Comparison of the Orientational Order of Lipid Chains in the L_α and H_{II} Phases†

Michel Lafleur,‡,§ Pieter R. Cullis,‡,∥ Bernard Fine,⊥ and Myer Bloom*,⊥

Department of Biochemistry, University of British Columbia, Vancouver, British Columbia, Canada V6R 2X7, and Physics Department, University of British Columbia, Vancouver, British Columbia, Canada V6T 2A6

Received March 23, 1990; Revised Manuscript Received May 23, 1990

ABSTRACT: The orientational order profile has been determined by using deuterium nuclear magnetic resonance (^{2}H NMR) for POPE in the lamellar liquid-crystalline (L_α) and the hexagonal (H_{II}) phases and is shown to be sensitive to the symmetry of the lipid phase. In the H_{II} phase, as compared to the L_α phase, the acyl chains are characterized by a greater motional freedom, and the orientational order is distributed more uniformly along the lipid acyl chain. This is consistent with a change from a cylindrical to a wedge-shaped space available for the lipid chain. ^{2}H NMR studies of POPE dispersions containing tetradecanol or decane, both of which can induce H_{II} phase structure, show very different behavior. Tetradecanol appears to align with the phospholipid chains and experience the L_α to H_{II} phase transition with a similar change in motional averaging as observed for the phospholipid chains themselves. In contrast, decane is apparently deeply embedded in the lipid structure and exhibits only a small degree of orientation. The L_α to H_{II} phase transition for systems containing decane leads to a dramatic increase of the motional freedom of decane which is more pronounced than that observed for the lipid chains. This is consistent with a preferential partition of the decane molecules into a disordered environment such as the intercylinder spaces in the H_{II} phase. The presence of decane in the H_{II} phase structure does not modify the order of the lipid chains. However, the L_α phase of POPE is slightly disordered by the addition of 9 mol % decane whereas it can accommodate as much as 20 mol % tetradecanol without a significant change of order. Finally, the concept of a stretching vector associated with the lipid acyl chain has been introduced to analyze the orientational order profile obtained in the H_{II} phase. With this model, the average order parameter of the H_{II} phase has been calculated and found to be in good agreement with experiment.

The polymorphic phase tendencies of lipid systems are dependent on the molecular properties of the lipid species and can be modulated by a wide variety of factors. However, the molecular basis for lipid polymorphism remains poorly understood. Several lines of investigation suggest that lipid polymorphism and hydrocarbon order are sensitive to the same forces and are intimately related [see Lafleur et al. (1990) and references cited therein]. Here, we attempt to provide insight into the effects of the different lipid phases, or the polymorphic tendencies of the lipid, on the orientational order distribution in the acyl chain. Deuterium nuclear magnetic resonance (^{2}H NMR)[1] is well established as a suitable technique for the characterization of the orientational order of lipid chains, primarily in the liquid-crystalline lamellar phase (L_α). An important contribution of these studies has been to show the existence of a characteristic variation of orientational order along the lipid chain (Seelig & Seelig, 1974, 1980). Recently it has been shown that the orientational order profile probed with perdeuteriated tetradecanol is considerably different in the H_{II} phase of a POPE lipid system than in the bilayer phase (Sternin et al., 1988). Instead of the relatively constant order in the methylene groups near the interface (the plateau region), which is characteristic of the L_α phase, a more uniform variation of the order along the tetradecanol chain is observed in the hexagonal (H_{II}) phase. In order to verify whether this accurately reflects the behavior of the phospholipid acyl chains themselves, we have characterized the variation of the orientational order profile during the L_α to H_{II} phase transition for the phospholipid chains using POPE in which the palmitoyl chain is fully deuteriated (POPE-d_{31}). Further, in a second part of this study, we have investigated the influence of certain H_{II} phase promotors on the order profile of the phospholipid acyl chains in the L_α phase as well as in the H_{II} phase. Tetradecanol and decane have been selected as H_{II} phase inducers because their different molecular nature suggests different mechanisms of H_{II} phase promotion. The response of the hydrocarbon order to the change of polymorphic tendencies is important for the understanding of the molecular events underlying a phase transition. In addition to examining lipid chain order, ^{2}H NMR studies on deuteriated tetradecanol and deuteriated decane in nondeuteriated POPE systems have been used to compare their motional freedom with that of the lipid chains, providing information concerning their location in the lipid matrix. Finally, to conclude, an analysis of the order profile in the H_{II} phase based on the *stretching vector approach* introduced by de Gennes (1974) is presented. Our results are well reproduced by this model taking in account some simple geometrical terms.

Materials and Methods

1-Palmitoyl-2-oleoylphosphatidylethanolamine-d_{31} (POPE-d_{31}) and 1-palmitoyl-2-oleoylphosphatidylcholine-d_{31} (POPC-d_{31}) and all other lipids were obtained from Avanti Polar Lipids

† Supported by the Natural Sciences and Engineering Research Council (NSERC) and the Medical Research Council (MRC) of Canada.

‡ Department of Biochemistry.

§ Recipient of a postdoctoral fellowship from the Medical Research Council of Canada.

∥ MRC Scientist.

⊥ Physics Department.

[1] Abbreviations: ACS, anisotropic chemical shift; DPPC, dipalmitoylphosphatidylcholine; EDTA, ethylenediaminetetraacetic acid; NMR, nuclear magnetic resonance; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine.

0006-2960/90/0429-8325$02.50/0

Inc. (Birmingham, AL). The deuteriated lipids showed a single spot on TLC analysis, and lipid chain analysis indicated an equimolar mixture of palmitoyl and oleoyl chains. The small peaks observed in the lipid spectra obtained in the L_α phase (e.g., see Figure 1; the small peaks correspond to the third and the fifth smallest quadrupolar splittings) suggest that acyl chain transmigration of about 20% has occurred during the synthesis of deuteriated lipids. These small peaks arise because the *sn*-1 and *sn*-2 lipid chains are not equivalent (Paddy et al., 1985). Decane-d_{22} was obtained from Cambridge Isotope Laboratories (Woburn, MA). Tetradecanol and decane were purchased from Sigma Chemical Co. (St. Louis, MO).

For pure lipid samples, 40 mg of dry lipid was hydrated with approximately 700 μL of 20 mM Hepes buffer, 300 mM NaCl, and 5 mM EDTA, pH 7.4, in deuterium-depleted water (Sigma Chemical Co.). The samples were then mixed at a temperature above the gel to liquid-crystalline phase transition in order to achieve a fully hydrated dispersion. For the samples of POPE containing tetradecanol, both constituents were dissolved in chloroform, and after evaporation of the organic solvent, the samples were hydrated in the same manner as the pure lipid. The vapor pressure of decane is too high to use a similar procedure for the sample preparation. In this case, the required amount of decane was added directly to the fully hydrated lipid dispersion. The sample was then incubated above the gel to liquid-crystalline phase transition for at least 2 days.

All the spectra were obtained on a home-built 46-MHz ^{2}H NMR spectrometer previously described (Davis, 1979; Sternin, 1985). The free induction decays were produced by a quadrupolar echo sequence with a τ value of 50 μs. After the second pulse, 2048 points were collected in quadrature with a dwell time of 5 μs, or 10 μs for spectra with narrow spectral width. The number of transients was between 42000 and 100000. The repetition time of the pulse sequence was at least 300 ms. The sample temperature was regulated by using a Bruker BV-T1000 temperature controller.

Results

Comparison of Orientional Order of the Lipid Chains in the L_α and H_{II} Phases. The first set of experiments was aimed at characterizing the influence of the lipid phase symmetry on the order profile of the lipid chains. The ^{2}H NMR powder pattern and the dePaked spectra of POPE-d_{31} in the L_α phase (60 °C), the H_{II} phase (75 °C), and when there is coexistence of both phases (68 °C) are shown in Figure 1. As can be seen, perdeuteriated chains lead to complex ^{2}H NMR spectra; the powder spectra of the lipid in a single phase are superpositions of 15 different powder patterns. No specific peak assignment can be made directly from these powder pattern spectra, particularly in the case of the H_{II} phase for which the doublets assigned to the methylene groups are not resolved. However, since the signal arises from the deuterium nuclei uniformly distributed along the lipid chain, a *smoothed* function for the variation of the orientational order along the acyl chains can be defined by exploiting the dePakeing method in a manner described in detail elsewhere (Sternin et al., 1988; Lafleur et al., 1989). Briefly, the numerical dePakeing procedure (Bloom et al., 1981; Sternin et al., 1983) is first used to separate the information concerning the orientational order of the C–D bond from the dependence of the quadrupolar splittings on the angle between the lipid symmetry axis and the magnetic field direction. The dePaked spectra presented in this paper correspond by convention to spectra characteristic of a sample oriented parallel to the magnetic field (the shoulder of the

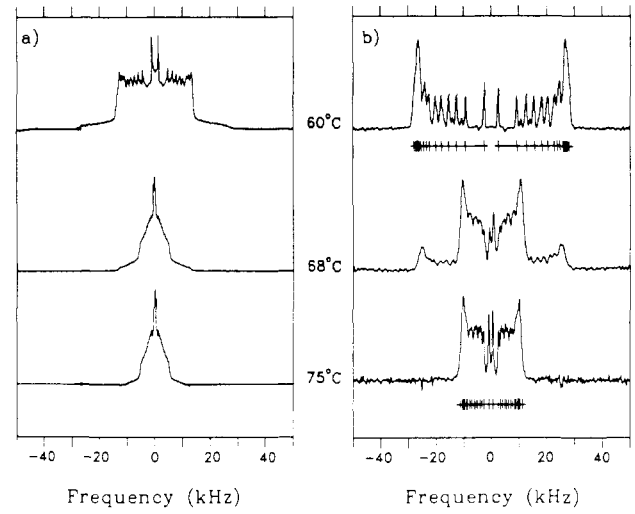

FIGURE 1: L_α to H_{II} phase transition of POPE-d_{31} as experienced by the perdeuteriated palmitoyl chain. (a) Powder pattern and (b) dePaked spectra of POPE-d_{31} in the L_α phase (60 °C), when the L_α and the H_{II} phases coexist (68 °C), and in the H_{II} phase (75 °C). The tick marks at the bottom of the dePaked spectra indicate the mean order parameters determined by the method described in the text.

powder spectra). The dePaked spectrum gives directly the probability distribution of the order parameters, and a mean value of the order parameter is calculated for each unit area associated with one methylene group (the quadrupolar splitting assigned to the terminal methyl is directly measured from the well-resolved innermost doublet). The tick marks at the bottom of the spectra represent these mean values. The order profiles presented in this paper are obtained from these order parameters, assuming a monotonic decrease of the order along the chain from the interface toward the middle of the bilayer (Sternin et al., 1988; Lafleur et al., 1989).

At 60 °C, the spectrum is typical of a phospholipid with a perdeuteriated chain in the liquid-crystalline phase (Davis, 1979; Paddy et al., 1985). The dePaked spectrum of POPE-d_{31} at 60 °C exhibits the fluid bilayer signature. A large fraction of the signal is associated with the largest quadrupolar splittings and is assigned to the methylene groups near the interface. The several resolved doublets with a smaller quadrupolar splitting correspond to methylene positions in the more disordered terminal part of the chain (Davis, 1979). The shape of the spectrum obtained in the H_{II} phase (at 75 °C) contrasts with that obtained for the L_α phase. First, the values of quadrupolar splittings of the deuteriated lipid in the H_{II} phase are smaller than those for the L_α phase, and, second, the distribution of order along the chain is modified by the change of the phase structure. In particular, the signal is distributed more uniformly along the acyl chain in the H_{II} phase than in the L_α phase, though the relative intensity remains somewhat larger for the largest quadrupolar splittings. It should be noted that the free induction decay for POPE-d_{31} at 75 °C was recorded for a longer time (by using a dwell time of 10 μs instead of 5 μs) in order to enhance the spectral resolution. Even under these conditions, no well-resolved doublets of the type observed in the L_α phase were detected, except for the doublet assigned to the terminal methyl. The *smoothed* order profile obtained from such a spectrum, in the manner described above, is not affected by the resolution. At 68 °C, the spectrum is a combination of the L_α and the H_{II} phase spectra, indicating that the lipid exchange between the two structures is slow on the NMR time scale.

These changes can be expressed in a quantitative way by calculating the moments of the spectra. As previously dem-

Table I: First and Second Moments, M_1 and M_2, and the Relative Mean Square Deviation of the Distribution of Quadrupolar Splittings, Δ_2, of POPE-d_{31}

temp (°C)	M_1 (s^{-1})	M_2 (s^{-2})	Δ_2
50	5.4×10^4	4.3×10^9	0.08
60	5.2×10^4	3.9×10^9	0.09
68	2.3×10^4	1.0×10^9	0.50
75	1.7×10^4	4.3×10^8	0.22

onstrated, the moments of a spectrum are a direct measurement of the moments of the distribution of quadrupolar splittings (Bloom et al., 1978; Davis, 1979). M_1 gives the mean value of the order parameters, $\langle|S_{C-D}|\rangle$, M_2 gives the mean value of the square order parameters, $\langle|S^2_{C-D}|\rangle$, etc. The values of M_1 and M_2 determined from the spectra of POPE-d_{31} at various temperatures are shown in Table I. The increase of temperature by 10 °C in the L_α phase leads to a decrease of M_1 by about 4%. M_1 decreases more drastically during the L_α to H_{II} phase transition. When all the lipids are in the H_{II} phase, at 75 °C, the value of M_1 is 1.7×10^4 s^{-1}; this is a reduction by a factor of 3 compared with the value calculated for the L_α phase at 60 °C. The relative mean square deviation of the order parameter distribution, Δ_2, is also influenced by the phase transition. In the L_α phase, values around 0.09 are obtained; this is of the same order as results previously published for DPPC-d_{62} (Davis, 1979). At 68 °C, Δ_2 reaches a maximum. As discussed by Davis (1979), this parameter is very sensitive to inhomogeneities in the sample. The coexistence of phases characterized by different quadrupolar splittings, such as the L_α and H_{II} phases, leads to a very wide distribution of order parameters. For example, a large value of Δ_2 is also observed for the coexistence of the L_β and L_α phases (Davis, 1979). Finally Δ_2 equals 0.22 at 75 °C, when POPE is in the H_{II} phase, indicating that the distribution of quadrupolar splittings is wider in the H_{II} phase than the L_α phase.

The smoothed orientational order profiles for the L_α and the H_{II} phases obtained from these spectra are shown in Figure 2a. The largest value of quadrupolar splitting has been normalized to unity in order to highlight the difference in the shape of the distribution of the orientational order. The order profile along the acyl chains is affected by the symmetry of the lipid phase as has been already observed using perdeuteriated tetradecanol as a probe (Sternin et al., 1988). For the L_α phase, a relatively flat plateau region extending for about six carbon positions is observed, followed by a rapid decrease of the order toward the end of the lipid chain (Seelig & Seelig, 1974, 1980). By contrast, a more monotonic decrease of $S(n)$ versus n is observed in the H_{II} phase. The redistribution of the order has as a consequence the increase of Δ_2 mentioned above. The plateau in the L_α phase corresponds to a substantial fraction of CD_2 groups having similar splittings, which gives rise to a reduced width of the order distribution. The more uniform distribution observed in the H_{II} phase leads to an increased value of Δ_2.

The reduction in order parameter observed as a result of the L_α to H_{II} phase transition is shown in Figure 2b. The ratio of the order parameters in the L_α phase to those of the H_{II} phase is larger than 2 for every position of the chain and reaches a value of 3.5 near the end of the chain. This decrease of order is due mostly to a change in the lipid phase symmetry. There is also a reduction of order due to the increase of temperature necessary to induce the phase transition. That this does not contribute significantly to the change observed is shown by the results obtained for lamellar POPC-d_{31}—the ratios measured over the same range of temperature in a single

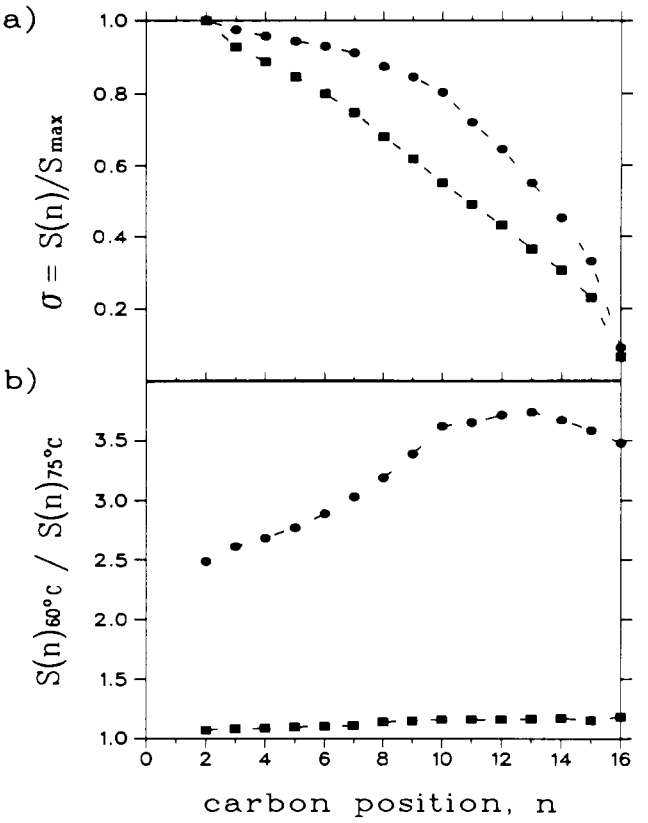

FIGURE 2: Influence of the lipid phase symmetry on the order profile. (a) Normalized orientational order profile of POPE-d_{31} in the L_α phase at 60 °C (●) and in the H_{II} phase at 75 °C (■). (b) Ratio of the order parameters $S(n)$ in the L_α phase (60 °C) to those in the H_{II} phase (75 °C) for POPE-d_{31} (●). The ratio for the same temperatures is plotted for POPC-d_{31} (■) which forms an L_α phase over the whole range of temperature.

lipid phase are close to unity (Figure 2b). Moreover, the ratio of quadrupolar splittings measured for the outermost doublet of each phase at a temperature at which both phases coexist (68 °C) is 2.4. This value reflects essentially the change in order experienced by the first few methylene groups of the lipid chain associated with the phase transition. The ratio of $S_{L_\alpha}/S_{H_{II}}$ versus n (Figure 2b) further indicates that there is no single scaling factor describing the change in order. The order is redistributed along the lipid chain in favor of a more uniform distribution in the H_{II} phase as shown above.

Influence of Tetradecanol and Decane on Lipid Order. In the next part of this study, we investigated the influence of tetradecanol and decane, two H_{II} phase inducers, on the orientational order profile of the lipid chain. The first step of this study was to characterize the effects of these agents on the polymorphism of POPE. The effects of tetradecanol and decane on the polymorphism of POPE-d_{31} were determined by plotting the order parameter of the outermost doublet, S_{max}, as a function of temperature. Figure 3 illustrates this plot for pure POPE-d_{31}, and for POPE-d_{31} containing 20 mol % tetradecanol or 9 mol % decane. The vertical dashed lines represent temperature where the L_α and the H_{II} phases coexist for a given system, although the coexistence is not limited to that temperature. For pure POPE, there is a progressive decrease of the order parameter with increasing temperature due to the increase of the motion of the acyl chains. Around 68 °C, both bilayer and H_{II} phases coexist, and at higher temperatures, POPE adopts the H_{II} phase characterized by a small order parameter. As shown in Figure 3, the addition of 20 mol % tetradecanol or of 9 mol % decane reduces the L_α to H_{II} phase transition temperature of POPE-

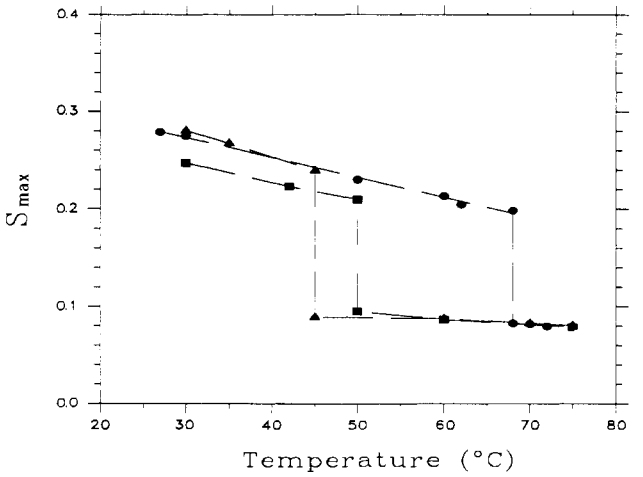

FIGURE 3: Thermotropism of pure POPE (●), POPE + 20 mol % tetradecanol (▲), and POPE + 9 mol % decane (■) probed by using S_{max}, the order parameter determined for the outermost doublet of the perdeuteriated palmitoyl chain spectra.

d_{31} by about 20 °C. It has been previously shown (Sternin et al., 1988) that the addition of 20% perdeuteriated tetradecanol induces a similar shift of the lamellar to hexagonal phase transition toward lower temperatures. Several other alkanes have also been shown to decrease the L_α to H_{II} phase transition temperature (Epand, 1985; Kirk & Gruner, 1985; Siegel et al., 1989; Sjölund et al., 1989).

An important question concerns how the shift of the transition temperature is reflected by the phospholipid chain order. It may be noted that S_{max} is not significantly influenced by the presence of tetradecanol over the experimental temperature range (see Figure 3). The values obtained for the POPE/tetradecanol system in the L_α and the H_{II} phases are close to those observed for pure POPE or those which could be extrapolated from the data of pure POPE in the H_{II} phase ($T \geq 68$ °C). Figure 4 shows a comparison of the whole order profile of POPE and POPE + 20% tetradecanol (a) at 30 °C when both systems are in the L_α phase and (b) at 75 °C for the H_{II} phase. As one can see, the presence of tetradecanol does not significantly modify the distribution of the order along the chain in either phase. Previous studies have shown that the presence of 1-octanol (25 mol%) or 1-decanol (25 mol %) does not affect significantly the order profile of DPPC bilayers (Thewalt et al., 1985). Our results indicate that the same is true for a longer alcohol in a POPE liquid-crystalline bilayer or H_{II} phase.

The order of the hydrophobic core of the POPE/tetradecanol system has been previously characterized by ^2H NMR where the long-chain alcohol bore the deuterium nuclei (Sternin et al., 1988). Figure 5 shows the order profile obtained by ^2H NMR for the system POPE/20 mol % tetradecanol in the L_α and H_{II} phases probed by the perdeuteriated palmitoyl chain of POPE or by perdeuteriated tetradecanol. Except for the chain terminal region, the general shape of the orientational order profiles for both phases is the same for both probes. An important difference between these probes is their chain length, the alcohol molecules being shorter than the palmitoyl chains by two carbon units. This is reflected in the profiles obtained with perdeuteriated lipid and perdeuteriated tetradecanol by the abrupt decrease of order at the end of the alcohol chain.

As can be seen in Figure 3, decane also shifts the L_α to H_{II} phase transition toward lower temperatures. The effects of this H_{II} phase inducer on the order profile of POPE-d_{31} are shown in Figure 4. In the L_α phase, a small decrease in order

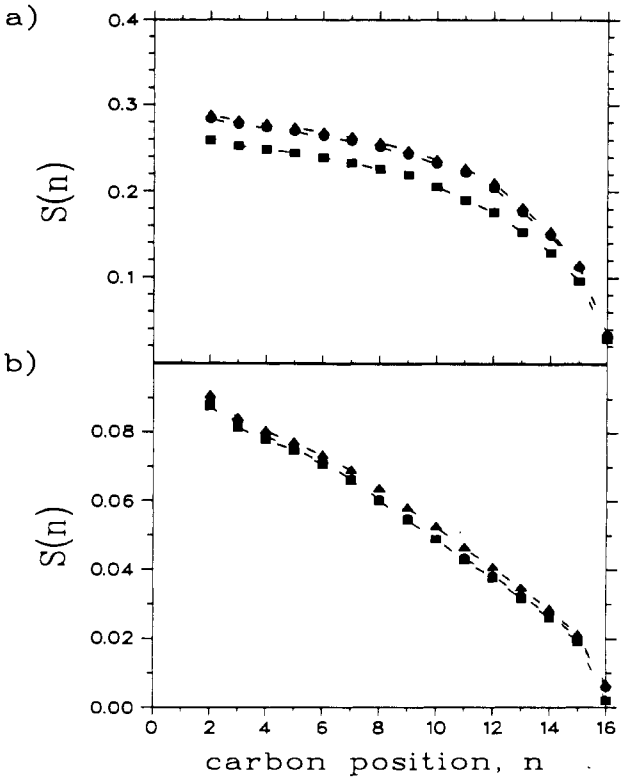

FIGURE 4: Comparison of the order profile probed by the perdeuteriated palmitoyl chain of POPE in pure POPE (●), POPE + 20 mol % tetradecanol (▲), and POPE + 9 mol % decane (■) in (a) the L_α phase (30 °C) and (b) the H_{II} phase (75 °C).

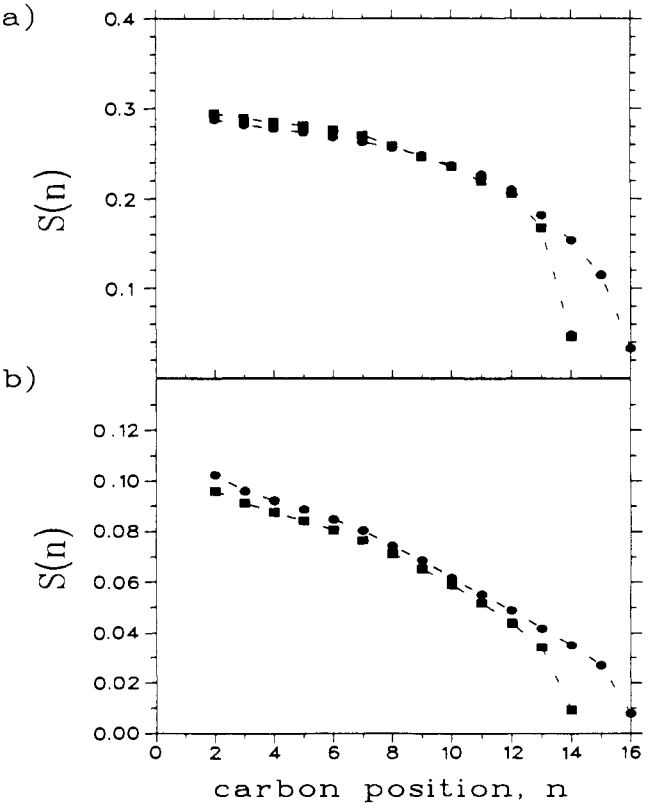

FIGURE 5: Comparison of the order profile obtained for POPE + 20 mol % tetradecanol system in (a) the L_α phase (30 °C) and (b) the H_{II} phase (50 °C) using POPE-d_{31} (●) and tetradecanol-d_{27} (■).

of about 7% is observed in the presence of 9 mol % decane for all the positions along the acyl chains. In the H_{II} phase, POPE accommodates 9 mol % decane without significant changes in the lipid chain order profile (Figure 5b).

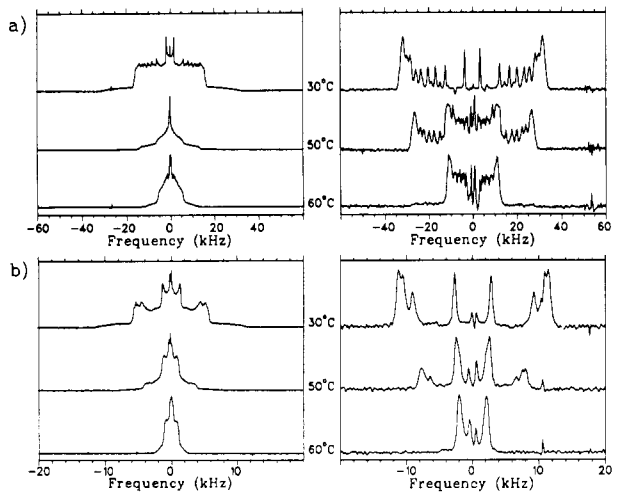

FIGURE 6: L_α to H_{II} phase transition of POPE + 9 mol % decane as experienced by (a) the perdeuteriated palmitoyl chain and (b) the deuteriated decane. Powder pattern (left panel) and dePaked (right panel) spectra of the sample in the L_α phase (30 °C), when the L_α and the H_{II} phases coexist (50 °C), and in the H_{II} phase (60 °C).

The nature of the interactions of decane and tetradecanol with POPE is likely different since tetradecanol is, like a phospholipid, an amphiphile whereas decane is totally apolar. The orientational order of deuteriated tetradecanol has been previously characterized (Sternin et al., 1988). Here, the POPE/decane system was studied using perdeuteriated decane. Figure 6 shows the spectra of POPE + 9 mol % decane prepared with (a) POPE-d_{31} or (b) decane-d_{22}. At 30 °C, the spectrum of the perdeuteriated lipid chain exhibits the typical shape of the L_α phase (Figure 6a). The powder pattern and the dePaked spectra of deuteriated decane in the lipid matrix at the corresponding temperature are shown in Figure 6b. The spectrum of decane-d_{22} in the L_α phase shows small quadrupolar splittings compared to those of the lipid chain. Narrow isotropic components are also observed in the middle of the spectra. One line is off-resonance and is likely associated with a small amount of deuteriated water. The other isotropic line likely originates from deuteriated decane but represents a very small fraction of the signal and was not considered in the analysis. The dePaked spectrum of decane-d_{22} in a POPE lamellar matrix at 30 °C is composed of a well-resolved doublet with a splitting of 2.8 kHz and a few doublets with closely spaced values of quadrupolar splittings in the range 9–12 kHz. These are typical values of splittings observed for alkanes embedded in lipid bilayers (Pope et al., 1984; Jacobs & White, 1984a,b; Sjölund et al., 1987; Siegel et al., 1989). On the basis of the fractional area, the well-known mobility of the terminal methyl group, and previous studies (Jacobs & White, 1984a,b; Pope et al., 1984), the innermost doublet is assigned to the terminal methyl groups and the rest of the signal to the methylene groups for which the order parameters vary within a limited range. Two doublets are partially resolved, implying that an order gradient also exists along the alkane chains. As already observed for other alkane molecules embedded in lipid bilayers (Jacobs & White, 1984a,b; Pope et al., 1984), a single signal is observed for the terminal methyl groups, indicating that both ends of the molecules experience, on average, the same motion.

As indicated by the change in the shape of the spectrum of POPE-d_{31} as a function of temperature, the POPE-d_{31}/decane system undergoes an L_α to H_{II} phase transition around 50 °C. For the spectrum of decane-d_{22} (Figure 6b), an increase of the proportion of the signal with a narrow quadrupolar splitting is observed during the L_α to H_{II} phase transition. A new component with a very small quadrupolar splitting of 0.4 kHz is also observed. At 60 °C, when the spectrum of POPE-d_{31} indicates that all the lipids form an H_{II} phase, only the narrow components are detected for decane-d_{22}. On the basis of the phase identification provided by the spectra of POPE-d_{31}, this narrow signal is assigned to deuteriated decane in the H_{II} matrix of POPE. As observed for the bilayer, only one doublet is obtained from the terminal methyls in the H_{II} phase. The rest of the deuterium nuclei give rise to a doublet with a quadrupolar splitting of 2 kHz. As previously observed for the lipid chains, the ratio of order parameters of deuteriated decane in the L_α over the H_{II} phase is significantly greater than 2. Measured at the maximum of intensity of each phase component at 50 °C when both phases coexist, the ratio is 3.1 for decane-d_{22}; this is significantly larger than the same ratio measured for the palmitoyl chain at the same temperature which is 2.4. If the ratio $S_{L_\alpha}/S_{H_{II}}$ is measured by using this time the spectra of decane-d_{22} recorded at 30 °C for the L_α phase and at 60 °C for the H_{II} phase, a value of 5.5 is obtained for the widest doublet of the decane spectra. This ratio is about 5.0 for the doublet which is partly resolved in the L_α phase. It represents the additive effects of the phase transition and the temperature increase. Since a single phase is observed at these temperatures, the order profile can be obtained for the lipid chains. The ratios $S(n)_{L_\alpha}/S(n)_{H_{II}}$ are calculated as a function of n, and the values obtained vary from 2.7 at the beginning of the lipid chain to 4.2 near its end (data not shown).

Discussion

The orientational order profile of the lipid acyl chain is sensitive to the phase symmetry and is considerably different for the H_{II} than for the L_α phase. First, the absolute values of order parameters of the H_{II} phase are considerably smaller than expected if the lipid experiences similar motions to the L_α phase and diffuses around the H_{II} cylinders. The lipid diffusion around the cylinders causes extra motional averaging leading to a reduction of the order parameters by a factor of 2 (Cullis & de Kruijff, 1979; Seelig, 1978). This factor of 2 is expected because the lipid molecule diffuses around the cylindrical axes in a time short ($\leq 10^{-5}$ s) on the NMR time scale, which projects the symmetry axis for the orientational order from the local surface normal to the axis of symmetry of the cylinder, which is at right angles to the surface normal. Then, the quadrupolar splittings are multiplied by $|(3\cos^2 90° - 1)/2| = {}^1/_2$. Since the radii of the hexagonally coordinated cylinders in the H_{II} phase are typically of order $R \approx 2$ nm (Seddon et al., 1984; Tate & Gruner, 1989) and the diffusion constants in fluid phases are of order $D \geq 4 \times 10^{-12}$ m^2 s^{-1}, the correlation time for diffusion around the cylinder axes, $\tau_c \leq R^2/2D \approx 10^{-6}$ s, is expected to be short on the NMR time scale. However, the change in order associated with the L_α to H_{II} phase transition is larger than this predicted factor of 2 for every position n along the lipid palmitoyl chain (Figure 2). This result indicates that the H_{II} phase is characterized by a more pronounced motional freedom of the lipid chain than observed in the L_α phase. Infrared spectroscopy has also shown that the proportion of gauche bonds over the whole chain is increased in the H_{II} phase as compared with the L_α phase (Mantsch et al., 1981). Second, the decrease of orientational order in the H_{II} phase cannot be expressed by a single scaling factor, but it is more pronounced toward the end of the chain. This leads to a more uniform distribution of order along the acyl chain in the H_{II} phase than in the L_α phase. This behavior is in good agreement with results previously obtained using specifically deuteriated POPE (Perly et al., 1985). These

changes are consistent with the change in geometry of the space available for the acyl chain motion resulting from the phase transition. In the bilayer structure, the cross-sectional hydrophilic area of the headgroups matches the cross-sectional hydrophobic area of the acyl chains. In the H_{II} phase, the lipid chains have access to a "cone" or "wedge"-shaped space, leading to an increase of available space toward the end of the chain. This is consistent with the large decrease of order parameters observed here for the carbon positions in this region. In the next section, a simple model is proposed to describe the orientational order of the lipid chain in terms of the phase symmetry, and a theoretical relation between orientational order parameters in the L_α and the H_{II} phases will be discussed.

The order distribution along the palmitoyl chain is more uniform for the H_{II} phase than for the L_α phase. This is illustrated directly by the dePaked results (Figure 1b) or by the normalized order profile for which a continuous decrease of $S(n)$ versus n is observed (Figure 2a). The plateau is reduced in the H_{II} phase, and this causes an increase in the width of the distribution of order parameters (Table I). As discussed previously (Lafleur et al., 1989), the use of perdeuteriated saturated chains gives a smoothed shape of the profile since the assumption of a monotonic decrease of the order along the chain is made. Therefore, local variations that might exist in the profile are not reproduced. The plateau of the L_α phase is actually a segment about six methylene groups long within which the order oscillates over a limited range (Seelig & Seelig, 1974). This is reflected in the smoothed order profile by a segment where $S(n)$ does not vary appreciably. For the H_{II} phase, such a segment is not as obvious. However, as seen on the dePaked spectra of the H_{II} phase, there is a larger intensity for the largest quadrupolar splitting, and this is expressed in the profile as a reduced slope of $S(n)$ versus n near the interface. This may be interpreted as a small plateau region, as suggested by Sankaram and Marsh (1989).

All the conclusions obtained here for the deuteriated lipid chain are in agreement with those obtained previously with perdeuteriated tetradecanol (Sternin et al., 1988). Tetradecanol also reflects the order in the bilayer since its chain aligns with the lipid chains. In order to examine the correlation between the two probes, it is of interest to compare the profiles obtained for the POPE/tetradecanol system using deuteriated lipid and deuteriated alcohol. However, certain comments should first be made about these profiles. As mentioned previously, the smoothed order profile does not describe the detailed structure of the order profile. The assumption of a monotonic decrease of the order may also introduce some systematic error in the order profile of the long-chain alcohol in a lamellar lipid matrix. For example, for decanol, the order parameter reaches a maximum value for carbon positions 4–5 (Thewalt et al., 1986). This is due to the fact that the hydroxyl group is situated at the interface in such a way that the first part of the chain is tilted to the bilayer normal or its motions are less restricted. The integration method smooths these variations to give a plateau followed by a rapid decrease of order (Sternin et al., 1988).

The remarkable coincidence of the profiles for the POPE/tetradecanol system obtained from the deuteriated POPE and the deuteriated tetradecanol indicates that, except for local geometric variations, both molecular species experience in a very similar way the anisotropic forces inside the lipid matrix. This is demonstrated for the L_α as well as the H_{II} phase. The maximum order parameter values observed in both cases are the same (within 5%), and the order varies in a very similar way along the chain except for the sharper decrease at the end of the chain observed for tetradecanol due to its smaller chain length. This is in agreement with a previous study on decanol in DPPC bilayers (Thewalt et al., 1986). Similar values of order parameters were observed for this system no matter whether the deuterium nuclei were located on the alcohol or on the chain of DPPC.

In contrast to tetradecanol, deuteriated decane does not exhibit order parameters similar to those observed for the lipid chain. As previously shown for other alkanes (Jacobs & White, 1984a,b; Pope et al., 1984; Sjölund et al., 1987), the results obtained for decane suggest that the alkane molecules are deeply embedded into the lipid structure in the lamellar phase. The methylene groups of deuteriated decane show small quadrupolar splittings in the range of 9–12 kHz. There is an order gradient existing along the decane molecules as shown by the distinguishable doublets of the dePaked spectra of decane-d_{22} in the L_α matrix. The methylene groups at the center of the chain are likely the ones which correspond to the doublet with the largest splitting. The methyl groups show a single doublet, indicating that both halves of the decane molecules experience the same motional averaging. This is expected because of the reflexion symmetry of the decane molecule and the short correlation time for the end-to-end flip-flops of the molecules (Pope et al., 1984). Though we cannot establish with certainty the location of decane on the basis of the quadrupolar splittings (we do not know whether the alkane molecules experience specific motions that average the NMR signal), the small splittings strongly suggest that they are mainly located near the end of the lipid chains which provide a more disordered environment.

As observed for the lipid acyl chains, there is a drastic decrease of the order parameters for the decane molecules during the L_α to H_{II} phase transition. Similar observations have been made for dodecane and hexadecane in a monomethylated DOPE matrix (Siegel et al., 1989) and for dodecane in DOPC (Sjölund et al., 1987). Three phenomena can give rise to the reduction of order: (i) the extra averaging caused by the rapid diffusion of decane around the H_{II} cylinders; (ii) an increase of motion due to the change of phase symmetry; and (iii) a relocation of the decane molecules to a region allowing greater motion during the phase transition. On the basis of our results, it is difficult to determine to what extent each of these factors contributes to the reduction of the quadrupolar splittings. However, a new aspect of that question can be addressed, which concerns the relative change of order observed as a result of the L_α to H_{II} phase transition for the phospholipid and the long-chain alkane or alcohol under the same conditions. Here, it is shown that decane experiences an increase of motional freedom as a result of the L_α to H_{II} phase transition which is more pronounced than any position of the lipid chain. As a result, this change *cannot* be simply explained by a reorganization similar to that of the lipid chains where the decane molecules remain roughly aligned with the lipid chains, diffuse around the H_{II} cylinders, and have more motional freedom due to the additional space available in the H_{II} phase. It has been suggested that in the H_{II} phase, decane partitions preferentially into interstitial spaces between the cylinders (Kirk & Gruner, 1985; Sjölund et al., 1987; Siegel et al., 1989). The drastic decrease of order that we observe for decane supports this suggestion. It is proposed that since the decane molecules partition preferentially into these intercylinder spaces, they release the packing tension and allow the intrinsic curling tendency of the lipid layer to be expressed without significant contribution of forces other than elastic.

Under these conditions, the measurement of the radius of the H_{II} cylinders should correspond to the spontaneous radius of curvature of the lipid layer, R_0 (Kirk & Gruner, 1985; Gruner, 1985). Our results also indicate that the lipid chains of H_{II} phase POPE are not on average affected by the presence of decane. This observation and the small orientational order of the decane molecules themselves suggest the preferential partition of decane into "low constraint" spaces without influencing the H_{II} phase structure, supporting the use of alkane for the measurement of R_0.

The H_{II} phase inducers have different effects on the lipid chain order profile in the L_α phase. The addition of decane leads to a small decrease of order, showing a direct effect of decane on the lipid bilayer structure. The increase in free energy associated with this destabilization of the bilayer structure can also contribute to the shift of the L_α to H_{II} phase transition. In contrast, the lipid packing can accommodate 20 mol % tetradecanol without a significant change in order even though the presence of 20 mol % tetradecanol shifts the L_α to H_{II} phase transition toward lower temperatures by about 20 °C. The presence of this H_{II} phase inducer produces, however, a change at the interface since the hydrophilic area occupied by the hydroxyl group of tetradecanol is relatively small and may lead to the destabilization of the bilayer. An X-ray diffraction study has shown that the distance between the centers of two adjacent cylinders at 80 °C decreases in the presence of 20 mol % tetradecanol in the POPE matrix to 62.5 Å from 69.5 Å, a change of about 10% (E. Eikenberry and S. Gruner, personal communication). We are presently investigating systems with a wider range of R_0 in order to verify the relationship between the order parameters, the curvature, and the polymorphic tendencies of lipids.

Theoretical Relationships between Orientational Order Parameters in the H_{II} and L_α Phases. (i) Review of ^{31}P NMR Results and Their Implications for ^2H NMR. It is customary in the analysis of ^{31}P NMR data to assume that the ^{31}P NMR anisotropic chemical shift (ACS) in the H_{II} phase is related to that in the L_α phase by a factor $-1/2$. This assumption is, in fact, normally quite well satisfied experimentally (Cullis & de Kruijff, 1976; Seelig, 1978). There is a well-defined theoretical basis for this factor of $-1/2$. If the packing of the polar headgroups of phospholipid molecules on the curved cylindrical surfaces of the H_{II} phase lipid–water interfaces is similar to that in the L_α phase, so that the local orientational order is the same for both phases, and the diffusion of the lipid molecules around the H_{II} phase cylinders is fast on the NMR time scale, then, the ACS is multiplied by $-1/2$. As described above, the lipid diffusion around the water core of the H_{II} phase cylinders is fast enough to cause extra motional averaging. The well-established result from ^{31}P NMR that the local orientational order is identical in the two phases could not so easily have been anticipated, especially since the molecular motions near the cylinder surface should not be locally axially symmetric. Indeed, we are unaware of any explicit theoretical explanation of this rather simple and pleasing result.

Our observation that the ^2H NMR quadrupolar splittings on the acyl chains of the lipid chains do *not* scale by a factor of $|1/2|$ (note that unlike ^{31}P NMR ACS measurements, ^2H NMR quadrupolar splittings only give the magnitude and not the sign of the orientational order parameters) on going from the L_α to the H_{II} phase is not surprising in view of the anticipated variation of the packing of the lipid chains in the interior of the hydrophobic region of the H_{II} phase. It would appear that the systematic variation with chain position n of the ratio of the order parameter $S(n)$ in the L_α phase to that in the H_{II} phase shown in Figure 2b can provide information on chain packing when a suitably detailed theory is developed. In the following section, a new theory of orientational order appropriate for this purpose is developed. Our theory is unconventional in that it relates the component $S_{ij}(n)$ of the orientational order tensor for the nth carbon position to a local, *position-dependent* orientational order tensor component, $S_{ij}(\mathbf{r})$.

(ii) The Concept of a "Stretching Vector" for the Acyl Chains. An interesting approach to the problem of orientational order in chains, one that is independent of a detailed description of molecular conformational geometry, has been developed by de Gennes (1974). He defines a *stretching* vector $\mathbf{J}(\mathbf{r})$ at each position $\mathbf{r}$ *in space* by

$$\mathbf{J}(\mathbf{r}) = \rho\langle \mathbf{R}_{m+1} - \mathbf{R}_m \rangle \quad (1)$$

where ρ is the monomer density of the methylene groups comprising the chain and $\mathbf{R}_1, \ldots, \mathbf{R}_N$ are the locations of the successive monomers (defined as midway along the C–C bonds) for one chain. The broken brackets represent an average over all possible values of m which can be found at the position $\mathbf{r}$. de Gennes introduced the *Ansatz* that since "... in most of the hydrophobic region, there is no end group..." i.e., the end group $\mathbf{R}_N$ is confined to a boundary layer of thickness $e \approx N^{1/2}a$, where a is the monomer–monomer interval, $\mathbf{J}$ satisfies the same conservation law:

$$\text{div } \mathbf{J} = 0 \quad (2)$$

as does the electric field in a charge-free region. Although the vector $\mathbf{J}$ is a measure of local orientational order, it is not *directly* related to the second-rank tensor $S(n)$ that is measured by ^2H NMR. Nevertheless, de Gennes was able to relate the plateau, i.e., the observed lack of variation of $S(n)$ with n for the top half of the acyl chain in the L_α phase with the constant value of $\mathbf{J}$ predicted by eq 2 for lamellar geometry, in analogy with the constant electric field obtained near an infinite, flat, uniformly charged plate. The argument is that if $\mathbf{J}$ is constant, so is the related second-rank tensor that is responsible for the nuclear electric quadrupolar splittings (de Gennes, 1974).

In order to relate $\mathbf{J}$ to our ^2H NMR measurements in the H_{II} phase, it is necessary to identify a second-rank tensor $S_{ij}(\mathbf{r})$ from which our measurements of $S(n)$ may be derived. In fact, a symmetric second-rank tensor associated with the vector $\mathbf{J}(\mathbf{r})$ may be defined in terms of the components $J_i(\mathbf{r})$ as follows [see, e.g., Tinkham (1964)]:

$$S_{ij}(\mathbf{r}) \approx (3/2)C\{J_i(\mathbf{r})J_j(\mathbf{r}) - \delta_{ij}(1/3)\sum_k [J_k(\mathbf{r})]^2\} \quad (3)$$

where C is a normalization constant.

The relatively rapid decrease of $S(n)$ in the L_α phase with n for the tail of the chain was then ascribed to the "end effects" neglected in the "derivation" of eq 2. It was suggested by de Gennes that a further test of eq 2, or an improved version of it, could be made in a different geometry. For example, in a system with cylindrical geometry, the solution to eq 2 is

$$\mathbf{J}(\mathbf{r}) = \text{constant} \times \mathbf{r}/r^2, \text{ i.e., } J \propto r^{-1} \quad (4)$$

in the lipid region. In the spirit of de Gennes' *Ansatz*, we can guess that the effects of local cylindrical geometry may be expressed in terms of a power series of the form

$$1/S(n) = a_0 + a_1 n + a_2 n^2 + \ldots \quad (5)$$

In order to test this conjecture, we have fitted the ratio of $S(n)$ in the L_α phase to that in the H_{II} phase in Figure 2b to a linear function of n for values of n in the range $2 \leq n \leq n_{max}$ and

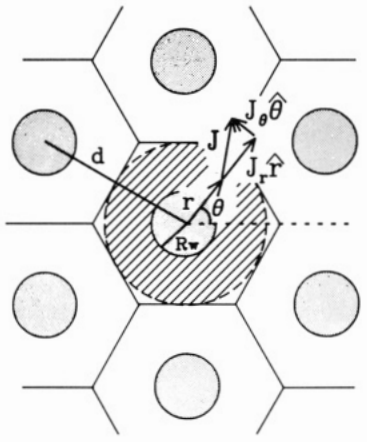

FIGURE 7: Schematic representation of the H_{II} lipid phase. The shaded regions represent the cylinder water cores, and the hatched area represents the area over which S_{zz} is averaged. The coordinates (r, θ) are illustrated in the figure, and the z coordinate is perpendicular to the (r, θ) plane.

extrapolated to find the value of $n = n_0$ for which this ratio is 2.0 for different values of n_{max}. As n_{max} was varied in the range $3 \leq n_{max} \leq 12$, values in the range $-1 \leq n_0 \leq -3$ were obtained. Since this range corresponds to the polar headgroup region, the result of this empirical analysis of the ^{2}H NMR data is consistent with the well-established results obtained by using ^{31}P NMR as described in the first part of this section.

(*iii*) *Theory of Orientational Order in the H_{II} Phase.* There exists no published theoretical orientational order parameter profile $S(n)$ for the H_{II} phase, even though the theory of orientational order, using acyl chain conformational averaging methods, in other amphiphilic phases having curved lipid–water interfaces has been treated and discussed [for a review, see Ben-Shaul and Gelbart (1985)]. Our extension of de Gennes' "stretching vector" model does provide a way of quantitatively analyzing measurements of orientational order in H_{II} phases. In order to relate $S_{ij}(\mathbf{r})$ given in eq 3 to the chain orientational order tensor $S_{ij}(n)$, it is necessary to know the spatial distribution function $P_n(\mathbf{r})$ of the nth carbon atom of the acyl chain. If this is known, $S_{ij}(n)$ can be calculated by using the obvious relation

$$S_{ij}(n) = \int \mathrm{d}V S_{ij}(r) P_n(\mathbf{r}) \tag{6}$$

We have not yet attempted to develop a theory for $P_n(\mathbf{r})$. However, it is clear that eq 6 will be useful in the interpretation of $S_{ij}(n)$ values for different molecules such as phospholipids and alkanes which are expected to have different spatial distribution in the H_{II} phase (Kirk & Gruner, 1985). For our present purpose, we shall simply make use of the fact that the average of $S_{ij}(n)$ over all carbon positions n is identical with the average over the entire volume of $S_{ij}(\mathbf{r})$. In this regard, the H_{II} phase does *not* have cylindrical symmetry. As shown in Figure 7, it consists of a two-dimensional lattice of hexagonally coordinated cylinders of radius R_w whose centers are a distance d apart so that only in the limit $R_w/d \to 0$ is the solution of eq 4 for cylindrical symmetry valid. The solution of eq 2 in the H_{II} phase, expressed in cylindrical coordinates $\mathbf{r} = (r, \theta, z)$ and their unit vectors $(\hat{r}, \hat{\theta}, \hat{z})$ (Figure 7) and including lowest order corrections in R_w/d is given by

$$\begin{aligned}\mathbf{J}(\mathbf{r}) &= J_r\hat{r} + J_\theta\hat{\theta} + J_z\hat{z} \\ &= (A/r) \times \\ &\quad \{1 + (2R_w/d)^6[(r/R_w)^6 + (r/R_w)^{-6}] \cos 6\theta\}\hat{r} - \\ &\quad (A/r)\{(2R_w/d)^6[(r/R_w)^6 - (r/R_w)^{-6}] \sin 6\theta\}\hat{\theta} + \\ &\quad \text{terms involving sine and cosine of } 12\theta, 18\theta, \text{ etc.}\end{aligned} \tag{7}$$

where A is a constant determined by boundary conditions. This is valid (approximately) between $r = R_w$ and values of r corresponding to the boundary of the hexagon formed by the bisectors of the vectors in the (r,θ) plane joining the cylinder axis at $r = 0$ with those of its nearest neighbors. Outside this hexagon, $\mathbf{J}(\mathbf{r})$ may be obtained from the translational symmetry properties of the H_{II} phase. The principal axes of $S_{ij}(\mathbf{r})$, defined by eq 3, are along the **u**, **v**, and **z** directions where $\hat{u}$ and $\hat{v}$ are, respectively, parallel and perpendicular to $J_r\hat{r} + J_\theta\hat{\theta}$. The **u** and **v** axes are in the (r, θ) plane and are rotated by an angle $\Delta = \tan^{-1}(J_\theta/J_r)$ from the $\hat{r}$ and $\hat{\theta}$ directions. The principal values of S_{ij} are easily shown to be

$$S_{uu} = S_\parallel = C(J_r^2 + J_\theta^2) \tag{8}$$

$$S_{vv} = S_{zz} = S_\perp = -(1/2)C(J_r^2 + J_\theta^2)$$

As discussed earlier, we can identify the *local* orientational order parameter S_{uu} in the immediate vicinity of the cylinder surface with that near the glycerol backbone in the L_α phase, $S_{L_\alpha}(0)$. Thus, for $(2R_w/d)^6 \ll 1$, we make the approximation

$$S_{L_\alpha}(0) = S_{uu}(r = R_w) \approx CA^2/R_w^2 \tag{9}$$

This enables us to compare the predictions of the model described above for the average ^{2}H NMR quadrupolar splitting in the H_{II} phase, which is governed by $\langle S_{zz}\rangle$, with that of the plateau in the L_α phase. It should be noted that the average $\langle S_{zz}\rangle$ over the volume occupied by the chains is identical with the average $\langle S(n)\rangle$ over all chain positions n. Again, neglecting terms of order $(2R_w/d)^6$ and averaging S_{zz} over an *assumed* uniform distribution of lipid chains in the annular region between $r = R_w$ and $r = \mathrm{d}/2 = R_w(1 + q)$, we obtain

$$\begin{aligned}\langle S_{zz}\rangle &\approx -CA^2 \ln(1 + q)/[R_w^2 q(2 + q)] \\ &\approx -S_{L_\alpha}(0) \ln(1 + q)/[q(2 + q)]\end{aligned} \tag{10}$$

It may be seen that the correct average of $\langle S_{zz}\rangle = -(1/2)S_{L_\alpha}(0)$ for ^{31}P NMR is obtained by taking the limiting value of eq 9 as $q \to 0$.

Though measurements of q have not been carried out for POPE, data on R_w and d are available as a function of temperature for DOPE (Tate & Gruner, 1989). These measurements show that R_w varies between 23 and 17 Å as the temperature is increased from 10 to 80 °C, while $(d/2) - R_w$ remains constant at about 16 Å. The value of d in DOPE at 45 °C is approximately the same as in POPE at 75 °C. At that temperature, $q \approx 0.84$ in DOPE, which gives a ratio $\langle S_{zz}\rangle/S_{L_\alpha}(0) \approx 0.26$. This ratio may be compared with the value of 0.27 obtained by identifying $S_{L_\alpha}(0)$ with $S_{max} \approx 0.20$ (see Figure 3) and estimating $\langle S_{zz}\rangle \approx 0.054$ from the data of Figure 4. Though we are comparing different systems (POPE and DOPE), when these systems have identical values of d, the close correspondence of their values of q obtained from two different types of measurements is impressive. It indicates that it may be possible to understand orientational order in the H_{II} phase, and other liquid-crystalline phases as well, in terms of simple geometrical features of the structure and independent of detailed models of conformational averaging. This possibility should now be explored theoretically and experimentally.

ACKNOWLEDGMENTS

We are indebted to Drs. E. F. Eikenberry and S. M. Gruner for providing us with the X-ray diffraction results. The figures of this paper were prepared by using the computer graphics software developed at the Tri-University Meson Facility (Vancouver, BC).

Registry No. POPE, 10015-88-0; tetradecanol, 112-72-1; decane, 124-18-5.

REFERENCES

Ben-Shaul, A., & Gelbart, W. M. (1985) *Annu. Rev. Phys. Chem. 36*, 179-211.

Bloom, M., Davis, J. H., & Dahlquist, F. W. (1978) in *Proceedings of the XXth Ampere Congress, Tallin* (Kundla, E., Lippmann, E., & Saluvere, T., Eds.) p 551, Springer-Verlag, Berlin.

Bloom, M., Davis, J. H., & MacKay, A. L. (1981) *Chem. Phys. Lett. 80*, 198-202.

Cullis, P. R., & deKruijff, B. (1976) *Biochim. Biophys. Acta 436*, 523-540.

Cullis, P. R., & deKruijff, B. (1979) *Biochim. Biophys. Acta 559*, 399-420.

Davis, J. H. (1979) *Biophys. J. 27*, 339-358.

de Gennes, P. G. (1974) *Phys. Lett. 47A*, 123-124.

Epand, R. M. (1985) *Biochemistry 24*, 7092-7095.

Gruner, S. M. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3665-3669.

Jacobs, R. E., & White, S. H. (1984a) *J. Am. Chem. Soc. 106*, 915-920.

Jacobs, R. E., & White, S. H. (1984b) *J. Am. Chem. Soc. 106*, 6909-6912.

Kirk, G. L., & Gruner, S. M. (1985) *J. Phys. (Paris) 46*, 761-469.

Lafleur, M., Fine, B., Sternin, E., Cullis, P. R., & Bloom, M. (1989) *Biophys. J. 56*, 1037-1041.

Lafleur, M., Bloom, M., & Cullis, P. R. (1990) *Biochem. Cell. Biol. 68*, 1-8.

Mantsch, H. H., Martin, A., & Cameron, D. G. (1981) *Biochemistry 20*, 3138-3145.

Paddy, M. R., Dahlquist, F. W., Dratz, E. A., & Deese, A. J. (1985) *Biochemistry 24*, 5988-5995.

Perly, B., Smith, I. C. P., & Jarrell, H. C. (1985) *Biochemistry 24*, 1055-1063.

Pope, J. M., Walker, L. W., & Dubro, D. (1984) *Chem. Phys. Lipids 35*, 259-277.

Sankaram, M. B., & Marsh, D. (1989) *Biophys. J. 56*, 1043-1044.

Seddon, J. M., Cevc, G., Kaye, R. D., & Marsh, D. (1984) *Biochemistry 23*, 2634-2644.

Seelig, A., & Seelig, J. (1974) *Biochemistry 13*, 4839-4845.

Seelig, J. (1978) *Biochim. Biophys. Acta 515*, 105-140.

Seelig, J., & Seelig, A. (1980) *Q. Rev. Biophys. 13*, 19-61.

Siegel, D. P., Banschbach, J., & Yeagle, P. H. (1989) *Biochemistry 28*, 5010-5019.

Sjölund, M., Lindblom, G., Rilfors, L., & Arvidson, G. (1987) *Biophys. J. 52*, 145-153.

Sjölund, M., Rilfors, L., & Lindblom, G. (1989) *Biochemistry 28*, 1323-1329.

Sternin, E. (1985) *Rev. Sci. Instrum. 56*, 2043-2049.

Sternin, E., Bloom, M., & MacKay, A. L. (1983) *J. Magn. Reson. 55*, 274-282.

Sternin, E., Fine, B., Bloom, M., Tilcock, C. P. S., Wong, K. F., & Cullis, P. R. (1988) *Biophys. J. 54*, 689-694.

Tate, M. W., & Gruner, S. M. (1989) *Biochemistry 28*, 4245-4253.

Thewalt, J., Wassall, S. R., Gorrissen, H., & Cushley, R. J. (1985) *Biochim. Biophys. Acta 817*, 355-365.

Thewalt, J., Tulloch, A. P., & Cushley, R. J. (1986) *Chem. Phys. Lipids 39*, 93-107.

Tinkham, M. (1964) *Group theory and quantum mechanics*, pp 124-130, McGraw-Hill, New York.

Chemiluminescence of the Mn^{2+}-Activated Ribulose-1,5-bisphosphate Oxygenase Reaction: Evidence for Singlet Oxygen Production†

Scott N. Mogel and Bruce A. McFadden*

Biochemistry/Biophysics Program, Washington State University, Pullman, Washington 99164-4660

Received March 14, 1990; Revised Manuscript Received June 12, 1990

ABSTRACT: Chemiluminescence has been observed during catalysis by Mn^{2+}-activated ribulose-bisphosphate carboxylase/oxygenase from spinach. The luminescence is ribulose 1,5-bisphosphate (RuBP) and O_2-dependent and is inhibited by 2-carboxyarabinitol 1,5-bisphosphate and high concentrations of bicarbonate; it is therefore ascribed to the RuBP oxygenase activity. The luminescence is inhibited by azide and enhanced in D_2O and in the presence of diazabicyclooctane. The emission maximum is between 620 and 660 nm. The initial rate of light emission is second order in enzyme concentration. The data strongly suggest that singlet oxygen is produced during turnover, that the observed chemiluminescence is due to dimol emission of singlet oxygen, and that this provides a basis for a highly sensitive assay for RuBP oxygenase.

The enzyme D-ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO),[1] as the name implies, catalyzes both the carboxylation and oxygenation of RuBP. These reactions are the initial steps in photosynthesis and photorespiration, respectively [for a review, see Miziorko and Lorimer (1983)]. Although the oxygenase activity competes for RuBP with the carboxylase activity and the resultant photorespiration appears to oppose photosynthesis, all RuBP carboxylases studied to date catalyze oxygenation. It has been proposed that the

† Supported in part by NIH Research Grant GM-19972.

* To whom correspondence should be addressed.

[1] Abbreviations: CABP, 2-carboxyarabinitol 1,5-bisphosphate; cps, counts per second; DABCO, diazabicyclooctane; MOPS, 3-(*N*-morpholino)propanesulfonic acid; $^1O_2$, singlet oxygen; $^3O_2$, triplet oxygen; RuBisCO, D-ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, D-ribulose 1,5-bisphosphate; SOD, superoxide dismutase.

0006-2960/90/0429-8333$02.50/0