Dynamics of the phosphate group in phospholipid bilayers
A ^{31}P-^{1}H transient Overhauser effect study

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ABSTRACT Two recent studies have addressed the question of the dynamics of the phosphate in egg phosphatidylcholine multilayers by measurement and interpretation of ^{31}P NMR spin-lattice relaxation. In the first (Milburn, M. P., and K. R. Jeffrey. 1987. *Biophys. J.* 52:791–799), the temperature dependences of the two contributions to the ^{31}P relaxation rate, a dipolar interaction of the phosphorus with neighboring protons and a time-dependent anisotropic chemical shielding interaction were separately measured. A further study (Milburn, M. P., and K. R. Jeffrey. 1989. *Biophys. J.* 56:543–549) incorporated the anisotropic nature of phospholipid motions into the dynamic model of the headgroup motion by measuring the ^{31}P spin-lattice relaxation time in oriented samples as a function of angle between the bilayer normal and the magnetic field. These angular dependent measurements were made at high field so that analysis could by made using the chemical shielding interaction because the ^{31}P-^{1}H dipolar interaction in phospholipid systems is complex and as such poorly understood.

Nuclear Overhauser effect (NOE) studies have attempted to identify the important proton species contributing to the ^{31}P-^{1}H dipolar interaction (Yeagle, P. L., W. C. Hutton, C. Huang, and R. B. Martin. 1975. *Biochemistry.* 15:2121–2124) and despite some controversy in interpretation (Burns, R. A., R. E. Stark, D. A. Vidusek, and M. F. Roberts. 1983. *Biochemistry.* 22:5084–5090), it was generally agreed that the choline methyl and methylene protons are the major contributors to the ^{31}P-^{1}H NOE. To further understand the nature of the ^{31}P-^{1}H dipolar interaction, we carried out ^{31}P-^{1}H Transient Overhauser effect (TOE) measurements on egg phosphatidylcholine multilayers. Protons from both the lipids and water are important in understanding the TOE measurements in both D_2O dispersions and H_2O dispersions of egg PC. A quantitative analysis of the TOE has enabled the cross-relaxation rate between the phosphorus and the two proton types to be determined. It is suggested that these TOE experiments are a direct observation of the interaction between the phospholipid phosphate and surrounding water protons. The correlation time describing the relative motion of the phosphate group and the water molecules is on the order of 10^{-11} s. The TOE measurements in phospholipid dispersions can be easily understood in terms of a straight forward model of the dipolar interaction and provide complementary information to NOE and T_1 measurements.

INTRODUCTION

Nuclear magnetic resonance has developed into a powerful technique for the investigation of the physical properties of cell membranes (1–3). ^{31}P NMR has been shown to be particularly useful when investigating the head group region of the phospholipid component (4, 5). Whereas NMR spectra give information about time averaged properties, such as the average molecular conformation, relaxation time measurements probe the amplitudes and time scales of the molecular motion. For measurements of the ^{31}P spin-lattice relaxation time to provide useful information and yield to a full quantitative analysis, the relaxation processes in such systems must be fully understood.

A recent series of papers has addressed the question of the dynamics of the phosphate group in phospholipids, by measurement of the ^{31}P NMR spin-lattice relaxation rate (6, 7) in egg phosphatidylcholine (PC) multilayers. An extensive study of the temperature and frequency dependence of the ^{31}P spin-lattice relaxation rate (6) has shown that two relaxation processes are important in phospholipid bilayers. At low NMR fields the dominant relaxation mechanism for the ^{31}P nucleus is the dipole–dipole coupling with surrounding protons while at high fields the anisotropic chemical shielding interaction is the important mechanism. The experimental results were described in terms of the reorientation of the headgroup phosphate moiety about a single axis and the assumption of an exponential correlation function for the motion. The strengths of the nuclear spin interactions were expressed in terms of the change in second moment of the lineshape resulting from the molecular motion responsible for the spin-lattice relaxation. The observation of a maximum in the spin-lattice relaxation rate as a function of temperature allowed the timescale for the reorientational motion of the phosphate segment to be well defined. A further study (7) refined this dynamical model by incorporating the anisotropic nature of the phospholipid motion through measurement of the ^{31}P spin-lattice relaxation rate as a function of the angle between the magnetic field and the bilayer normal in oriented multilayers. Because this study

was carried out at high NMR frequencies, the angular dependent relaxation rate could be analyzed solely in the terms of the anisotropic chemical interaction but the model now had to include the orientation of the axis of rotation in the principal axis coordinate system of the chemical shift tensor. If there had been a contribution to the relaxation from the ^{31}P-^{1}H dipolar interaction an explicit calculation in terms of the angular parameters would not have been possible because the nature of the ^{31}P-^{1}H dipolar interaction is poorly understood.

The ^{31}P-^{1}H dipolar coupling in phospholipid multilayers is complex due to the abundance of proton species with which the ^{31}P spin can interact. Further, these dipolar couplings may be intramolecular (for example with the choline methylene protons) or intermolecular (for example with the surrounding water protons). A number of investigators[8–12] have looked at these problems by using the ^{31}P-^{1}H Nuclear Overhauser effect (NOE), where the enhancement of the ^{31}P magnetization is measured while the proton resonance is saturated. The selective irradiation of the proton lineshape allowed the contributions of the various protons to be evaluated. This technique, however, has proven to be controversial (8). Discrepancies in these ^{31}P-^{1}H NOE frequency profiles have been attributed to differences in decoupling power. In fact the usefulness of this technique has been called into question (8) because the methylene and methyl (choline) resonances are separated by ~100 Hz while the breadth of the NOE frequency profile is up to 350 Hz wide. Despite these limitations, there seems to be a consensus that the choline methyl and choline methylene protons are the mmin contributors to the ^{31}P-^{1}H NOE (8).

In an effort to gain a more complete understanding of the ^{31}P-^{1}H dipolar interaction in phospholipid systems, measurements of the ^{31}P-^{1}H transient Overhauser effect (TOE) were undertaken on egg PC multilayers. The TOE is sensitive to parameters similar to the NOE and has been shown (13, 14) to be a useful but little used technique giving complementary information to the NOE. The TOE experiment is carried out by observing the phosphorus magnetization as a function of time following an inversion of the proton magnetization. Both the magnitude and time dependence of the change in phosphorus magnetization due to the inverted proton magnetization are able to provide information on the system being studied.

Results of the ^{31}P-^{1}H TOE experiments carried out on egg PC multilayers dispersed in both D_2O and H_2O show that both water and lipid protons contribute to the dipolar relaxation of the ^{31}P nucleus and their contributions can be separately determined. The experimental results are well understood in terms of a straight forward analysis despite the complexity of the system under study. It is suggested that the ^{31}P-^{1}H TOE experiment in egg PC multilayers results in a direct observation of the interaction between the PC phosphate and surrounding water molecules. Although a correlation time for the interaction cannot be precisely determined by this technique, the experimental data are consistent with a correlation time on the order of 10^{-11} s. In light of this information, small differences in the ^{31}P spin-lattice relaxation rate of egg PC multilayers as measured in D_2O compared to H_2O dispersions, can be attributed to the water protons whereas previous studies had suggested that the water protons play no role in phospholipid ^{1}H-^{31}P dipole–dipole interactions (10).

MATERIALS AND METHODS

Egg phosphatidylcholine was extracted from hen egg yolks using the method of Singleton et al. (15) and its purity was checked by thin layer chromatography. The lipid was stored in ethanol under nitrogen atmosphere at −18°C. To prepare an NMR sample the solvent was evaporated from the desired quantity of lipid (~200 mg) and a homogeneous dispersion was formed in the NMR sample tube by the addition of excess H_2O or D_2O. (D_2O at 99.8% purity was obtained from Stohler Isotope Chemicals, Waltham, MA).

The NMR measurements were carried out at a ^{31}P resonance frequency of 61.2 MHz using a superconducting magnet and a home-built FT-NMR spectrometer. A double-resonance probe was constructed for this experiment using transmission line tuning (16). One modification was made: the $\lambda/4$ open circuit cable was replaced by an LC trap tuned to the proton resonant frequency.

The ^{31}P and ^{1}H T_1 measurements were made with a saturation recovery sequence (6). The ^{31}P-^{1}H TOE experiment was carried out in a manner similar to that of a standard inversion recovery pulse sequence except that the 180° inversion pulse was applied to the proton magnetization and the observe (90°) pulse, at a time τ after the 180° pulse, applied to the phosphorus magnetization. A recovery time of at least five times the ^{31}P T_1 was allowed before repetition of the pulse sequence. A typical experiment averaged 400 transients for each τ value.

RESULTS

^{1}H and ^{31}P relaxation rates

Fig. 1 shows the measurement of the ^{1}H spin-lattice relaxation rate as a function of temperature for dispersions of egg PC in H_2O and in D_2O. The measurements in H_2O are the spin-lattice relaxation rates of the water protons only because this proton species is greatly in excess of the lipid protons. Due to the large ratio of water/lipid, no "bound" water is observed and the saturation recovery data fit well to a single exponential recovery. The measurements of the ^{1}H spin-lattice relaxation rate of egg PC in D_2O are dominated by the lipid protons. Because this study used unsonicated lipid dispersions the ^{1}H spectrum is not of high resolution and the relaxation time for each proton species could not be separately measured. The saturation recovery data were well fit by a

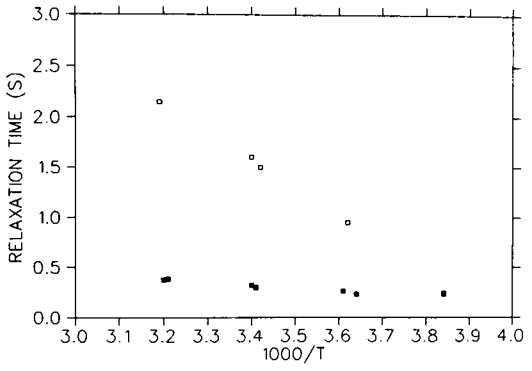

FIGURE 1 ^1H T_1 of egg PC dispersions in H_2O (□) and D_2O (⊠) as a function of temperature at 151 MHz. In both cases the saturation recovery data were well described by a single exponential curve. The results (□) for H_2O dispersions measure ^1H T_1 for the water protons, whereas the results (⊠) for D_2O dispersions are a measure of the ^1H T_1 of the lipid protons.

single exponential indicating that the relaxation times of the different proton species in the lipid do not differ significantly. Studies of ^1H spin-lattice relaxation rates using sonicated dispersions where small vesciles are formed (17–19) and where the high resolution spectra identify different proton species have shown that the various lipid ^1H spin-lattice relaxation rates vary by ~20%. Although spin diffusion has been suggested to be important in understanding ^1H relaxation in lipid molecules (17), intramolecular ^1H-^1H dipolar interactions are thought to be the dominant relaxation mechanism (18, 19).

Fig. 2 shows the ^{31}P spin-lattice relaxation rate as a function of temperature for egg PC multilayers in H_2O and D_2O. A comparison of the ^{31}P spin-lattice relaxation

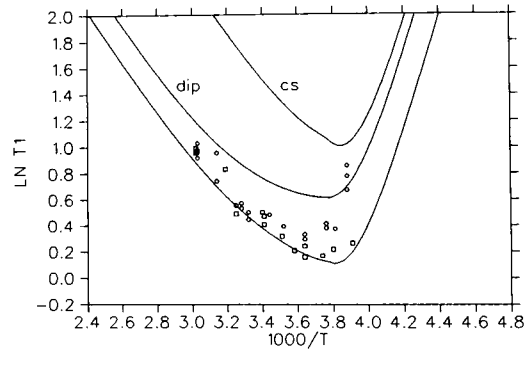

FIGURE 2 ^{31}P T_1 results for egg/PC dispersions in H_2O (□) and D_2O (○) as a function of temperature at 61.2 MHz. Calculations for the ^{31}P relaxation at this resonant frequency using the model of Milburn and Jeffrey (6) are shown as solid lines. The two sources of ^{31}P spin-lattice relaxation, the ^{31}P-^1H dipolar interaction (dip) and the chemical shielding anisotropy (cs) are shown.

rate for the H_2O and D_2O dispersions shows no difference at high temperatures but at low temperatures the ^{31}P spin-lattice relaxation rate in D_2O becomes markedly shorter than the spin-lattice relaxation rate in H_2O. The solid line in Fig. 2 is the result of a calculation based on the model outlined in reference 6 for the ^{31}P spin-lattice relaxation rate at 61.2 MHz. At this frequency, the ^{31}P-^1H dipolar interaction (dip) is responsible for much of the ^{31}P relaxation rate although the chemical shielding (CS) anisotropy makes a significant contribution. Although the parameters used for the model calculation were obtained from the previous fit to data at four different resonant frequencies (6), the model describes the present data taken at 61.2 MHz.

^{31}P-^1H transient Overhauser effect

The transient Overhauser effect (TOE) measures the time-dependent changes of the magnetization of a particular nucleus after the inversion of the magnetization of a neighboring nucleus. In this study, the ^{31}P magnetization has been monitored at times subsequent to the inversion of the proton magnetization for egg PC multilayers dispersed in both H_2O and D_2O. Fig. 3 *A* shows the results of such an experiment at 0°C, for egg PC dispersed in D_2O (□) and H_2O (▲). Fig. 3 *B* shows the data for the TOE experiment at 20°C. The results are plotted as the percentage change in phosphorus magnetization (P) from its equilibrium value (P_0), $(P - P_0) \times 100/P_0$), as a function of time after the inversion of the proton magnetization. The relative intensity of the phosphorus magnetization is seen to reach a maximum of ~14% in the H_2O dispersion 1 s after the proton inversion. In D_2O the phosphorus relative intensity reaches a maximum of ~7% at about 0.6 s. The maximum intensity change is much greater for the H_2O experiment than for the D_2O case and the maximum occurs at a later time following the proton inversion for H_2O lipid dispersions than for the same experiment in D_2O. Unlike the NOE experiments (10), the ^{31}P-^1H TOE gives different results for experiments carried out in D_2O as compared to H_2O. Thus, the ^{31}P-^1H TOE provides a means of observing the interaction of the protons in water with the phospholipid phosphate moiety.

Modeling the transient Overhauser effect

To interpret the ^{31}P-^1H TOE in a complex biomolecule such as a lipid, where the ^{31}P-^1H dipolar interaction may be of an inter or intra molecular nature and where a number of different proton species may be responsible for the ^{31}P-^1H dipolar coupling, it is instructive to use as simple a model as is necessary to describe the data. To model the ^{31}P-^1H TOE in PC multilayers it may be

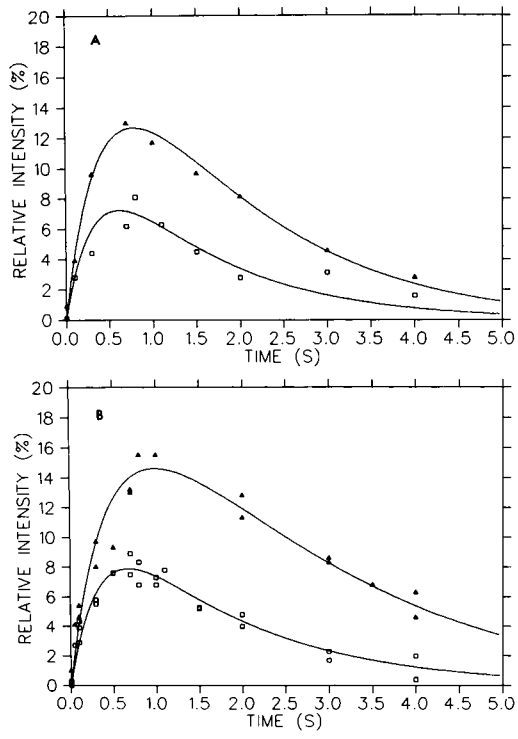

FIGURE 3 (*A*) ^{31}P-^{1}H TOE measurements showing the change in phosphorus signal intensity M_z^P relative to its equilibrium value M_z^{Po} as a function of time following the inversion of the proton magnetization, $[(M_z^P - M_z^{Po}) \times 100/M_z^{Po}$ vs. $t]$. Results are shown for egg PC dispersions in H_2O (▲) and D_2O (□) at 0°C obtained at a ^{31}P resonant frequency of 61.2 MHz. The solid lines show fits based on the model described in text using the following parameters for the D_2O dispersions: $\rho_1 = 0.74\ s^{-1}$, $\rho_2 = 3.03\ s^{-1}$ and $\sigma = 0.07\ s^{-1}$ and the H_2O dispersions: ρ_1, ρ_2, and σ as in D_2O, $\rho_3 = 1.11\ s^{-1}$ and $\beta = 0.03^{-1}$. (*B*) ^{31}P-^{1}H TOE measurements repeated at 20°C in H_2O (▲) and D_2O (□). The fitting parameters used to obtain the solid lines in D_2O: $\rho_1 = 0.63\ s^{-1}$, $\rho_2 = 2.86\ s^{-1}$ and $\sigma = 0.07\ s^{-1}$, and in H_2O: ρ_1, ρ_2, and σ as in D_2O, $\rho_3 = 0.666\ s^{-1}$ and $\beta = 0.03\ s^{-1}$.

assumed that the phosphorus spin interacts with two distinct proton species, the lipid protons and the water protons. The justification for not differentiating between various lipid protons is based on the fact that the ^{1}H spin-lattice relaxation rates of the various lipid protons do not differ by more than ~20% (17) and that the TOE is sensitive to the proton relaxation time. Thus, the TOE results are interpreted in terms of the interaction of the ^{31}P magnetization with slowly relaxing (~1.5 s) water protons and with faster relaxing (~0.3 s) lipid protons. Further, the experiments in H_2O and D_2O provide a means of separating these two contributions to the TOE.

First, consider the D_2O experiment where only one species of protons interacts with the phosphorus. Equations describing the motion of the magnetizations in such a two-spin system may be written (13) as:

$$dM_z^P/dt = -\rho_1(M_z^P - M_z^{Po}) - \sigma(M_z^H - M_z^{Ho}) \quad (1)$$

$$dM_z^H/dt = -\sigma(M_z^P - M_z^{Po}) - \rho_2(M_z^H - M_z^{Ho}), \quad (2)$$

where M_z^P and M_z^H describe the phosphorus and proton magnetizations, respectively. The symbol ° denotes the equilibrium value; ρ_1, the relaxation rate of the phosphorus; ρ_2, the relaxation rate of the protons, and σ, the so-called cross-relaxation rate. The parameters ρ_1, ρ_2, and σ are functions of the possible transition probabilities of the two-spin system (see reference 13) which may incorporate other relaxation mechanisms. In fact, ρ_2 is determined by a dipolar interaction with other protons and ρ_1 has a contribution from the chemical shielding interaction. Solution of the coupled system of Eqs. 1 and 2 may be further simplified by neglecting the term $-\sigma(M_z^P - M_z^{Po})$ in Eq. 2 because in thermodynamic terms, the proton spin reservoir is large compared to the phosphorus reservoir. In other terms, each phosphorus is expected to interact with many protons (4 methylene- and 9 methyl- choline protons if only the headgroup protons are considered) so that a small change in phosphorus magnetization from its equilibrium value would not be expected to affect the proton magnetization to any significant extent. Thus, the equations

$$dM_z^P/dt = -\rho_1(M_z^P - M_z^{Po}) - \sigma(M_z^H - M_z^{Ho}) \quad (3)$$

$$dM_z^H/dt = -\rho_2(M_z^H - M_z^{Ho}) \quad (4)$$

must be solved with the following initial conditions for the TOE experiment;

$$(M_z^P - M_z^{Po})_{t=0} = 0$$

and

$$(M_z^H - M_z^{Ho})_{t=0} = -2M_z^{Ho}.$$

The solution of Eq. 2 for the standard inversion recovery experiment is

$$(M_z^H - M_z^{Ho}) = -2M_z^{Ho} \exp(-\rho_2 t), \quad (5)$$

where the proton magnetization is observed to recover exponentially to its equilibrium value with the time constant ρ_2. The solution of Eq. 3 with a substitution from Eq. 5 gives,

$$M_z^P - M_z^{Po} = -M_z^{Ho}(2\sigma/(\rho_1 - \rho_2)) \exp(-\rho_1 t) + 2M_z^{Ho}(\sigma/(\rho_1 - \rho_2)) \exp(-\rho_2 t).$$

Since $M_z^{Ho} = \gamma M_z^{Po}$ where γ is the ratio of the gyromag-

netic ratios of the proton and phosphorus nuclei, this equation becomes

$$(M_z^P - M_z^{Po})/M_z^{Po} = [-2\gamma\sigma/(\rho_1 - \rho_2)] [\exp(-\rho_1 t) - \exp(-\rho_2 t)], \quad (6)$$

which describes the relative intensity of the phosphorus magnetization as a function of time for the TOE experiment.

The curve representing Eq. 6, which describes the ^{31}P-^1H TOE experiment in D_2O dispersions of egg PC, is shown in Fig. 3 for two temperatures, 0 and 20°C. The experimental data are well modeled by Eq. 6 using the experimentally determined value of ρ_1 at the appropriate temperature, a value of ρ_2 20% below the measured lipid proton relaxation rate and a value of $\sigma = 0.07 \pm 0.01\ s^{-1}$ at both 0 and 20°C. Adjustment of ρ_2 from the experimentally measured proton relaxation rate was necessary to give the correct shape and position of the TOE maximum. The parameter σ has a negligible effect on these features of the TOE curve but instead determines the maximum intensity of the TOE. Because the experimentally measured value of ρ_2 represents an average over the different lipid protons, a value of ρ_2 20% below that determined directly from the proton T_1 experiment shows that the proton species responsible for the TOE has a slightly longer T_1 than the average for all the lipid protons. This is consistent with the fact that the T_1's of the various lipid protons are not all equivalent (see previous discussion).

The ^{31}P-^1H TOE experiment of dispersions of egg PC in H_2O can be understood in terms of the interaction of the ^{31}P nucleus with two proton species: the lipid protons, and the water protons where the TOE interaction parameters for the lipid protons have been elucidated through the D_2O experiments. The water and lipid protons can be considered to be separate spin reservoirs with no coupling between them. Again, it is assumed that a change in the phosphorus magnetization does not influence the water protons because the phosphorus reservoir is much smaller than the water proton reservoir. Thus, the combined spin system can be described by the equations,

$$\begin{aligned} dM_z^P/dt &= -\rho_1(M_z^P - M_z^{Po}) - \sigma(M_z^H - M_z^{Ho}) - \beta(M_z^W - M_z^{Wo}) \\ dM_z^H/dt &= -\rho_2(M_z^H - M_z^{Ho}) \\ dM_z^W/dt &= -\rho_3(M_z^W - M_z^{Wo}), \end{aligned} \quad (7)$$

where M_z^W has been introduced as the magnetization of the water protons, ρ_3 the relaxation rate of the water protons, and β the cross-relaxation rate describing the coupling between the phosphorus and water protons. Solution of these equations for the initial conditions

$$\begin{aligned} (M_z^P - M_z^{Po})_{t=0} &= 0 \\ (M_z^H - M_z^{Ho})_{t=0} &= -2M_z^{Ho} \\ (M_z^W - M_z^{Wo})_{t=0} &= -2M_z^{Wo} \end{aligned}$$

gives

$$\begin{aligned} (M_z^P - M_z^{Po})/M_z^{Po} = &-[2\gamma\sigma/(\rho_1 - \rho_2) \\ &+ 2\gamma\beta/(\rho_1 - \rho_3)] \exp(-\rho_1 t) \\ &+ [2\gamma\sigma/(\rho_1 - \rho_2)] \exp(-\rho_2 t) \\ &+ [2\gamma\beta/(\rho_1 - \rho_3)] \exp(-\rho_3 t), \end{aligned} \quad (8)$$

which describes the relative intensity of the phosphorus magnetization as a function of time after inversion of the proton magnetization. The fitting of Eq. 7 to the TOE experiment in H_2O is shown in Fig. 3 for 0 and 20°C. Parameters ρ_1, ρ_2, and σ were obtained as described in the analysis of the D_2O experiments, the value of ρ_3 was measured experimentally as a function of temperature (Fig. 1) and β was adjusted to best fit the maximum relative intensity of the phosphorus magnetization. Values of $\beta = 0.03\ s^{-1}$ were obtained for both temperatures. It should be noted that the parameter β has a negligible effect on both the shape and the position of the maximum of the modeled curve. These are determined by the other previously determined parameters, lending credibility to the model calculation.

DISCUSSION

Understanding the dipolar interaction between protons and the phosphorus nucleus of the phospholipid phosphate is a complex problem owing to the abundance of protons. Previous studies have suggested that the choline methyl and methylene protons are most important and that both inter- and intramolecular dipolar interactions with the phospholipid head group are present (10). However, measurement of the ^{31}P-^1H TOE in phospholipid dispersions has shown that water protons must also be considered. In fact, this little used technique is very sensitive to these protons, the maximum enhancement of the phosphorus magnetization being larger by a factor of two when the TOE experiment is carried out in H_2O dispersions of PC as compared to D_2O dispersions.

Interpretation of the ^{31}P-^1H TOE experiment in egg PC dispersions is straight forward despite the numerous possibilities for ^{31}P-^1H dipolar interactions. For the purpose of analysis of the TOE, the phosphorus can be considered to interact with two groups of protons: a faster relaxing lipid proton and a much slower relaxing water proton. The effect of these two groups of protons can be

separated by measuring the TOE in H_2O and in D_2O dispersions of egg PC because replacing the water hydrogen with a deuterium effectively reduces this dipolar interaction by a factor of $(\gamma_H/\gamma_D)^2 = 42.4$. Modeling the TOE experimental data involves the determination of only a single parameter in each case, the cross-relaxation rate. The shape of the TOE is most sensitive to the various relaxation rates which can be measured separately, and adjustment of the cross-relaxation rate affects only the maximum intensity change in the phosphorus lineshape. This simple picture of ^{31}P-^1H interactions in the phospholipid headgroup gives an excellent understanding of the TOE given the fact that only a limited parameter space is available to fit the data. In a sense, the measurement of the TOE can be considered a direct measure of the cross-relaxation rate.

From measurements of the ^{31}P-^1H TOE in D_2O dispersions of egg PC a cross-relaxation rate of 0.07 ± 0.01 s^{-1} has been obtained for the interaction of the ^{31}P nucleus with the lipid protons at both temperatures (0 and 20°C). The experimental uncertainty in the TOE data prevented the temperature dependence of the cross-relaxation rate from being observed. A value for the strength of the dipolar interaction between the lipid protons and the ^{31}P nucleus and a value for the activation energy of the molecular motion has been obtained by measuring the ^{31}P spin-lattice relaxation rate at four resonance frequencies (6). Using these parameters a second determination of the cross-relaxation rate can be obtained as a function of temperature using the appropriate transition probabilities (13). From these calculations the cross-relaxation rate $\sigma = 0.12$ s^{-1} at 20°C and σ decreases to 0.07 s^{-1} at 0°C which is consistent with the value of 0.07 ± 0.01 s^{-1} for σ obtained from the TOE experiment. Using the TOE determined value of σ, the expected steady-state NOE can also be computed because the ratio σ/ρ_1 is necessary to determine the increase in amplitude of the ^{31}P resonance if the proton resonance is saturated. This calculation shows that a NOE of ~1.3 should be observed for this system. Studies of ^{31}P-^1H NOE's of sonicated PC vesicles in D_2O (8, 10) have obtained maximum NOE's in the range 1.3–1.4 (with quoted experimental uncertainties of ±15%) which are consistent with the calculation using the TOE results.

Using the parameters obtained for the dipolar interaction between the phosphate phosphorus and the lipid protons from the D_2O experiments, the TOE experiment for samples in H_2O yielded a cross-relaxation rate of 0.030 ± 0.005 s^{-1} for the interaction of the phosphorus with the water protons. Because this cross-relaxation rate is ~40% of that obtained for the phosphorus-lipid proton interaction it must be concluded that there is a significant interaction between the phosphorus and the protons of the water. Because the cross-relaxation rate is a function of both the correlation time for the relative motion of the phosphorus-water proton system and the interaction strength of the phosphorus-water proton dipolar coupling, these quantities cannot be obtained independently. However, it is possible to estimate the strength of the ^{31}P-^1H dipolar interaction, as this strength is a function of the internuclear distance. In a lipid dispersion water molecules would be in close proximity to the choline phosphate. A ^{31}P-^1H internuclear separation for phosphate phosphorus-water proton would be comparable to phosphorus-proton distances for nondirectly bonded protons (such as choline methylenes). Thus, an interaction strength for the phosphorus-water proton coupling on the order of that determined for the phosphorus-lipid proton can be postulated.

An estimate for the phosphorus-water proton coupling constant allows an order magnitude determination for the correlation time of the relative motion of the phosphorus-water proton system. The phosphorus-lipid proton interaction strength was found to be equivalent to that obtained for a single proton a distance of 0.22 nm from the phosphorus given isotropic motion of the proton with respect to the phosphorus (6). From this ^{31}P-^1H interaction strength, the TOE determined β of 0.030 s^{-1}, is obtained for correlation times either longer or shorter than the inverse of the phosphorus Larmor frequency. However, given the fact that a negligible contribution from the water protons to the ^{31}P spin-lattice relaxation rate is observed (Fig. 2), a correlation time in the short correlation time regime is suggested. This can be seen by noting the behavior of the function β/ρ' where ρ' is the contribution of the dipolar coupling defined by the cross-relaxation rate β, to the ^{31}P relaxation rate. This function is large (>0.45) in the short correlation time regime and small (<0.05) in the long correlation time regime. Using $\beta = 0.03$ s^{-1}, a value of ρ' well within experimental uncertainty is calculated for $\beta/\rho' = 0.45$, whereas $\beta/\rho' = 0.05$ would predict that the water protons give rise to an important contribution to the phosphorus relaxation rate. These arguments suggest a correlation time for the phosphorus-water proton interaction on the order of 10^{-11} s because both an appropriate value of β and a negligible contribution of the water protons to the phosphorus relaxation rate are allowed in this regime.

This TOE study of PC dispersions suggests that the phosphorus interacts with any particular water proton for only a very short period of time. The estimated correlation time of 10^{-11} s presents a number of possibilities for the physical nature of the phosphorus-water proton interaction. This interaction may be viewed as a hydrogen bond between the water molecules and the phospholipid phosphate with a lifetime on the order of 10^{-11} s. The phosphate group on phospholipids has often been considered a potential site of hydrogen bonding (20, 21) and a

water-phosphate hydrogen bond has been proposed to explain the results of ^2H T_1's in egg PC nucleus in CCl_4/D_2O mixtures (22). The physical data of the TOE experiment also support a picture of the phosphorus-water proton interaction where the spin interaction is modulated by the rotational diffusion of the water molecules in proximity to the phospholipid phosphate. A correlation time of $\sim 0.5 \times 10^{-12}$ s can be computed for the rotational diffusion of H_2O at ambient temperatures (23), although it is difficult to define such a correlation time for water at the surface of a lipid bilayer.

The TOE measurements show that there is a strong interaction between the phosphorus and water protons, however, the contribution of the water protons to the ^{31}P T_1 is negligible because of the very short correlation time for the modulation of the spin interaction as compared to the inverse of the phosphorus Larmor frequency. It is expected that as the temperature is lowered and the correlation time begins to approach the inverse Larmor frequency, the water protons would become increasingly important in determining the ^{31}P T_1. Fig. 1 shows that the ^{31}P T_1 is much longer in D_2O dispersions of egg PC than in H_2O dispersions only at low temperatures and this difference increases as the temperature is decreased. The difference in the ^{31}P T_1 for H_2O as compared to D_2O dispersions at low temperatures can be attributed to the water protons. Also, the difference in the ^{31}P T_1 between H_2O and D_2O dispersions seems to increase dramatically at the freezing point of water (3.8 on the 1000/temperature scale) indicating a possible change in the correlation time of the phosphorus-water proton interaction at this temperature.

From Eq. 7 it can be seen that the water protons are expected to contribute to the experimentally determined ^{31}P-^1H NOE. However, it has been reported previously (10) that the NOE is independent of the H_2O/D_2O ratio in egg PC dispersions but the large experimental uncertainties associated with these experiments may have prevented the observation of the water proton contribution to the ^{31}P-^1H NOE. TOE experiments are more sensitive to the water protons than the NOE showing a large increase in the maximum enhancement of the phosphorus magnetization when the lipid is dispersed in H_2O compared to D_2O. This sensitivity is due, in part, to the fact that the water protons relax in a time comparable to that of the phosphorus nucleus, whereas the lipid protons have a much shorter relaxation time.

In summary, the observation of the ^{31}P-^1H TOE in phospholipid dispersions shows that the water protons have a significant dipolar interaction with the phosphate phosphorus. The TOE experiment can be understood in terms of a simple picture of phosphorus-proton dipolar interactions and the cross-relaxation rates between the phosphorus and the various protons can be directly inferred. Although similar to the NOE, the TOE experiments provide information not observed from measuring the nuclear Overhauser enhancement or the spin-lattice relaxation rate and should be considered as a potentially useful complementary technique when investigating the dipolar interaction especially in complex biomolecules.

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