotational motion of the molecule we make use of the temperature dependence of deuterium spectral splittings and the anisotropic contribution to the chemical shift for ^{31}P from the head group of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) reported by Brown and Seelig (3). We apply an order parameter-based approach found to be successful in other liquid crystal phases (4). We find that these spectra and their temperature dependence are governed by three orientational order parameters. These order parameters are a direct reflection of the symmetry of the orientational order of the phase and are completely

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consistent with the existing model of the bilayer membrane. Furthermore, it is shown that the head group does not appear to change its preferred conformation any measurable amount as the temperature is varied, even when the liquid crystal-gel phase transition is traversed.

MOTIONAL AVERAGING IN THE BILAYER MEMBRANE

In this paper, we are concerned about the motional averaging of both the deuterium quadrupole interaction and the anisotropic contribution to the ^{31}P chemical shift. Both of the quantities time average in the same way. The quadrupole interaction is observed as a splitting of the Zeeman line, which can be expressed as (4)

$$\Delta\nu_i = \frac{3}{2} \nu_Q^i \left\langle \frac{3}{2} \cos^2 \theta_i - \frac{1}{2} \right\rangle, \quad (1)$$

where $\nu_Q^i = e^2 q_i Q/h$ is the coupling constant associated with a deuterium spin at the i^{th} site in a molecule measured in a solid where the orientational motion of the molecule is, for the most part, frozen out. The principal z axis of the electric field gradient for that interaction is normally taken to be along the i^{th} carbon-deuteron bond. In Eq. 1 the angle θ_i is the instantaneous angle between that bond direction and the direction of the magnetic field. In a liquid crystal the orientation of the molecule fluctuates in time and the splitting is reduced because of the time averaging indicated by the brackets in Eq. 1. The anisotropic contribution to the chemical shift averages in a manner analogous to the quadrupole interaction. The anisotropic contribution to the chemical shift σ of a specific spin can be expressed as (5,6):

$$\Delta\sigma_i = \frac{2}{3} (\sigma_{\parallel} - \sigma_{\perp})_i \left\langle \frac{3}{2} \cos^2 \theta_i - \frac{1}{2} \right\rangle, \quad (2)$$

where $(\sigma_{\parallel} - \sigma_{\perp})_i$ represents the total anisotropic range of the shift for the i^{th} spin as measured in a solid where the orientational motion has been mostly frozen out. The angle θ_i is the instantaneous angle between the principal z axis for the shielding interaction and the magnetic field. The brackets in Eq. 2 express the time averaging resulting from orientational motion in the liquid crystal. Eqs. 1 and 2 are written for the case where the electric field gradient tensor and chemical shift tensor as measured in the solid crystal are axially symmetric. Writing these expressions for the most general nonaxial case would not change the argument that follows, but would only complicate the equations (10). Some discussion of this is given in the last section of the paper.

Since both the quadrupole splitting and the chemical shift depend on the time average of $\langle (3/2) \cos^2 \theta_i - 1/2 \rangle$, it is useful to express this average in terms of the motions expected in a liquid crystal. It is common procedure to twice transform the expression in the time average brackets. The first transformation is to a molecular frame M_x , M_y , and M_z , where M_z is the long axis of the molecule. The second transformation is to a director, N_z , which is the preferred direction of orientation that M_z assumes, on the average, as the molecule reorients in the liquid crystal. Under these transformations (7)

$$\left\langle \frac{3}{2} \cos^2 \theta_i - \frac{1}{2} \right\rangle = \sum_{p,q} \left\langle (\cos \alpha_p^i \cos \alpha_q^i) \left(\frac{3}{2} \cos \theta_p \cos \theta_q - \frac{1}{2} \delta_{pq} \right) \right\rangle, \quad (3)$$

independence will be verified for the membrane molecules in this paper. Under this independence, Eq. 3 can be expressed as (4)

$$\left\langle \frac{3}{2} \cos^2 \theta_i - \frac{1}{2} \right\rangle = a_{zz}^i S_{zz} + \frac{1}{3} (a_{xx}^i - a_{yy}^i) (S_{xx} - S_{yy}) + \frac{4}{3} a_{xy}^i S_{xy} + \frac{4}{3} a_{xz}^i S_{xz} + \frac{4}{3} a_{yz}^i S_{yz}. \quad (4)$$

The quantities $a_{pq}^i = \langle 3 \cos \alpha_p^i - \delta_{pq} \rangle / 2$ depend on the time average conformation of the head group, as illustrated in Fig. 1 *c*. The order parameters $S_{pq} = \langle 3 \cos \theta_p \cos \theta_q - \delta_{pq} \rangle / 2$ describe the orientational order of the molecular axes system about a space-fixed director normal to the bilayer, as illustrated in Fig. 1 *c*. Note that the first term in Eq. 4 is the term considered by Petersen and Chan (8), who also treated a_{zz} and S_{zz} as independent parameters.

It is normally believed that the phospholipids can rotate in some fashion about their long molecular axis, M_z . If this rotation is two-fold, as implied by the rectangular shape of the molecule (Fig. 1 *a,b*) the order parameters are averaged under the invariant operations $\theta_x \rightarrow \pi - \theta_x$ and $\theta_y \rightarrow \pi - \theta_y$ giving $S_{yz} = S_{xz} = 0$ and consequently three surviving order parameters S_{zz} , $(S_{xx} - S_{yy})$, and S_{xy} . A three-fold or higher fold rotation about M_z (freely rotating molecule) would leave only the S_{zz} term; we will show this is not the case. If M_z is a two-fold rotation axis and if one of the other molecular axes, say M_x , is also a two-fold rotation axis (a phase apolar in M_z , for example), then only the two-order parameters S_{zz} and $(S_{xx} - S_{yy})$ survive. It is noted that this would require diffusion of the molecular across the bilayer membrane during the time scale of the NMR measurement (time of the free induction decay). Literature on diffusion does not indicate, however, that this is the case (9).

On the other hand, if diffusion from one side of the bilayer to the other were rapid enough, there is another way one could have three surviving order parameters. M_x could be a two-fold rotation axis for the flip-flop motion of the molecule, leaving S_{zz} , $(S_{xx} - S_{yy})$, and S_{yz} . An observation of these three order parameters would then imply that M_z was less than a two-fold rotation axis ("partial rotational freeze-out," as it is sometimes referred to in liquid-crystal literature). Surviving order parameters under these symmetries are summarized in Table I.

Note that all of the order parameters in Eq. 4 are uniaxial in that only one director is required in their definition. Eq. 4 is valid only for uniaxial liquid crystals or for biaxial liquid

TABLE I
ORDER PARAMETERS CHARACTERIZING VARIOUS SYMMETRIES AND MOTION

Case	Surviving order parameters*	Symmetry operations on molecular axes
I	S_{zz}	Free rotation about M_z or n -fold rotation about M_z for $n \geq 3$.
II	S_{zz} and $(S_{xx} - S_{yy})$	Two-fold rotation about M_z as well as M_x or M_y . (Rotation about the long axis biased in two orientations, with diffusion of the molecule across the bilayer.)
IIIa	S_{zz} , $(S_{xx} - S_{yy})$, and S_{yx}	Two-fold rotation about M_z only. (Rotation about long axis biased in two orientations but no diffusion across bilayer.)
IIIb	S_{zz} , $(S_{xx} - S_{yy})$, and S_{yz}	Two-fold rotation about M_x only. (Diffusion across bilayer and rotation about long axis biased in one direction only.)

*The magnitude of the surviving parameters reflects the degree to which a particular bias in the rotation about the molecular axes is present.

crystals aligned such that the director of the long axis is parallel to the magnetic field direction (10).

INTERPRETATION OF THE SPECTRA

We now turn to the data of Brown and Seelig (3), which we have reproduced in Fig. 2 *a*. There are two uses we wish to make of these data: to determine the number of surviving order parameters in Eq. 4; and to examine the validity of Eq. 4, i.e., the independence of the a_{pq} and S_{pq} .

Case I, One Order Parameter, S_{zz}

We start by assuming there to be only one order parameter, namely S_{zz} , involved in temperature dependence of the quadrupole spectra of Fig. 2 *a*. In the development described above, the parameter S_{zz} is common to each site in the head group, in which case one splitting, say $\Delta\nu_\alpha$, from the α site, can be expressed in terms of the splitting from any other site, say $\Delta\nu_\beta$ of the β site:

$$\Delta\nu_\alpha = (\nu_q^\alpha a_{zz}^\alpha / \nu_q^\beta a_{zz}^\beta) \Delta\nu_\beta, \quad (5)$$

giving a proportional relationship between $\Delta\nu_\alpha$ and $\Delta\nu_\beta$ if the term $(\nu_q^\alpha a_{zz}^\alpha / \nu_q^\beta a_{zz}^\beta)$ is constant with temperature. However, the temperature dependence curves of these splittings in Fig. 2 *a* are clearly not proportional to one another and one is forced into one of two conclusions: either the time average conformation of the head group, as indicated by the ratio $(\nu_q^\alpha a_{zz}^\alpha / \nu_q^\beta a_{zz}^\beta)$, is changing with temperature, or the assumption of one dominant order parameter, S_{zz} , is not valid and more order parameters must be considered. We can examine which of these two conclusions is correct by testing for the presence of two order parameters.

Case II, Two Order Parameters

These could be S_{zz} and $(S_{xx} - S_{yy})$, which are the dominant parameters in a smectic A-like liquid crystal phase (4). Since these parameters are averages of two different functions, they have different temperature dependences and can provide for the different variations of the curves. Again, the order parameters are common to each site in the molecule and as such can be eliminated from a set of three equations leaving, for example, the equation

$$(\Delta\nu_\alpha / \Delta\nu_\beta) = C_\gamma (\Delta\nu_\gamma / \Delta\nu_\beta) + C_\beta. \quad (6)$$

The C 's are conformation coefficients that depend on the a_{pq} 's of Eq. 4. For the spectral splittings α , β , and γ , they are $C_\gamma = \nu_q^\alpha (a_\alpha b_\beta - a_\beta b_\alpha) / \nu_q^\gamma (a_\gamma b_\beta - a_\beta b_\gamma)$ and $C_\beta = \nu_q^\alpha (a_\gamma b_\alpha - a_\alpha b_\gamma) / \nu_q^\beta (a_\beta b_\gamma - a_\gamma b_\beta)$, where $a_i = a_{zz}^i$ and $b_i = (a_{xx}^i - a_{yy}^i)$, ($i = \alpha, \beta, \gamma$). If the values of the C 's are independent of temperature, then ratio plots such as $\Delta\nu_\alpha / \Delta\nu_\beta$ vs $\Delta\nu_\gamma / \Delta\nu_\beta$ should yield a straight line. Such plots shown in Figs. 2 *b*, *c*, and *d* show no such straight lines. As before, this would suggest either a conformation change in the head group as the temperature is varied (C 's temperature dependent) or that there are more than two order parameters dominant. The latter possibility is quite likely, as Cases IIIa or *b* in Table I are strong possibilities. We therefore apply the test for three order parameters.

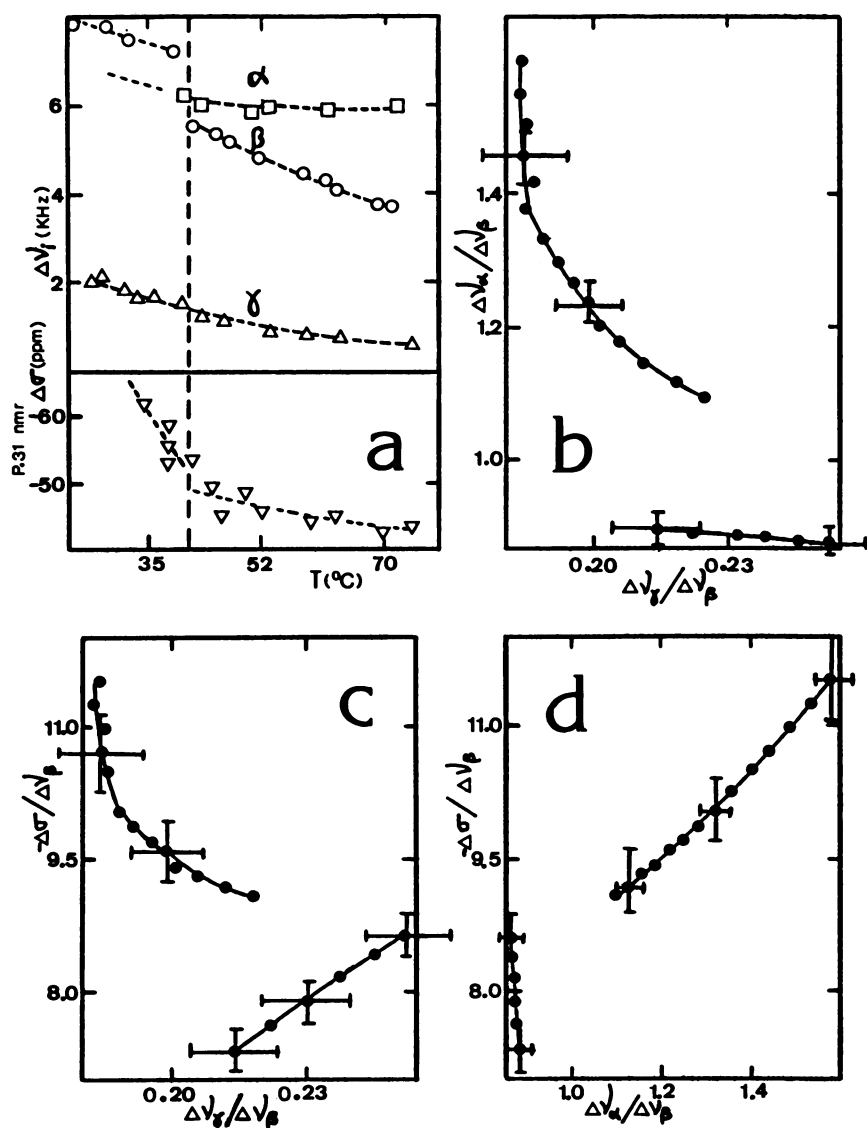


FIGURE 2 (a) A reproduction of the data of Brown and Seelig (3) showing the temperature dependence of the deuterium splittings from the α , β , and γ sites of the head group, as well as the anisotropic contribution to the chemical shift of the ^{31}P spins. Graphs (b), (c), and (d) show two-dimensional plots of ratios of the splittings and shift anisotropies at various temperatures. The plotted points (solid dots) were obtained from the dashed lines in Fig. 2 a instead of the actual data points to reduce the scatter. The error bars are discussed in the text.

Case IIIa or IIIb, Three Order Parameters

In this case, three splittings or shifts are required to determine a fourth and one can write

$$(\Delta\sigma/\Delta\nu_\beta) = D_\alpha(\Delta\nu_\alpha/\Delta\nu_\beta) + D_\gamma(\Delta\nu_\gamma/\Delta\nu_\beta) + D_\beta, \quad (7)$$

where the D 's are the conformation coefficients that depend upon the a_{pq}^i , ν_q^i , and $(\sigma_{\parallel} - \sigma_{\perp})$. In this equation we have made use of the chemical shift anisotropy as well as the quadrupole splittings. A test of the validity of this expression for constant conformation coefficients D can be made by use of a three-dimensional Cartesian plot of the ratios $(\Delta\sigma/\Delta\nu_\beta, \Delta\nu_\alpha/\Delta\nu_\beta, \Delta\nu_\gamma/\Delta\nu_\beta)$ to see if a plane is formed. It is not a trivial process to determine if the plotted ratios all lie on a common plane. An analytical construction must be made (see Appendix A). The procedure involves constructing a line between two of the plotted ratios and extending that line to the point of interception with one of the three Cartesian planes in which $\Delta\sigma/\Delta\nu_\beta = 0$, $\Delta\nu_\alpha/\Delta\nu_\beta = 0$, or $\Delta\nu_\gamma/\Delta\nu_\beta = 0$. This is done for all pair combinations of ratios. If all of the ratios lie in the same plane, the constructed points of interception with each Cartesian plane will form a straight line. Each of these lines is the line where the plane containing the plotted ratios intersects that particular Cartesian plane.

Fig. 3 shows the constructed points of interception with each of the Cartesian planes. This figure shows that indeed a single plane is formed consistent with the fact that three and only three order parameters are required to describe the orientational order of the head group in both the gel and liquid crystal phase. As we obtain the same plane for both phases of the system, this suggests that the head group does not change its preferred conformation, even across the phase transition. This result is consistent with a recent NMR study (11) and a recent neutron-scattering study (12) on the structure of the head group in both phases.

PREDICTED TEMPERATURE DEPENDENCE OF THE CHEMICAL SHIFT

Another illustration of the validity of the above arguments is to show that the temperature dependence of the ^{31}P chemical shift anisotropy can be predicted by the temperature dependence of the three deuterium splittings. From the plane construction in Fig. 3 *a* the values of the three conformation coefficients are $D_\alpha = -6.2$, $D_\beta = 2.9$, and $D_\gamma = -24.5$. With these values, Eq. 7 and temperature dependence of the quadrupole splittings in Fig. 2 *a* the temperature dependence of the chemical shift anisotropy, $\Delta\sigma$, can be calculated. Fig. 3 *b* shows the calculated temperature dependence of the chemical shift along with the measured values of Brown and Seelig (3). The agreement is good and consequently consistent with the three-order-parameter concept.

ERRORS

The analysis presented in this paper requires data with small relative error in the temperature dependence of the splittings. It is important that the relative splittings at each temperature be determined from the spectra in a consistent manner. In this regard, the deuterium spectral patterns from unoriented uniaxial samples are exceedingly helpful, in that the splittings are taken from edge singularities. These provide well-defined positions on the patterns from which one can obtain the relative splittings. In fact, unoriented samples appear to be better for this study than uniformly aligned samples, as the position of the center of a spectral line may not

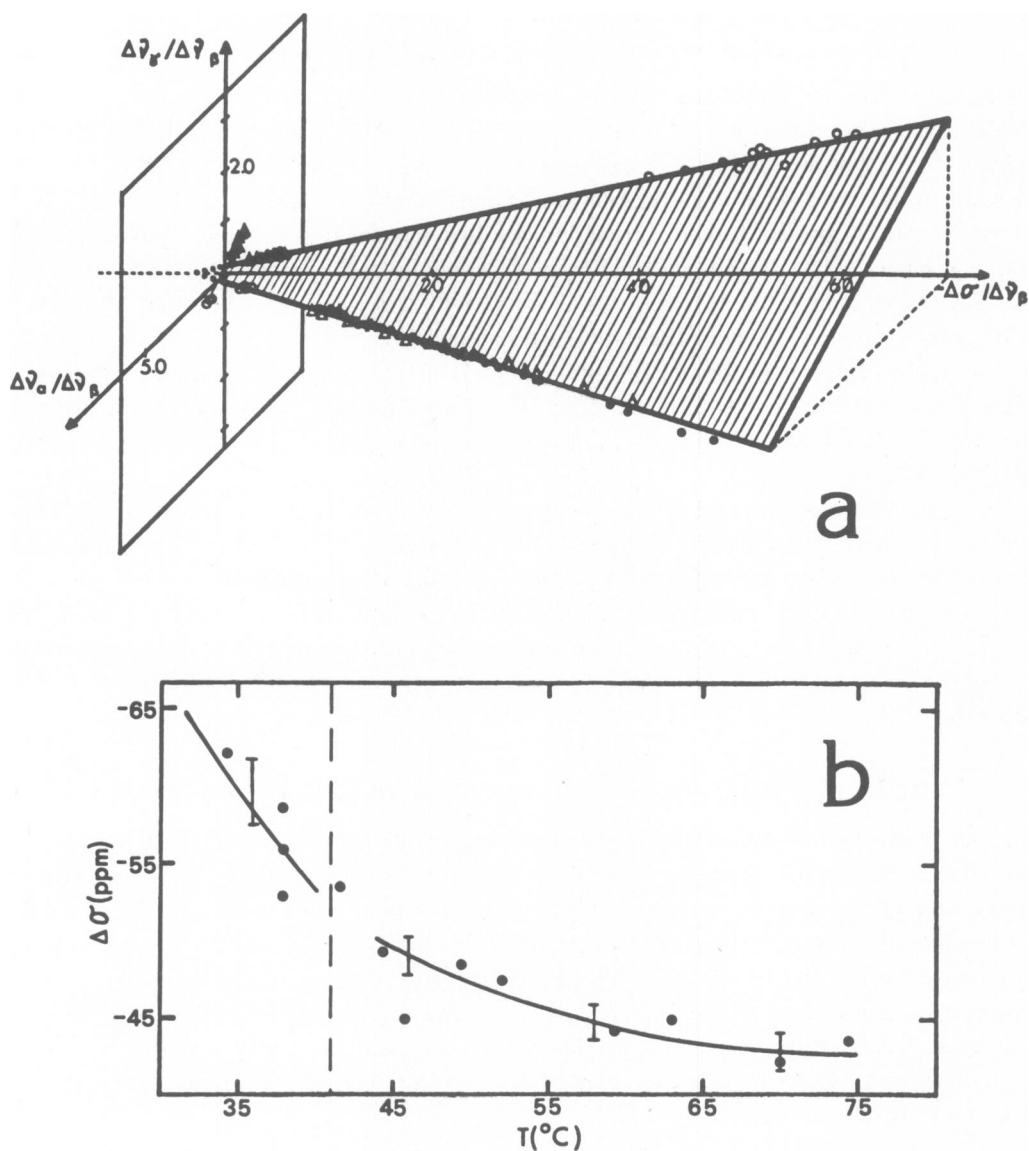


FIGURE 3 (a) Construction showing the plane formed by a three-dimensional plot of ratios obtained at different temperatures. To avoid confusion, the actual ratios in the plane are not shown. Shown are the constructed points of interception with each Cartesian plane $\Delta\sigma/\Delta\nu_\beta = 0$, $\Delta\nu_\alpha/\Delta\nu_\beta = 0$, and $\Delta\nu_\gamma/\Delta\nu_\beta = 0$, as described in the text where: open circles are points constructed from ratios in the gel phase; closed circles are from ratios in the liquid crystal phase; open triangles are points constructed from ratios in one phase and those in another. (b) Calculated temperature dependence of the chemical shift anisotropy from the equation $\Delta\sigma = -6.2 \Delta\nu_\alpha + 2.9 \Delta\nu_\beta - 24.5 \Delta\nu_\gamma$ (solid line); plotted points from Fig. 2 a. Error bars on the calculated curve are discussed in the text.

be as well defined as an edge singularity. Although it is not a problem in this paper, overlapping spectral lines can give a false temperature dependence if not properly deconvoluted.

The error involved in the interpretation presented in this paper is best illustrated in Fig. 3 *b*, in which the measured and calculated temperature dependence of the chemical shift anisotropy is presented. The solid curve is calculated from the quadrupole splittings as discussed above. The error bar on the solid curve was determined from uncertainties in the relative (not absolute) splittings of: $\Delta\nu_\alpha$ and $\Delta\nu_\beta$, ± 70 Hz (L_α phase), ± 100 Hz (gel phase); $\Delta\nu_\gamma$, ± 25 Hz (L_α phase), ± 50 Hz (gel phase). While these errors may be small for absolute splittings, they are not unreasonable for relative splittings in view of the larger number of data points and of the appearance of the published spectral patterns (13). A large number of data points on a temperature dependence plot is a form of signal averaging, in that a smooth curve can be constructed through the points, provided a phase transition does not occur. The error bar on the calculated curve for the chemical shift anisotropy of ^{31}P is less than the scatter of the measurements because the width of these spectral patterns are less well defined, i.e., a shoulder instead of a singularity is involved (3, 14).

The error bars on the ratio plots of Fig. 2 were determined from the uncertainties listed above for the relative quadrupole splittings and from an uncertainty of ± 1 ppm for the relative values of $\Delta\sigma$. The maximum scatter on the reported data is ± 2 ppm, but this can be reduced by the large number of data points which establishes the trend for the temperature dependence of that quantity.

The uncertainties in the splittings and shift anisotropies listed above introduce error in the plane in Fig. 3 *a* by providing uncertainty in the intersection of the plane with the various axes. In that plot these uncertainties were determined to be: ± 0.05 on the $\Delta\nu_\gamma/\Delta\nu_\beta$ axis, ± 0.15 on the $\Delta\nu_\alpha/\Delta\nu_\beta$ axis, and ± 2 on the $-\Delta\sigma/\Delta\nu_\beta$ axis.

DISCUSSION

First, we would like to comment on the shapes of the spectral patterns from which the splittings were obtained. In the liquid crystal phase the spectral patterns appear void of an asymmetry parameter, as indicated by the edge singularity (15). In liquid crystals this means that the phase is uniaxial (10). A spectral pattern will be axially symmetric in the uniaxial liquid crystal even though it may not be in the solid crystal. The asymmetry parameter associated with the chemical bonds will motionally average via the same orientational order parameters discussed in this paper by changing only the effective values of the a_{pq} coefficient (10). This is why it can be ignored in the use of Eqs. 1 and 2 made in this paper.

A biaxial liquid crystal phase is indicated by the presence of an asymmetry in the spin interaction and hence in the spectral pattern. In this case the asymmetry parameter is created by asymmetry in the molecular orientational order rather than in the chemical bonds (16, 17). The broad peaks on the gel phase spectral patterns (13) could be the result of an asymmetry, in that the edge singularity becomes a logarithmic singularity and consequently less defined on such a pattern (15). If biaxial effects are present they are weak and had little effect on the work described in this paper.

The central feature of this paper has been to show that while the molecule is rotating about its long axis, the rotation is not free but biased. This is expected, since some of the orientations

that the head group assumes as the molecule rotates about its long axis may be preferred over others due to coulomb attractions or packing in the bilayer. Whether the rotational motion is biased to a single direction or two equally likely directions opposite to one another (two-fold) hinges on motional averaging of the quadrupole spectra due to diffusion across the bilayer (flip-flop motion). If diffusion across the bilayer is rapid enough, then the mechanism for three order parameters is IIIb. For this to happen a molecule, on the average, must diffuse across the membrane $\sim 40 \text{ \AA}$ in a time $\sim \Delta\nu^{-1} \sim (1 \text{ kHz})^{-1} = 1 \text{ ms}$. Using the expression of $D_{\parallel} \approx x^2/2\tau$ for the diffusion constant parallel to the long axis of the molecule (across the bilayer) where x is the distance the molecule moves, on the average, and τ the time, one obtains an estimated value $D_{\parallel} \lesssim 10^{-10} \text{ cm}^2/\text{s}$ necessary for Case IIb. Unfortunately, D_{\parallel} has not been as well studied as that for lateral diffusion D_{\perp} ($D_{\perp} \sim 10^{-7} \text{ cm}^2/\text{s}$) (18). Pulsed gradient NMR methods¹ show D_{\perp}/D_{\parallel} for water in multilayer systems to be ~ 40 ; however, only a lower limit of 100 has been established for the lipids by this technique.¹ One technique (9) has given a value for $D_{\parallel} \lesssim 10^{-16} \text{ cm}^2/\text{s}$. The latter measurement would therefore suggest Case IIIa to be the most likely case. More studies of D_{\parallel} would be helpful in this regard.

A secondary but important feature of this paper is the rather strong effect that the biased rotational motion has on the temperature dependence of the deuterium spectra and chemical shifts. This appears to be a powerful tool in the study of membrane systems. An interesting study might be that involving deuterium spectra from the end-chains. The temperature dependence of quadrupole spectra in DMPC has recently been reported by Oldfield et al. (19). A similar study of these spectra is in progress.

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APPENDIX A

Procedure for Determining the Planarity of a Collection of Points

Consider three points A , K , and B , defined by their rectangular coordinates (X, Y, Z) as illustrated in Fig. 4. They define a plane (plane π in Fig. 4 a). The central problem is to find that plane. This can be accomplished if we know the (vertical) intersection of the plane π with the plane $[Z, 0, Y]$ and its (horizontal) intersection with plane $[X, 0, Y]$. If we take the projections of points A , K , and B , then points A' , K' , and B' are projections on plane $[Z, 0, Y]$ and A'' , K'' , B'' projections on plane $[X, 0, Y]$. Points A and K define a straight line, \overline{AK} , with projections $\overline{A'K'}$ and $\overline{A''K''}$. Likewise, K and B define \overline{KB} with projections $\overline{K'B'}$ and $\overline{K''B''}$. To find the horizontal intersection of plane π we may start with projection $\overline{A'K'}$ and find its interception with $(0, Y)$ axis. We then construct a line perpendicular to this axis on plane $[X, 0, Y]$ and find the common point of this line with the other projection $\overline{A''K''}$ (follow arrows on Fig. 4). This will clearly be a point, (1), of the horizontal intersection of plane π . In a similar way, we can find point (2) using $\overline{K'B'}$ and $\overline{K''B''}$. Points (1) and (2) define completely the horizontal intersection. Conversely, starting with $\overline{A''K''}$, we may find point (3). It would be possible to get yet another point to define the vertical intersection independently of the horizontal intersection. However, since point P has to be a common point, we use it instead of a fourth point, leaving Fig. 4 a not too cluttered.

While the above method for determining a plane is illustrative, it is nonetheless quite cumbersome to use in practice. We now consider the following procedure: rotate plane $[X, 0, Y]$ and the projections on

¹Ukleja, P., and J. W. Doane. 1979. Anisotropy of translational diffusion in lipid bilayers. Submitted for publication.

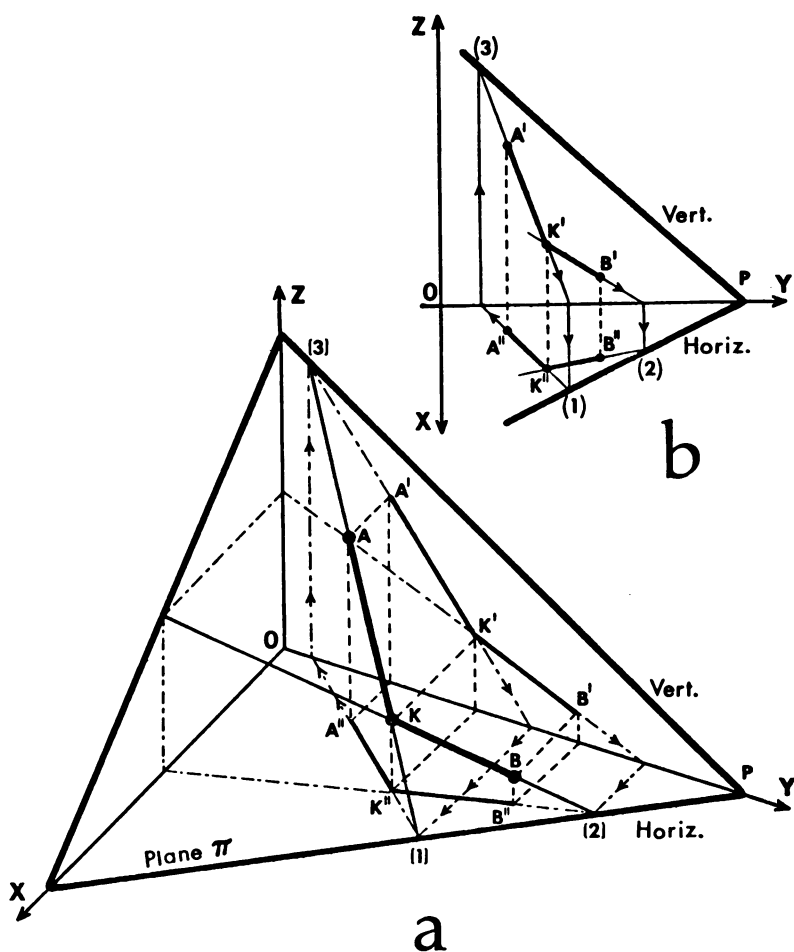


FIGURE 4 (a) Three-dimensional construction in a rectangular coordinate system of axes (x, y, z) of the vertical and horizontal intersections of a plane (π) containing three given points A , K , and B . (b) Two-dimensional representation of the same construction.

this plane about the $(0, Y)$ axis by 90° . In this way the plane $[X, 0, Y]$ will coincide with plane $[Z, 0, Y]$, but $(0, X)$ and $(0, Z)$ axes will have opposite directions (Fig. 4 b). With the axes as drawn in Fig. 4 b, one can plot the points A' , K' , and B' , as well as A'' , K'' , and B'' directly. The intercepts of the plane π with the $[Z, 0, Y]$ plane and $[X, 0, Y]$ plane can be constructed in two instead of three dimensions, simplifying the procedure. This procedure is also workable on computers.

We now make the following observations: (a) All points that lie on plane π will construct into straight lines defining the horizontal and vertical intersections. (b) If the points on plane π lie on a straight line, they will all construct as a point or cluster on each of the intersections.

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