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Molecular Motion And Phases In An Equimolar Phosphatidylcholine/ethylene Glycol System

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632.8-nm probe. These numbers may depend somewhat on experimental conditions and protein separation procedures, but we believe that they give clues about the properties of the diffracting species. Recall that the absorption maximum for spiropyran depends strongly on the local environment. Spiropyran in aqueous buffer has an absorption maximum at 505 nm while in dioxane the maximum occurs at 600 nm. The absorption maximum for spiropyran covalently bound to BSA lies at 517 nm which should be compared with 520 nm for spiropyran in a water dioxane mixture having 0.93 mole fraction water. One possibility is that the slowest species is a BSA dimer (n-mer?) which is photobleached by the writing laser. The photoexcitation of bound spiropyran may result in the dissociation of the dimer to give BSA monomers. The monomer would, of course, have concentration maxima at the positions where the dimer population was depleted, i.e. the two resulting concentration gratings would be phase shifted by π. The free spiropyran is known to be photobleached by the writing beam at 488.0 nm, and the grating would be expected to diffract with approximately the same phase as the dimer grating since both result from bleaching. Another possibility is that photoexcitation of spiropyran bound to BSA causes the BSA to unfold so that the hydrodynamic radius is greatly increased. As in the first case, this would be consistent with the similar magnitudes of a_1 and a_2 and the observed phase shift. A third, less likely, possibility is that normal monomers and the slow species are initially in equilibrium and that both species are photoexcited to produce gratings which are phase shifted with respect to each other. In contrast to classical light scattering, these possibilities cannot be distinguished on the basis of scattering intensities since in HRS the diffraction amplitude is directly proportional to the number of photoexcited molecules independent of the state of molecular aggregation.

The relative amplitudes indicate that diffraction from the free spiropyran is considerably enhanced by using the shorter wavelength probe as expected from the absorption spectrum of this species. There is also an enhancement of the component which we have assigned to BSA monomers relative to the slow component when the probe wavelength is increased. This would be consistent with a less polar environment for the spiropyran label in BSA than in the slow species, which would tend to favor the suggested unfolding mechanism. However, we hasten to add that the situation may be complicated by undetected polydispersity and rapid exchange reactions involving free spiropyran.

The studies reported here revealed unexpected effects and interesting possibilities with HRS. The diffracting power of a single label molecule may be as large as that for a labeled macromolecule, and as shown here, free labels may be detected in trace quantities. Multiple gratings are expected even for simple systems since a depletion grating is created for the ground state at the same time that a grating of excited states is formed. These gratings can be resolved when the ground and photoexcited molecules have different diffusion coefficients. Also, we have found that phase differences for various diffracting gratings may aid in the separation of components. More experience must be gained with other labels and other proteins before HRS can become a standard method for the determination of tracer diffusion coefficients. We believe that the separation of components, the determination of phase shifts, the study of association reactions, and the investigation of the frequency dependence of resonance enhancement in multicomponent mixtures can be most effectively studied through the combination of electrophoresis with HRS.[16] The HRS experiment readily lends itself to this application since the signals are large and coherent mixing of diffracted and reference beams is readily controlled.

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Molecular Motion and Phases in an Equimolar Phosphatidylcholine/Ethylene Glycol System

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An equimolar mixture of phosphatidylcholine and ethylene glycol was studied by pulsed proton NMR at temperatures between 100 and 430 K. Relaxation times T_1, $T_{1\rho}$, and T_{1D}, and second moments, M_2, were measured. The system is lamellar liquid crystalline at room temperature, but at least four phases are present within the temperature range studied. Phase transitions were confirmed by DSC. Activation barriers were estimated for methyl reorientation, choline group motion, and chain motion. Models to describe the various motions and the role of spin diffusion are discussed. The relationship of the parameters for the present system to those for the corresponding aqueous phosphatidylcholine phase is briefly considered.

Introduction

The widely studied liquid crystalline phase formed by phosphatidylcholines (lecithins) and water has recently been shown to have a nonaqueous counterpart. Diols, such as ethylene glycol, in combination with lecithins, also from lamellar liquid crystalline phases, and a structural comparison between the aqueous and nonaqueous lyotropic liquid crystalline phases has been reported.[1] This study showed marked differences between the two different systems.

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Following this, we reported a study[2,3] of solvent mobility in the liquid crystalline phase. The study was based on ^2H NMR quadrupole splittings and spin–lattice relaxation times. These studies suggested that the solvent (ethylene glycol) exists in three different forms in the phase. These are labeled "bound" for solvent forming a 1:1 complex with lecithin, "solvating" for solvent in close proximity to the head-group plane and "isotropic" for solvent further removed from the head-group plane.

Following the preliminary structural report,[1] we have also studied the lamellar interlayer spacings for a series of straight-chain diols in combination with lecithin. A more detailed interpretation of the results[4] indicated that the phases prepared from short-chain diols are similar to aqueous phases, whereas the longer chain diols exhibit different behavior.

Aspects related to the above studies are the mobility of the lecithin chains and the phase behavior as a function of temperature for the short-chain diol system. These properties can be studied by use of proton NMR relaxation parameters. The equimolar aqueous lecithin system has been studied[5] by the T_1 technique[6] over the temperature range 63–443 K. Anhydrous lecithins have been studied by proton second moments,[7] and T_1 and T_{1D} techniques.[8] Molecular motion in lecithin–water systems has also been studied as a function of water composition.[9] we have made a thorough proton NMR relaxation study of the equimolar ethylene glycol/soya lecithin (EG/L) system, and we present relaxation data (T_1, $T_{1\rho}$, T_{1D}) and second moments for the system over the temperature range 100–433 K. From these studies, we have determined the temperature ranges over which the various phases are present. The phase transition temperatures were confirmed by DSC studies. We have also discussed the identification and theoretical description of the various processes controlling relaxations over the temperature range of the study.

Experimental Section

Materials. Lecithin of vegetable soybean origin, Epikuron 200 (Lucas Meyer, Hamburg, West Germany), was purified on an alumina column. The eluting solvent was chloroform/methanol (9:1 by volume). The fractions containing only lecithin were vacuum distilled (50 °C) with 2 mg of antioxidant Progallin P added per 10 g of lecithin.

Ethylene glycol (Fisher certified) was dried over sodium sulfate and then fractionally distilled under vacuum with dry nitrogen. This was done repeatedly until a 0.04% water content was attained.

Sample Preparation. The dried components, lecithin and ethylene glycol, were weighed into a small glass vial with a screw top, flushed with nitrogen, and centrifuged. They were mixed in a Vortex vibromixer with intermittent heating (50 °C) to facilitate mixing and then centrifuged (7000 rpm) to remove air bubbles. The sample was examined, between slide and cover, on a microscope between crossed polarizers to ensure proper mixing and absence of air. It was then transferred to an NMR tube, dried, and flushed with nitrogen and the tube sealed. Finally, it was left to equilibrate overnight in a water bath slowly cooling from 50 to 21 °C.

Measurements. NMR relaxation rates (T_1, $T_{1\rho}$, T_{1D}) were measured by use of a Polaron high-powered pulsed spectrometer operating at 60 MHz.[10] T_1 values were also measured at 17 MHz by use of a home-built pulsed spectrometer. Second moments were obtained by analysis of the Bloch decays.

Calorimetric data were obtained by use of a Perkin-Elmer DSC-2 differential scanning calorimeter.

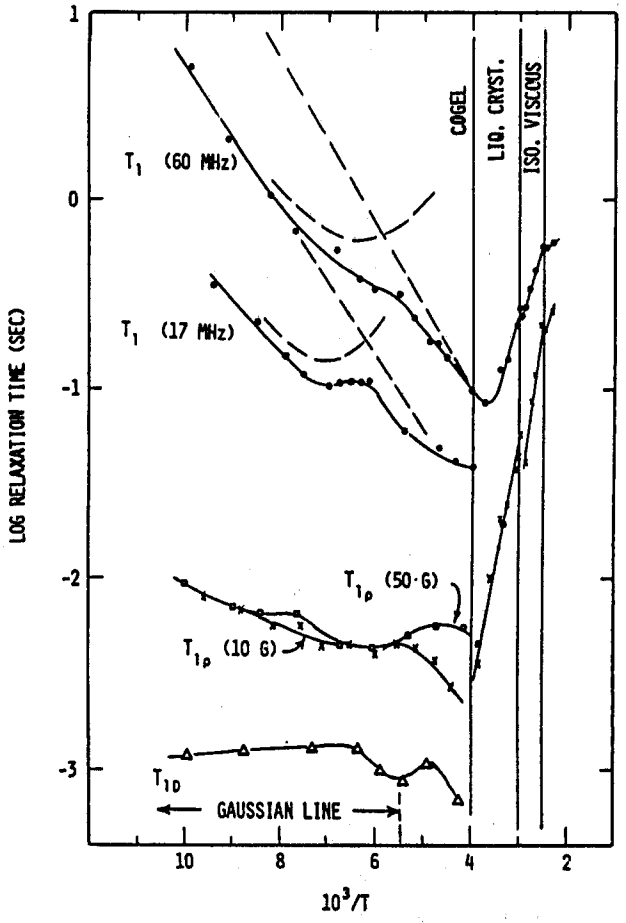

Figure 1. Proton NMR relaxation times vs. reciprocal temperatures for equimolar ethylene glycol/lecithin. Phase transitions were identified by discontinuities in NMR data and by DSC data. T_1 values were measured at two spectrometer frequencies (17 and 60 MHz). $T_{1\rho}$ values were measured at two radiofrequency field strengths (10 and 50 G).

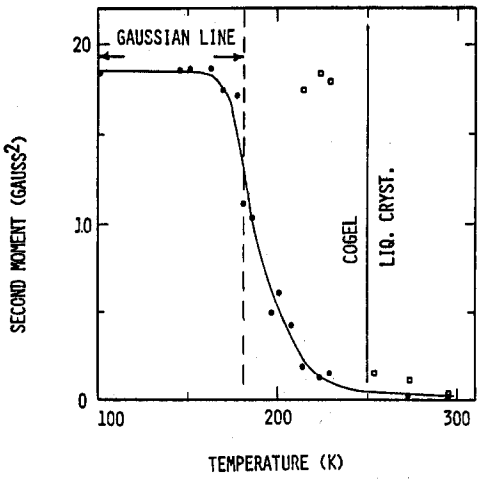

Figure 2. Proton NMR second moments vs. temperature for equimolar ethylene glycol/lecithin. The second moment was separated into two components (●, principal component; □, smaller component) as described in the text.

Results and Discussion

Proton relaxation times T_1, $T_{1\rho}$, and T_{1D} for the equimolar EG/L system are plotted on a log scale vs. reciprocal temperature in Figure 1. Discontinuities in the NMR parameters and confirmatory calorimetric data indicate that there are at least four (and probably five) phases within the temperature range studied.

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By comparison with previous studies on aqueous systems[5,7-9,11-13] and by consideration of the second moment data to be presented below, we label the cogel, liquid crystalline, and isotropic viscous phases as indicated in Figure 1. The highest temperature phase is probably isotropic liquid, and there is most likely a cogel/crystalline transition[14] at low temperature.

The second moment data are presented in Figure 2; M_2 is plotted vs. temperature between 100 and 300 K. Below 180 K, the line is Gaussian as indicated in the plot. Between 180 and 250 K, the line shape is non-Gaussian. We have interpreted the Bloch decays schematically in terms of two components, with a line-width transition attributed to the principal component. An alternate interpretation would attribute the observed effects to a phase transition at 180 K. Second moments for temperatures above 300 K were not measured since the NMR lines are very narrow and structure is resolved.

Cogel Phase. This phase is stable below about −24 °C. There is probably a transition to crystalline solid or gel at low temperature. The NMR line has Gaussian shape below 182 K, as indicated in Figures 1 and 2, and this may represent the solid or gel phase.

At lowest temperatures, there is evidence for a T_1 minimum at both 17 and 60 MHz. The lower frequency data show the minimum more clearly resolved. Dashed lines show a likely resolution of the data into two contributions. The lowest temperature mechanism shows a minimum at $10^3/T \sim 6$, and the gradient corresponds to an activation barrier of somewhat less than 2 kcal/mol. This low-temperature minimum is most likely attributable to methyl group reorientation. The ratio of the T_1 minima at 60 and 17 MHz is ~4 and there is an ω^2 dependence on the low-temperature sides of the minima; this agrees with the BPP theory.[15] The depths of the minima suggest that only two methyls of the five present in the lecithin molecule dominate the relaxation.[10] We assign this minimum to chain methyl reorientation.

The remaining three methyls are present in the choline group, and we believe that these methyls are reorienting slowly compared to the chain methyls. Separate ^{31}P NMR studies[16] indicate that the choline group is "rigid" in this temperature range, and crystal structures[17] indicate that the *N*-methyl groups interact strongly with neighboring PO_4 groups. This interaction persists to much higher temperature, into the lamellar phase.[17] A complex motion, highly coupled to the choline group and described by a distribution of correlation times,[18] would be an appropriate model for *N*-methyl motion. Such a motion could result in the broad, featureless $T_{1\rho}$ and T_{1D} behavior in this temperature region. No effects attributable to this motion can be seen in T_1.

There may be an additional contribution to $T_{1\rho}$ and T_{1D} in this region due to another process. This is suggested by the M_2 data. Note that the rigid-lattice M_2 value[11] is ~30 G^2, and the largest value of M_2 obtained by us is ~18 G^2 (at 100 K). This reduction in M_2 cannot be accounted for by methyl reorientation alone; there must be other motions. Possible motions include local torsional oscillation[19] and segmental motions in the form of defects.[20]

The low-temperature M_2 plateau (~18 G^2) in Figure 2 is an interesting feature. This plateau is also observed[11] with distearoyllecithin but over only a small temperature range (~160–200 K). Below ~150 K, M_2 for DSL increases with decreasing temperature to ~30 G^2. This transition is attributed to methyl reorientation and other processes, as discussed above. No evidence for this anticipated transition was obtained with the present nonaqueous system. These observations are probably attributable to the fact that DSL contains saturated chains with consequent better packing as opposed to soya lecithin, which contains primarily linoleyl chains. The unsaturation produces a structure with considerable motion, even at 100 K.

As mentioned above, at $10^3/T \sim 5.5$, there is a possible line-width transition or phase transition. There is also some indication of a weak minimum in T_{1D} at this temperature. This behavior has also been observed with pure lecithin just prior to melting, and it has been assigned to diffusion of lipid molecules in the bilayer plane in the cogel phase. From the value of the correlation time at a T_{1D} minimum (~10^{-4} s) and assuming a diffusion step length of 10^{-6} cm, one estimates $D \sim 10^{-8}$ cm^2/s at 180 K. This value agrees with that obtained directly by pulsed field gradient measurements[21,22] on lipids in gel phases. There may also be a contribution to T_{1D} from molecular reorientation about the long molecular axis.

As the temperature is increased above 200 K, all relaxation times exhibit behavior consistent with low-temperature arms of motional minima. A line-width transition reduces M_2 to almost zero. The line shape has the expected form described by Bloom,[23] and a phase transition to liquid crystalline is observed at ~250 K. This is the familiar chain melting process, in which the entire molecule becomes thermally activated and the crystalline lattice is loosened.

For $10^3/T \sim 4$, just below the phase transition, we may expect that the motions leading to chain melting will affect the relaxation processes. In fact, it appears that all the relaxation processes may be controlled by the same mechanism. If we assume that this is the case, then we can examine the frequency dependence. For over 4 orders of magnitude, it is approximately $\omega^{1/2}$. There are two possible mechanism that could account for this observed dependence. They are (i) orientational director fluctuations[24-26] (ODF) and (ii) spin diffusion limited relaxation. The detailed discussion of the mechanisms is beyond the scope of this paper and will be presented separately. We favor an explanation inolving the defect diffusion model of Powles[20] applied to the effects of kinks and jogs[27,28] moving up and down the chains. If we assume that this model describes the motion, then we can estimate the activation barrier for the motion. The gradient in Figure 1 corresponds to ~2 kcal/mol. This would be equal to the activation barrier for a BPP motion; this value is too low to characterize chain motions.

To adequately describe this relaxation, we consider rapidly relaxing local segments coupled to the remainder of the molecule by spin diffusion. The problem is analogous to relaxation caused by paramagnetic impurities in solids as treated originally by Bloembergen,[29] with various limiting cases being treated by Khutsishvili,[30] deGennes,[31] and Blumberg.[32] The approach was successfully applied to relaxation in alkanes at low temperature.[33] We use the expression[34] for the relaxation rate given in terms of

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TABLE I: Observed Activation Barriers[a] for Equimolar Ethylene Glycol/Lecithin

phase	T_1 data	$T_{1\rho}$ data
cogel[b]	2[c] (14 ± 2)[d]	(14 ± 2)[d]
liq cryst	4.9[e]	6.5[f]
iso viscous	3.6[e]	9.2[e]

[a] In kcal/mol. [b] Includes probable crystalline solid phase at low temperature. [c] Methyl minimum observed at low temperature. [d] Estimated by asuming model described in text for chain motion. [e] Unassigned motion. [f] Choline group motion.

the spin-diffusion coefficient, D, and the spectral density function, $J(\omega)$.

$$1/T_1 \propto [J(\omega)]^{1/4}D^{3/4} \quad (1)$$

For simplicity, we assume D is constant and the spectral density is given by the defect diffusion model,[20] in the low-temperature limit

$$J(\omega) \propto \omega^{-2/3}\tau_c^{-1/2} \quad (2)$$

This predicts, in the low-temperature limit

$$1/T_1 \propto \tau_c^{-1/8} \quad (3)$$

and the activation barrier for the motion is thus ~12–16 kcal/mol. This value is fairly representative of what one anticipates for the chain melting process.

Liquid Crystalline Phase. There is an apparent T_1 minimum centered just above the cogel/liquid crystalline phase transition. This minimum has been observed[35] previously in similar systems, and it has been suggested that this minimum is associated with head-group motion. We believe that this assignment is an oversimplification. The ^{31}P NMR line becomes slightly narrowed[16] near room temperature, indicating a correlation time $\sim 10^{-4}$ s for the PO_4 group. In light of this observation, it is hard to imagine choline group motion with a correlation time $\sim 10^{-8}$ s, to be consistent with the T_1 data. The T_1 data show an $\omega^{1/2}$ dependence on the high-temperature side of the minimum; however, the origin of this dependence is unknown, since we can rule out spin-diffusion effects in the presence of the strong motional narrowing. There may be contributions from ODF or the defect motions are discussed above. The gradient in Figure 1 corresponds to 4.9 kcal/mol.

The behavior of $T_{1\rho}$ in this region is entirely different from that of T_1; $T_{1\rho}$ is frequency independent and its behavior appears to be the high-temperature arm of a $T_{1\rho}$ minimum describable by standard BPP theory. Thus, there is an abrupt change in the motion governing $T_{1\rho}$ across the phase boundary. The time scale for the motion ($\tau_c \sim 10^{-6}$ s) agrees with that of the PO_4 group from ^{31}P studies[16] and this suggests that choline group motion could govern the relaxation. The gradient in Figure 1 corresponds to 6.5 kcal/mol; this also corresponds to the activation barrier in this case.

High-Temperature Phases. In the phase labeled "Isotropic Viscous", T_1 and $T_{1\rho}$ both exhibit portions of high-temperature arms of relaxation minima. The fact that $T_1 \neq T_{1\rho}$ indicates that two different processes control the separate relaxations and that there is not rapid isotropic motion in this phase. T_1 dependence corresponds to 3.6 kcal/mol, while $T_{1\rho}$ dependence corresponds to 9.2 kcal/mol. $T_{1\rho}$ may have contributions from molecular reorientation about the amphiphile long axis and/or diffusion on the aggregate surface. There are slight discontinuities as well as changes in slope at the phase transition at $10^3/T \sim 2.5$ (130 °C).

The activation barriers for all phases obtained in the context of the present theoretical discussion are summarized in Table I.

An interesting aspect of this study is that certain features of the ethylene glycol system can be compared with those of the aqueous system. Activation barriers for aqueous lecithin systems have not been determined systematically so as to be compared with the presently reported values. The parameter that can be directly compared with that for the aqueous system is the temperature of the cogel to liquid crystalline phase transition, since these have been widely studied for aqueous systems. For example, equimolar aqueous dipalmitoyllecithin (DPL) shows a number of line-width transitions[9] in the gel phase, and these have been attributed[9] to motions of various segments of the lecithin molecule. Distearoyllecithin (DSL) also shows several line-width transitions[11] in the gel phase, with two component lines observed just below the phase transition temperature. The onsets of line-width transitions occur[11] at ~−50 °C and the phase transitions occur[9] between ~40 and 70 °C, depending upon water content. These effects occur ~90 °C above corresponding effects in the present system. However, it has been shown that the transition temperatures also depend strongly upon chain length and degree of unsaturation.[36] Our system should be compared with egg yolk lecithin; the latter exhibits a phase transition[37] at −22 °C, which is identical with that of our system. Thus, we conclude that ethylene glycol based and aqueous based systems are identical in this respect, and the amphiphile chains appear to be the crucial factor that determines the phase transition temperature.

Registry No. Ethylene glycol, 107-21-1.

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