DYNAMICS OF THE PHOSPHATE GROUP IN PHOSPHOLIPID BILAYERS

A ^{31}P Nuclear Relaxation Time Study

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ABSTRACT The spin-lattice relaxation time of the ^{31}P nucleus in the phosphate group of egg yolk phosphatidylcholine multilamellar dispersions has been investigated at four resonant frequencies (38.9, 81.0, 108.9, and 145.7 MHz) in the temperature range from −30° to 60°C. The observed frequency dependence of the relaxation indicates that both dipolar relaxation and relaxation due to anisotropic chemical shielding are significant mechanisms. The experimental data have thus been modeled assuming both mechanisms and the analysis has allowed the contribution of each to the relaxation to be determined along with the correlation time for the molecular reorientation as a function of temperature. Dipolar relaxation was found to dominate at low nuclear magnetic resonance frequencies while at high frequencies the anisotropic chemical shift dominates. The correlation time of the phosphate group is on the order of 10^{-9} s at 60°C and increases to $\sim 10^{-7}$ s at −30°C. It is observed that the freezing of the buffer which occurs at ~ -8°C has a significant effect on the phosphate group reorientation. This effect of the freezing is to change the activation energy for the phosphate group reorientation from 16.9 KJ/mol above −8°C to 32.5 KJ/mol below −8°C.

INTRODUCTION

As part of any effort to understand the physical properties of biological membranes at the molecular level, it is essential to study the motional properties of the various constituent lipid and protein molecules as well as their ordering and average conformation (1). Nuclear magnetic resonance (NMR) has proved to be a valuable tool to investigate both the structural and dynamical properties of biological and model membrane systems (1–5). While NMR spectra give information about the time averaged properties such as the average molecular conformation and order parameters, relaxation time measurements, with the aid of a suitable model, can probe the amplitudes and time scales of molecular motions in membranes (3). Much of the work to date on the lipid component has involved the phospholipids (6) and ^{31}P-NMR has proven to be particularly useful for such research (7–9).

^{31}P spin-lattice relaxation time (T_1) measurements of phospholipids were first reported by Horwitz and Klein (10) but an incomplete knowledge of the relaxation mechanism prevented a quantitative analysis. Dipolar relaxation and relaxation due to the anisotropic chemical shift have been proposed as the dominant mechanisms in determining the linewidth (effectively the spin–spin relaxation time, T_2) of the isotropic ^{31}P spectrum of sonicated phospholipid vesicles (11–13). The analysis was complicated, however, by the rapid tumbling of the vesicles in addition to the internal motion of the phosphate segment. Spin-lattice relaxation of the ^{31}P spins was examined in the course of evaluating results from ^{31}P [^{1}H] Nuclear Overhauser Effect experiments on phospholipid systems (14–16). Measurements of the ^{31}P T_1 at one temperature were compared at two frequencies and from the apparent lack of a frequency dependence, it was concluded that only the nuclear dipole–dipole interaction was significant since the spin-lattice relaxation due to the time dependent chemical shielding anisotropy varies as the frequency squared in the short correlation time limit (17). In a later study (18), T_1 was measured at four different frequencies but at a single temperature again with no apparent frequency dependence.

The most extensive measurements of ^{31}P relaxation in phospholipid dispersions were reported by Seelig and his collaborators (19). The temperature dependence of the ^{31}P spin-lattice relaxation was measured at two resonance frequencies. It was found at the higher frequency, that as the temperature was varied, T_1 went through a minimum (no minimum was seen at the lower frequency). The existence of a T_1 minimum enables a direct determination of a motional correlation time, since at the temperature of the minimum, the correlation time, τ_c, is on the order of the inverse of the Larmor precessional frequency, ω_0, that is, $\omega_0\tau_c = 1$. The T_1 data of Seelig et al. (19) were analyzed to yield a correlation time as a function of temperature and an activation energy for the molecular motion based on the

assumption that at the lower frequency only a dipolar relaxation mechanism was present while at the higher frequency both the dipolar and chemical shielding interaction influenced the measured results.

In the present study the ^{31}P nuclear spin-lattice relaxation has been measured as a function of temperature at four resonant frequencies to evaluate the relative contributions of the various relaxation mechanisms at each frequency and in this way gain a more complete picture of the ^{31}P spin-lattice relaxation and hence of the molecular motion of the phosphate moiety in phospholipid dispersions. The measurements were carried out on aqueous dispersions of egg yolk lecithin because of the wide range of temperature available for study within the liquid crystalline phase. The results of this study show that a complete analysis of relaxation time data requires an extensive study of both the temperature and frequency dependence. Ideally measurements of T_1 as a function of the angle between the bilayer normal and the applied magnetic field should also be made, however this study shows that the angular dependence of T_1 cannot be obtained from the ^{31}P-NMR powder pattern but must await experiments carried out on aligned samples.

MATERIALS AND METHODS

Sample Preparation

Egg phosphatidylcholine (PC) was extracted from hen egg yolks using the method of Singleton et al. (20) and its purity was checked by thin layer chromatography. The lipid was stored in ethanol under nitrogen atmosphere at −18°C. To prepare a lipid sample for an NMR experiment, the desired quantity of lipid solution was pipetted into a round bottom flask and the ethanol removed by evaporation. The dried lipid was then scraped from the flask and mixed with a 10 mM Hepes buffer to form an aqueous dispersion. Helium gas had previously been bubbled through the buffer to remove any paramagnetic oxygen. The lipid dispersion was stirred, vortexed, and then alternately frozen and thawed to produce multilamellar liposomes. Typically 300 μl of buffer were mixed with 200 mg of lipid which corresponds to a ratio of ~60 mol of water per mole of lipid. The sample tube was flushed with nitrogen gas before sealing.

NMR Measurements

Experiments were carried out at four ^{31}P resonant frequencies, 38.9, 81.0, 108.9, and 145.7 MHz. Superconducting magnets and a home-built FT-NMR spectrometer were used at all frequencies with the exceptions that the 81.0 MHz experiments were carried out on a CXP-200 spectrometer (Bruker Instruments, Inc., Bilkrica, MA) and an electromagnet was used at 38.9 MHz. ^{31}P T_1 measurements were made with a saturation recovery sequence (21) and no proton decoupling in order that the state of the nuclear spin system be very well defined at the beginning of the relaxation period. Selective excitation experiments were carried out using the DANTE sequence (22) to show that diffusion of the lipid molecules over the curved surfaces of the multilamellar dispersion prevented measurements of the angular dependence of T_1 from the powder pattern spectrum. The DANTE sequence consists of a group of pulses; each pulse has a width much <90°. The pulses are separated by a time t_s and the entire sequence has a duration t_w. The frequency domain excitation spectrum of such a sequence corresponds to sinc (sin x/x) functions of width $1/t_w$ separated by a frequency $1/t_s$. Selective excitation of a specific part of the powder pattern spectrum was accomplished by choosing the appropriate values of t_s and t_w.

RESULTS

Holeburning Experiments

The characteristic shape of ^{31}P-NMR spectrum (9) of egg PC is a result of the orientational dependence of the anisotropic chemical shielding interaction and the broadening due to the dipolar interaction with protons surrounding the phosphorus nucleus (23). The orientational dependence of the chemical shielding effect gives rise to this so-called powder pattern spectrum. Molecular motion of the lipid molecule within the bilayer averages the anisotropic chemical shift resulting in a tensor interaction, which is axially symmetric about the bilayer normal, but because the sample is a multilamellar dispersion there is no preferred orientation of the bilayer normal with respect to the magnetic field resulting in a powder pattern spectrum. Each frequency region of the ^{31}P spectrum arises from a part of the sample where the bilayer normal is oriented at a particular angle with respect to the applied magnetic field (9). There is no a priori reason to expect that for the anisotropic motion of the lipid molecule in a bilayer that the measured T_1 will be the same for all orientations of the bilayer normal with respect to the magnetic field. A uniform T_1, however, is observed across the powder pattern. This is clear from Fig. 1, where the ^{31}P spectrum is

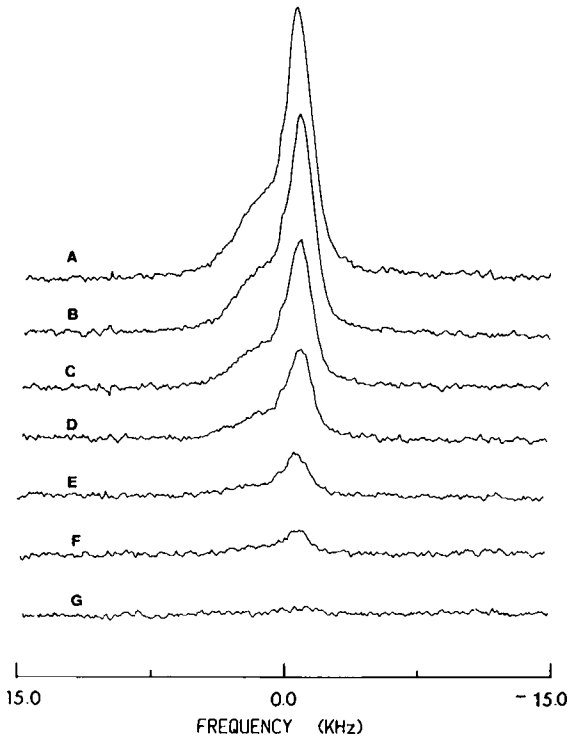

FIGURE 1 A sequence of spectra of egg PC recorded at 81.0 MHz showing the ^{31}P-NMR lineshape at various times after complete saturation of the resonance. The time delays after the saturating sequence are: (*A*) 5.0 s, (*B*) 2.0 s, (*C*) 1.0 s, (*D*) 0.5 s, (*E*) 0.2 s, (*F*) 0.1 s, and (*G*) 0.01 s.

shown to recover during a saturation-recovery experiment with little change in line shape.

To investigate the apparent lack of an angular dependence to T_1 further, the ^{31}P powder pattern was selectively excited using a DANTE sequence and the recovery of the spectrum monitored at times after such an excitation. Fig. 2 shows the results of such an experiment where two "holes" are burned into the ^{31}P spectrum. These experiments show that the excited regions of the spectrum recover on the order of 10 ms, a time short compared with T_1.

T_1 Experiments

Fig. 1 shows the recovery of the nuclear signal during a saturation-recovery T_1 experiment. The recovery of the integrated intensity as a function of time after the saturation sequence was found to be well described by a single exponential decay. The temperature dependence of the ^{31}P T_1 was measured at four resonance frequenceis (38.9, 81.0, 108.9, and 145.7 MHz) over a temperature range of $-30°$ to 60°C. The data are shown in Fig. 3. At each frequency results were obtained from a number of different samples prepared from different batches of egg lecithin. No significant differences are seen to result from sample preparation. The uncertainty in a measurement of T_1 was found to be on the order of 5%. Data at each frequency show a minimum in the value of T_1 and the minimum occurs at a higher temperature with increasing frequency. The minima occurred at -10, -2, 3, and 10°C for the frequencies 38.9, 81.0, 108.9, and 145.7 MHz, respectively. Furthermore, T_1 is seen to be frequency dependent on both sides of the T_1 minimum. At a particular temperature on the low temperature side of the T_1 minimum, T_1 increases with frequency. This behavior is characteristic of dipolar relaxation (17). However, a purely dipolar relaxation mechanism would not give rise to a frequency dependence at temperatures above the T_1 minimum. In Fig. 3, a frequency dependence is also observed on the high temperature side of the T_1 minimum; T_1 decreases with increasing frequency at a given temperature. This behavior is characteristic of relaxation due to a time-dependent anisotropic chemical shielding interaction (17).

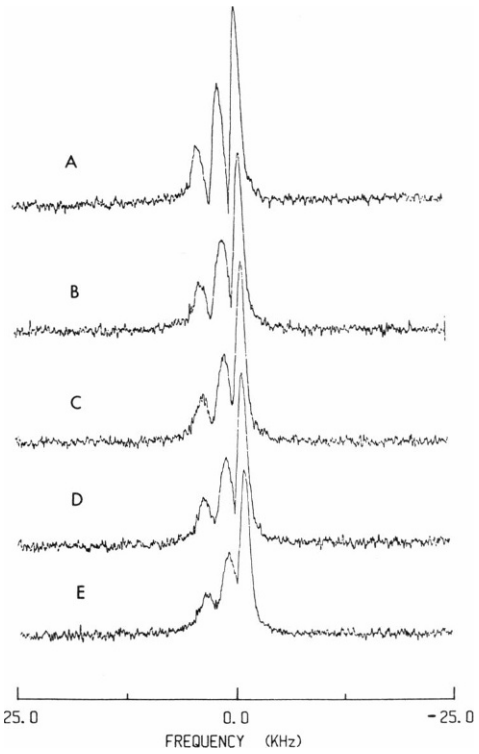

FIGURE 2 A sequence of spectra showing the recovery of a selectively excited phosphorus spectrum of egg PC. The spectra were taken at 108.9 MHz and 20°C. The selective excitation was achieved with a sequence of 2.5 μs wide radiofrequency pulses separated by 0.44 ms and of total duration 3.3 ms. The spectra were recorded with delay times after the application of the DANTE sequence equal to (*A*) 0.0 ms, (*B*) 0.2 ms, (*C*) 0.7 ms, (*D*) 1.2 ms, and (*E*) 2.2 ms.

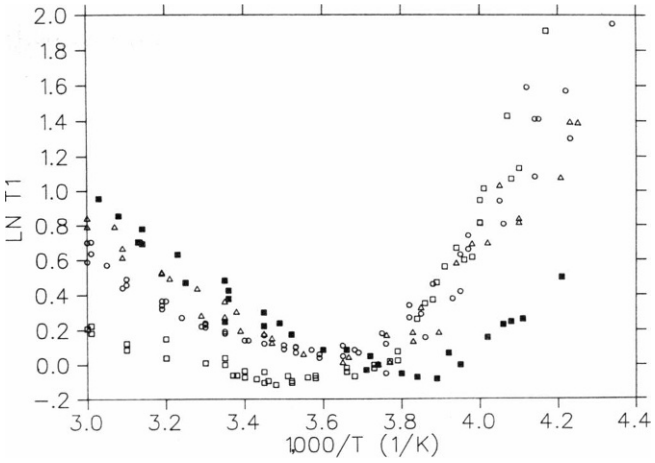

FIGURE 3 The ^{31}P spin-lattice relaxation time of egg PC as a function of temperature showing the results of measurements at four resonant frequencies: (■) 38.9 MHz, (Δ) 81.0 MHz, (O) 108.9 MHz, and (□) 145.7 MHz. A minimum in the relaxation time is seen in each curve and there is a frequency dependence on both sides of the minimum.

Modeling the Spin-Lattice Relaxation

Phosphorus spin-lattice relaxation in model membrane systems is complex not only because of the possibility of at least two relaxation mechanisms but because the molecular motion of the phosphate group arises from changes in conformation of the lipid molecule itself and reorientation of the molecule as a whole. Brown (24, 25) has recently given a very complete description of spin-lattice relaxation in lipid bilayers for the situations where either the nuclear dipolar or quadrupolar relaxation mechanism is effective. In this analysis the model used to describe the relaxation was kept as simple as possible to avoid the introduction of parameters that could not be defined by the experimental results. The T_1 data shown in Fig. 3 suggest that both dipolar relaxation and relaxation due to time-dependent chemical shielding are responsible for spin-lattice relaxa-

tion of egg PC dispersions. An overall relaxation rate can thus be written as the sum of two contributions,

$$1/T_1 = 1/T_{1\text{dip}} + 1/T_{1\text{csa}}, \quad (1)$$

where dip and csa indicate dipolar and time-dependent anisotropic chemical shielding contributions to the relaxation rate. Nuclear relaxation arises from that part of the spin-lattice interaction made time dependent by the molecular motion. A measure of the strength of the interaction responsible for the relaxation is the change in second moment (3) of the observed lineshape due to the motion. The following expressions for the two relaxation mechanisms were used in the data analysis:

$$1/T_{1\text{dip}} = (1/2)\,\Delta M_{2\text{dip}}\,[J(\omega_0 - \omega_p) + 3J(\omega_0) + 6J(\omega_0 + \omega_p)] \quad (2),$$

(17) and

$$1/T_{1\text{csa}} = (3/4)\,\Delta M_{2\text{csa}}\,J(\omega_0) \quad (3)$$

(26). Under certain restricted circumstances exact expressions can be derived for the changes in second moments ΔM_2 but in this analysis these are taken as parameters to be determined by the experimental results. The spectral density functions (17) $J(\omega)$ are assumed to have a Lorentzian form

$$J(\omega) = \tau_c/(1 + \omega^2 \tau^2{}_c) \quad (4)$$

where

$$\tau_c = \tau_0 \exp(E_a/kT). \quad (5)$$

It was assumed that the molecular motion responsible for relaxation can be described by a single effective correlation time τ_c which has an Arrhenius relationship as a function of temperature. Thus, using Eqs. 1–5 to fit the data involves four fitting parameters: (*a*) $\Delta M_{2\text{dip}}$, the change in dipolar second moment, (*b*) $\Delta M_{2\text{csa}}$, the change in second moment due to time dependent anisotropic chemical shielding, (*c*) τ_0, the correlation time at infinite temperature, and (*d*) an activation energy E_a.

Each of the four T_1 versus temperature curves was fit to a function derived from Eqs. 1–5, using a Marquardt nonlinear least squares algorithm (27). The parameters generated by this fitting routine were not identical at each frequency. It was found, however, that a single set of parameters could be chosen using the computer fitting as a guide that would describe the experimental data. Initial attempts to follow this procedure were unsuccessful due to the asymmetry of the relaxation curves in the region of the T_1 minimum. That is, the slopes of the relaxation curves of the high and low temperature regions were not equivalent and as such, the curves could not be fit successfully with one set of parameters. To obtain a successful fit the data were broken into two regions about an apparent discontinuity at −8°C, and each region ($T > -8$°C and $T < -8$°C) was fit independently.

Fig. 4 shows the fitting to the high temperature region, $T > -8$°C. Both the dipolar and anisotropic chemical shielding contributions to the total relaxation are shown. At 38.9 MHz the dipolar interaction dominates the relaxation while at 145.7 MHz the anisotropic chemical shift relaxation is the more important mechanism. At the intermediate frequencies of 81.0 and 108.9 MHz both mechanisms contribute significantly to the observed results. The relative importance of the two mechanisms is the same for the low temperature region, $T < -8$°C. These fittings are shown in Fig. 5. The difference between the low and high temperature regions is that the slope of the relaxation curve away from the minimum in the low temperature region is greater than that of the high temperature region as is evident by comparing Figs. 4 and 5. The slope of the relaxation curve is a reflection of the activation energy and the steeper slope of the low temperature region indicates that the activation energy is higher in this region. The high temperature and low temperature regions were fit with the same ΔM_2 parameters but E_a and τ_0 were allowed to change with the constraint that the two regions have the same value of the correlation time at −8°C. The values obtained for the changes in second moment were:

$$\Delta M_{2\text{dip}} = 1.8 \times 10^8\ \text{s}^{-2},$$

and

$$\Delta M_{2\text{csa}}/\omega_0^2 = 1.3 \times 10^{-9}.$$

The change in second moment due to the anisotropic chemical shift has been divided by ω_0^2 to remove the dependence on the Larmor frequency. In the high temperature region, the correlation time at infinite temperature was 8.75×10^{-13} s and the activation energy 16.9KJ/mol, while values of 1.2×10^{-16} s and 32.5 KJ/mol were obtained for these parameters in the low temperature region.

DISCUSSION

Since all regions of the phosphorus spectrum (each reflecting a particular angle of the bilayer normal with respect to the magnetic field) were observed to recover at the same rate, there appears to be no angular dependence to the relaxation rate. Further investigations of the phenomenon by saturation of a small region of the spectrum showed that magnetization was effectively transferred from one part of the sample to another in a time short compared with T_1. This is in agreement with similar experiments carried out by Brown and Davis (28). These investigators used a selective inversion recovery pulse sequence on the deuterium spectrum of dipalmitoylphosphatidylcholine that had been selectively deuterated at a number of positions in the fatty acyl chains and found that a hole burnt in the center

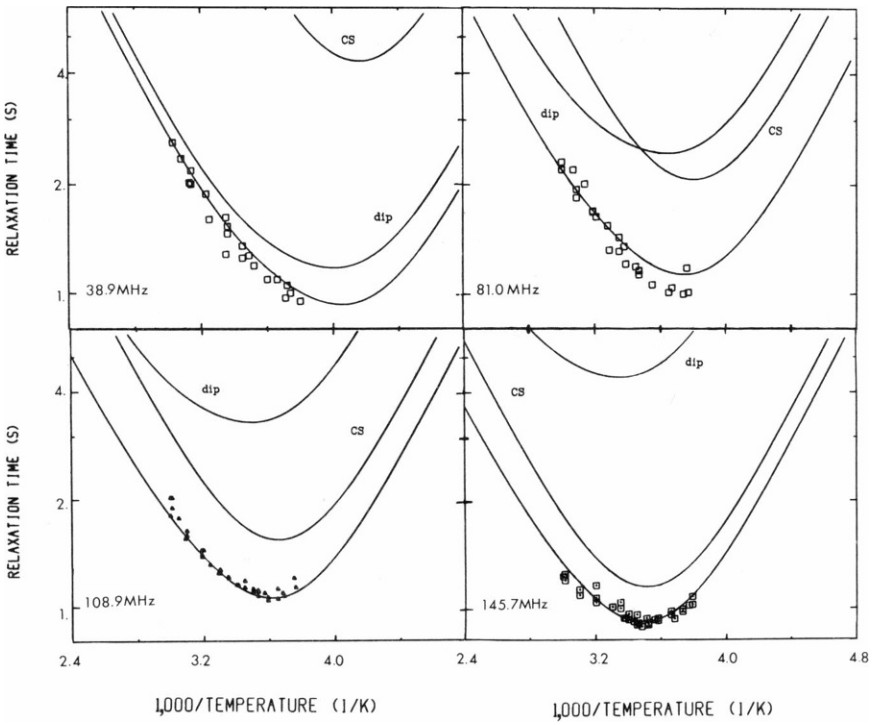

FIGURE 4 ^{31}P spin-lattice relaxation time data in the region $T > -8°C$ showing the theoretical fits for each frequency and the contributions to the relaxation from both the dipolar (dip) and chemical shielding (CS) mechanisms at each frequency. The dipolar relaxation mechanism is seen to dominate at low frequency while the chemical shift anisotropy dominates at higher frequencies.

of the spectrum recovered in a time of the order of 5 ms. In this paper it was proposed that lipid diffusion over the curved surfaces of the multilamellar dispersion effectively carried magnetization from one part of the powder pattern spectrum to another resulting in the rapid recovery of the signal in the selectively saturated region. Because the lipids experienced significant angular excursions on the order of 5 ms, the observation of any orientational dependence to the deuterium relaxation rate was prevented. The important conclusion then is that although there may be an angular dependence to the spin-lattice relaxation, the lipid molecule samples all orientations with respect to the magnetic field over the time T_1 by diffusing around the curved surface of the liposome effectively averaging any

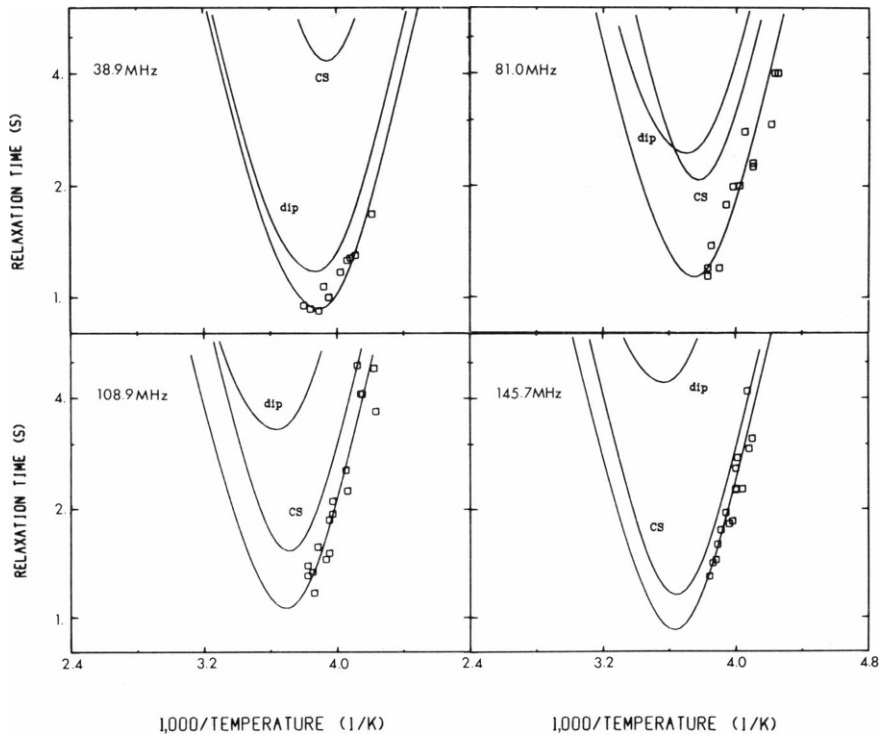

FIGURE 5 ^{31}P spin-lattice relaxation time data in the region $T < -8°C$ showing the theoretical curves for each frequency and the contributions to the relaxation from both the dipolar (dip) and chemical shielding (CS) mechanisms at each frequency. The dipolar relaxation mechanism dominates at low frequency while at higher frequency the chemical shift anistropy is dominant.

orientational effects that may be present. Orientational dependence can, therefore, only be measured by using aligned samples that have only one orientation of the bilayer normal with respect to the main magnetic field.

The results of the T_1 experiments illustrate that the relaxation is frequency dependent on both sides of the T_1 minimum. This behavior was modeled assuming two mechanisms played a role in the relaxation; a dipolar mechanism through the interaction of the phosphorus nucleus with neighboring protons and a mechanism involving the modulation of the anisotropic chemical shielding interaction. It is essential to have both the frequency and temperature dependence of the spin-lattice relaxation to carry out a complete analysis of the data. If only a limited temperature range is examined incorrect conclusions are possible. For example, if only a narrow temperature region near the T_1 minimum were examined, little frequency dependence would be observed since in this region the frequency dependencies of the two mechanisms have opposing effects.

The mathematical model used to analyze the experimental data was purposely kept as simple as possible to minimize the number of adjustable parameters. In addressing the validity of this model three points must be discussed: the magnitudes of the ΔM_2 parameters, the correlation time for the molecular motion, and the discontinuity that occurs at -8°C. First consider the value of the ΔM_{2dip} obtained in the modeling of the relaxation curves. This value would be obtained in the circumstance where there were a dipolar interaction between a phosphorus nucleus and a neighboring proton separated by 0.22 nm and motion of the proton with respect to the phosphorus nucleus were isotropic. Since there is possibly more than one proton involved, given the number of equivalent *N*-methyl and methylene protons in a PC molecule, this would be a minimum distance of interaction. Such a distance for the separation of phosphorus and protons is reasonable for such a molecular system. It should be noted that a more refined model would have to consider both intramolecular and intermolecular dipolar interactions as well as anisotropic reorientation. Second, consider the change in second moment due to the time-dependent anisotropic chemical shielding. The principal values of the shielding tensor for the phosphate group in the rigid phosphatidylcholine molecule are ~, $\sigma_{xx} = -81$ ppm, $\sigma_{yy} = -25$ ppm and $\sigma_{zz} = 110$ ppm (29) and the chemical shielding anisotropy observed in the ^{31}P spectra of the liquid crystalline phase is 50 ppm (8). The second moment of a line broadened by the anisotropic chemical shift is given by

$$M_2 = (1/5)\,\delta^2\,\omega_0^2\,(1 + \eta^2/3)$$

where

$$\delta = \sigma_{zz} \quad \text{and} \quad \eta = (\sigma_{xx} - \sigma_{yy})/\sigma_{zz}.$$

The calculated change in second moment, M_2/ω_0^2, is 2.3×10^{-9} as compared with the measured value of 1.3×10^{-9}. The calculated change assumes that all of the change in M_2 is due to the motion responsible for the spin-lattice relaxation. The factor of two difference indicates that the motion primarily responsible for the spin-lattice relaxation does not cause the entire observed change in the ^{31}P lineshape.

Now the question arises as to the nature of the molecular motion causing the spin-lattice relaxation. Fig. 6 shows the temperature dependence of the correlation time for reorientation of the phosphate moiety of the PC molecule as derived from modeling the T_1 data. The slope of this semilogarithmic plot is proportional to the activation energy and the discontinuous change of the slope at -8°C demonstrates the change of activation energy from 16.9 to 32.5 KJ/mol at this temperature. As seen in Fig. 6 the correlation time for the motion is on the order of 10^{-9} s at 60°C and it increases as the temperature is lowered by two orders of magnitude, being on the order of 10^{-7} s at -30°C. No discontinuity in the relaxation rate was observed at the gel-liquid crystalline phase-transition temperature at ~ -20°C. However, few data points were obtained in the gel phase so that no conclusions about changes due to the phase transition can be made.

The results given in this paper agree with a similar study by Seelig et al. (19). These investigators calculated an activation energy of 17.1 KJ/mol for the headgroup reorientation and computed a correlation time versus temperature in close agreement with the results obtained here. Yeagle et al. (14) obtained a correlation time of 1.4 ns at 23°C by assuming a motionally limited Overhauser enhancement, again in agreement with this study. The ^{31}P-NMR data have shown that chemical shielding contributes very little to the relaxation rate at the frequencies used by Yeagle et al. (14), thus the conclusion that dipolar

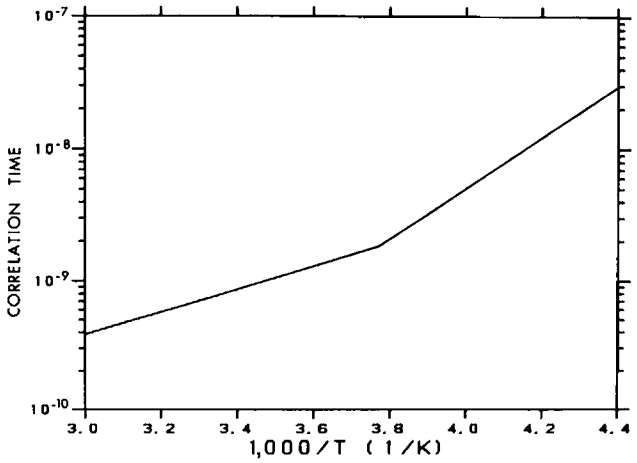

FIGURE 6 Semilogarithmic plot of the correlation time of the phosphate group reorientation as a function of temperature as derived from modeling the spin-lattice relaxation data. The slope of the plot is indicative of the activation energy for the reorientation.

relaxation dominates is good to a first approximation and the calculations of the above investigators are in agreement with the present results. Dielectric relaxation measurements of Shepherd and Buldt (30) are also consistent with the present results. In the liquid crystalline phase of dipalmitoylphosphatidylcholine it was found that the activation energy for the headgroup reorientation was 16.6 KJ/mol and that the dielectric relaxation time was in the order of 2.3 ns at 50°C in reasonable agreement with the correlation time determined from ^{31}P-NMR in egg PC.

In each of the experiments mentioned above the motion of the phosphate group was modeled in terms of a single effective correlation time. However, given the fact that there can be reorientation about several bonds in the region of the phosphate group as well as motion of the lipid molecule as a whole, the use of a single effective correlation time is an over simplification. Spin-lattice relaxation time measurements are particularly sensitive to motion described by a correlation time that has a value of the order of the reciprocal of the Larmor frequency. The success of the simple model in fitting the experimental data and the observation of the minimum in the T_1 data is a result of the T_1 measurements selecting out motion of the phosphate group where $\tau_c \sim 1/\omega_o$.

A picture has emerged of the phosphocholine headgroup being on average oriented parallel to the plane of the bilayer (31) but there is considerable flexibility about nearly each bond in the headgroup. The phospholipid molecule as a whole executes anisotropic reorientation which is axially symmetric about an axis parallel to the bilayer normal. Based on electron spin resonance studies on dipalmitoylphosphatidylcholine (32) the fatty acid chains have been found to have a rotational correlation time about a factor 3 less than the correlation times of the phosphate moiety obtained in this study. A detailed study of the NMR spectra and relaxation times (33) of deuterons selectively substituted at the 6- and 14-carbon positions on the sn-2 acyl chain of dimyristoylphosphatidylcholine has been modeled in terms of three correlation times describing chain rotation, τ_r, chain fluctuation, $\tau_{r\perp}$, and *trans*-gauche isomerization, τ_J. The rotation and fluctuation of the acyl chains can be taken as a measure of the motion of the lipid molecule as a whole. The reported analysis indicates that in the liquid crystal phase $\tau_{r_\parallel}$ and $\tau_{r_\perp} \sim 10^{-8}$ s while $\tau_J \sim 10^{-10}$ s. The activation energies for the chain motions are both ~50 KJ/mol while the *trans*-gauche isomerization has $E_a \sim 14$ KJ/mol. A comparison of these results with the ^{31}P relaxation time analysis indicates that the effective correlation time determined from the ^{31}P data are not describing motion of the molecule as whole but probably motion of the phosphate group with respect to the glycerol backbone of the lipid. Trahms (34) argues that a rotation about the P—O bond adjacent to the glycerol backbone is most consistent with the experimental data. This is consistent with the analysis of Strenk et al. (35) who postulate a rigid glycerol backbone based on deuterium NMR measurements in this region. Since more than one model may be constructed that will fit the data, such models must be based on as many experimental results as possible. In addition to ^{31}P-NMR studies, many ^{13}C- and ^{2}H-NMR studies have been carried out on the headgroup of phospholipids (6) and a more complete picture of the headgroup conformation and dynamics is emerging based on data from NMR studies and other techniques (36).

Lastly, account must be made for the discontinuity in the relaxation rate at −8°C. The data have been described in terms of a model where the amplitudes of the two relaxation mechanisms are constant over all temperatures and where the activation energy changes discontinuously at −8°C (there is no discontinuity in the correlation time at this temperature). This change in activation energy at −8°C can be attributed to restriction in the motion of the headgroup caused by freezing of the buffer in the headgroup region. A certain amount of water is normally bound to the lipid molecule in such systems (37), however, these experiments were carried out with enough buffer to be well in excess of this amount. Thus the reorientation of the phosphate moiety is restricted by the freezing of the excess water resulting in a change in the activation energy of the phosphate reorientation by almost a factor of two. The freezing of the excess buffer was monitored by ^{2}H-NMR of egg PC dispersions in a $^{2}H_2O$ buffer and the severe motional restriction of the water molecules expected upon freezing were observed to occur in the region of −8°C from the changes in the ^{2}H spectrum. This gives an experimental basis for breaking the ^{31}P data into two distinct regions, one corresponding to temperatures above the freezing point and one below the freezing of the excess buffer.

The simple model chosen to describe the spin-lattice relaxation seems to fit the experimental data very well despite the assumption that the amplitudes of the two relaxation mechanisms (ΔM_{2dip} and ΔM_{2csa}) remain constant independent of temperature over the entire range studied. It was observed that there were changes in the ^{31}P-NMR lineshape over the temperature range studied indicating some change in the order parameter tensor describing the average orientation and amplitude of the motion of the phospholipid headgroup. From ^{31}P-NMR it is not possible to determine the entire order parameter tensor, but rough arguments can be used to estimate the possible changes in the ΔM_2 parameters that could take place. The parameter ΔM_2 is roughly proportional to $(1 - S^2)$ (38) where S is the order parameter. From deuterium NMR results (3), it is expected that $S < 0.25$ and the changes in S are <10% so that changes in ΔM_2 are expected to be <1%. Therefore, to a good approximation, the changes that are known to occur in the ordering of the phosphate group are not of sufficient size to be reflected in changes in the amplitudes of the relaxation mechanisms.

In summary, the measurement of the ^{31}P spin-lattice

relaxation rate of egg PC as a function of temperature carried out at four resonant frequencies has revealed that both the chemical shielding and dipolar relaxation are important mechanisms for spin-lattice relaxation in these systems. The analysis has allowed the contribution of each mechanism at each frequency to be evaluated. The molecular motion that dominates the spin-lattice relaxation is not motion of the lipid as a whole but reorientation of the phosphate group with respect to the glycerol moiety. In addition it has been found that the freezing of the excess water in the multilamellar dispersions restricts the headgroup reorientation to an extent that an increase in the activation energy by a factor of two is observed at the freezing point.

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