A PROTON NMR MOMENT STUDY OF THE GEL AND LIQUID-CRYSTALLINE PHASES OF DIPALMITOYL PHOSPHATIDYLCHOLINE

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ABSTRACT Measurements of the proton second and fourth moments have been undertaken for multilamellar dispersions, in excess water, of protiated and chain-perdeuterated dipalmitoyl phosphatidylcholine (DPPC) in the temperature range −20 to 50°C. The comparison of the measured moments with the rigid lattice M_2 and the calculated second moment values in the presence of certain motions gives insight into the dynamic structure of the methylene chains of DPPC. This study demonstrates that at −15°C there is still a significant amount of methylene chain motion or disorder in DPPC. At 35°C the moment values indicate that the methylene chains are not in the fully extended all-*trans* conformation and they may also be rotationally disordered. At the pretransition there is a decrease in magnitude of the proton second moments, which can be accounted for by an increase in the lateral diffusion rate to a value greater than 10^{-11} cm^2/s. This work suggests that at temperatures just below the main transition the chain conformation is considerably different from the common model structure (P_β') in which the chains are fully extended. In the liquid-crystalline phase the proton moment data are in agreement with data from other techniques on the liquid-like nature of the methylene chains. It is demonstrated how the values of the moment ratio $M_{4r}/(M_{2r})^2$, which are relatively constant within each phase, can be used to calculate the molar fractions of coexisting liquid-crystalline and gel-phase phospholipid at temperatures near the main transition.

INTRODUCTION

It has been demonstrated (Stockton et al., 1977; Gally et al., 1979; Rance et al., 1980) that multilamellar dispersions of synthetic phospholipids in the liquid-crystalline phase are good model systems for the physical state of phospholipids in natural biological membranes above their phase transition. The phospholipid model membrane system of dipalmitoyl phosphatidylcholine (DPPC) in water has recently been studied by a considerable number of physical techniques, including Raman spectroscopy (Pink et al., 1980; Yellin and Levin, 1977), infrared spectroscopy (Cameron and Mantsch, 1978; Cameron et al., 1980), x-ray diffraction (Janiak et al., 1976; Brady and Fein, 1977; Inoko and Mitsui, 1978), neutron diffraction (Zaccai et al., 1979), ultrasonic studies (Mitaku et al., 1978), dielectric studies (Shepherd and Büldt, 1978), calorimetry (Albon and Sturtevant, 1978; Lentz et al., 1978), electron paramagnetic resonance (Luna and McConnell, 1977; Delmelle et al., 1980; Marsh, 1980), deuteron magnetic resonance (Davis, 1979), and proton magnetic resonance (Bloom et al., 1978; Pope and Cornell, 1979).

Magnetic resonance techniques have played an important role in the understanding of the motional properties of DPPC dispersions. From deuteron magnetic resonance (^{2}H NMR) of a

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series of samples containing DPPC selectively deuterated at different sites, the motional state of the hydocarbon chains in the liquid-crystalline phase has been described quantitatively (Seelig and Seelig, 1974) by the variation of the methylene order parameters, S_{CD} with chain position where $S_{CD} = \frac{1}{2} \langle 3 \cos^2 \theta - 1 \rangle$ and θ is the angle between the C—D bond vector and the symmetry axis of the motion. In a recent ^{2}H NMR study of multilamellar chain-perdeuterated DPPC (Davis, 1979), the order parameter profile for the liquid-crystal phase was derived from a spectrum simulation, and from the values of the spectral moments it was deduced that the gel phase has considerable disorder at 20°C but is more solid-like at 0°C.

Although the proton magnetic resonance (^{1}H NMR) spectra of multilamellar lipid dispersions are in principle understood in terms of dipolar interactions between many pairs of nuclear spins (Wennerström and Lindblom, 1977; Bloom et al., 1977), it is difficult to derive quantitative information from them. The broad, featureless ^{1}H NMR spectra of multilamellar DPPC have no resolved splittings or chemical shifts, and in general their linewidth does not relate directly to molecular order (Wennerström, 1973). A set of useful quantitative parameters of the ^{1}H NMR lineshape for lipid systems are the spectral moments and these have been used in several studies of multilamellar dispersions (Jantzen and Dunell, 1963; Andrew, 1950; Veksli et al., 1969; Peters and Kimmich, 1978; Jeffrey et al., 1979; Trahms and Boroske, 1979; Bloom et al., 1978). In particular, the second moment for multilamellar DPPC was found to decrease considerably in the temperature range 20° to 100°C and to undergo a sharp drop at the gel to liquid-crystalline phase transition at 41°C (Veksli et al., 1969).

In the present work, detailed careful measurements have been made of the ^{1}H NMR moments M_2 and M_4 as a function of temperature for DPPC dispersed in excess water. To separate the contributions of the hydrocarbon chains from that of the head groups, two samples were used: protiated DPPC and chain-perdeuterated DPPC. The proton moments have been analyzed with the objective of understanding more about the phase behavior of DPPC in excess water, and comparisons are made with the ^{2}H NMR spectral moments for chain-perdeuterated DPPC (Davis, 1979), illustrating how the two techniques complement each other.

MATERIALS AND METHODS

Protiated L-α-phosphatidylcholine, β,γ,dipalmitoyl and chain-deuterated L-α-phosphatidylcholine, β,γ,diplamitoyl (~ 98% deuterated) were obtained from Calbiochem-Behring Corp., San Diego, Calif. and Lipid Specialties, Boston, Mass., respectively, and were used without further purification. D_2O (99.7% deuterated) was purchased from Merck Sharp & Dohme Canada Ltd., Montreal.

The protiated DPPC and chain-deuterated DPPC lipids were mixed with D_2O (50–60% by weight) by stirring in 7.5-mm NMR sample tubes and were annealed at 50°C for at least 1 h before use. The purity of each sample was verified by thin layer chromatography both before and after the NMR measurements. This step was particularly important because very small amounts of lyso-phosphatidylcholine were found to have a marked effect on the ^{1}H NMR moments especially in the vicinity of the phase transitions. Samples left at room temperature for a few days were found to contain sufficient lyso-phosphatidylcholine to alter seriously the values of the ^{1}H NMR moments. Between ^{1}H NMR measurements the samples were stored at −20°C.

The ^{1}H NMR results were acquired with a Bruker SXP 4-100 NMR spectrometer operating at 90 MHz (Bruker Spectrospin Ltd., Canada). The free induction decays were digitized by a Nicolet 1090AR dual channel digital oscilloscope (Nicolet Instrument Corporation, Madison, Wis.), signal averaged in an Intel 8080A microprocessor-based computer (Intel Corp., Santa Clara, Calif.), and

Fourier transforms and moment calculations were made with a Nicolet B-NC-12 minicomputer. Temperature control was achieved with an airflow device, with an accuracy of 0.5°C, although a temperature gradient of ~ 1°C existed across the sample. After each temperature change, there was a waiting period of 20 min to 2 h, depending on the temperature difference, which was employed for sample equilibration.

The spectrometer frequency was set exactly on resonance and the in-phase and out-of-phase free induction decays (FID) after a 90° pulse were collected with a 2-μs dwell time in 2K data points. Digitization was started precisely at the center of the 1-μs 90° pulse (Barnaal and Lowe, 1963). To remove coherent receiver and pulse noise, a 180° pulse was applied 20 ms before every other 90° pulse, and alternate scans were subtracted from the accumulative data memory.

The first 8 μs of the FID was unobtainable because of the dead time of the receiver. Since this information is essential for high-fidelity Fourier transform spectra, the in-phase FID was extrapolated to $t = 0$ by means of the function $S(t) = S(o)(1 - M_2 t^2/2)$, and the out-of-phase FID was extrapolated using the function $S(t) = S(o)M_1 t$. These functions were observed to fit all the FID out to times of at least 14 μs from the pulse. The extrapolated FID were Fourier transformed and M_2 and M_4 were calculated from the spectra. The validity of this procedure was illustrated by the integrity of the spectrum, the flatness of the base line, and the comparison between the calculated M_2 and that used for extrapolation. The receiver linearity at short times was found to be better than 0.1% in the observation of the FID for H_2O. The error in the measurement of M_2 was estimated to be <5%.

MOMENTS

For any rigid solid the rigid lattice proton dipolar second moment, M_2, may be calculated from (Abragam, 1961, p. 112)

$$M_2 = \frac{3}{4}\gamma^4 h^2 I(I+1)\frac{1}{N}\sum_{j=1}^{N}\left[\sum_{j\neq k}\frac{(1-3\cos^2\theta_{jk})^2}{r_{jk}^6}\right], \tag{1}$$

where the summation in M_2 is over all dipolar coupled proton pairs, r_{jk} is the separation between the protons, and θ_{jk} is the angle between r_{jk} and the external magnetic field. Although the rigid-lattice M_2 is independent of molecular motion, in the presence of a motion with a correlation time $\tau_c \ll 1/\sqrt{M_2}$, the experimentally measured second moment is smaller than M_2 and is called the residual second moment, M_{2r}.

For the methylene chains of a phospholipid molecule in a multilamellar dispersion, two classes of dipolar coupled proton pairs can be distinguished: intramolecular and intermolecular. In general these types of interactions will be affected differently by molecular motion. In the presence of rapid axial rotation of the phospholipids, the intramolecular dipolar interactions are projected onto the rotation axis so that $M_{2r}(\theta)$(intramolecular) = $M_{2r}(0)$(intramolecular)$(P_2[\cos\theta])^2$, and $\langle M_{2r}$(intramolecular)$\rangle = 1/5\, M_{2r}(0)$(intramolecular), where θ is the angle between the rotation axis and the external magnetic field (Wennerström, 1973) while the magnitude of the intermolecular dipolar interactions is reduced considerably (Andrew, 1950). When rapid lateral diffusion occurs as well, the intermolecular M_{2r} is reduced to zero.

In the liquid-crystalline phase of DPPC, where M_{2r} (intermolecular) is averaged out by the rapid lateral diffusion, the methylene proton M_{2r} may be separated into an intramethylene contribution and an intermethylene contribution (Bloom et al., 1978):

$$M_{2r}(\text{methylene}) = \frac{1}{N}\sum_{j=1}^{N} m_2 S_{j\text{HH}}^2 + M_2(\text{intermethylene}), \tag{2}$$

where m_2 is the evaluation of the angular independent part of Eq. 1 for a methylene proton pair and $S_{j\mathrm{HH}}$ is the proton dipolar order parameter of the jth proton pair. The methyl contribution to M_{2r} is treated in a similar way using parameters appropriate for that group. For comparison the second moment for deuteron NMR spectra from methylene chains in the L-α phase is given by:

$$M_{2r} = \frac{1}{5}\left(\frac{3}{4}e^2qQ/h\right)^2 \frac{1}{N}\sum_{j=1}^{n} S_{j\mathrm{CD}}^2, \tag{3}$$

where $e^2q\,Q$ is the electric quadrupole coupling constant for a methylene C—D group, and the dipolar contribution to the deuteron M_{2r} is neglected relative to the much larger quadrupolar term given by Eq. 3.

RESULTS

Proton NMR spectra have been acquired for a dispersion of protiated DPPC in D_2O (50% by weight) in the temperature range −30 to 55°C. From each spectrum the residual second and fourth moments were calculated using the relation:

$$M_{2nr} = \int_{-\infty}^{\infty} \omega^{2n}\, \mathrm{f}(\omega)\mathrm{d}\omega \Big/ \int_{-\infty}^{\infty} f(\omega)\mathrm{d}\omega, \tag{4}$$

and adjusting the frequency scale so that $f(0)$ was at the center of the spectrum (i.e., $M_1 = 0$). The actual integration limits used were the points where the signal disappeared into the noise. As shown in Fig. 1 *a*, M_{2r} decreases by more than an order of magnitude in this temperature range, and there are two sharp drops—one presumably from the pretransition at 35.5°C and the other due to the gel to liquid-crystalline phase transition at 41°C. To examine the behavior of the headgroup alone and to derive the moments for the methylene chains, ^{1}H NMR measurements were carried out on chain-deuterated DPPC in D_2O (50% by weight) in the temperature range −20 to 50°C. For this sample the chain melting transition occurs at 37°C, 4°C lower than that in protiated DPPC. The headgroup second moment, plotted in Fig. 1 *b*, is about one quarter as large as that for the whole molecule at the same temperature, and it also exhibits a drop at the pretransition temperature. From the experimental moments of the headgroup and the whole molecule, the residual moments for the methylene chains alone were derived from the relation:

$$M_{2nr}\,(\text{chains}) = \frac{N_\mathrm{H} + N_\mathrm{C}}{N_\mathrm{C}} M_{2nr}\,(\text{molecule}) - \frac{N_\mathrm{H}}{N_\mathrm{C}} \mathrm{M}_{2nr}\,(\text{headgroup}), \tag{5}$$

where N_H (=18) and N_C (=62) are the number of protons in the headgroup and the methylene chains of DPPC, respectively. The temperature scale for M_{2nr} headgroup was shifted up by 4°C to adjust for the difference in phase transition temperatures for the two samples. The temperature dependence of the methylene chain M_{2r} is plotted in Fig. 1 *c*.

The Gel Phase

Several recent x-ray and neutron diffraction studies on acqueous dispersions of DPPC (Tardieu et al., 1973; Janiak et al., 1976; Zaccai et al., 1979) have been interpreted to indicate that below the pretransition, the methylene chains are fully extended in planer bilayers, tilted

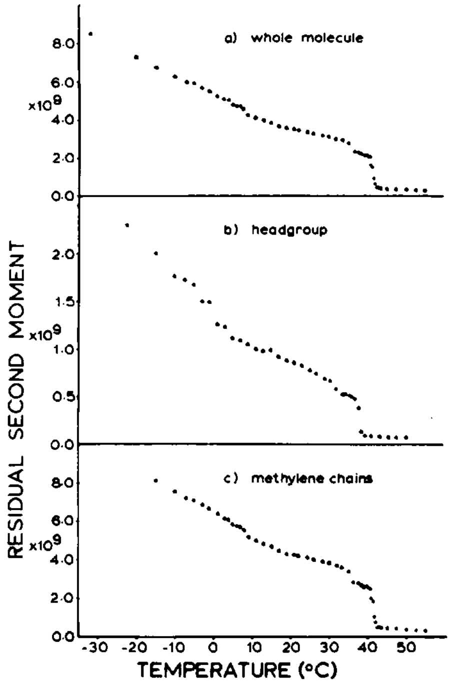

FIGURE 1 Proton residual second moments (second^{-2}) are plotted as a function of temperature for: *a*, the whole molecule; *b*, the headgroup; and *c*, the methylene chains of DPPC dispersed in excess water. The error in the moment measurements is 5% or less.

relative to the bilayer normal, and possess rotational disorder. This has been called the L'_β phase. In another x-ray study (Brady and Fein, 1977) on more dilute DPPC dispersions, it was concluded that the methylene chains possessed some liquid-like character in the gel phase. Although NMR on unoriented samples cannot easily give information on the molecular tilt angle, the hypothesis that the methylene chains are in a fully extended all-*trans* conformation can be tested with the aid of the values of the ^{1}H NMR residual second moments.

The calculated rigid-lattice M_2 for palmitic acid chains in the all-*trans* conformation, on the assumption of C—H and C—C distances of 1.094 and 1.54 Å and tetrahedral angles, is $2.0 \times 10^{10}\ s^{-2}$ (Lawson and Flautt, 1965). If the terminal methyl groups undergo rapid rotation, an M_{2r} of $1.7 \times 10^{10}\ s^{-2}$ should be observed (Lawson and Flautt, 1965); and if the whole molecule rotates about its long axis, M_{2r} should decrease to $5.3 \times 10^9\ s^{-2}$ (Jeffrey et al., 1979). The additional presence of rapid lateral diffusion, which averages all the intermolecular interactions, further reduces M_{2r} to $3.8 \times 10^9\ s^{-2}$ (Jeffrey et al., 1979). The interaction of the two chains on each molecule, which introduces a small error when relating these calculations to phospholipids, shall be neglected.

At 35°C, just below the pretransition, the methylene chain proton M_{2r} for DPPC is $3.4 \times 10^9\ s^{-2}$—slightly less than the value $3.8 \times 10^9\ s^{-2}$ which one would expect for fully extended chains rotating about their long axis and undergoing rapid lateral diffusion. In fact, the experimental M_{2r} should be compared with a larger number since, for reasons which shall be elaborated in the next section, it is unlikely that lateral diffusion is sufficiently fast to completely eliminate M_{2r} (intermolecular) at temperatures below the pretransition. If the methylene chains remain in the fully extended all-*trans* conformation, they must, to account for the small M_{2r} value, undergo not only rapid long axis rotation disorder but also rapid rigid body motion about an axis perpendicular to the long axis. This seems improbable, and a more plausible interpretation of the M_{2r} data at 35°C is in terms of a model where the DPPC methylene chains are not in the all-*trans* conformation and they may also be rotationally disordered.

As the temperature is decreased in the gel phase, the measured M_{2r} increases monotonically, but at -15°C, M_{2r} is 8×10^9 s^{-2}, only 40% of the rigid lattice value, which indicates that a considerable amount of motion and/or disorder still occurs in the system. Remarkably, in another study (Peters and Kimmich, 1978), it was discovered that at temperatures well below 200°K the proton second moment of a DPPC dispersion in 40% D_2O was still appreciably less than the rigid lattice value.

The results from several other physical techniques have led to similar conclusions about the structure of gel-phase DPPC in excess water. ^{2}H NMR studies (Davis, 1979) also support a model where the gel-phase DPPC chains are not in the all-*trans* conformation. These studies further indicate the presence of rotational disorder at temperatures well below the pretransition temperature. Raman scattering studies (Yellin and Levin, 1977; Pink et al., 1980) have been interpreted to indicate the presence of gauche conformations in gel-phase DPPC. This interpretation is questioned by Cameron et al. (1980), who on the basis of infrared spectroscopy studies propose the existence of a broad phase transition for fully hydrated DPPC from a nearly hexagonal chain packing structure that permits long axis chain motions at 10°C to an orthorhombic or monoclinic crystal structure that prevents long axis motions at -60°C. It is interesting to note the presence of a change in the slope of the ^{1}H M_{2r} vs. temperature curves for DPPC (Fig. 1) at ~10°C, but any connection between this feature and the proposed phase transition is pure conjecture. Using saturation transfer electron paramagnetic resonance, Marsh (1980) measured the temperature dependence of the mobility of spin-labeled probe molecules dissolved in fully hydrated DPPC. He observed a substantial increase in molecular reorientation rate along the long methylene chain axis occurring over a range of temperature centered at ~25°C. Thus for fully hydrated gel-phase DPPC there is agreement on the presence of rapid long axis rotational disorder, but there is still some controversy over the presence of gauche conformers at temperatures below the pretransition temperature.

The Pretransition Region Between 35° and 41°C

Differential scanning calorimetry curves for multilamellar DPPC in excess water (Suurkuusk et al., 1976) reveal a relatively low enthalpy phase transition ($\Delta H \sim 2$ kcal/mol) at 35°C, which is known as the pretransition. As the temperature is increased through the pretransition, a number of physical properties of DPPC dispersions are observed to change: the thickness of the water layer increases by 4 Å (Inoko and Mitsui, 1978), the density decreases by ~1.5% (Mitaku et al., 1978), the permittivity and conductivity at 50 MHz show large increases (Shepherd and Büldt, 1978), EPR spin-label studies show an increase in the spectral component normally associated with fluid-state lipid (Luna and McConnell, 1977), a discontinuous decrease occurs in the intensity of certain Raman lines (Pink et al., 1980; Yellin and Levin, 1977, the intermethylene proton second moment drops by 20% (Trahms and Boroske, 1979), and x-ray studies reveal an increase in bilayer thickness and the appearance of additional reflections corresponding to distances of order 140 Å (Janiak et al., 1976). The most popular interpretation of the structure of DPPC multilamellar dispersions in the pretransition region, based on x-ray studies (Janiak et al., 1976; Tardieu et al., 1973), is the P'_β structure in which the lamella are distorted by a periodic ripple and the hydrocarbon chains are fully extended in their all-*trans* conformation and tilted relative to the bilayer normal.

In the pretransition region, the proton M_{2r} for the DPPC methylene chains varies from 3.2 to 2.7 × 10^9 s^{-2} which should be compared with the value 3.8 × 10^9 s^{-2} calculated for extended hydrocarbon chains undergoing rapid axial rotation and lateral diffusion (Jeffrey et al., 1979). Clearly, the hydrocarbon chains are not in the above configuration, so that any realistic model for the DPPC multilamellar structure in the pretransition region must include a considerable amount of additional motion or disorder.

Comparison of the data in Fig. 1 *a* and *b* indicated that both the headgroup and the methylene chains of DPPC have a reduction in the value of M_{2r} by ~20% at the pretransition. When the proton second moment for the whole molecule is measured for both directions of temperature change at very slow rates (~1°–2°C/h) (Fig. 2), a hysteresis of 2.5°C is observed in the pretransition temperature. A similar hysteresis was found in the temperature dependence for the permittivity and conductivity of DPPC (Shepherd and Büldt, 1978), for the intensity of the EPR spectral component normally identified with fluid-state lipid (Luna and McConnell, 1977), and in the microviscosity as measured by fluorescence depolarization (Lentz et al., 1978). It is interesting that in these cases the hysteresis at the pretransition is much greater than that at the gel-to-liquid crystalline phase transition. A pretransition rate constant of 5 ± 2 min has been measured by fluorimetric studies (Lentz et al., 1978). The hysteresis had been attributed to the slow redistribution of water after the change in water layer thickness that occurs at the pretransition (Inoko and Mitsui, 1978) and to the slow transition of the chains to a different packing arrangement (Lentz et al., 1978).

Although the ^{1}H NMR M_{2r} drops by 20% at the pretransition, there is no corresponding discontinuity in the temperature dependence of the ^{2}H NMR second moment (Davis, 1979) in the same temperature region. In general, a change in the average intramolecular proton-proton dipolar interaction should be reflected in the order parameters for the C—D bond vectors. One must then, in order to make the two measurements compatible, invoke either a special internal motion of the molecule which occurs at a frequency intermediate between the ^{1}H and ^{2}H resonance line widths, or some other motion that leaves the ^{2}H NMR moments unchanged; or one must assign the drop in ^{1}H second moment to a change in M_{2r} (intermolecular). The latter explanation is much more probable.

There are a limited number of mechanisms which can change M_{2r} (intermolecular) without affecting M_{2r} (intramolecular). Minor structural changes such as the establishment of a rippled bilayer or a change in tilt angle or chain–chain spacing could have this effect, but it is very unlikely that they could account for the large drop in the values of the proton M_{2r} at the pretransition: 7 × 10^8 s^{-2} for the chains and about 1 × 10^8 s^{-2} for the headgroup. The onset, however, of lateral diffusion to a dispersion of DPPC molecules already rapidly rotating can diminish the proton M_{2r} by as much as 1.5 × 10^9 s^{-2} (Jeffrey et al., 1979). The actual

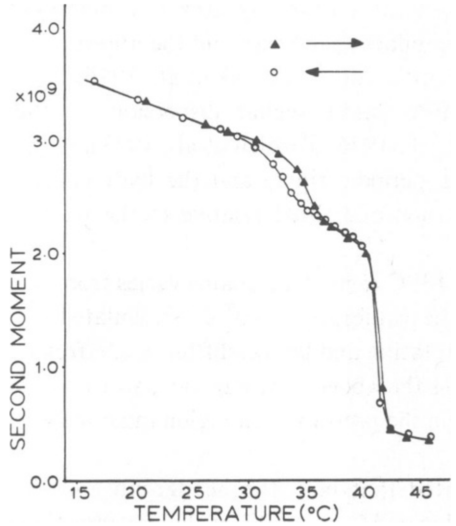

FIGURE 2

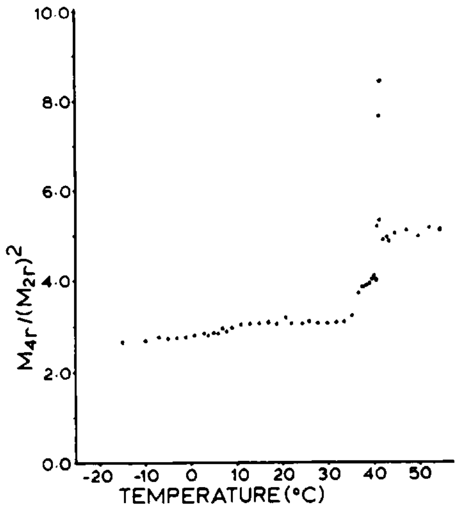

FIGURE 3

FIGURE 2 The plot of residual second moment (second^{-2}) for multilamellar protiated DPPC is expanded in the transition region to illustrate the hysteresis in the two phase transitions.

FIGURE 3 The moment ratio M_{4r}/M_{2r}^2 for the methylene chains of multilamellar DPPC is plotted as a function of temperature.

reduction in M_{2r} depends on the deviation of the hydrocarbon chains from the all-*trans* conformation oriented normally in a planar bilayer. Thus, lateral diffusion is the most likely mechanism for the observed changes in M_{2r} at the pretransition.

It is interesting to note that the decrease in M_{2r} at the pretransition occurs over a range of several degrees. If this reduction in M_{2r} is a result of a continuous increase in lateral diffusion rate, the diffusion rate at the midpoint of the pretransition can be estimated from the relation $M_{2r}\tau_C^2 = 1$ (Gutowsky and Pake, 1950; Andrew and Lipofsky, 1972). From the value of M_{2r} for the methylene chains at 35.5°C, one calculates a characteristic time $\tau_C \sim 2 \times 10^{-5}$ s for the DPPC molecule to move a distance $r(= \sqrt{4D\tau_C})$ of about one molecular diameter, which corresponds to a lateral diffusion constant, D, of order 10^{-11} cm^2/s. An alternate model for the variation of M_{2r} at the pretransition is that there is a discontinuous increase in the value of D for each molecule but, owing to sample inhomogeneities, different regions of the sample undergo the pretransition at different temperatures. This hypothesis is supported by the fact that both the headgroup and the hydrocarbon chains, which have different values of M_{2r}, undergo the pretransition at the same temperature relative to the main transition temperature. For this model one would expect the correlation time due to diffusion to vary from a value much longer than that derived from $M_{2r}\tau_C^2 = 1$ at temperatures below the pretransition to a much shorter value above the pretransition. The lateral diffusion constant calculated previously of 10^{-11} cm^2/s would then be a lower limit for the diffusion rate above the pretransition. The lateral diffusion rates of multilamellar DPPC in the gel phase have been measured by fluorescence photobleaching recovery (Wu et al., 1977; Fahey and Webb, 1978), and the experimental values obtained for D in the pretransition region are of order 10^{-11} to

10^{-10} cm^2/s, in agreement with that estimated by either model, but the data are not sufficiently accurate to distinguish between the two models.

The Chain-melting Transition and the Liquid-Crystalline Phase.

It is well known that multilamellar dispersions of DPPC in excess water undergo at 41°C a first-order phase transition in which the hydrocarbon chains become much more disordered. Above this chain-melting transition the DPPC molecules are in the liquid-crystalline ($L\alpha$) state, which has been studied by many physical techniques and is well characterized by the variation of deuteron order parameters with chain position (Seelig and Seelig, 1974; Davis, 1979) determined from ^{2}H NMR. The proton methylene M_{2r} changes from 2.5×10^9 to 5.0×10^8 s^{-2} (a factor of 5) at the chain melting transition, and at 50°C the methylene M_{2r} is 3.9×10^8, a value one-tenth that expected for extended chains in the presence of rapid rotation and lateral diffusion, so the ^{1}H NMR moment data is in good agreement with that obtained by other techniques on the liquid-like nature of the $L\alpha$ phase of multilamellar DPPC.

The proton order parameter, $S_{j\mathrm{HH}}$, and the deuteron order parameter, $S_{j\mathrm{CD}}$, for the same position on a methylene chain in an $L\alpha$ phase lipid are not expected to differ significantly (Higgs and Mackay, 1977). Then, using Eqs. 2 and 3 and the values of $S_{j\mathrm{CD}}$ for DPPC at 50°C (Seelig and Seelig, 1974) one estimates M_{2r} (intramethylene) $= 0.57$ M_{2r}, and from the deuteron second moment for chain-deuterated DPPC at the equivalent reduced temperature of 46°C (Davis, 1979) one estimates M_{2r} (intramethylene) $= 0.51$ M_{2r}. These calculations are consistent with the value of the proton M_{2r} (intermethylene) $= 2.46 \times 10^8$ s^{-2} (which yields M_{2r} (intramethylene) $= 0.46$ M_{2r}) obtained for DPPC at 45°C using a solid echo technique (Trahms and Boroske, 1979).

Analysis of the Moment Ratio M_4/M_2^2

The moment ratio (M_{4r}/M_{2r}^2), which can give information about the proton NMR lineshape (Abragam 1961) is plotted as a function of temperature in Fig. 3 for the methylene chains of the multilamellar dispersion of DPPC. As observed for M_{2r}, M_{4r}/M_{2r}^2 undergoes discontinuous changes at each phase transition. The peak at the chain-melting transition is of special interest and yields additional information on the nature of the transition. Furthermore, the moment ratio is relatively constant within each of the three phases of DPPC observed in this temperature study.

For a rigid solid with an approximately isotropic angular distribution of dipolar couplings, the proton NMR lineshape is often approximated by a Gaussian function $\{f(\omega) = f(\omega_0) \exp(-[\omega - \omega_0]^2/2M_2)\}$ for which one can easily calculate $M_4/M_2^2 = 3$. Below the pretransition value of M_{4r}/M_{2r}^2 for the methylene chains of DPPC in the gel phase is close to 3. It should be pointed out that while a Gaussian lineshape always has $M_4/M_2^2 = 3$, in general a lineshape with $M_4/M_2^2 = 3$ is not necessarily Gaussian. In Fig. 4, however, where the proton free induction decays at four temperatures are compared with Gaussian FID having the same values of M_{2r}, the FID at −1°C is indeed nearly Gaussian, but at 34°C, where the chains have acquired more motion and disorder, deviations from a pure Gaussian occur at times longer than ~30 μs. In multilamellar DPPC in the pretransition region, the moment ratio $M_{4r}/M_{2r}^2 = 4$. The divergence between the experimental FID at 39°C and a Gaussian simulation is illustrated in Fig. 4 *c*. In the liquid-crystalline phase, the ^{1}H NMR lineshape is clearly not

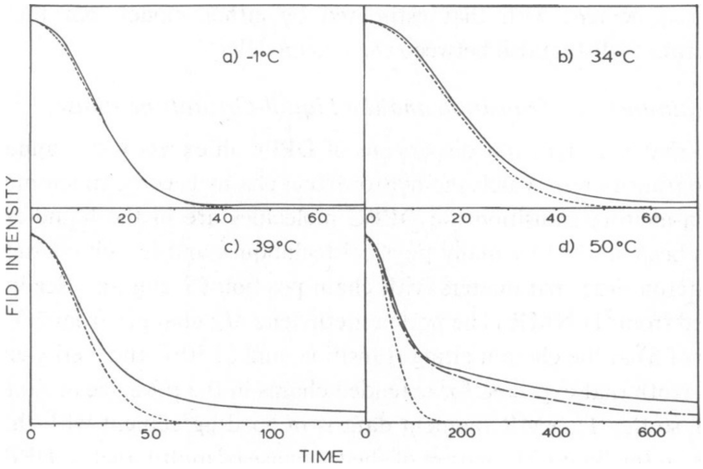

FIGURE 4 The experimental derived in-phase proton FID (—), a Gaussian simulation having the same M_{2r} (. . .), and a simulation made up of a superposition of Gaussian doublets (Bloom et al., 1977) (— · —) for the methylene chains of multilamellar DPPC are illustrated at four temperatures: *a*, −1°C in the gel phase; *b*, 34°C in the gel phase; *c*, 39°C in the pretransition region; and *d*, 50°C in the liquid-crystalline phase. Time, microseconds.

Gaussian, as is demonstrated in Fig. 4 *d*. The proton lineshape for methylene chains in the Lα phase has been discussed in detail in two recent studies (Bloom et al., 1977; Bloom et al., 1978) and a computer-simulated FID based on these studies is plotted in Fig. 4 *d*.

The sharp peak in M_{4r}/M_{2r}^2 at the chain-melting transition arises from the coexistence of gel and liquid-crystalline phase DPPC. From the values of M_{4r}/M_{2r}^2 for the gel (R_g) and the liquid-crystalline (R_L) phases, and the ratio of second moments of the two phases, R_m (= M_{2r} gel/M_{2r} liquid-crystal), at the transition temperature, the value of M_{4r}/M_{2r}^2 in the transition region may be related to the molecular fraction of gel, X_g, as follows:

$$M_{4r}/M_{2r}^2 = \frac{X_g(R_g R_m^2 - R_L) + R_L}{X_g^2(R_m - 1)^2 + 2X_g(R_m - 1) + 1}. \qquad (6)$$

This relation assumes that the gel to liquid-crystalline phase transition involves no intermediate phases; i.e., during the main transition, DPPC molecules exist in one of only two phases: gel with moments M_{2nr} gel and liquid crystalline with moments M_{2nr} liquid-crystal.

Using $R_g = 4.0$, $R_L = 5.0$ and $R_m = 5.0$, one finds that at 42°C, where the peak in the M_{4r}/M_{2r}^2 curve occurs, the molecular fraction of gel in multilamellar DPPC is 15%. For this sample some of the coexistence of the two phases is due to the thermal gradient of ~1°C across the sample; this data therefore should not be taken as quantitative evidence for the coexistence of the two phases in a sample with a homogeneous temperature.

CONCLUSIONS

In this paper it has been demonstrated that in spite of the complexity of ^{1}H NMR spectra and the lack of specificity of the probe, the comparison of experimental proton second moments with the rigid lattice M_2 and with calculated residual moments can yield considerable knowledge on the physical state of multilamellar DPPC. In the liquid-crystalline (Lα) phase of DPPC, which is relatively well understood, the proton moment data are in accord with that from other physical techniques but do not render as much detailed structural information as some of the other techniques notably ^{2}H NMR and x-ray diffraction. In the gel phase of DPPC, however, where there is disagreement on the interpretation of data from different techniques, the proton moment study is much more valuable.

The proton moment data give evidence that DPPC chain methylenes are almost definitely not in the fully extended all-*trans* conformation at temperatures higher than about 15°C, and that they exhibit a considerable amount of motion and/or disorder down to very low temperatures. Surprisingly, this behavior is not observed for the methylene chains or paraffins which have an experimental second moment close to the rigid lattice value at temperatures just below their main chain-melting transition (Peters and Kimmich, 1978). This striking disparity may be a result of the different constraints imposed on phospholipid methylene chain structure by the headgroup arrangement in the bilayer.

It is worthwhile to speculate on what disorder or motions occur in the DPPC hydrocarbon chains in the gel phase that result in the remarkably low values of M_{2r}. As already discussed, the discontinuity in the proton moment data at the pretransition can be accounted for by the onset of rapid lateral diffusion, and it is probable that at temperatures $> \sim 15°C$ at least some of the DPPC molecules are rotating. There may also be gauche conformations at the C—C bonds; in fact Raman scattering studies (Pink et al., 1980; Yellin and Levin, 1977) have indicated the presence of an appreciable number of gauche rotations in the hydrocarbon chains of gel-phase DPPC dispersions. One model,[1] which could account for some of the discrepancy of gel-phase proton moments from that calculated for chains in the fully extended all-*trans* conformation, is the presence of small twists about the long chain axis at each C—C bond by a few degrees. Although such twists would not necessarily have a large effect on the chain length or require much energy they could presumably occur on a time scale rapid enough to cause motional narrowing of the ^{1}H NMR spectra. The actual physical state of multilamellar DPPC in the gel phase probably involves a complex temperature-dependent superposition of several different types of conformation and motions of this nature.

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