Lateral Diffusion of the Phospholipid Molecule in Dipalmitoylphosphatidylcholine Bilayers. An Investigation Using Nuclear Spin–Lattice Relaxation in the Rotating Frame†

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ABSTRACT: Measurements of the proton NMR spin–lattice relaxation time in the rotating frame ($T_{1\rho}$) have permitted the explicit determination of the lateral diffusion coefficient of phospholipid molecules in the lamellar mesophase of dipalmitoylphosphatidylcholine at temperatures above the phase-transition temperature. The experimentally observed temperature and frequency dependence of $T_{1\rho}$ for the dipalmitoylphosphatidylcholine protons suggest that intermolecular dipole–dipole relaxation contributions are important. Proton $T_{1\rho}$ experiments involving dilution with deuterated dipalmitoylphosphatidylcholine support the premise that intermolecular dipolar interactions are significant and, concomitantly, that lateral diffusion is the motion modulating that interaction. The lateral diffusion coefficient is determined directly from the dependence of the rotating frame spin–lattice relaxation rate ($1/T_{1\rho}$) on the strength of the applied radiofrequency field in the spin-locking experiment. A series of experiments with varying concentrations of dipalmitoylphosphatidylcholine in the lamellar mesophase indicates that the lateral diffusion coefficient varies as a function of phospholipid concentration.

The sites of action for many biological reactions are located within cell membranes, while the passage of molecules through the membrane is a prerequisite for other processes. The kinetics and mechanisms of molecular processes associated with membranes such as active-transport systems, hormone–receptor interactions, and drug–receptor interactions as well as the mobility of the molecular species involved are dependent upon the composition and structure of the membrane and, consequently, upon the dynamic state of the membrane. The integrity and functional diversity of membranes are reflected in the different membrane compositions of various tissues (Chapman, 1975; Tanford, 1973). X-ray diffraction (Wilkins et al., 1971) and neutron scattering (Schoenborn, 1976) studies suggest that a phospholipid bilayer forms a major structural feature of biological membranes.

The fluidity of the lipid core within the phospholipid bilayer affects the partitioning of small molecules into the bilayer (Hubbell and McConnell, 1971). Lipid composition, e.g., fatty acyl chain length, degree of unsaturation, and the presence of molecules such as cholesterol and its derivatives, also influences the permeability of phospholipid liposomes to nonelectroytes such as glycerol and erythritol (DeGier et al., 1968). Thermal effects (Chapman et al., 1967; Reinert and Steim, 1970), the antagonism of pressure on anesthetic-mediated effects (Johnson and Miller, 1970; Seeman and Roth, 1972; Machleidt et al., 1972), and the effects of anesthetics by themselves (Vanderkooi et al., 1977; Hubbell and McConnell, 1968) have been shown to impose similar changes in properties for both natural membranes and phospholipid bilayer systems. Studying static and dynamic structural features and conditions affecting the fluidity within phospholipid bilayers as model membrane systems should yield information about the dynamics of membranes and their associated biological processes.

Several molecular motions collectively determine the dynamic behavior in a phospholipid bilayer. The lateral self-diffusion coefficient (D, cm^2/s) is a dynamic parameter representing translational mobility of a phospholipid molecule in the plane of the bilayer. Values of the diffusion coefficient for phospholipid and other related amphiphilic molecules have been reported for model lipid bilayer systems of varied composition (Roeder et al., 1976; Lindblom et al., 1976; Devaux and McConnell, 1972; Träuble and Sackmann, 1972; Tiddy and Everiss, 1976; Fahey et al., 1977).

Electron paramagnetic resonance (EPR)[1] experiments with spin-labeled probes (Hubbell and McConnell, 1969, 1971), fluorescent spectral studies of dyes, and nuclear magnetic resonance (NMR) studies have been employed to investigate molecular dynamics in various phospholipid bilayer model systems (James, 1975a; Brûlet and McConnell, 1975; Seiter and Chan, 1973; Michaelson et al., 1973; Edidin, 1974; Wahl et al., 1971). However, no method for determining the diffusion coefficient has gained general acceptance due to either technical and experimental limitations or the potential perturbation of the bilayer by foreign probes. Pulsed field gradient NMR spin–echo experiments have been applied to the study of self-diffusion in phospholipid and lipid bilayers; however, these experimental systems push the technique's capabilities to their limits. Other investigations of self-diffusion in these systems, e.g., NMR spectral and relaxation time measurements as well as EPR and fluorescence spectroscopic studies, yield only estimates of the diffusion coefficient. To derive the diffusion coefficient from strictly measurable quantities is desirable.

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[1] Abbreviations used are: EPR, electron paramagnetic resonance; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DPPC-d_{62} di(perdeuteriopalmitoyl)-phosphatidylcholine.

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It has been shown that in a viscous system such as glycerol, if certain conditions prevail (vide infra), the diffusion coefficient may be determined directly from NMR relaxation data (Burnett and Harmon, 1972). If intermolecular dipolar relaxation due to translational diffusion gives rise to a significant contribution to the spin–lattice relaxation rates in the Zeeman field or stationary frame ($1/T_1$) or in the spin-locking field or rotating frame ($1/T_{1\rho}$), a radiofrequency dependence exists from which the self-diffusion coefficient may be obtained directly.

We have examined the proton spin–lattice relaxation rates in the rotating frame as a function of radiofrequency, concentration, and temperature for dipalmitoylphosphatidylcholine (DPPC) in the homogeneous multilamellar dispersion above the Chapman phase-transition temperature, i.e., in the smectic liquid-crystalline mesophase. DPPC is abundant in natural membranes, has no unsaturation, and there is no heterogeneity in the lipid chain lengths, thus yielding a model membrane system with a minimum of complications.

Theory

Intramolecular dipolar spin–lattice relaxation has been described in detail (Abragam, 1961a). It is well known that under motional narrowing conditions, i.e., $\omega\tau \leq 1$ where ω is the Larmor angular frequency and τ is the correlation time for the motion causing relaxation, there is no frequency dependence of the spin–lattice relaxation rates $(1/T_1)_{\text{INTRA}}$.

Treatment of the intermolecular case, being diffusion-model dependent, is more complex (Bloembergen et al., 1948; Torrey, 1953; Abragam, 1961b). Under the motional narrowing conditions, the intermolecular spin–lattice relaxation rate $(1/T_1)_{\text{INTER}}$ has been shown to be related to the self-diffusion coefficient (Abragam, 1961b):

$$\left(\frac{1}{T_1}\right)_{\text{INTER}} = \frac{2\pi}{5}\frac{N\gamma^4\hbar^2}{\sigma D} \tag{1}$$

where N is the spin density, γ is the gyromagnetic ratio for the nucleus observed, σ is the molecular diameter, D is the diffusion coefficient, and $\hbar$ is Planck's constant divided by 2π. Torrey's theory of nuclear spin relaxation (Torrey, 1953) via translational diffusion which employed the theory of random jumps has been extended by Harmon and Muller (1969) to include an expanded radial distribution function. Their development showed that in the low-frequency limit the intermolecular spectral density "rolls off" with increasing frequency in the region $\omega < 1/\tau$. Thus, if the overall relaxation rate is dominated by intermolecular effects, the relaxation rates exhibit a frequency dependence in the extreme narrowing region.

For spin 1/2 systems, the relaxation rate, $(1/T_1)_{\text{INTER}}$, is (Harmon and Muller, 1969)

$$\left(\frac{1}{T_1}\right)_{\text{INTER}} = \frac{2\pi N\gamma^4\hbar^2}{5\sigma D}\left[1 + A(\langle r^2\rangle, \sigma) - B\left(\frac{\omega\sigma^2}{D}\right)^{1/2}\right] \tag{2}$$

with $\langle r^2\rangle$ being the mean-squared jump distance, A and B representing numerical constants, and the other symbols are as described above. Evaluation of the only frequency-dependent term of eq 2 in terms of jump diffusion parameters shows that the size of ω becomes important when the time between diffusive jumps, τ, becomes comparable to the Larmor period:

$$\left(\frac{\omega\sigma^2}{D}\right)^{1/2} = \left(\omega\tau\frac{6\sigma^2}{\langle r^2\rangle}\right)^{1/2} \tag{3}$$

The parameters of eq 3 are as described above, and τ, the mean time between diffusive jumps, can be used as a correlation time for the diffusive motion causing relaxation. It is apparent from eq 2 that measurements of the spin–lattice relaxation rate as a function of frequency can lead to a determination of the diffusion coefficient.

Diffusion in viscous or low-temperature systems will involve motions on a much slower time scale. Consequently, it is advantageous to measure the spin–lattice relaxation time in the rotating frame ($T_{1\rho}$) as a function of $\omega_1 (= \gamma H_1)$, the angular frequency for H_1, the applied rf field; H_1 is more than three orders of magnitude smaller than ω_0 $(= \gamma H_0)$, the Larmor precessional frequency in the laboratory frame. In addition to having a much smaller frequency, which is appropriate for studying slower motions, the applied rf field H_1 is more readily varied than is the stationary magnetic field H_0. According to Burnett and Harmon (1972), for intermolecular dipole–dipole spin–lattice relaxation in the rotating frame,

$$\frac{1}{T_{1\rho}} = C\omega_1^{1/2} + \frac{1}{T_2} \tag{4}$$

where the constant C contains the diffusion coefficient and $1/T_2$ is the spin–spin or transverse relaxation rate. Taking the derivative of eq (4) with respect to $\omega_1^{1/2}$, one obtains

$$\frac{\mathrm{d}(1/T_{1\rho})}{\mathrm{d}(\omega_1^{1/2})} = -\sqrt{2}\gamma^4\hbar^2\pi N/20D^{3/2} \tag{5}$$

The limitation on ω_1 that $\gamma H_1 > \gamma H_{\text{loc}}$, where H_{loc} is the local dipolar field strength, is satisfied for a system in the weak collision limit.

The above theory is based on isotropic diffusion and is not directly applicable to the system in which we are interested. For the case of translation in two dimensions only (diffusion parallel to the plane of phospholipid bilayer), the lateral diffusion coefficient may be obtained by a minor modification of eq 5 (Harmon, personal communication):

$$\frac{\mathrm{d}(1/T_{1\rho})}{\mathrm{d}(\omega_1^{1/2})} = -3\sqrt{3}\gamma^4\hbar^2N/40D^{3/2} \tag{6}$$

Brûlet and McConnell (1975) have considered nuclear spin relaxation (T_1 and T_2) in which the dipolar interactions governing relaxation are modulated by translational motion of molecules restricted to the plane of a model membrane. They derived expressions for nuclear T_1 and T_2 relaxation, via electron–nuclear dipole interactions arising from membrane-bound spin labels, which show that the two-dimensional character of the molecular motions influences the nuclear spin-relaxation rates. That we have observed experimentally the frequency of the rotating frame spin–lattice relaxation rates as predicted by eq 6 and that the diffusion coefficients calculated via eq 6 agree reasonably well with independent spin-label results (vide infra) support the use of eq 6 for the determination of lateral diffusion coefficients. To ensure, however, that the application of eq 6 to the lateral or two-dimensional diffusion of phospholipid molecules within a bilayer is indeed proper, it is necessary to prove unequivocally that the frequency-dependent term $(\omega\sigma^2/D)^{1/2}$ is not altered (but for a possible numerical factor) under the transformation from isotropic three-dimensional to restricted two-dimensional translational freedom. An analysis of the derivation by Harmon and Muller (1969) in terms of the effects of two-dimensional molecular translation on nuclear spin relaxation and the resulting expression for $1/T_{1\rho}$ is expected to yield this proof in future work.

Materials and Methods

Chemicals. Dipalmitoyl-DL-α-phosphatidylcholine (DPPC), grade I, crystalline synthetic, was used as obtained from the manufacturer without further purification. Di(perdeuteriopalmitoyl)phosphatidylcholine (DPPC-d_{62}) was purchased as a solution dissolved in 15% chloroform and 85% benzene sealed in a glass ampule. Deuterium oxide, 100 atom %, was used in the preparation of all samples. Dr. S. I. Chan kindly loaned us a prepared sample of DPPC/DPPC-d_{62} with a 50-50 weight composition.

Homogeneous Solutions. The normal DPPC samples were prepared by dissolving the crystalline phospholipid in a minimal amount of analytical reagent grade chloroform, transferring the solution to a preweighed sample tube, and then removing the solvent. The sample tube was then evacuated to 0.1–0.01 mmHg at 70–90 °C for 14–16 h to remove any traces of solvent. The tubes were reweighed, the appropriate quantity of D_2O was micropipetted into the sample tube, nitrogen was flushed through the tube, and the tubes were sealed. The solvent was removed from the DPPC-d_{62}, and the resulting crystalline solid was redissolved in chloroform. The partially deuterated samples were prepared by dissolving the appropriate amount of normal DPPC in the DPPC-d_{62} solution. The rest of this preparation parallels the above description, except the sample was left at room temperature while under vacuum. Homogeneous multilamellar dispersions were formed by centrifuging the samples through a constriction in the sample tube at least 9–10 times while maintaining the sample above the phase transition temperature.

Sample Stability. The normal DPPC samples were stable over long periods of time, but the deuterated samples had a lifetime which seemed to be a function of temperature and/or purity of the sample. We were unable to achieve reproducible results after 3 weeks with one of the deuterated samples.

NMR Equipment. The collective proton resonance of DPPC was monitored at 44.376 MHz using a CPS-2 pulsed NMR spectrometer and PGS-2 pulse programmer built by Spin-Lock Electronics Ltd. of Canada in conjunction with a JEOL Model 4H-100 electromagnet and power supply. Variable-temperature capability was supplied by a JEOL Model JES-VT-3 temperature controller, enabling the use of a constant temperature within ±0.5 °C. The free induction decay (FID) was sampled with a Princeton Applied Research CW-1 boxcar integrator, and the resulting output was read on a Hewlett-Packard Model 3034A digital voltmeter.

NMR Experiment. Rotating frame spin-lattice relaxation time ($T_{1\rho}$) experiments were performed according to the spin-locking method described by Solomon (1959). Immediately after application of a 90° pulse, a second rf pulse of magnitude H_1, but phase shifted by 90° with respect to the initial pulse, is applied for a time t. The magnitude of the FID is then monitored as a function of t, the length of time the phase-shifted "spin-locking" pulse is applied. The time constant for the resulting exponential decay of the FID magnitude is $T_{1\rho}$:

$$M(t) = M_0 \exp\{-t/T_{1\rho}\} \tag{7}$$

The strength of the "spin-locking" field H_1 was determined by measuring the pulse length (t_w) required for a single pulse of magnitude H_1 to produce a null in the FID, the condition for the null being $n\pi = \gamma H_1 t_w$. Successive increments in t_w were used corresponding to pulse lengths of π, 2π, etc., up to 7π for most cases, and then the average time $\langle t_w \rangle$ was used for the calculation of H_1. The uncertainty in H_1 is on the order of 5%, the greatest probable error occurring for small values of H_1. The values of H_1 experimentally employed in this study were at least a factor of ten larger than the local dipolar field H_{loc} which can be calculated to be 0.36 G from the second moment data reported by Veksli et al. (1969) for DPPC/D_2O.

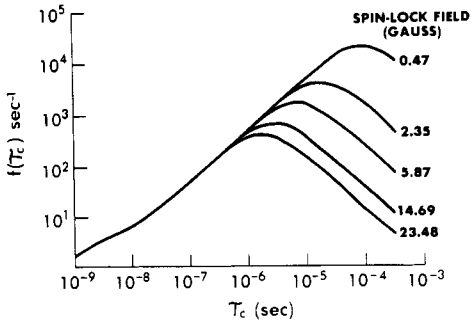

FIGURE 1: Log-log plot of $f(\tau)$, which represents the term in brackets on the right-hand side of eq 8, vs. the correlation time τ for intramolecular rotational motions causing relaxation. The Larmor precession frequency, ω_0, is 2π (44.376 MHz), corresponding to the frequency of our pulsed NMR spectrometer, and the family of curves represent various values of ω_1, the spin-locking rf field angular frequency ($\omega_1 = \gamma H_1$), representative of those values used in our experiments.

The proton FID of DPPC in the smectic liquid-crystalline phase decays extremely rapidly. The FID appears to decay with two components, but $T_{1\rho}$ values, determined from sampling the purely fast, purely slow, and then at the intersection of the two components, are virtually identical. In all subsequent experiments, the boxcar integrator was gated on for 50 μs, starting 20 μs after the receiver was gated on following the rf pulses; this procedure enabled us to obtain maximum signal amplitude.

Results

Prerequisite to utilizing the frequency dependence of the spin-lattice relaxation rate for determining the diffusion coefficient D, it must be demonstrated that a significant contribution to the observed relaxation rates arises from translational diffusion. The rotating frame spin-lattice relaxation rate due to intramolecular dipole-dipole interactions is given by (Jones, 1966):

$$\left(\frac{1}{T_{1\rho}}\right)_{\text{INTRA}} = K\left[\frac{3}{2}\left(\frac{\tau}{1+\omega_1^2\tau^2}\right) + \frac{5}{2}\left(\frac{\tau}{1+\omega_0^2\tau^2}\right) + \left(\frac{\tau}{1+4\omega_0^2\tau^2}\right)\right] \tag{8}$$

in which ω_0 is the Larmor precessional frequency ($\omega_0 = \gamma H_0$), ω_1 is the angular frequency of the spin-locking field H_1 ($\omega_1 = \gamma H_1$), τ is the correlation time for the rotational motion causing relaxation, and K is a constant that depends on the nucleus being studied. Figure 1 gives the functional forms of eq 8 for $\omega_0 = 2\pi \times 44.376$ rad/s and several values of ω_1. Of interest is the lack of a dependence on ω_1 in the motional narrowing limit, $\omega_1^2\tau^2 \ll 1$. Because ω_0 remains constant, eq 8 becomes, in this region

$$\frac{1}{T_{1\rho}} \simeq K'\tau \tag{9}$$

The temperature dependence of the relaxation rate is reflected by the temperature (T) dependence of the correlation time which may be expressed in terms of the activation energy (E_{act}) for the motion effecting relaxation in an Arrhenius equation (James, 1975b):

$$\tau = \tau_0 \exp\{E_{act}/RT\} \tag{10}$$

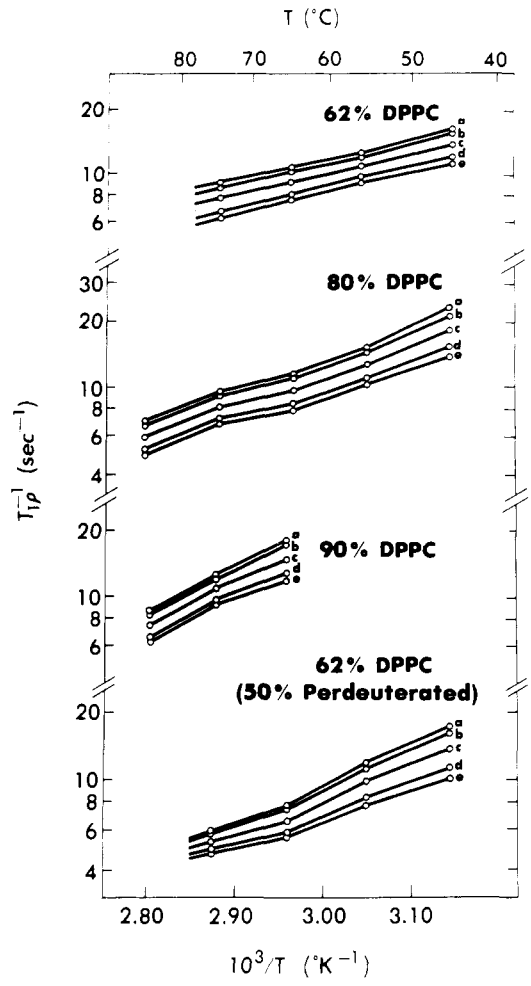

FIGURE 2: Temperature dependence of the proton rotating frame spin-lattice relaxation rate ($1/T_{1\rho}$) showing the variation with spin-locking field strength H_1. The dependence is shown for three normal dipalmitoylphosphatidylcholine (DPPC) samples and one 50% perdeuterated dipalmitoylphosphatidylcholine sample. The spin-locking field strengths H_1 (G) are (a) 2.3, (b) 3.36, (c) 5.97, (d) 9.33, and (e) 11.29.

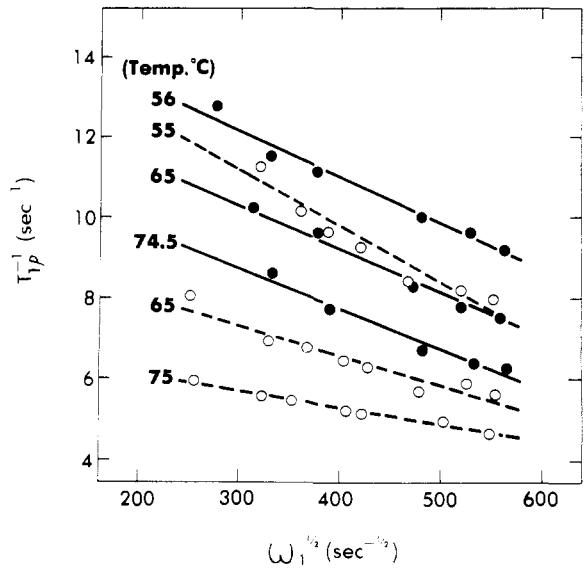

FIGURE 3: Proton rotating frame spin-lattice relaxation rate ($1/T_{1\rho}$) dependence on the spin-locking field angular frequency ω_1, where $\omega_1 = \gamma H_1$, at three temperatures for 62% (w/w) (= 4.3 mol %) DPPC (solid line and filled circles) and 2.1 mol % DPPC/2.2 mol % DPPC-d_{62} (corresponding to 62% w/w) (dashed line and empty circles).

Figure 2 shows the temperature dependence of the rotating frame spin-lattice relaxation rate determined experimentally for the protons in DPPC and DPPC/DPPC-d_{62} in the liquid-crystalline lamellar mesophase with deuterium oxide. The behavior is that described by eq 9 and 10 which indicates we are observing relaxation in the extreme narrowing limit. However, a frequency dependence of the relaxation rates is also apparent in Figure 2, suggesting that the intramolecular relaxation picture is not compatible with the observed results and, therefore, that intermolecular effects may be important.

Figure 3 represents the experimental results for deuterium dilution experiments, which add further support to the case for intermolecular relaxation. Samples in which the phospholipid is comprised of 50 mol % of DPPC-d_{62} and 50 mol % of DPPC were studied with the expectation that if, in fact, intermolecular relaxation is important the experimental $T_{1\rho}$ relaxation rates would be diminished with respect to those of the normal protonated DPPC sample. The results in Figure 3 show that the $T_{1\rho}$ relaxation rates, for the sample in which half the DPPC molecules are perdeuterated, are 25–35% lower than the relaxation rates for the sample with normal DPPC, from which the contribution to the overall relaxation rate due to intermolecular effects is estimated to be ca. 50%. Thus, the relative motion of protons due to translational diffusion of adjacent DPPC molecules in the lamellar mesophase is a significant mechanism for longitudinal nuclear spin relaxation in the rotating frame. The contribution is well resolved from other relaxation contributions and appears to arise experimentally with a linear dependence of the relaxation rate on $\omega_1^{1/2}$ for the DPPC samples studied, as shown in Figures 3 and 4.

There remains the possibility that the observed frequency dependence of the $T_{1\rho}$ values arises from mixing of the dipolar and Zeeman fields (Thompson and Kydon, 1974):

$$\frac{1}{T_{1\rho}} = \frac{1}{T_{1X}}\left[\frac{H_1^2}{H_{loc}^2 + H_1^2}\right] + \frac{1}{T_{1D}}\left[\frac{H_{loc}^2}{H_{loc}^2 + H_1^2}\right] \quad (11)$$

where T_{1D} is the spin-lattice relaxation time in the local dipolar field. However, utilizing a value for H_{loc} calculated from the second moment data of Veksli et al. (1969) and the T_{1D} data presented by Valic et al. (1976), it is found that less than 10% of the experimentally observed change in $T_{1\rho}$ with H_1 variation could be ascribed to this source. Consequently, the observed frequency dependence of $T_{1\rho}$ for the protons in DPPC in the liquid-crystalline lamellar phase is attributed to translational motion.

The least-squares slope, C, of eq 4 was evaluated for each experiment and then used to calculate the lateral self-diffusion coefficients via eq 6. Figure 5 is an Arrhenius plot of the resulting diffusion coefficients from which activation energies, E_{act}, for the diffusive process are calculated. Table I lists the values of the diffusion coefficients and activation energies calculated for each sample at the various temperatures at which the experiment was done.

The frequency dependence of the relaxation rates is accurate to within 5% if $(\omega_1\sigma^2/D)^{1/2} \leq 2$ (Burnett and Harmon, 1972). Using an estimate of 8 Å for σ, this condition is satisfied for all cases reported here. Our estimate of σ was obtained using known values of the DPPC bilayer dimensions (Levine et al., 1968; Schoenborn, 1976) and the apparent molar volume of unsonicated DPPC solutions, as extrapolated from results given by Sheetz and Chan (1972).

Discussion

The lateral diffusion coefficient is a physically measurable parameter representing the overall molecular mobility of phospholipid molecules in a membrane system. The fluidity

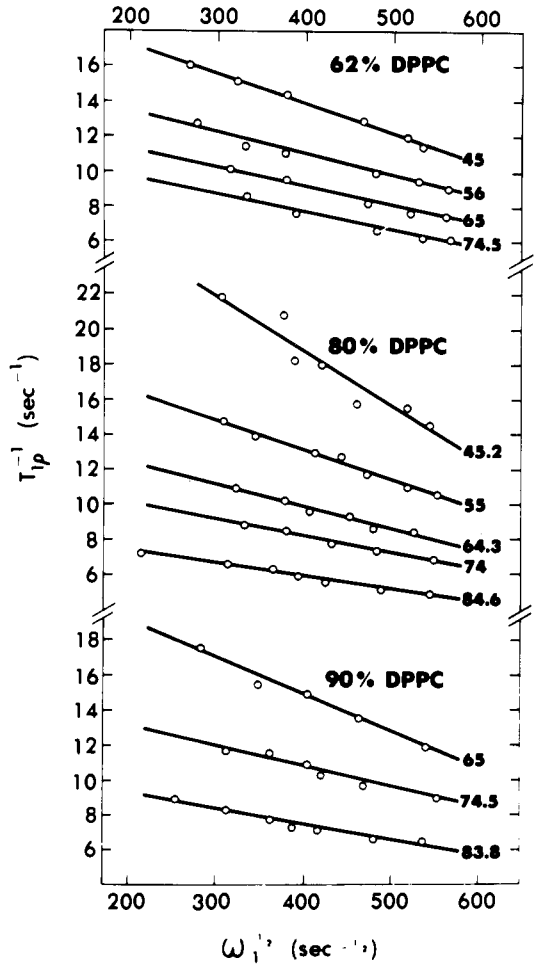

FIGURE 4: Proton rotating frame spin-lattice relaxation rate ($1/T_{1\rho}$) dependence on the spin-locking field angular frequency ($\omega_1 = \gamma H_1$) for 62, 80, and 90% (w/w) DPPC. Each sample was examined at several temperatures.

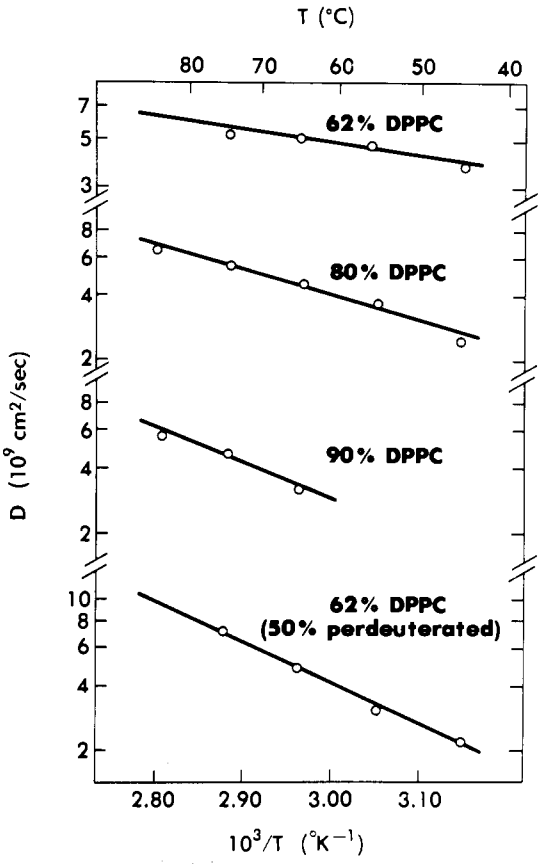

FIGURE 5: Lateral self-diffusion coefficient (D, cm^2/s) as a function of inverse temperature for 62, 80, and 90% (w/w) DPPC and 2.1 mol % DPPC/2.2 mol % DPPC-d_{62} [which corresponds to 62% (w/w) DPPC] dispersed in the lamellar mesophase.

TABLE I: Lateral Self-Diffusion Coefficients and Calculated Activation Energies for the Diffusion Process for Dipalmitoylphosphatidylcholine Multilamellar Dispersions.

% DPPC (w/w)	Temp (°C)	$D \times 10^9$ (cm^2/s)	E_{act} (kcal/mol)
62[a]	45	3.8	
	56	4.7	
	65	5.1	2.7
	74.5	5.3	
80	45.2	2.5	
	55	3.7	
	64.3	4.6	5.3
	74	5.5	
	84.6	6.5	
90	65	3.2	
	74.5	4.7	7.5
	83.8	5.8	
62[b] (half perdeuterated)	45	2.2	
	55	3.1	
	65	4.8	8.5
	75	7.1	

[a] 62% (w/w) DPPC is equivalent to 4.3 mol %. [b] 2.2 mol % DPPC plus 2.1 mol % DPPC-d_{62}, yielding a total of 4.3 mol % of the phospholipid in this sample.

of a bilayer is more a composite description, than a physical parameter, of the effects from all determinants contributing to the dynamic state of the membrane system. The diffusion coefficient, as studied in artificial and natural membranes, has proven useful in providing insight into the dynamics of molecular events in these systems. Diffusion coefficients have been determined for lecithin molecules and other related molecules distributed in natural membrane bilayers and for proteins residing on the surface of the membrane of cells (Stier and Sackmann, 1973; Lee et al., 1973; Scandella et al., 1972; Schlessinger et al., 1977). This information itself is useful in understanding the mechanisms by which these cell-surface molecules act and allows more detail to be assigned to membrane model theories. There is also, provided that a suitable model is chosen to describe the system's behavior, information contained within the diffusion coefficient pertaining to the microscopic events of translational motions, which yield on the macroscopic level the process of translational diffusion.

Assuming the DPPC molecules in the bilayer are arrayed in a hexagonal lattice (Levine et al., 1968; Devaux et al., 1973), the diffusion coefficient can be related to the mean time between jumps τ or the diffusive jump frequency ν by eq 12:

$$\tau = \sigma^2/4D \text{ or } \nu = 4D/\sigma^2 \qquad (12)$$

Mean jump times were calculated for our system using the estimated value of 8 Å for σ and the experimentally determined diffusion coefficients; the values range from 2.3×10^{-7} to 7.3×10^{-7} s. Thus, the DPPC molecules will be exchanging lattice sites, on the average, at a rate $\geq 1.4 \times 10^6$ s^{-1}.

The significance here is that we are able to extract the lateral self-diffusion coefficient directly from the experimental NMR data without making any a priori assumptions about molecular

geometries or motional time scales. For the most part, diffusion studies by EPR spin-label methods, NMR relaxation-time measurements, or observation of the fluorescence of optically excited probes involves estimations and assumptions about correlation times and molecular geometries and which electromagnetic interactions dominate the observed spectral results (Edidin, 1974). In fact, with these techniques eq 12 is employed in reverse to estimate the diffusion coefficient. Values calculated for the diffusion coefficient are then only limits.

Träuble and Sackmann (1972), using EPR methods, investigated the lateral diffusion of a spin-labeled steroid molecule in aqueous dispersions of DPPC at temperatures above the phase transition. The diffusion coefficient they report for the steroid molecules in these multilayers is 10^{-8} cm^2/s. The diffusion coefficients we have determined from the frequency dependence of $T_{1\rho}$, as reported in Figure 5 and Table I, can be compared with the value obtained from the above-mentioned EPR spin-label technique. The diffusion coefficient obtained via the EPR spin-label technique implies that the diffusion is faster than we measure. Although it is likely that the different values obtained from the two techniques are due to inherent differences in the methods and because the molecules whose diffusion is studied in each case are different, it is possible that the diffusion may indeed be faster in the presence of the spin labels which could perturb the bilayer.

With the implementation of this novel technique for the direct determination of lateral diffusion coefficients in a model membrane system, it is necessary to consider how bilayer fluidity is reflected in the diffusion coefficient. Our preliminary investigations into this matter deal with the temperature and concentration dependence of the diffusion coefficient for DPPC bilayers above the phase-transition temperature. Over the temperature range studied, a moderate dependence of the diffusion coefficient on temperature does exist (see Figure 5) as one would intuitively expect. It is also evident that over the temperature range examined the diffusive mechanism does not change, as only a single Arrhenius activation energy is obtained from the plots of D vs. $1/T$ (see Table I). There is not enough data to justify a detailed interpretation of any variation of the diffusion coefficient with concentration of the DPPC samples, although there does appear to be a trend that the diffusion coefficient decreases with increasing concentration. This type of trend may be speculated to represent changes in the lattice arrangement possibly due to effects of differential amounts of water around the polar head groups, but more work must be done to rigorously support any interpretation at all.

We hope to utilize this technique in the study of the dynamic state of the membrane system by characterizing physical changes that may take place within a bilayer in terms of the effects on the diffusion coefficient. To evaluate how changes in the diffusion coefficient may be used in understanding fluidization and other changes in bilayer dynamics, it is necessary to perform a variety of the conventional experiments which have been used in the study of bilayer fluidity, for example, observation of the effects on phospholipid translational diffusion in the bilayer due to molecules such as cholesterol and its derivatives, from variations in fatty acyl chain length, or of the presence of unsaturation in the fatty acyl chains.

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Kinetics of Lipid–Protein Interactions: Interaction of Apolipoprotein A-I from Human Plasma High Density Lipoproteins with Phosphatidylcholines†

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ABSTRACT: We have studied the interaction of liposomes of dipalmitoyl- and dimyristoylphosphatidylcholine (DPPC and DMPC, respectively) with apolipoprotein A-I (apoA-I) from human plasma high density lipoproteins (HDL) by chromatography on Sepharose 4B and kinetic turbidimetric methods. The incubation and chromatography of apoA-I and highly turbid DMPC mixtures at the phospholipid gel→liquid crystalline transition temperature, T_c, revealed the complete incorporation of DMPC into a lipid–protein complex which scattered very little light. Similar experiments conducted above (30 °C) and below (22 °C) T_c showed much less complex formation even when incubated for much longer times. Over similar time periods DPPC failed to associate completely with apoA-I even at its T_c (41.5 °C). We have determined the rates of association of DPPC and DMPC with apoA-I as a function of temperature by measuring the rate of disappearance of liposomal turbidity. Below and above the T_c of DMPC, the rate of its association with apoA-I was slow, but increased by a factor of 500 to 1000 at T_c. A similar set of experiments substituting DPPC for DMPC showed very slow reaction rates even at the T_c of the former lipid. The relatively high rate of interaction of apoA-I and DMPC at T_c was assigned to the increased permeability of the DMPC matrix produced by a high percentage of boundary lipid which we viewed as a lattice defect between coexisting gel and liquid crystalline phases. The slow decrease in rate between T_c and T_c + 15 °C was assigned to the retention lattice defects composed of ordered and disordered populations of DMPC. The absence of a fast reaction between DPPC and apoA-I was probably due to its lower permeability even at its T_c. These results were suggested to be important in predicting the rate of transfer of apoA-I from HDL to phospholipids and may be important in regulating the activity of the enzyme, lecithin:cholesterol acyltransferase.

Apolipoprotein A-I (apoA-I), the major protein of the human plasma high density lipoproteins (HDL)[1] (Jackson et al., 1976), has been the subject of numerous structural studies in several laboratories. Its amino acid sequence has been reported by Baker et al. (1974). ApoA-I readily self-associates (Vitello & Scanu, 1976) and undergoes a helix→random coil transition upon heating (Tall et al., 1976; Gwynne et al., 1975), solute perturbations (Gwynne et al., 1974; Reynolds, 1976), or pH shifts (Gwynne et al., 1974, 1975). Apo-I interacts, presumably via the hydrophobic effect, with alkanes (Stone & Reynolds, 1975), lysophosphatides (Verdery & Nichols, 1974; Haberland & Reynolds, 1975), sodium dodecyl sulfate (Reynolds & Simon, 1974), and certain phosphatidylcholines. Assman & Brewer (1974) observed negligible interaction of apoA-I with egg phosphatidylcholine whereas Middelhoff et al. (1976) isolated substantial quantities of lipid–protein complexes from mixtures of apoA-I and DMPC or DPPC. However, they found that a large fraction of apoA-I did not associate with the lipids. Rosseneu et al. (1976) found a large negative enthalpy of interaction of apoA-I with DMPC and lysolecithin which they assigned to the enthalpy of binding;

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[1] Abbreviations used: PC, phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; T_c, gel → liquid crystalline transition temperature; apoA-I, apolipoprotein A-I, the major protein of human high density lipoproteins; HDL, high density lipoprotein; Ans, 8-anilino-1-naphthalenesulfonate; Tempo, 2,2,6,6-tetramethylpiperidinyl-1-oxy.

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