Dynamics of the phosphate group in phospholipid bilayers
A ^{31}P angular dependent nuclear spin relaxation time study

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ABSTRACT To understand ^{31}P relaxation processes and hence molecular dynamics in the phospholipid multilayer it is important to measure the dependence of the ^{31}P spin-lattice relaxation time on as many variables as the physical system allows. Such measurements of the ^{31}P spin-lattice relaxation rate have been reported both as a function of Larmor frequency and temperature for egg phosphatidylcholine liposomes (Milburn, M. P., and K. R. Jeffrey. 1987. *Biophys. J.* 52:791–799). In principle, the spin-lattice relaxation rate in an anisotropic environment such as a bilayer will be a function of the angle between the bilayer normal and the magnetic field. However, the measurement of this angular dependence has not been possible because the rapid (on the timescale of the spin-lattice relaxation rate) diffusion of the lipid molecules over the curved surface of the liposome average this dependence (Milburn, M. P., and K. R. Jeffrey. 1987. *Biophys. J.* 52:791–799; Brown, M. F., and J. H. Davis. 1981. *Chem. Phys. Lett.* 79:431–435). This paper reports the results of the measurement of the ^{31}P spin-lattice relaxation rate as a function of this angle, β', (the angle between the bilayer normal and the external magnetic field) using samples oriented between glass plates. These measurements were made at high field (145.7 MHz) where the spin-lattice relaxation processes are dominated by the chemical shielding interaction (Milburn, M. P., and K. R. Jeffrey. 1987. *Biophys. J.* 52:791–799). A model of molecular motion that includes a fast axially symmetric rotation of the phosphate group ($\tau_i \approx 10^{-9}$ s) and a wobble of the head group tilt with respect to this rotation axis has been used to describe both the angular dependence of the spin-lattice relaxation and the spectral anisotropy. Cholesterol is seen to have a negligible effect on the motional properties of the phospholipid phosphate segment as measured by the orientation dependence of the spin-lattice relaxation.

INTRODUCTION

Nuclear magnetic resonance has been used extensively to investigate the physical properties of cell membranes (1–5). ^{31}P NMR has been shown to be particularly useful in giving information about the head-group region of the phospholipid component (5–7). Whereas NMR spectra give information about the time averaged properties such as the average molecular conformation, relaxation time measurements, with the aid of a suitable model, can probe the amplitudes and time scales of molecular motions in membranes. ^{31}P NMR spin-lattice relaxation time measurements have been used to investigate both model and biological membranes. For such measurements to provide useful information and yield a full quantitative analysis, the relaxation processes in such systems must be fully understood.

In a recent paper (8) ^{31}P NMR spin lattice relaxation time measurements have been reported in egg phosphatidylcholine model membranes. These measurements were made as a function of both resonant frequency (38.9, 81.0, 108.9, and 145.7 MHz) and temperature (−30–60°C). This extensive study of the temperature and frequency dependence of ^{31}P relaxation has shown that two relaxation processes are dominant in lipid membranes. Relaxation due to anisotropic chemical shielding dominates at high NMR frequencies (145.7 MHz), whereas at low NMR frequencies relaxation due to a ^{1}H-^{31}P dipolar interation is important. At intermediate frequencies both mechanisms contribute to the measured relaxation time. A minimum occurred in the relaxation time measured as a function of temperature, and the position of this minimum was seen to be a function of frequency (8).

In an effort to characterize the various motions of lipids in membrane systems it is important to have models of the molecular dynamics based on as many experimental results as possible. In addition to being a function of temperature and frequency, the ^{31}P spin-lattice relaxation time is, in principle, a function of the angle (β') between the bilayer normal and external magnetic field. Measurement of this spin-lattice relaxation rate anisotropy is a useful source of information on molecular dynamics and has been used recently in the case of ^{2}H NMR in model membrane systems (9, 10). In a multilamellar lipid dispersion, it is not possible to measure this orientation dependence of the spin-lattice relaxation rate from a powder pattern spectrum because of rapid (on the time-

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scale of the spin-lattice relaxation time) diffusion of the lipid molecules over the curved surface of the lamellae (8, 11). (In fact, selective excitation of a narrow region of the ^{31}P lineshape has been used to measure diffusion times [12].) However, through the use of samples that are oriented between glass plates, the spin-lattice relaxation rate can be measured as a function of the angle β'. This paper reports the results of measurements of the angular dependence of the ^{31}P-NMR spin lattice relaxation time which have been obtained by using dispersions of egg phosphatidylcholine aligned between glass plates. These measurements have been carried out at a NMR frequency of 145.7 MHz, in the frequency region where the dominant relaxation process is anisotropic chemical shielding, at a number of temperatures. Results show an angular dependence that varies by a factor of 1.5 as the orientation changes from $\beta' = 0°$ to $\beta' = 90°$. The angular dependent values of the spin-lattice relaxation time are seen to be temperature independent within experimental error.

The experimental results are analyzed in terms of a specific model of molecular motion, and an expression for relaxation rate is calculated explicitly for this model. A fast axially symmetric rotation of the phosphate segment is included which requires that the angles α and β be specified where these angles refer to the orientation of the axis of rotation in the frame of reference of the molecular chemical shielding tensor. In addition to this fast axially symmetric rotation a much slower wobble in the tilt of the lipid phosphate segment with respect to the rotation axis has also been included. The parameters of the model are obtained by requiring that the model describe both the angular dependence of the spin-lattice relaxation as calculated explicitly for this model and the spectral anisotropy. This model is compared to models that have been used to simulate the ^{31}P spectral parameters of phospholipid liposomes (13–15).

MATERIALS AND METHODS

Egg phosphatidylcholine was extracted from hen egg yolks using the method of Singleton et al. (16) and its purity was checked by thin layer chromatography. The lipid was stored in ethanol (180 g/liter) under nitrogen atmosphere at −18°C. To prepare samples oriented between glass plates, 35 μl of lipid stock solution was applied to a cover glass slide (20 × 7 mm). The ethanol was removed by evaporation and 25 plates were stacked. The plates were then incubated at 40°C under a humid atmosphere for 3–4 h until hydrated. The plates were then placed in a goniometer and the alignment of the samples was observed using ^{31}P NMR.

It was observed that alignment of samples was better than 90%. That is, a small unaligned component remains and it has been suggested (17) that this small unoriented component is due to packing defects.

Cholesterol was obtained from Sigma Chemical Co., St. Louis, MO, and was further purified by recrystallization.

The NMR measurements were carried out at a ^{31}P resonant frequency of 145.7 MHz using a superconducting magnet and a home-built FT-NMR spectrometer. ^{31}P T1 measurements were made with a saturation recovery sequence (8).

The experimental uncertainty in the measurement of T_1 was ~10%. This is a factor of two larger than the uncertainty in the ^{31}P spin lattice relaxation times reported previously (8) for multilamellar dispersions of egg lecithin. The increase is a result of changing hydration of the samples during a measurement on a single sample and from sample to sample. The fully hydrated samples were sealed with an extra drop of water, but some hydration effects were noticed for samples which were used for several days especially at temperatures above 30°C.

RESULTS

Orientation dependence

Fig. 1 shows the oriented ^{31}P spectrum of egg PC as a function of β', the angle between the bilayer normal (which in this case is perpendicular to the surface of the glass plates) and the main magnetic field. This behavior has been described previously (18, 19) and it is observed that there is a linear relation between the chemical shift and $(3\cos^2\beta' - 1)$. The width of the oriented spectrum is seen to be reduced at the "magic angle" where part of the dipolar interaction is averaged to zero by the axially

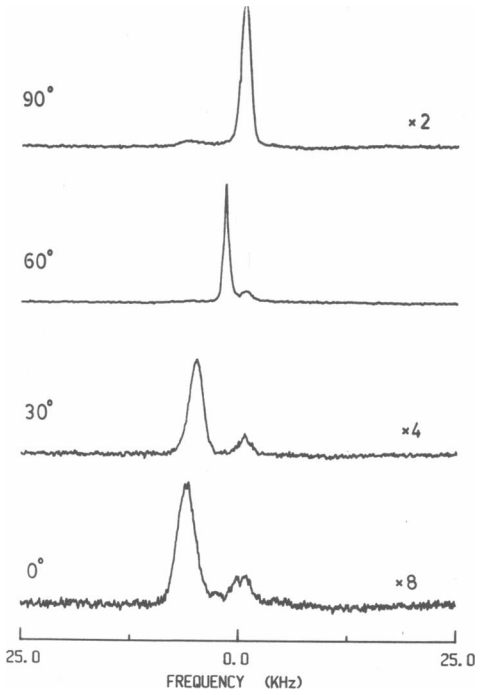

FIGURE 1 ^{31}P spectra of egg phosphatidylcholine bilayers oriented between glass plates shown as a function of orientation. The NMR frequency is 145.7 MHz. The chemical shift and linewidth are both functions of orientation.

symmetric motion so that the linewidth Δ can be described by a function $\Delta = \Delta_0 + \Delta_1 (3 \cos^2 \beta' - 1)$, where Δ_0 and Δ_1 characterize the constant and angular dependent parts of the linewidth, respectively (18). The chemical shielding anisotropy as measured from the oriented spectra is -48 ppm.

Fig. 2 shows the ^{31}P spin lattice relaxation time measured as a function of the angle β', the angle between the bilayer normal and the applied magnetic field, obtained at four temperatures. A maximum value of ~1.2 s is observed for the spin-lattice relaxation time at an angle $\beta' = 0$. The spin-lattice relaxation time is seen to decrease as β' increases, reaching a value of ~ 0.8 s at $\beta' = 90°$.

Fig. 3 shows the ^{31}P spin-lattice relaxation time measured as a function of the angle β' for a 50 mol% mixture of egg phosphatidylcholine and cholesterol measured at three temperatures. The ^{31}P spin-lattice relaxation time is observed to decrease from a value of ~1.2 s at $\beta' = 0$ to a value of ~0.8 s at $\beta' = 90°$. Within experimental uncertainty these results show that there is little change in the motional characteristics of the phospholipid phosphate segment affecting the spin-lattice relaxation as a result of adding cholesterol.

It is observed in Fig. 2 that the angular dependence of the relaxation rate has a negligible temperature dependence. The temperature dependence of the ^{31}P spin-lattice relaxation rate at 145.7 MHz has been measured previously (8) and a variation of <5% was observed for temperatures betwen 6 and 22°C. Fig. 2 shows that the value of the spin-lattice relaxation rate does appear to increase by ~20% at the temperature of 40°C compared with the lower temperatures, at least for orientations

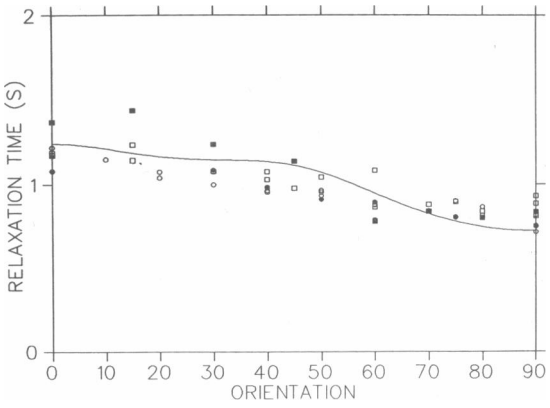

FIGURE 2 ^{31}P relaxation time as a function of orientation for aligned egg phosphatidylcholine bilayers (□ 6.0°C, ○ 15°C, ● 22°C, ■ 40°C) showing the prediction (*solid line*) of a model using an axially symmetric rotation about the direction (42°, 69°) in the frame of the chemical shielding tensor and a wobble in the headgroup tilt of ±15°. A correlation time of 3.5 ns and the values $\sigma_{11} = -80$ ppm, $\sigma_{22} = -20$ ppm, and $\sigma_{33} = 110$ ppm were used.

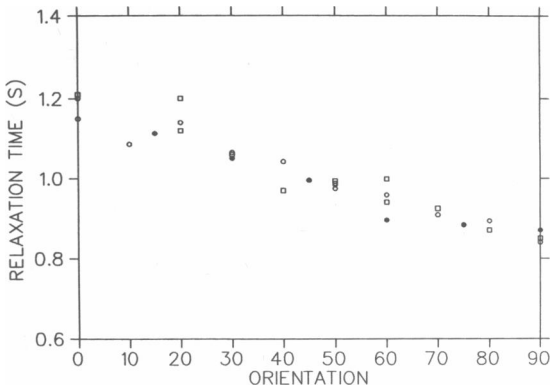

FIGURE 3 ^{31}P spin-lattice relaxation time as a function of the angle between the bilayer normal and the external magnetic field taken at 145.7 MHz for samples of egg phosphatidylcholine/cholesterol (50 mol%) oriented between glass plates. (□ 7.0°C, ○ 21°C, ● 40°C).

below $\beta' = 50°$. From Fig. 3 of reference 8, it is observed that the spin-lattice relaxation time has increased by ~20% as the temperature rises from 10 to 40°C. The value of spin-lattice relaxation computed by taking a weighted average over the orientation (see reference 7) using the data of Fig. 2 agree within experimental uncertainty to the values of spin-lattice relaxation obtained from using multilamellar dispersions (8).

Modeling the relaxation

Because measurements of the ^{31}P spin-lattice relaxation have been carried out at 145.7 MHz, only the chemical shielding contribution to the spin-lattice relaxation need be considered (8). It has been shown that in these lipid bilayer systems that fast axially symmetric rotation of the lipid phosphate segment gives rise to the ^{31}P spectral features (19). Although the chemical shielding tensor of the phospholipid ^{31}P is nonaxially symmetric with principal components $\sigma_{11} = -80$, $\sigma_{22} = -20$, and $\sigma_{33} = 110$ (7), the ^{31}P spectrum of unoriented bilayers shows an axially symmetric lineshape characterized by the chemical shielding anisotropy, $\Delta\sigma$. The anisotropy, $\Delta\sigma$, is a function of the principal components of the shielding tensor and the angles α and β which describe the orientation of the axis of rotation in the molecular frame/principal axis system.

$$\Delta\sigma = \sin^2 \beta \cos^2 \alpha \sigma_{11} + \sin_2 \beta \sin_2 \alpha \sigma_{22} + \cos^2 \beta \sigma_{33} - \sigma_i, \quad (1)$$

where $\sigma_i = (\sigma_{11} + \sigma_{22} + \sigma_{33})/3$ (7). The observed anisotropy may be reduced further from the expression 1 by additional motions such as a wobble of the lipid headgroup with respect to the rotation axis, fluctuation of the

rotation axis with respect to the magnetic field, diffusion of the lipid molecule around the curved surface of a liposome, or rapid tumbling of small vesicles.

The analytical form of the angular dependence of the spin-lattice relaxation is sensitive to the details of molecular motion. An explicit expression for the ^{31}P spin-lattice relaxation can be computed with a working model of the molecular dynamics of the phosphate group. Because many motions are present in a macromoleculae such as a lipid (8), in this paper the approach is to utilize as simple a model as necessary to account for the measured data, taking into account the fact that the spin-lattice relaxation rate will be sensitive to motions with correlation times on the order of the inverse of the Larmor frequency. The model presented here includes two motions: a fast axially symmetric rotation and a variation of the rotation axis with respect to the frame of the chemical shielding tensor (effectively a restricted wobble in the tilt of the headgroup). The timescale of the axially symmetric rotation is on the order of the Larmor frequency (8) and therefore contributes to the relaxation. The model assumes that the wobble motion does not contribute to the nuclear spin relaxation so that the timescale of this motion is much slower than the Larmor frequency. Instead the effect of the wobble is to average the orientation dependence by modulating the angles α and β which define the orientation of the axis of rotation in the frame of the molecule. It is also assumed that this wobble is fast enough to average the spectral anisotropy so that the wobble timescale is intermediate between the Larmor frequency and the timescale defined by the inverse of the spectra width.

The chemical shielding Hamiltonian for this system, H_{CS}, can be written as

$$H_{CS} = \hbar\omega_0 \sum_L (-1)^L T_{2L} \sum_{p,N} \rho_{2p} D_{N-L}(\Omega') D_{pN}[\Omega(t)],$$

where ω_0 is the larmor frequency, L is summed from -1 to 1, p, N are summed from -2 to 2, the T_{2L} are spin operators in spherical tensor notation, ρ_{2p} are parameters of the shielding tensor in spherical tensor notation, and $D_{N-L}(\Omega')$ and $D_{pN}[\Omega(t)]$ terms are Wigner rotation matrices describing transformations involving rotations Ω' and Ω. The T_{2m} and ρ_{2p} are defined in terms of spin operators I_0, $I_{\pm1}$, and the parameters δ and η describing the chemical shielding interaction in the principal axis system (20).

$$T_{20} = \frac{2}{\sqrt{3}} I_0$$

$$T_{2\pm1} = \frac{1}{\sqrt{2}} I_{\pm1}$$

$$T_{2\pm2} = 0,$$

and

$$\rho_{20} = \frac{3}{\sqrt{2}}\delta$$

$$\rho_{2\pm2} = \tfrac{1}{2}\delta\eta$$

$$\rho_{2\pm1} = 0.$$

The transformation from the principal axis system of the chemical shielding interaction to the bilayer normal is described by a rotation $\Omega(t)$ and specified by $D_{pN}[\Omega(t)]$ and the transformation, $D_{N-L}(\Omega')$, from the bilayer normal to the laboratory frame defined by the externally applied magnetic field is described by the rotation Ω'.

The spin-lattice relaxation rate may be calculated by

$$\frac{1}{T_1} = \frac{1}{\hbar^2}\int_0^\infty Tr\,\overline{[I_Z, H_{CS}^+(\tau)]\,[H_{CS}(0), I_Z]}\,d\tau / Tr I_Z^2,$$

where H_{CS}^+ denotes the Hamiltonian in the rotating frame and where the bar denotes an ensemble average. H_{CS}^+ is defined as

$$H_{CS}^+(t) = e^{-iH_Z t} H_{CS} e^{iH_Z t},$$

where

$$H_Z = \hbar\omega_0 I_Z.$$

Applying the appropriate commutation relations and traces,

$$\frac{1}{T_1} = 2\omega_0 \sum_{\substack{pp' \\ NN'}} \rho_{2p}\rho_{2p'} D_{N1}(\Omega') D_{N'-1}(\Omega') \cdot \int_0^\infty \overline{D_{pN}[\Omega(\tau)] D_{p'N'}[\Omega(0)]} e^{i\omega_0\tau}\,d\tau.$$

At this point it is necessary to calculate the correlation function $\overline{D_{pN}[\Omega(\tau)] D_{p'N'}[\Omega(0)]}$ for an axially symmetric rotation which is considered the dominant motion in determining the spin-lattice relaxation rate. Thus, the angles α and β are time independent on the timescale defined by the inverse of the Larmor frequency and describe the direction of the axis of rotation in the molecular frame of the shielding tensor. The transformation from the bilayer frame to the lab frame is described through the angle β'. Thus,

$$D_{N1}(\Omega')\, D_{N'-1}(\Omega') = d_{N1}(\beta')\, d_{-N-1}(\beta'),$$

and

$$\overline{D_{pN}[\Omega(\tau)] D_{p'N'}[\Omega(0)]} = \langle e^{-p\alpha(\tau)} e^{ip'\alpha(0)} \rangle \cdot \langle d_{pN}[\beta(\tau)] d_{p'N'}[\beta(0)] \rangle \langle e^{iN\gamma(\tau)} e^{iN'\gamma(0)} \rangle.$$

Combining these results,

$$\frac{1}{T_1} = 2\omega_0 \sum_{Npp'} \rho_{2p}\rho_{2p'} d_{N1}(\beta') d_{-N-1}(\beta')\, e^{i(p+p')\alpha} d_{pN}(\beta) d_{p'-N}(\beta) \cdot \int_0^\infty G_N(\tau) e^{i\omega_0\tau} d\tau,$$

where

$$G_N(\tau) = \langle e^{iN\gamma(\tau)} e^{-iN[\gamma(0)]} \rangle = e^{-\tau/\tau_c}.$$

This is an explicit result, where $\Sigma_{Npp'}$ leaves 45 nonzero terms.

Fig. 4 shows the orientation dependence of the spin-lattice relaxation time predicted by this model. It is seen that the angular dependence of the spin-lattice relaxation rate is a sensitive function of the angles α and β which describe the "tilt" of the phosphate segment with respect to the rotation axis. Curves 1 and 4 of Fig. 4 show the prediction for the case of a fixed headgroup tilt over the sample for two positions of the rotation axis. It is expected that in addition to the fast axially symmetric rotation there exist a rich array of slow motions of the lipid molecule as a whole and of various segments of the lipid macromolecule (17, 21–23). The effects of slower motions are included in this model by allowing the values of α and β to vary over a specified range with equal weighting for simplicity. Curves 2 and 3 of Fig. 4 show the sensitivity of this model to the addition of this slower motion. In addition to predicting the angular dependence of the spin-lattice relaxation rate it was required that the fitting parameters predict the spectral anisotropy, with the assumption that the slower motions are fast enough to average the spectral anisotropy. With this simple model it was possible to predict both the spectral anisotropy and the angular dependence of the spin-lattice relaxation rate making the inclusion of any further motions ambiguous.

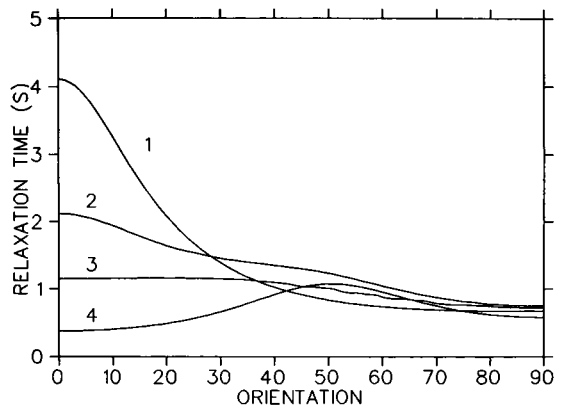

FIGURE 4 ^{31}P relaxation time as a function of the orientation of the axis of motion with respect to the magnetic field for an axially symmetric rotation. This is a sensitive function of the angles (α, β) which describe the orientation of the axis of rotation in the frame of the chemical shielding tensor. Curve 1 shows the predicted orientation dependence for α = 36° and β = 90°; curve 4: α = 45°, β = 45°. Curves 2 and 3 show the orientation dependence for a model that allows an equally weighted distribution of headgroup tilts with respect to the rotation axis over a square region of (α, β) space. Curve 3: (40°, 72°), ±3°, predicted $\Delta\sigma$ of −66 ppm. Curve 2: (40°, 72°), ±30°, predicted $\Delta\sigma$ of −46 ppm.

Fig. 2 (*solid line*) shows the angular dependence of the spin-lattice relaxation rate of the phosphate ^{31}P nucleus predicted for this model with the rotation axis centered at α = 42° and β = 69° and both angles allowed to vary over a range of ±15°. Using Eq. 1 these parameters predict a value of −49 ppm for the spectral anisotropy which is in agreement with the measured value.

Due to the fact that the variation in spin-lattice relaxation time with temperature was on the order of the experimental uncertainty, the correlation time could not be obtained as a function of temperature. The values of spin-lattice relaxation predicted by the motional model were computed using a correlation time of 3.5×10^{-9} s. This agrees with the correlation time determined by measurement of the temperature and frequency dependences (8) where τ_c was seen to increase from $\sim 1 \times 10^{-9}$ s to 5°C to 5×10^{-9} s at 40°C.

DISCUSSION

The motional model used to analyze the experimental data was purposely kept as simple as possible. The molecular motion of a lipid molecule is expected to be quite complex, and many possible motions can be envisaged, including bond rotations, isomerizations, rotation of the molecule as a whole, and various collective and noncollective bilayer motions. Given this seemingly complex picture it is important to appreciate that the experimental results of a given technique will be sensitive only to motions that fall within a given timescale. For example, spin lattice relaxation will be sensitive to motions with a correlation time close to the inverse of the Larmor frequency. The simple model used in this paper includes a fast axially symmetric motion with a correlation time of 3.5×10^{-9} s which gives rise to the relaxation. That the characteristics of the spin-lattice relaxation can be described by a single correlation time is consistent with the observation of a minimum in the Arrhenius plot of data from egg phosphatidylcholine liposomes (8). It seems that this effective correlation times does not describe the motion of the lipid molecule as a whole which has a correlation time an order of magnitude smaller (22) but probably describes motion of the phosphate group with respect to the glycerol backbone of the lipid.

To predict both the angular dependence of the spin-lattice relaxation rate and the spectral anisotropy the effects of slower motions have been included in this model by allowing a wobble in the tilt of the phosphate group with respect to the rotation axis. The effect of the slow motions was also included in the computation of the spectral anisotropy where it was assumed that this motion was fast enough to cause motional narrowing. A timescale of the slow motion is thus defined as $10^{-9} \ll \tau_c \ll 10^{-4}$ s.

Motions on this timescale have been observed in the fatty acid region of the lipid (17, 22), but as motion of the headgroup has a large degree of independence from the chains (8) it would be instructive to investigate the headgroup motions with techniques sensitive to motions in this timescale. It should be noted that the amplitude of the wobble may be temperature dependent, however this information could not be obtained because the temperature dependence of the relaxation rate was on the order of the experimental uncertainty.

A number of models of phospholipid head group motion have been proposed based on simulation of the ^{31}P spectrum phospholipid membranes. Kohler and Klein (14) discuss two models, one that includes a fast rotation about the P—O bond followed by a slower rotation about the molecular z axis, and a second that consists of a fast rotation about the glycerol C1—C2 bond, followed by a yet slower rotation about the molecular z axis. To simulate the temperature dependence of the spectrum, a motion of the long axis of the phospholipid motion about the bilayer normal was included. Because in the fast correlation time regime the spectrum is not sensitive to the rate of motion, no correlation times were obtained. These models are consistent with the present model sharing the essential features of a fast axially symmetric rotation and a slower motion, and from the present data it is not possible to distinguish between the various possibilities of bond rotations.

Seelig and Gally (25) have proposed a similar model of headgroup motion in phosphatidylethanolamine. They assume a free rotation about the C1—C2 bond (of the glycerol group), conformational jumps about the P—O bond (adjacent to the glycerol group), and a wobble implicit in the use of a C1—C2 order parameter. This model is also consistent with the model of the present work where a single effective correlation time describes the result of various bond rotations and jumps.

Campbell et al. (13) have simulated the ^{31}P NMR spectra of phospholipids and have found that the spectra can be described using the angle α in the range $0° < \alpha < 65°$ and β in the range $60° < \beta < 90°$. The ranges $27° < \alpha < 57°$ and $54° < \beta < 84°$ derived from modeling the orientation dependence of the relaxation rate are consistent with these ranges. Campbell et al. (13) have given a limit for two rotational diffusion coefficients that they have included in their model, $R_{11} \geq 2 \times 10^7\ s^{-1}$ (representing the rapid internal motion[s]) and $R_1 \leq 5\ s^{-1}$ (representing the net effect of slow motions). The correlation time of 3.5×10^{-9} s for the effective correlation time of the axially symmetric rotation obtained from the results of the orientation dependence agree with the limits placed on R_{11}.

Cholesterol is known to increase the order parameter of lipid molecules when included into the bilayer (3) as observed using deuterium magnetic resonance in the hydrocarbon chain region. In the case of ^{31}P, there is no apparent effect of cholesterol on the orientation dependence or the values of spin-lattice relaxation as seen in comparing Figs. 2 and 3. This seems to imply that the ordering of the phosphate segment is quite independent of the chains so that the variation in headgroup tilt is not affected by an increase in chain order. Such a result is consistent with previous investigations of the effect of cholesterol. The changes in order parameter and bulk phase in the hydrocarbon chains due to cholesterol addition have been well documented (3), whereas it has been reported that cholesterol is seen to have a negligible effect on the headgroup conformation (27) and dynamics (28).

In summary, the ^{31}P spin-lattice relaxation time shows a dependence on the orientation of the bilayer normal to the main magnetic field and this can be measured using samples oriented between glass plates. Our approach to fitting the experimental data to theory has been to start with as simple a model as possible and only refine the model when forced to do so by new data. A model of molecular motion that includes a fast axially symmetric rotation and a wobble of the headgroup tilt has been used to describe the orientation dependence. The parameters of the present model were determined by requiring that the model describes both the orientation dependence of T_1 and the spectral anisotropy. These two constraints are sufficient to limit the range of parameters so that the uncertainty in the parameters are determined primarily by the experimental uncertainty in the original data. Cholesterol is seen to have a negligible effect on the motional properties of the phospholipid phosphate segment as measured by ^{31}P NMR. At the present time there are few measurements exploring the slow motions within the headgroup region of the lipid bilayer. Measurements of such quantities as $T_{1\rho}$ (the spin lattice relaxation time in the rotating frame) would be very useful in refining the model further.

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