the Rouse models provides a general description of the dynamical behavior on length scales up to 40 Å and time scales to the 10⁻³-s region. Deviations of the experimental line shape from that predicted by the Rouse model cannot be attributed to local reorientation. Thus, an influence of entanglement constraints is not visible on the experimental length and time scales. The experiments in semidilute solutions led to the direct observation of hydrodynamic screening on a microscopic scale and allowed an evaluation of the hydrodynamic screening length $\xi_H$. Its magnitude is close to that of the excluded volume screening length $\xi_v$, which we have determined recently on the similar system PDMS/C₆D₆. This result disagrees with the theoretical prediction $\xi_H \approx 4\xi_v$ supposedly valid in the semidilute regime. Perhaps the discrepancy results from using too concentrated systems in our study. More experiments in the semidilute regime are necessary in order to solve this problem and to extract the concentration exponent for $\xi_H$. Line-shape arguments as well as the quantitative evaluation of our data support strongly the model of incomplete screening. The viscosity $\eta$ which appears in incomplete screening is not yet interpreted on a microscopic level. It exhibits startling similarities to the macroscopic viscosity of an equivalent but nontangled PDMS solution of smaller molecular weight. In concentrated solution above $\epsilon = 45\%$ enhanced Rouse relaxation prevails. Microscopically determined segmental friction coefficients compare well with those taken from zero-shear viscosity measurements. This agreement demonstrates the basic correctness of the molecular foundations of the Rouse model.

Acknowledgment. We thank Dr. B. Lehnen for providing the PDMS and Dr. J. B. Hayter for assistance during the experiments. This work was supported in part by the Bundesministerium für Forschung und Technologie and by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 41).

Registry No. Neutron, 12586-31-1.

Electron Spin Resonance Studies on Ordering and Rotational Diffusion in Oriented Phosphatidylcholine Multilayers: Evidence for a New Chain-Ordering Transition

Hisao Tanaka and Jack H. Freed

Baker Laboratory of Chemistry, Cornell University, Ithaca, New York 14853 (Received: June 12, 1984)

An alignment technique based on compression has been developed for preparation of planar oriented lipid samples for ESR. The ESR spectra of defect-free samples prepared by this technique could be studied over a wide temperature range, showing gel and liquid-crystalline phases of DPPC and DMPC. The ESR spectra of spin-labeled DPPC’s and cholestane clearly indicate a new phase transition occurring at 100–110 °C in the liquid-crystalline phases of DPPC and DMPC hydrated to 3 wt % water with somewhat higher temperatures for higher water content. The extensive spectra that were obtained could be analyzed in detail with a model of anisotropic molecular rotation in a mean orienting potential by using the methods of spectral analysis previously developed by Freed and co-workers. On the basis of the results on molecular ordering and rates of rotational diffusion, the new phase transition is characterized as one in which orientational order is significantly reduced but the rate of molecular motions is not drastically changed. This is compared with a recent theoretical model, which predicts a similar high-temperature transition. The present results also reveal some new aspects regarding hydrocarbon chain motion and disorder in the crystalline phase.

I. Introduction

In recent years, some new phase transitions have been found for dimyristoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC). These consist of weak transitions occurring below¹ and above² the temperature of the main transition, $T_c$ (gel–liquid crystalline transition), as well as a metastable transition below $T_c$³ that are observed with lipid systems containing excess water. Fine structure⁴ (i.e., successive pretransition phenomena) occurring in the range of the main transition has also been observed with lipids hydrated to low water content. However, phase diagrams for the higher temperature range (≥100 °C) have not been studied as extensively.⁵–¹⁰ We have found, in this study, a new phase transition occurring at 100–110 °C in the liquid-crystalline phase of DPPC and DMPC hydrated to low content (≤5%).

The purpose of this study with spin-labeled DPPC and with cholestane (CSL) is to assign DPPC and DMPC phases (and phase transitions) found from ESR spectra and to characterize them with respect to ordering of the chain and its motion. This is accomplished by determining the ordering and diffusion constants of the nitroxide spin-labels using the ESR line-shape analysis valid over the slow- and fast-motional regime that has been developed in this laboratory.¹¹–¹⁵ The present work was motivated by an effort¹⁶ to study the protein–lipid interaction with the present objective being to obtain precise data on the pure lipids as standard systems to compare with the complex ESR spectra¹⁶ of samples containing protein (polypeptide).

In order to accomplish the above objectives, we sought a reliable alignment technique for preparation of oriented ESR samples.

Systems

¹Permanent address: Josai University, Saitama, Japan.


0022-3654/84/2088-6633$01.50/0 © 1984 American Chemical Society
One limitation of the evaporation technique used in previous ESR studies is that the macroscopic orientation of the samples prepared as films becomes unstable at higher temperatures. We developed a compression technique on the basis of Asher's alignment method. The technique consists of repeating compressions on lipids just above the main transition temperature. The quality of sample orientation was determined by use of polarizing microscopy. We have confirmed that the morphology (as well as the transmitted photointensity) of an oriented sample detected under crossed polarizers closely correlates with the degree of order manifested in the ESR spectra. The procedure of sample preparation and quality control is described in the Experimental Section.

The use of defect-free oriented samples enabled us to clearly observe lipid-phase transitions through the appearance of composite spectra in the transition (two-phase) region. In general, the well-oriented ESR spectra were analyzed with significantly less ambiguity than is possible for those from unoriented systems. From ESR observations on DPPC and DMPC hydrated to 3% water, three phase transitions were found in the temperature range ≤180 °C: two were assigned to the main transition and to the isotropic transition by reference to the transition temperatures in the literature. The remaining one, at 100–110 °C, which was observed most distinctly in the present experiments using oriented lipid samples and hydrocarbon chain (or phosphatidylcholine) spin-labels was characterized as a "chain-orientational" transition from the data analyzed. By this we mean a transition in which the mean ordering of the lipid chains is significantly reduced but with less dramatic effects on the chain dynamics. In a recent theory, Kimura and Nakano have predicted the appearance of what may be a similar orientational transition in the liquid-crystalline phase of DPPC, DMPC, and other lipids. Thus, we believe our observations provide direct evidence for the presence of this orientational transition, as discussed in this paper.

Another aspect of this work focuses on a current topic in magnetic resonance studies on lipid dispersions regarding the presence and type of hydrocarbon chain motion and/or disorder in the gel phase, for which the model of a rigid and extended conformation (e.g. all-trans) was established by previous X-ray studies. In the present work, more detailed aspects of such matters were revealed by our systematic study of ordering, anisotropic diffusion, and their temperature dependence using the different spin probes.

Our experimental methods are described in section II, while our results and spectral analyses are given in section III. These results are discussed in section IV, with a summary and concluding remarks in section V.

II. Experimental Section

Materials. The phosphatidylcholines DPPC and DMPC were purchased from Sigma and checked for purity by thin-layer chromatography according to standard methods. The nitroxide CSL (3-doxyl derivative of cholest-3-one) was obtained from

\[ \text{CSL, SPC, and 16PC. The relation between the molecular axes systems of the ordering axes (x',y',z') and magnetic tensor axes (x'',y'',z'') is found by spectral simulation to be } \]

\[ z' = z'' \text{ for 16PC and 5PC and } z' = z'' \text{ for CSL.} \]

Synvar, and the DPPC derivative nitroxides 5PC and 16PC (DPPC's bonded at 5th and 16th carbon of the hydrocarbon chain by the nitroxide moiety) were a gift from G. W. Feigenson, Department of Biochemistry, Cornell University. These nitroxides are shown in Figure 1. Hexadeoxytrimethylammonium bromide (HTAB), purchased from Aldrich and recrystallized from toluene, was used to coat the glass plates used for a sample sandwich. The glass plates (0.8 × 2.2 cm² in area and 130 μm in thickness) were cleaned first with chloroform, then hot concentrated sulfuric acid, and then deionized water and were treated with HTAB just before use to promote homeotropic alignment.

Mixtures of lipid with nitroxide were prepared by a freeze–dry method in the following procedure. Typically 25 mg of lipid was put in a 2-mL glass flask with a ground joint. A given volume of benzene–methanol (95:5 v/v) solution of nitroxide was added to the flask to form 0.5 mol % nitroxide mixture with lipid. After complete dissolution of the solutes, the flask was linked to a vacuum line, and the solution was quickly frozen with liquid N₂. The frozen solution in a dry ice–2-propanol cold bath was evacuated for 2 days at 10⁻³ torr, followed by further evacuation for 2 days at room temperature to ensure solvent removal. The dry solids were crushed to a fine powder and were hydrated to a water content below 20 wt %. A homogeneous distribution of water in the lipid layer was promoted by gas–solid absorption. It consists of separately placing a proper amount of deionized water and the powdered mixture inside the flask sealed under Ar atmosphere. After being kept for a week at room temperature in the dark, the hydrated mixture was aligned as described below.

Alignment Procedure with the Compression Technique. Sample holders for compression were made of a Mylar sheet stuck on the surface of a microscope glass slide and designed to form a cavity at the center. A surfactant-treated glass plate was placed in the cavity of the holder fixed onto the heating stage. Subsequently, 3 mg of hydrated lipid mixture was spread on the glass, away from the edge, and was covered by the second glass plate and then by the top holder. The assembly was heated to above the main transition temperature (Tₒ) while pressed from the top. The optimal temperature for compression, corresponding to the softening temperature of the hydrated lipid, depends on water content (65–80 °C on going from 15 to 2 wt % water in DPPC). At the appropriate temperature, the sandwiched sample was compressed with pressure applied from above but translated in strokes of several seconds duration across the surface. After the process was repeated for several minutes, the lipid layer became spread to a thin layer (5–20 μm). After the above procedure, the sample assembly, with pressure applied from the top, was cooled to room temperature.
temperature at a rate of 1–2 °C/min. An excess of the starting lipid material leaking out of the sample sandwich was removed, and the sandwich was sealed on its edges with epoxy or some other adhesive.

Skillful use of this technique can provide substantially defect-free (≤3% of defect area) oriented lipid samples at comparatively low temperatures (≤80 °C) and in a short period of time (≤1 h). Furthermore, the planar orientation of the lipid multilayer between the glass plates remains stable as the ESR spectra are recorded, without the appearance of any defects, over the full temperature range including the gel and liquid-crystalline phases. The defect-free samples used for ESR measurements were prepared as multilayers with a typical thickness of 10 μm (~2000 bilayers) containing a monodomain with at worst a few small air bubbles.

The water content in a sample was calibrated with 1H NMR measurements of a nitroxide-free reference sample prepared in the same manner as the ESR samples. The oriented sample was crushed in a small vial and extracted with 400 μL of deuterated benzene–chloroform (2:1 v/v) mixture. The 1H NMR spectrum of extract was recorded on a Varian 90-MHz CFT-20 pulse spectrometer. The water content was determined from the relative intensity of the water signal (δ: 2.3–2.8 ppm, referred to Me2Si) vs. the trimethylammonium group of the lipid (cf. Figure 1) (δ = 3.3 ppm). The main transition temperature (Tm) was determined from the ESR spectrum of the corresponding nitroxide sample prepared at the same time and in the same manner. Thus, the water content of subsequent ESR samples was determined according to the relation Tm/°C = -1.5 × [water] + 77 (for samples ≤15 wt % of water). This relation was established from the sets of NMR and ESR samples of different water content. Accuracy is expected to be ±1 to ±2 wt %.

Characterization of Oriented ESR Samples. An incomplete procedure for sample alignment leaves unoriented areas (defects) in the sample. Defects are also produced through dilations of the sample sandwich, rapid change of temperature, etc. In the course of establishing our alignment technique and the conditions for ESR measurements, we observed several types of defects using a polarizing microscope. These are, according to the morphological nomenclature used in the previous literature,11 amorphous type (i.e. starting with unoriented materials), polygonal-array type, oily streak type, and strandlike type. These defects were observed under crossed polarizers to study the conditions of their appearance and evolution and also to estimate their area relative to the remaining oriented region by utilizing measurement of the transmitted photointensity.11 In general, defects in thin samples (<30 μm) were observed as white (bright) areas. The ESR spectra of samples containing defects are composed of two components of which the one assigned to defects exhibits no detectable angular dependence of the hyperfine splittings on rotation of the plate sample. Such spectral features were observed only under the conditions where the respective type of defect could be observed under crossed polarizers. For example, the amorphous type of defect is stable below and above Tm, while the polygonal type only appears above Tm. Although clear observation of the defect signal depended on such factors as the particular nitroxide used, the temperature, water content, etc., the signal intensity observed with CSL samples was found to be proportional to the defect area estimated optically. From these facts as well as some others, it was judged that the quality of the ESR sample alignment is decisively determined by the presence of defects detected optically. The samples which contained substantially no defects (≤3%) produced a simple triplet pattern32 of nitroxide over the temperature range studied except in the phase transition region, as discussed in the next section. Because of the importance of recognizing and eliminating defects for any careful ESR studies

![Figure 2](https://example.com/figure2.png)

**Figure 2.** ESR spectra of 16PC in oriented DPPC multilayers with 3 wt % hydration. Phase transitions are observed at 75 and 100 °C. Phase I, below 75 °C, is the biaxial gel phase. Phases II and III are liquid-crystalline Lc phases.

on oriented samples, we provide further discussion on these matters in the Appendix. Polarizing Microscopy and ESR Measurements. Oriented lipid samples were observed by a polarizing microscope, a Nikon OPTIPHOTO-POL, before and after ESR measurement. A Mettler FP5-FP52 thermostat control accessory attached to the microscope was used for observation of defects at higher temperatures. Transmitted photointensity under crossed polarizers was measured with a Mettler 18100 photorecorder unit attached to the microscope.

For ESR measurements, a sample attached to a goniometer was placed in a Varian TE 011-mode cavity. The ESR spectra were recorded on a Varian E-12 spectrometer at X band with 100-kHz field modulation. The temperature of the sample was controlled with a Varian 257 accessory and was read from an OMEGA 410A-TC digital thermometer with a fine copper–constantan thermocouple, one terminal of which was placed at the center of sample plate. The estimated uncertainty in temperature was ±1 °C. The temperature of sample was only changed slowly to avoid producing defects. The ESR spectra were usually taken every 5 °C, at θ = 0 and 90° (the normal to the sample plate parallel and perpendicular to the external magnetic field, respectively) and at intermediate values of θ at some temperatures in each phase. The reproducibility of the ESR spectra was checked by comparing the spectra taken at the same temperature in both heating and cooling cycles. The main transition temperature (Tm) observed in both processes was used to estimate any loss of hydrated water at higher temperatures. The microwave power and the modulation amplitude employed were low enough so as not to affect the ESR line shapes. We confirmed that there was no broadening for the nitroxide concentration (0.5 mol %) used from intermolecular spin interactions (e.g., Heisenberg spin exchange interaction).

III. Experimental Results and Spectral Analysis

The temperature dependence of the experimental spectra of the oriented lipid samples is shown for 16PC in DPPC and CSL in DMPC hydrated to 3%, in Figures 2 and 3, respectively. In the former spectra, phase transitions are observed at 75 and 100 °C, where two-phase spectra due to components from the upper and
Tanaka and Freed

Figure 3. ESR spectra of CSL in oriented DMPC multilayer hydrated to 3 wt.%. Phase transitions occur between 50 and 60 °C and between 95 and 115 °C.

Figure 4. Temperature dependence of the apparent line splittings of 16PC in oriented DPPC multilayer hydrated to 3 wt. %. 1, biaxial gel phase; II and III, liquid-crystalline phases; IV, isotropic phase. The splittings were measured between the centers of $M_1 = 0$ and 1 lines.

lower temperature ordered phase(s) may be seen. The apparent hyperfine splittings are plotted as a function of temperature up to 180 °C in Figure 4. From this figure, we can find the third transition in the range of 140–160 °C, leading to angular ($\theta$)-independent ESR spectra. In DMPC hydrated to 3% (Figure 3), the first and second transitions were observed between 50 and 60 °C and between 95 and 115 °C, respectively. In the respective ordered phases I, II, and III (with increasing temperature), the observed line splitting was a maximum for CSL and a minimum for 5PC and 16PC at $\theta = 90^\circ$ (i.e. normal to the bilayer plane perpendicular to the external magnetic field) with an experimental error ($\pm 2^\circ$) in sample setting. Figure 5 shows plots of splitting with $\theta$ for CSL and 16PC in phase I. The ESR spectra of 5PC in phase II at different $\theta$'s are shown in Figure 6.

With an increase of the water content in DPPC, the temperature of the first transition decreased monotonically to 50 °C; i.e. $T_s$ is $75 \pm 3$ °C at 3% water, $66$ °C at 7%, $57$ °C at 12%, and $52$ °C at 15%. The temperature of the second transition tended to increase from 100 °C at 3% water to 110 °C at 7%. The second transition temperature in samples hydrated at higher water content could not be measured because of the greater loss of water from the sample sandwich at the high temperatures. Where no loss of the water occurred (i.e. at lower hydration), reproducibility of the spectra and transition temperatures was observed in the heating and cooling cycles. Judging from spectral characteristics on phase transitions, the two-phase range (transition range) was wider in the second transition ($\sim 20^\circ$, e.g. 90–110 °C for 3 wt % H$_2$O-DPPC) than in the first one ($\sim 10^\circ$). From comparison of the above results on transition temperatures with the phase diagrams of DPPC and DMPC hydrated at low water contents, the first transition can be assigned to the main transition (gel–liquid crystalline transition; we shall follow Powers and Pershan who classified the low-temperature phase as a biaxial gel phase). The isotropic transition was previously observed with a conoscopic birefringence method as a transition from an optically uniaxial phase to an isotropic phase (135–160 °C in DPPC hydrated to 2–8%). Thus, the third transition in this work is regarded as identical with the optically isotropic transition. In section IV, we will discuss the character of the second transition found in this study.

The ESR spectra of the oriented samples were analyzed by use of the method of line-shape analysis described in detail elsewhere. This analysis involves the following coordinate systems: the first ($x,y,z$) is the laboratory frame with the $z$ axis along the external magnetic field. A second ($x'',y'',z''$) is the principal axis system of the magnetic tensor of the molecule A (with an arbitrary tilt of $g$ relative to A allowed but not needed in this work). A third ($x',y',z'$) is the ordering axis (which is taken as the same as the rotational diffusion axis) system of the molecule. The last

Table of Contents

1. Introduction
2. Experimental
3. Results and Discussion
4. Conclusion

1. Introduction

The effects of temperature and hydration on the alignment and diffusion of oriented phosphatidylcholine multilayers were studied. The constants used for the simulation are listed in Table I. The calculated spectra at 60 °C exemplify an effect of temperature (33.5 G for CSL and 14.4 G for 16PC and 5PC) found in the spectra of CSL in oriented DPPC hydrated to 3 wt %.

2. Experimental

The potential function determining molecular ordering was introduced into the simulations (mainly for the parallel and perpendicular rotational diffusion coefficients). The potential is expanded in a series of Wigner rotation matrix elements.

3. Results and Discussion

The principal values of the potential function were determined as follows. The potential, \( \lambda D_{00} + \rho(D_{00} + D_{02}) + \epsilon D_{02} + \ldots \), where \( C_{MX} \), \( \lambda, \rho, \) and \( \epsilon \) are dimensionless coefficients of the respective terms and \( \Omega \) represents the Euler angles between the \((x',y',z')\) and \((x'',y'',z'')\) molecular axis systems.

The potential function function determines molecular ordering is expanded in a series of Wigner rotation matrix elements.

4. Conclusion

The calculated spectra of 16PC in oriented DPPC hydrated to 3 wt %, shown at 60 °C (phase I), 80 °C (phase II), and 120 °C (phase III). The constants used for the simulation are listed in Table I. The calculated spectra at 60 °C exemplify an effect of temperature (33.5 G for CSL and 14.4 G for 16PC and 5PC) found in the spectra of CSL in oriented DPPC hydrated to 3 wt %.

### Table I

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda )</td>
<td>5.0 G</td>
</tr>
<tr>
<td>( \rho )</td>
<td>2.0058</td>
</tr>
<tr>
<td>( \epsilon )</td>
<td>0.5 G</td>
</tr>
</tbody>
</table>

### Table II

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A_{11} )</td>
<td>33.5 G</td>
</tr>
<tr>
<td>( A_{12} )</td>
<td>14.6 G</td>
</tr>
<tr>
<td>( A_{13} )</td>
<td>14.4 G</td>
</tr>
</tbody>
</table>

Figure 7. Comparison of the experimental (---) and best-fit calculated (-----) spectra of CSL in oriented DPPC hydrated to 3 wt %, shown at 60 °C (phase I), 80 °C (phase II), and 120 °C (phase III). The calculated spectra at 60 °C exemplify an effect of temperature (33.5 G for CSL and 14.4 G for 16PC and 5PC) found in the spectra of CSL in oriented DPPC hydrated to 3 wt %.

Figure 8. Water content dependence of the ESR spectra of CSL in oriented DPPC, recorded at 70 °C (phase II): (---), experimental spectrum; (- - -), best-fit calculated spectrum. The other constants are as follows: \( D_{00} = 0.01 \) at 7 and 12 wt % water and 0 at 15 and 20 wt %; \( R_{12} = 9.0 \times 10^7 \) at 7%, 1.7 \times 10^7 at 12%, 4.0 \times 10^7 at 15%, and 6.0 \times 10^7 at 20%; \( N = 50 \) and \( T_2^{*-1} = 1.2 \) G at all the water contents.

Figure 9. Water content dependence of the ESR spectra of CSL in oriented DPPC, recorded at 70 °C (phase II): (---), experimental spectrum; (- - -), best-fit calculated spectrum. The other constants are as follows: \( D_{00} = 0.01 \) at 7 and 12 wt % water and 0 at 15 and 20 wt %; \( R_{12} = 9.0 \times 10^7 \) at 7%, 1.7 \times 10^7 at 12%, 4.0 \times 10^7 at 15%, and 6.0 \times 10^7 at 20%; \( N = 50 \) and \( T_2^{*-1} = 1.2 \) G at all the water contents.

phys. J. motions is, however, limited, and we find, in general, that simpler moiety is attached. The spectral sensitivity to such complex overall motion of the labeled lipid with many internal modes of motion of the flexible hydrocarbon chain to which the nitroxide moiety is attached. It was difficult to evaluate the subtle effect of spreading parameter $\langle D_{22} \rangle$ by the following expression:

$$
\langle D_{22} \rangle = \frac{1}{6} R L,
$$

and $\langle D_{22} \rangle = \int P(\theta, \phi) \sin \theta^2 d\theta^2 d\phi$, where $\theta$ denotes the angle between the principal axis $z'$ of the ordering tensor and the principal axis $z''$ of the director frame. $P(\theta, \phi) \sin \theta^2 d\theta^2 d\phi$ is the distribution of $z'$ relative to $z''$ given by $P(\theta, \phi) = \exp[-\lambda/(3 \cos^2 \theta - 1)] + \{6(1/2)\sin^2 \theta \cos 2\theta\}$. 

![Figure 10](image10.png)

**Figure 10.** Plots of the ordering $\langle D_{22} \rangle$ vs. temperature for CSL (■) in oriented DPPC hydrated to 3 wt % and 16PC (○) in oriented DPPC hydrated to 3%; I, biaxial gel phase; II and III, liquid-crystalline phases; IV, isotropic phase.

![Figure 11](image11.png)

**Figure 11.** Arrenius plots of the mean diffusion constant $R$ (●) in oriented DPPC hydrated to 3 wt % and ○ 16PC in oriented DPPC hydrated to 3%; I, biaxial gel phase; II and III, liquid-crystalline phases; IV, isotropic phases.

![Figure 12](image12.png)

**Figure 12.** Arrhenius plots of the mean diffusion constant $R$ of 16PC in oriented DPPC hydrated to 7% based on values in Table III-B.
Oriented Phosphatidylcholine Multilayers

Table II: Parameters for Molecular Ordering and Anisotropic Rotation for 16PC in DPPCa

<table>
<thead>
<tr>
<th>t, °C</th>
<th>phase</th>
<th>(D_e2)</th>
<th>(D_e2 + D_z2)</th>
<th>R_1, s^-1</th>
<th>R_1, s^-1</th>
<th>N</th>
<th>E_a, kcal/mol</th>
<th>T_2* s^-1, G</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Hydrated to 3 wt %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>I</td>
<td>0.32</td>
<td>-0.17</td>
<td>9.3 x 10^7</td>
<td>1.9 x 10^8</td>
<td>2.0</td>
<td>6.1</td>
<td>1.4</td>
</tr>
<tr>
<td>40</td>
<td>I</td>
<td>0.32</td>
<td>-0.17</td>
<td>1.8 x 10^8</td>
<td>3.6 x 10^8</td>
<td>1.2</td>
<td>(3.0)</td>
<td>0.8</td>
</tr>
<tr>
<td>50</td>
<td>I</td>
<td>0.32</td>
<td>-0.17</td>
<td>2.4 x 10^8</td>
<td>4.8 x 10^8</td>
<td>1.0</td>
<td>5.9</td>
<td>0.5</td>
</tr>
<tr>
<td>60</td>
<td>I</td>
<td>0.32</td>
<td>-0.17</td>
<td>3.3 x 10^8</td>
<td>6.6 x 10^8</td>
<td>1.0</td>
<td>5.9</td>
<td>0.5</td>
</tr>
<tr>
<td>70</td>
<td>I</td>
<td>0.32</td>
<td>-0.17</td>
<td>4.2 x 10^8</td>
<td>8.4 x 10^8</td>
<td>1.0</td>
<td>5.9</td>
<td>0.5</td>
</tr>
<tr>
<td>80</td>
<td>I</td>
<td>0.19</td>
<td>-0.12</td>
<td>8.0 x 10^8</td>
<td>9.6 x 10^8</td>
<td>1.0</td>
<td>5.9</td>
<td>0.5</td>
</tr>
<tr>
<td>90</td>
<td>III</td>
<td>0.04</td>
<td>0.00</td>
<td>1.2 x 10^9</td>
<td>1.2 x 10^9</td>
<td>-0.10</td>
<td>2.0 x 10^9</td>
<td>2.1 x 10^9</td>
</tr>
<tr>
<td>120</td>
<td>III</td>
<td>0.03</td>
<td>0.00</td>
<td>1.4 x 10^9</td>
<td>1.4 x 10^9</td>
<td>-0.10</td>
<td>2.0 x 10^9</td>
<td>2.1 x 10^9</td>
</tr>
<tr>
<td>140</td>
<td>III</td>
<td>0.02</td>
<td>0.00</td>
<td>2.1 x 10^9</td>
<td>2.1 x 10^9</td>
<td>-0.10</td>
<td>2.0 x 10^9</td>
<td>2.1 x 10^9</td>
</tr>
<tr>
<td>(B) Hydrated to 7 wt %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-20</td>
<td>I</td>
<td>0.33</td>
<td>-0.11</td>
<td>2.7 x 10^7</td>
<td>5.4 x 10^7</td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>-10</td>
<td>I</td>
<td>0.33</td>
<td>-0.11</td>
<td>3.8 x 10^7</td>
<td>7.6 x 10^7</td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>0</td>
<td>I</td>
<td>0.33</td>
<td>-0.11</td>
<td>4.7 x 10^7</td>
<td>9.4 x 10^7</td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>10</td>
<td>I</td>
<td>0.33</td>
<td>-0.11</td>
<td>5.6 x 10^7</td>
<td>1.1 x 10^8</td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>50</td>
<td>I</td>
<td>0.33</td>
<td>-0.11</td>
<td>9.0 x 10^7</td>
<td>1.8 x 10^8</td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>70</td>
<td>II</td>
<td>0.19</td>
<td>0.00</td>
<td>8.5 x 10^8</td>
<td>1.0 x 10^9</td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>80</td>
<td>II</td>
<td>0.17</td>
<td>0.00</td>
<td>1.0 x 10^9</td>
<td>1.2 x 10^9</td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>120</td>
<td>III</td>
<td>0.02</td>
<td>0.00</td>
<td>2.0 x 10^9</td>
<td>2.0 x 10^9</td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
</tr>
</tbody>
</table>

a Estimated errors: ±2% in (D_e2), ±30% in (D_e2 + D_z2), ±10% in R_1, ±20% in N, ±0.1 G in T_2* s^-1. b See footnotes to Table IIA.

Table III: Parameters for Molecular Ordering and Anisotropic Rotation for 5PC in DPPC Hydrated to 3 wt %

<table>
<thead>
<tr>
<th>t, °C</th>
<th>phase</th>
<th>(D_e2)</th>
<th>(D_e2 + D_z2)</th>
<th>R_1, s^-1</th>
<th>R_1, s^-1</th>
<th>N</th>
<th>T_2* s^-1, G</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>I</td>
<td>0.64</td>
<td>6.0 x 10^6</td>
<td>6.0 x 10^6</td>
<td>6.0 x 10^6</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>60</td>
<td>I</td>
<td>0.64</td>
<td>1.2 x 10^7</td>
<td>1.2 x 10^7</td>
<td>1.2 x 10^7</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>70</td>
<td>I</td>
<td>0.64</td>
<td>2.0 x 10^7</td>
<td>2.0 x 10^7</td>
<td>2.0 x 10^7</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>80</td>
<td>II</td>
<td>0.46</td>
<td>1.2 x 10^8</td>
<td>2.4 x 10^8</td>
<td>2.4 x 10^8</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>90</td>
<td>III</td>
<td>0.41</td>
<td>1.6 x 10^8</td>
<td>3.2 x 10^8</td>
<td>3.2 x 10^8</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>110</td>
<td>III</td>
<td>0.21</td>
<td>5.0 x 10^8</td>
<td>5.0 x 10^8</td>
<td>5.0 x 10^8</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>130</td>
<td>III</td>
<td>0.16</td>
<td>6.0 x 10^8</td>
<td>6.0 x 10^8</td>
<td>6.0 x 10^8</td>
<td>10</td>
<td>1.5</td>
</tr>
</tbody>
</table>

a Estimated errors: 4% in (D_e2), ±20% in R_1, and ±50% in N. b See footnotes to Table IIA.

Figure 13. ESR spectra of 16PC in unoriented DPPC dispersions with 7 wt % water in a tube: (---), experimental spectra; (----), simulations based upon the parameters in Table IIB.

IV. Discussion

As is easily discerned from the structures of the nitroxides CSL, 5PC, and 16PC illustrated in Figure 1, these nitroxides are expected to report directly on the respective hydrocarbon chains of the lipid in different ways. The rigid and elongated nitroxide CSL, which is also hydrophobic, probes the average ordering of the chain skeleton (major part of chain) by aligning its long axis relative to the chains. This is thus a measure of the overall molecular alignment. The DPPC derivatives 5PC and 16PC report directly on the respective nitroxide moiety about an (composite) internal axis collinear with the z' axis. This is because R_1, the diffusion coefficient for internal rotation, has a nearly equivalent effect on the spectrum as R_1. The choice whether to ascribe an R_1 > R_1 to the effects of internal motion or to the overall motion must then be based on physical insight. For 16PC there is sufficient flexibility that both R_1, R_1 could be significantly determined by the modes of chain motions, while taking these features of the respective nitroxides into account.

The rigid and elongated nitroxide CSL, which is also hydrophobic, probes the average ordering of the chain skeleton (major part of chain) by aligning its long axis relative to the chains. This is thus a measure of the overall molecular alignment. The DPPC derivatives 5PC and 16PC report directly on the respective nitroxide moiety about an (composite) internal axis collinear with the z' axis. This is because R_1, the diffusion coefficient for internal rotation, has a nearly equivalent effect on the spectrum as R_1. The choice whether to ascribe an R_1 > R_1 to the effects of internal motion or to the overall motion must then be based on physical insight. For 16PC there is sufficient flexibility that both R_1, R_1 could be significantly determined by the modes of chain motions, while taking these features of the respective nitroxides into account.

Biaxial Gel Phase. Recent NMR studies5,15 of DPPC dispersions have presented some features differing from the previously held view6,7,15 of an extended all-trans conformation with no...
disordered segmental motion of the hydrocarbon chain in the crystalline phase. A deuterium NMR study\(^{24}\) of perdeuterated DPPC dispersions does not support a model of the gel phase wherein the hydrocarbon chains are fully extended in a rigid all-trans form. This study also indicates that a large fraction of the lipid molecules cease their rotation about their long axes at a temperature near 0 °C. Corresponding to these results, saturation transfer ESR\(^{25}\) shows a rapid decrease in the rate of rotational diffusion of the chain at temperatures below 20 °C. However, it is reported from a proton NMR study\(^{26}\) of DPPC dispersions that at −15 °C there is still a significant amount of methylene chain motion or disorder. The present results listed in Tables I–III are consistent with the above results on the presence of chain motion and disorder but, in addition, reveal some detailed aspects regarding the following matters. (1) In the temperature range of ~40–70 °C in the gel phase, the chain motions of 5PC and 16PC are still significant (with rates of 10^9–10^8 s^−1). However, anisotropy in the rotational diffusion is highest in this phase. (2) The rates of diffusional motion of the hydrocarbon chain differ by an order of magnitude between the central (5th) position and terminal position as represented by values of \(R = 2.4 \times 10^8 \) and \(2.4 \times 10^7 \) s^−1 at 50 °C for 16PC and 5PC, respectively. The high value of \(R\) for 16PC indicates fast segmental motion around the terminal C−C bond. Thus, a relatively high all the spin probes remain constant throughout the gel phase, in contrast to the motions which are thermally activated processes (Figures 10 and 11). This could be explained in terms of modes of motion in the gel phase which remain unchanged. (4) The chain disorder and/or distortions in the local positions (5th and terminal position), probably related to segmental chain motions, can be inferred from \(S\) values that are lower in 5PC and 12PC than in the crystalline phase (50 °C). The disorder is largest at the terminal position as further evidenced by the significant asymmetry term in the ordering for 16PC.\(^{27}\) However, the lipid as a whole is still (in the time average of the chain motions) close to an extended structure, as inferred from the very high \(S\) and \(N\) values of CSL, \(N = (R_1/R_2) = 150\). Figure 12 shows the Arrhenius plot of \(R\) for 16PC in DPPC hydrated to 7%, ranging from 60° (just below \(T_g\)) to a lower temperature (~20 °C). It is seen in this figure that the slope changes at ca. 20 °C, suggesting a change in the mode of chain diffusion at this temperature. The activation energy was determined to be 7.1 ± 1.5 kcal/mol in the upper temperature range (30–60 °C) and 3.0 ± 0.6 kcal/mol in the lower range (~20 to +10 °C), keeping \(N = 2\) over the whole range. On the other hand, in this lower temperature range, the spectra for 5PC are almost unchanged, implying motions too slow to be detected. In previous \(^2\)H NMR\(^{24}\) and STESR\(^{25}\) studies, it was concluded that hydrocarbon chain motions at 0–20 °C. Our results on 5PC are consistent with this provided we regard the motions as too slow to be detected rather than "frozen out". However, our results with 16PC show that the terminal methyl group still exhibits motion even at −20 °C. The reduced activation energy might imply that rotations about the terminal C−C bond can occur more easily once the main parts of the chains become more rigid. (By the major groups of the palmitoyl chains\(^{38a}\).) A significant amount of methylene motion at −15 °C observed by \(^1\)H NMR\(^{28}\) may thus reflect the rotation of the terminal methyl group.

The New Phase Transition in the Liquid-Crystalline Phase. As already noted, we have found a phase transition occurring at 100–110 °C. In order to characterize this transition (second transition), we compare the change in the ordering \(S\) and diffusion constant \(R\) at temperatures just below and above the two-phase region for this second transition, vs. the temperature induced by the first or main transition. From the data at 70 and 80 °C and also 90 and 110 °C in Tables I–III, it is found that the ordering parameter \(S\) experiences a more significant relative reduction at the second transition compared to that at the main transition (i.e. by factors of 0.42, 0.51, and 0.27 for CSL, 5PC, and 16PC, respectively, at the second transition and by 0.84, 0.72, and 0.59, respectively, at the main transition). On the other hand, the diffusion coefficient \(R_1\) for the chain probes (i.e. 5PC and 16PC) experiences a more significant relative increase (i.e. by factors of 6 and 2.7 for 5PC and 16PC, respectively, at the main transition and by 3.2 and 1.3, respectively, at the second transition). We thus regard the second transition as an "orientational type" leading to a significant "melting" of the orientational order of the lipid hydrocarbon chains but a smaller increase in fluidity, as measured by \(R_1\) in comparison to the main transition. The main transition, on the other hand, shows only a moderate decrease in ordering but a more substantial increase in \(R_1\) for the chain probes. Thus, a relatively high all the spin probes experience a more effectively "unfreezes" the chain motions at the main transition. The relative increase in \(R_1\) for CSL at the two transitions is, however, comparable (i.e. by factors of 8.2 and 11 at the main and second transitions, respectively), suggesting that while local chain motion increases more significantly at the main transition, the overall molecular motions exhibit comparable relative changes at both phase transitions. Also, while \(R_1\) shows substantial change at the phase transitions, \(R_1 = NR_1\), which measures the motion about the long chain axis, is much less affected by the phase transitions. This undoubtedly reflects the existence of significant amount of this type in the gel phase, which may be due to its relatively unhindered nature. In fact, \(R_1\) values for CSL and 5PC are comparable (with that for 16PC only about 3 times faster) in phases I and II even though their \(R_1\)s are at least an order of magnitude different (i.e. the respective \(R_1\)s obey CSL < 5PC < 16PC) in these phases.\(^{39}\)

We can also comment on the relative differences of the phase transitions with respect to position along the chain. At both phase transitions there is a more significant relative reduction in ordering at the end of the chain but a smaller increase in fluidity (as measured by \(R_1\)). Thus, while there is greater "melting" of orientational order at the end of the chain, the end-chain motions are not as significantly tied to the ordering.

Lastly, we comment on the liquid-crystalline phase III vs. the isotropic one-phase (phase IV). We have also extensively studied this transition, except for results with CSL shown in Table I. At this transition, the residual ordering is, of course, lost, but there is hardly any change in motional rates. Thus, liquid-crystalline phase III appears already to be very fluid with its fluidity largely unaffected by the small amount of ordering in this phase, except perhaps for an estimated value of \(N\) somewhat larger than the value \(N = 5\), characteristic of CSL in isotropic fluids (and for the isotropic phase, phase IV). These trends bear a striking similarity to observations typical of isotropic–nematic phase transitions in thermotropic liquid crystals, and one wonders whether there might be some validity to a comparison of liquid-crystalline phase III to a thermotropic nematic while phase II is to be compared to a thermotropic smectic. While nematics show


\(^{37}\) This flexibility gradient is, of course, well-known (cf.: Berliner, L. J., Ed., Spin-Labelling—Theory and Applications, Academic Press, New York, 1976; Chapters 12 and 13). We are able to quantify the flexibility gradient in terms of the reduced ordering and its symmetry, as well as the increased motional rate and its reduced asymmetry (cf. also ref 15c). These authors criticize the determination of ordering by ESR methods, presumably because of the perturbing effect of the nitroxide, which could lower the ordering. The corresponding reference noted above (ref 15c) is not rigorous ESR analysis of well-oriented samples suggests that perhaps at least part of the previously published discrepancies may be due to imprecise methods used to interpret the ESR spectra.

\(^{38}\) (a) Seelig, A.; Seelig, J. Biochemistry 1974, 13, 4839. (b) These authors also comment on the liquid-crystalline phase III vs. the isotropic one-phase (phase IV). We have also extensively studied this transition, except for results with CSL shown in Table I. At this transition, the residual ordering is, of course, lost, but there is hardly any change in motional rates. Thus, liquid-crystalline phase III appears already to be very fluid with its fluidity largely unaffected by the small amount of ordering in this phase, except perhaps for an estimated value of \(N\) somewhat larger than the value \(N = 5\), characteristic of CSL in isotropic fluids (and for the isotropic phase, phase IV). These trends bear a striking similarity to observations typical of isotropic–nematic phase transitions in thermotropic liquid crystals, and one wonders whether there might be some validity to a comparison of liquid-crystalline phase III to a thermotropic nematic while phase II is to be compared to a thermotropic smectic. While nematics show
a substantial temperature-dependent variation in ordering similar to that in phase III, the ordering in phase III (as evidenced by CSL) appears to be significantly smaller than typically found for nematics. Also, the second transition, in showing a very substantial change in $S$, is unlike a nematic-smectic transition, wherein $S$ hardly changes. Furthermore, we have no evidence to suggest that the multilayer (or smectic) ordering is lost.\footnote{Added in Proof: It appears to}

Our observations on the second transition in DMPC are similar to those for DPPC. For example, the second transition starts to occur at $S = 0.57$ for CSL in DPPC and when $S = 0.60$ for CSL in DMPC. (For CSL in DMPC hydrated to 3%, $S = 0.86$ in the crystalline phase; it ranges from 0.75 to 0.60 over the temperature range 60–95 °C and is $<0.20$ above 120 °C.)

We would now like to point out interesting similarities between our observations and recent theoretical predictions of Kimura and Nakano\footnote{Kimura and Nakano may have over} on the basis of their simplified model for orientational phase transitions in systems of flexible molecules. They present a simplified, hence more convenient, model than that used by Marcella.\footnote{This is a calorimetrically weak transition occurring at 62 °C in the liquid-crystalline phase of DPPC bilayers and monolayers under conditions of excess water. In their model there is a large drop in $S$ (going to zero) but a small change in $\sigma$, the conformation parameter, at the second transition. We observe a small residual ordering ($S \neq 0$) at the second transition, but Kimura and Nakano may have oversimplified their model in ignoring effects of polar head groups and of repulsive forces. Their prediction of only a small change in $\sigma$ might be correlated with the rather small changes in motional order we observed for the chain probes at the second transition. Their model, however, does not clarify any difference between the second transition and the transition to an isotropic phase. They are thus led to a different correlation between lipid-phase transitions and those of thermotropic liquid crystals from what we have suggested above.}

Dependence of Ordering and Diffusion on Water Content. We have already discussed our results on the DPPC samples hydrated to 3%. Here, we comment on our results analyzed for samples hydrated to higher water content (≤15%). In the gel phase, correlation of ordering $S$, vs. temperature was also found for 7% water ($S = 0.88$ for CSL, 0.64 for 5PC, and 0.32 for 16PC; cf. Tables IB and IIIB). The value of $S$ was moderately reduced with an increase of the water content, as represented by the values for CSL (0.90 to 0.78 on going from 3% to 15%). The $R$'s for 7% water (3.7 × 10$^8$ s$^{-1}$) for CSL and 1.8 × 10$^8$ s$^{-1}$ for 16PC at 40 °C) were slightly higher than those for 3% as shown in Tables I and II. Such small effects of water content on the chain ordering and diffusion in the crystalline phase can be understood in terms of just the small increase of the surface area per lipid molecule in its hexagonal packing. In the liquid-crystalline phase, the $S$ and $R$ values are strongly dependent on water concentration, as shown by the data for CSL in DPPC at 70 °C in Figure 9. However, when compared at the same $\Delta T = T - T_c$, they were found to closely approach each other. For example, the $S$'s of CSL in DPPC converge to 0.73–0.76 at $\Delta T = +10$ °C in the range of 2%–20% water investigated. This indicates that the change in molecular ordering brought about by the main transition is almost independent of the water content. The $S$ values of 5PC and 16PC at 80 °C, i.e., just above $T_c$, are 0.46 and 0.19, respectively. These agree well with 0.46 and 0.21 at 41 °C just above $T_c$ for the 5C and 15C selectively deuterated DPPC's, respectively, determined by $^3$H NMR on dispersions with excess water. This correspondence may confirm our view that at $T_c$ the equilibrium properties of the lipid chains are essentially independent of water content.\footnote{Added in Proof: It appears to}

V. Summary and Concluding Remarks

The purpose of this study, viz. the assignment and characterization of the phases and phase transitions of DPPC and DMPC in the low water content region (≤15%) from the viewpoint of the hydrocarbon chain orientation and diffusion as probed with
Transmitted photointensities vs. temperature for defect-free samples and samples with defect (a) and defect (b).

Figure 15.

ESR spectra of CSL in oriented samples at 3% water ($t_{II} = 75 \, ^\circ C$) with defects for $\theta = 0^\circ$: (a) unoriented starting materials (area 65% at room temperature), (b) polygonal-array defects (area 37% at 80 $^\circ C$).

Figure 16.

ESR spectra of CSL in 3 wt % H$_2$O-DPPC samples with and without defects for $t = 110 \, ^\circ C$ and $\theta = 0^\circ$ in phase III. Other details as in Figure 17.

The above remarks apply over the low water content region ($\leq 15\%$) which we investigated in this work. A more detailed picture of the chain order and diffusion may be obtained by further studies including the use of additional selectively nitroxide-labeled DPPC's. Also, in this work, we have not dealt with any (presumably small) variations in the local director from the mean macroscopic director, although some deviations between predicted and observed spectra in the crystalline phase (cf. Figure 8) might be due to such effects. The behavior of the polar head groups of lipids within the phases and at the phase transitions is a very interesting subject which may be investigated by the use of head-group spin probes.42

We have demonstrated, throughout this study, the great value of utilizing oriented lipid samples of well-defined quality in magnetic resonance studies combined with accurate spectral simulations.
Acknowledgment. We thank Professor G. W. Feigenson of Cornell University for gifts of 5PC and 16PC, Dr. Leoa Kar for many helpful discussions, and Dr. Yuhie Shimoyama for a critical reading of the manuscript. This work was supported by NIH Grant GM 25862.

Appendix: Analysis of Defects and Their Effects on ESR Spectra

Some additional results related to this paper are presented in this section.

1. Types of Defects Observed. The following types of defects were observed under crossed polarizers with our samples.

(a) Unoriented Starting Materials. This type with no regular and repeated texture was assigned to the starting polycrystalline materials left unoriented after the alignment procedure (thus it does not belong to the category of defects in a true sense). This type occurs through insufficient compression with the compression technique and also through rapid change of temperature and water content. The large domains are often observed on boundaries between air (or steam) bubbles and the surrounding oriented areas when Powers annealing technique is applied to our ESR sample preparation. The other types of defects are ultimately changed to this type in their time evolution. A photograph of this defect is shown in Figure 14a.

(b) Polygonal-Array Defects. On raising the temperature, these defects first appear at \( T_r \), and then the individual polygons grow in size and often change in type (a) in the upper temperature range (\( >100 \, ^\circ\text{C} \)) of the liquid-crystalline phase (\( L_\alpha \) phase) (cf. Figure 14b). With our ESR samples, this type often appears in a few days after alignment. It is also produced by detaching the glass plate of the sample sandwich from the oriented lipid layer on a part of the sample area, indicating sample dilation as a major origin for its appearance.

(c) Oily Streak Defects. This type appears through insufficient compression and also rapid cooling after the alignment procedure (cf. Figure 14c).

(d) Strandlike Defects. This type is sometimes produced in an oriented region by the rapid change of temperature. These appear as just a few lines under the microscope.

(e) Fine Dot Defects. Only this type is not listed in Asher's paper.\(^{21}\) We have found this type a few times when the water content of the multilayers has been increased by use of a humidity technique or by a submersion technique. This type is similar morphologically with the polygonal-array type (b) but can exist also in the crystalline phase. It appears as fine white dots on an otherwise black background.

2. Major Defects and Alignment Techniques. Major defects occurring in our ESR samples are types (a) and (b) from the view of both frequency and extensiveness. These defects seem to occur more frequently and extensively in our ESR samples than in the optical samples (Asher's samples\(^{20}\) and Powers' samples\(^{23}\)), even if the alignment principles are the same for the ESR and optical samples. This comes from the difference in the geometry and form of the sample used: they (Asher,\(^{20}\) mechanical alignment technique, and Powers,\(^{22}\) annealing technique) used a big sample assembly consisting of slide glasses, spacer, and mechanical press. The use of the assembly enables them to keep uniform thickness and proper pressure over an entire sample region and hence to suppress the occurrence of defects. We used thin glass plates (cover glass), no spacer, and an adhesive instead of their corresponding substances. Therefore, in our sandwich form, the defects occur easily due to nonuniform thickness, distorsion (or strain) of glass plates, and/or dilation of the sample sandwich. With the compression technique used in this study, the difficulty was conquered by preparation of thin-layer samples (10 \( \mu \text{m} \) in typical thickness). If the sample conditions similar to those for the original samples could be realized for ESR sample preparation, it should be possible to prepare thicker defect-free monodomains of oriented ESR samples. Significantly thicker samples have been produced in our laboratory but with small amounts (\(<10\%\)) of defects.\(^{43}\)

3. Factors Leading to the Instability of Macroscopic Orientation. The following factors make oriented samples unstable through reduction of ordering interactions.

(a) Increase of Temperature. In general, the macroscopic orientation of lipid samples is less stable above \( T_r \). The multilayer form sandwiched between glass plates is better than the film form produced by an evaporation technique in temperature stability.

(b) Increase of the Water Content.

(c) Introduction of Polypeptide (Granuladin A). Therefore, to prepare defect-free samples containing water at higher contents and/or GA, one requires a skillful technique during the alignment procedure.

4. Factors Leading to Reduced Reproducibility of ESR Spectra of Oriented Samples. (a) The Presence of Defects. This can be detected optically with a polarizing microscope (i.e., morphology and transmitted photointensity).

(b) Change of the Water Content. The sample preparation and measurement at higher temperatures (\( \geq 100 \, ^\circ\text{C} \)) cause a reduction of the water content. The water content should be calibrated by a proper method. In the low water content region (\( \leq 15\% \)), the main transition temperature can be used as a measure of the water content involved between bilayers, because the temperature is sensitive to the water content.

(c) Chemical Quality of Lipid. We have observed two-component ESR patterns a few times when we used, for sample preparation, commercial lipids that have stood for a long time after being unsealed. In this case, the whole sample area appeared black under crossed polarizers. It was inferred that the oriented samples consisted of two regions of which one was rich in the decomposed products of lipid and the other was deficient in them. The areas rich in the decomposed products may form an isotropic phase which also appears black under crossed polarizers. Therefore, lipids should be checked for chemical quality before use and should not be kept, for a long time, at higher temperatures (\( \geq 210 \, ^\circ\text{C} \)) in the process of sample alignment.

5. Limitations of ESR Measurements at Higher Temperatures (\( >100 \, ^\circ\text{C} \)). The following factors imposed restrictions on the ESR measurements of higher water content (\( >10\% \)) samples at higher temperatures (\( >100 \, ^\circ\text{C} \)).

(a) Escape of the Water from the Bilayers. When performing ESR measurements above 100 \( ^\circ\text{C} \), the water concentration is reduced usually to below 8%. This problem may not be improved by use of an Asher- or Powers-type sample assembly (cf. ref 20 and 2).

(b) Chemical Decomposition of Nitroxide. The ESR signals of nitroxides (CSL and 16PC) were rapidly reduced through the higher temperature measurements of higher water content samples. This may be caused by hydrolysis of the N-O bond at higher temperatures. A similar problem was brought about by the presence of GA.

6. Correspondence between Polarizing Microscopy and ESR. Results with respect to Defects Involved in Oriented Samples. We investigated the correspondence between polarizing microscopy (PM) and ESR, using the samples containing the “most popular defects”, i.e., unoriented starting materials (a) and polygonal-array defects (b). Figure 15 shows transmitted photointensities vs. temperature for our thin-layer samples (20-30 \( \mu \text{m} \)). A jump in the intensity at \( T_r \) observed for defect (b) corresponds to first occurrence of the defect. The rapid decrease at \( T_r \) observed for defect (a) does not mean a decrease in the quantity of the defect. This can be explained in terms of a change in the optical birefringence at \( T_r \). The quantity of defect (a) of the sample used for this measurement was not changed over the whole temperature range (\( \leq 120 \, ^\circ\text{C} \)). Figure 16 shows the ESR spectra of the corresponding samples. We see two-component patterns in the whole temperature range (\( \leq 120 \, ^\circ\text{C} \)) for the sample with defect (a) and one pattern only in the liquid-crystalline phase (\( 80-120 \, ^\circ\text{C} \)) for the sample with defect (b). Thus, PM and ESR results correspond qualitatively to each other. The outer and inner components in the ESR spectra can be assigned respectively to the defects and oriented areas. This was confirmed semiquantitatively by measurements of the transmitted photointensity. In
thick samples, defects, irrespective of the type, appear white (bright) under crossed polarizers. This phenomenon could be used to estimate the area occupied by defects in the entire sample region, through averaging the photointensities measured at local areas. The defect-free sample and nearly "100%-defect" (i.e., unoriented starting material) sample were used as standards. The results showed that the outer component is reduced in ESR signal intensity with decrease in the defect area determined optically. A simple relation was found for pure lipid samples (CSL and 16PC); viz, defect-free samples yield a simple triplet and defect-containing samples yield a two-component type of spectrum.

The observation of defect signals depends on the line width and ordering and so on the water content, temperature, and nitroxide. The defect signals were best observed in the liquid-crystalline phase II (80-90 °C at 3% water) of CSL samples. In the case of 16PC, the defect signals from samples containing as much as 50% of their area as defects were not resolved near room temperature. Therefore, a polarizing microscope is in general better for defect detection than ESR. The ESR spectra of defect-free samples exhibited good reproducibility when the conditions of water content, lipid quality, etc. were well-defined.

Figures 17 and 18 show a comparison of the ESR signals from defects (a) and (b) in the liquid-crystalline phases II and III. The respective types of defects produced slightly different signals in their apparent splitting and broadening, which reflect on the macroscopic structure intrinsic to the respective defect (cf. ref 20). However, to a rough approximation, the angular (9) dependence of the apparent splitting in the spectrum from the defects appears to be negligible.

Registry No. DPDC, 2644-64-6; DMPC, 13699-48-4; cholesterol-3-one, 15600-08-5.

Analysis of a Phase Transition at 134 K in Decanoic Acid by Infrared Spectroscopy

D. B. West and H. L. Strauss*

Department of Chemistry, University of California, Berkeley, California 94720 (Received: June 22, 1984)

Using infrared spectroscopy, we have discovered a small phase transition at 134 K in pure decanoic acid (n-C10H20O2). Differential scanning calorimetry shows it to be a sharp, first-order transition with ΔH ~ 15 cal/mol. Examining the effects of the transition on the vibrational modes of the hydrocarbon chain, we can determine the slight change in the crystal structure resulting.

Two other possible phase transitions occurring below 100 K are discussed, one of them related to the bizarre infrared spectral changes occurring at low temperatures which were explained by Umemura and Hayashi. The phase behavior discovered here has a strong dependence on the water content of the decanoic acid, and it may be possible to relate it to the more complicated phase behavior of lipid systems and biological membranes.

Introduction

In recent years it has become apparent that the solid-to-liquid-crystal phase transition discovered in model lipid systems is important in biological membranes. In the same way, the elucidation of solid-solid phase transitions in fatty acids may be useful in suggesting what sort of temperature-dependent structural changes are possible in membranes or other lipid-containing systems. Specifically, the crystal structure of decanoic acid, with its planes of carboxylic acid groups and hydrocarbon tails extending out from these planes, looks very much like the lipid bilayers in membranes and so may serve as a model for them. In this paper we report the discovery of a small phase transition (and two other probable phase transitions) in decanoic acid and analyze the associated structural change in detail using infrared spectroscopy. In addition to allowing understanding of the structural changes described in this paper, our interpretation of the infrared measurements on decanoic acid may aid the interpretation of vibrational measurements on complicated biological systems containing fatty acids or fatty-acid-like components.

Long-chain fatty acids form dimers in the solid state, the two head groups making an eight-membered ring with two hydrogen bonds. The crystal structure studied here is the C form of the polymorphic normal fatty acids which is metastable (obtained from London, the melt irreversibly) for the acids having even numbers of carbons between the head groups of the normal fatty acids (described in infrared spectrum associated with the strong hydrogen bonding of the head group perpendicular to the bonds. The crystal structure studied here is the head groups making an eight-membered unit cell. The hydrocarbon chain is essentially parallel to this plane of carboxylic head groups and hydrocarbon tails extending out from these planes. This structure, shown in Figure 1, has the axis a qualitative way, the changes in the molecular structure at the phase transition. Further, by a detailed analysis of the different bands of a particular progression, local information can be obtained. That is, we can determine whether changes in the conformation occur in the chain near the head group, in the middle of the chain, or at the methyl group.

Experimental Section

The decanoic acid used was Aldrich Gold Label 99+%, which was further purified by zone refining. Subsequent GC and TLC


0022-3654/84/2088-6644$01.50/0 © 1984 American Chemical Society