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Solid-State NMR Study of Trehalose/1,2-Dipalmitoyl-*sn*-phosphatidylcholine Interactions†

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ABSTRACT: ^{31}P and ^{2}H solid-state NMR studies of dry trehalose (TRE) and 1,2-dipalmitoyl-*sn*-phosphatidylcholine (DPPC) mixtures are reported. ^{31}P spectra are consistent with a rigid head group above and below the calorimetric phase transition for both dry DPPC and a dry 2:1 TRE/DPPC mixture. In addition, ^{2}H spectra of DPPC labeled at the 7-position of the *sn*-2 chain (2[7,7-$^{2}H_2$]DPPC) show exchange-narrowed line shapes with a width of 120 kHz over the temperature range 25–75 °C. These line shapes can be simulated with a model involving two-site jumps of the deuteron. In contrast, the ^{2}H NMR spectrum of a dry 2:1 TRE/2[7,7-$^{2}H_2$]DPPC mixture above the phase transition (T_c = 46 °C) is narrowed by a factor of ~4 to a width of 29 kHz. Simulation of this spectrum requires a model involving four-site jumps of the deuteron and is indicative of highly disordered lipid acyl chains similar to those found in the L_α-phases of hydrated lipids. Thus, TRE/DPPC mixtures above their transition temperatures exist in a new type of liquid crystalline like phase, which we term a λ-phase. The observation of the dynamic properties of this new phase indicates the mechanism by which anhydrobiotic organisms maintain the integrity of their membranes upon dehydration.

When most cells are dehydrated, extensive damage occurs due to membrane fusion and to scrambling of membrane surfaces. This in turn leads to a loss of membrane function and cell viability. However, it has been recently reported that trehalose (TRE),[1] a nonreducing disaccharide of glucose that is found at ~20 wt % concentration in anhydrobiotic organisms, can be employed to stabilize membranes in the dry state (Crowe & Crowe, 1982; Crowe et al., 1983a,b). For example, electron micrographs of muscle microsomes dehydrated and rehydrated in the presence of TRE do not show cell damage normally associated with dehydration (Crowe et al., 1983a). It is postulated that this effect arises because the disaccharide can replace the water of hydration in the bilayer. Furthermore, similar results have been obtained in model membrane systems. Specifically, 2:1 stoichiometric mixtures of TRE and a model phospholipid, 1,2-dipalmitoyl-*sn*-phosphatidylcholine (DPPC), exhibit a phase transition close to 41 °C, which is the T_c of hydrated DPPC (Crowe et al., 1984). In contrast, dry DPPC[2] has a phase transition of 65 °C (Chapman et al., 1967). It is also known from monolayer studies that trehalose in the aqueous phase will expand lipid monolayer films (Johnson et al., 1984) and IR spectra show shifts associated with hydrogen bonding between trehalose and the head group of DPPC (Crowe et al., 1984). Thus, this evidence suggests that a sugar-to-head-group interaction is responsible for these effects. Nevertheless, molecular details concerning the nature of this interaction are scant.

In this paper, we present results of a solid-state NMR study aimed at elucidating the dynamic structure of TRE/DPPC mixtures. ^{31}P spectra of dry DPPC and 2:1 TRE/DPPC

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[1] Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-phosphatidylcholine; 2-[7,7-$^{2}H_2$]DPPC, 1,2-[2-*palmitoyl*-7,7-$^{2}H_2$]dipalmitoyl-*sn*-phosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; TRE, trehalose.

[2] For a discussion of our use of the term "dry DPPC" and "dry TRE/DPPC" mixtures, see Experimental Procedures.

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mixtures taken both below and above the calorimetric transitions consist of axially asymmetric powder patterns of ~200 ppm total breadth, indicating there is little motion of the PO_4 part of the head group and that long axis diffusion of the lipid molecules is effectively absent. ^2H quadrupole echo spectra of dry 2[7,7-$^2H_2$]DPPC show broad, featureless line shapes of ~120-kHz breadth that exhibit little change in either their shape or breadth upon passing through the calorimetric transition at 65 °C. In marked contrast is the behavior of the 2:1 TRE/2[7,7-$^2H_2$]DPPC spectra. Below the transition temperature of 46 °C in the phase that we refer to as the κ-phase,[3] we find line shapes similar to those observed in dry DPPC. However, after passing through the transition, the spectrum narrows by a factor of ~4 to 29 kHz. Furthermore, the shape of this spectrum is quite different from that observed in the L_α-phase and can be simulated with a model involving four-site gauche-trans isomerization. Thus, this new "L_α-like" phase, which we hereafter refer to as a λ-phase, arises strictly from internal modes of chain isomerization rather than isomerization combined with axial diffusion (Blume et al., 1982; Blume & Griffin, 1982). We believe the unique dynamical properties of the λ-phase may play a major role in maintaining membrane stability in dehydrated organisms.

Experimental Procedures

Materials. TRE was obtained from Sigma Chemical Co. (St. Louis, MO) and unlabeled DPPC from Avanti Polar Lipids (Birmingham, AL). 2[7,7-$^2H_2$]DPPC was synthesized by Avanti Polar Lipids (Birmingham, AL) by using 2[7,7-$^2H_2$]palmitic acid prepared in this laboratory according to previously published methods (Das Gupta et al., 1982). The purity of all starting materials and synthesized phospholipids was checked by standard methods (Blume et al., 1982).

Sample Preparation. Samples for both DSC (2–5 mg of lipid) and NMR (50–100 mg) were prepared by lyophilization with 2:1 MeOH/benzene as solvent. The lyophilized material was placed in 7-mm glass tubes, pumped at 10^{-5} torr overnight, and then heated under vacuum for 1 h at 10 °C above the phase transition temperature, and the tubes were immediately sealed. All samples were checked by DSC (Perkin-Elmer DSC-2C, Norwalk, CT) at each step of the sample preparation procedure. In the following we refer to these samples as "dry DPPC" and "dry TRE/DPPC" mixtures. The ^{31}P spectra of DPPC presented below are consistent with this preparation being "anhydrous" (Griffin, 1976). At the moment we do not know how much H_2O, if any, is bound to the TRE/DPPC mixtures. Consistency in sample preparation is difficult to achieve, as will be discussed elsewhere. Here we simply note that this leads to the necessity of quality control by DSC at each step of sample preparation.

NMR Spectroscopy. ^{31}P and ^2H NMR spectra were obtained on a home-built solid-state pulse spectrometer operating at 9.35 T (160.99 MHz for ^{31}P, 61.047 MHz for ^2H, and 397.7 MHz for ^1H). ^{31}P spectra were recorded by using Hahn echo or cross-polarization with a 180° refocusing echo to minimize base-line distortion (Pines et al., 1973; Griffin, 1981). ^2H spectra were recorded by using the quadrupole echo with a pulse spacing of 40 μs (Davis et al., 1976), and the 90° pulse width was typically 1.8–2.5 μs. Temperature control was achieved with a gas flow system described elsewhere (Wittebort et al., 1981).

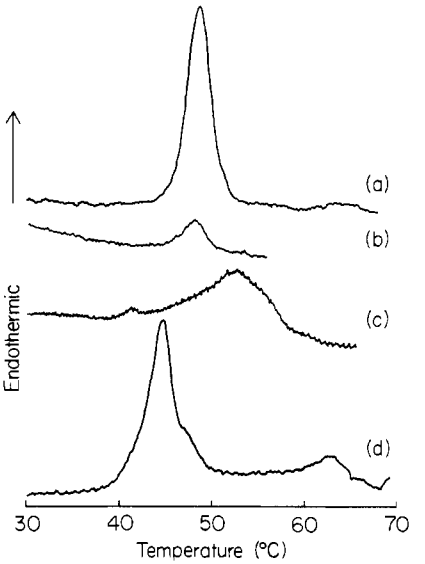

FIGURE 1: DSC thermograms of a dry 2:1 TRE/DPPC mixture showing metastability of the main transition. All scans were recorded at a heating rate of 5 °C/min. (a) Initial heating run. T_c = 48.9 °C; ΔH = 6.9 kcal/mol. (b) Second heating run immediately following first run. T_c = 48.5 °C; ΔH = 0.95 kcal/mol. (c) Sample annealed for 2 days at 25 °C. T_c = 52.0 °C; ΔH = 3.91 kcal/mol. (d) Sample annealed for 5 days at 25 °C. T_c = 45.9 °C; ΔH = 6.45 kcal/mol.

Results

DSC. Using the procedure described above, it is possible to duplicate the concentration-dependent DSC results of Crowe et al. (1984) up to 2:1 TRE/DPPC (not shown). Since the T_c of this particular dry sugar/lipid mixture nearly equals that of hydrated DPPC, it was used for all NMR studies.[4] We have found that the $\kappa \rightarrow \lambda$ transition in dry 2:1 mixtures is conditionally reversible; i.e., following the initial heating run, samples require annealing before the phase transition is observed again. This is illustrated in Figure 1a, where a transition with an enthalpy of 6.91 kcal/mol is observed for the first heating. This is diminished by rescanning (Figure 1b), and by the third heating, the phase transition disappears altogether (not shown). After 2 days of storage at room temperature, the mixture has partially reannealed, giving rise to a ΔH of 3.91 kcal/mol (Figure 1c), and after 5 days, the sample is fully recovered and exhibits a ΔH of 6.45 kcal/mol (Figure 1d). Note that in Figure 1d there is a minor peak at about 65 °C that reveals slight phase separation in the sample from the repeated heating and cooling runs. A similar conditional reversibility has been observed in formation of the L_c-phase in DPPC (Chen et al., 1980). In contrast to the behavior shown in Figure 1, dry DPPC has a fully reversible phase transition and does not exhibit metastability in its phases.

^{31}P NMR. The ^{31}P solid-state NMR spectra of dry DPPC, 2:1 TRE/DPPC, and hydrated DPPC are shown in Figure 2. Although both the TRE/DPPC mixture and DPPC exhibit rigid lattice powder line shapes, the widths of the powder patterns are not the same. Dry DPPC has a spectral width of 235 ppm, whereas 2:1 TRE/DPPC has a spectral width of 195 ppm. This amounts to a 17% narrowing of the TRE/DPPC spectra and probably indicates that the nature of the hydrogen bonding at the PO_4 is different from that of dry DPPC. A very similar difference in spectral width is observed

[3] In analogy with common practice we employ Greek letters to identify these new lipid/sugar phases. We have chosen κ and λ for the low- and high-temperature phases, respectively, since they do not seem to have been used previously. At the moment, X-ray experiments have not been performed on TRE/DPPC mixtures, so it is unclear if these phases should be labeled L, P, etc.

[4] We could not reproduce the thermal behavior of 3:1 TRE/DPPC mixtures reported by Crowe et al. (1984).

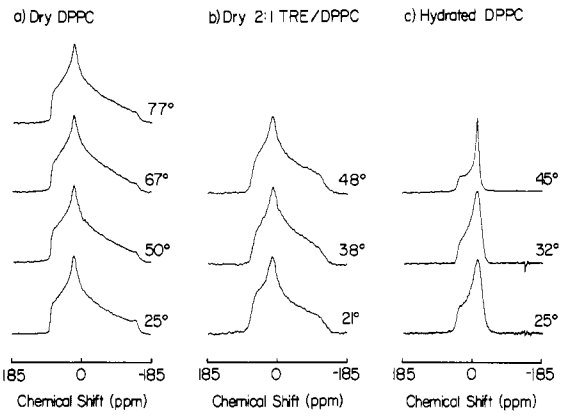

FIGURE 2: Experimental 160.99-MHz proton-decoupled ^{31}P NMR spectra obtained as a function of temperature. (a) Dry DPPC; cross-polarization with 180° echo; T_c = 67 °C. (b) Dry 2:1 TRE/DPPC mixture; cross-polarization with 180° echo; T_c = 46 °C. (c) DPPC in 50 wt % H_2O; Hahn echo; T_c = 41 °C.

between anhydrous DPPC and DPPC·H_2O or DPPE (Griffin, 1976; Herzfeld et al., 1978).

As the samples are heated above their respective phase transitions, there is no change in the ^{31}P NMR spectra of the dry lipid mixtures as shown in Figure 2. If the phase transition increases mobility of the lipid molecule, then the change is not occurring at the phosphate part of the head group. The spectra strongly suggest an absence of long axis diffusion since that would lead to motional averaging of the chemical shift tensor. This is in direct contrast to hydrated DPPC where an axially symmetric tensor is detected in the gel phase down to −10 °C, i.e., 50 °C *below* the main transition. Thus, the ^{31}P spectra indicate that fast-limit long axis diffusion, which is present in hydrated DPPC bilayers, is absent for the dry lipid and TRE/lipid mixtures.

^2H NMR. DPPC ^2H-labeled at the 7-position of the *sn*-2 chain was used to examine the dynamic structure of the hydrocarbon chain in the presence and absence of TRE. Temperature-dependent ^2H NMR spectra of dry 2[7,7-$^2H_2$]DPPC, 2:1 TRE/2[7,7-$^2H_2$]DPPC, and hydrated 2[7,7-$^2H_2$]DPPC are shown in Figure 3. Below the phase transition, all spectra show broad exchange-narrowed line shapes. Note, however, that there are discernible differences among these spectra. For example, in dry DPPC at 25 °C the line shape exhibits a flat top, whereas in both the 2:1 TRE/DPPC mixture and hydrated DPPC the center of the spectrum is narrowed. In addition, in the spectrum of hydrated DPPC at 16 °C ($L_{\beta'}$) there are clearly well-developed parallel edges. The line shape at higher temperatures in the TRE/DPPC mixtures becomes triangular at 35 °C while for hydrated DPPC at 37 °C ($P_{\beta'}$) the parallel edges continue to develop and the central portion of the spectrum begins to resemble parts of a Pake pattern. These features are absent in the spectrum of dry DPPC, even at 55 °C. At yet higher temperatures, some narrowing of the dry DPPC spectrum is observed, but even above the calorimetric transition temperature of 65 °C, this is not dramatic.

In contrast, spectra of both the 2:1 TRE/DPPC and, as is well-known, the hydrated DPPC sample narrow by a factor of ~4 upon passing through the thermal phase transition. In the case of hydrated DPPC, the spectrum is an axially symmetric powder pattern with $\Delta\nu_{Q\perp}$ = 29 kHz. For 2:1 TRE/DPPC mixtures in the λ-phase the spectrum transforms to a featureless spike with a half-width of ~29 kHz. The shape of this spectrum as well as its width can be simulated with a model based on restricted gauche-trans isomerization. The difference in the shapes of these two spectra is due to the absence of rapid axial diffusion in the TRE/DPPC samples.

Computer Simulations of the ^2H Line Shapes. Computer simulations of the ^2H line shapes in dry 2[7,7-$^2H_2$]DPPC and 2:1 TRE/2[7,7-$^2H_2$]DPPC are shown in Figure 4. The model used in the calculations consists of a deuteron jumping among defined sites with a particular jump rate and a certain probability in the occupation of a site (R. J. Wittebort, E. T. Olejniczak, and R. G. Griffin, unpublished results). All simulations are corrected for spectral distortions due to finite pulse

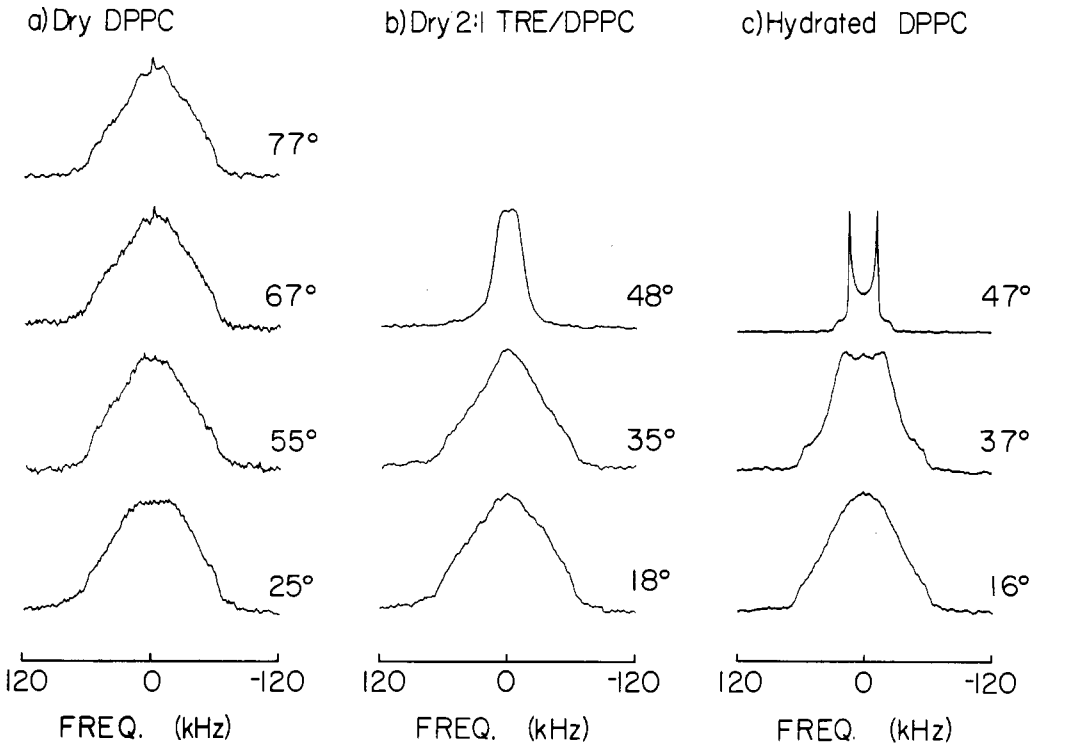

FIGURE 3: Experimental 61.047-MHz ^2H NMR spectra obtained as a function of temperature. DPPC is labeled at the 7-position of the *sn*-2 chain. Quadrupole echo with τ = 40 μs. (a) Dry 2[7,7-$^2H_2$]DPPC; T_c = 67 °C. (b) Dry 2:1 TRE/2[7,7-$^2H_2$]DPPC; T_c = 46 °C. (c) 2[7,7-$^2H_2$]DPPC in 50 wt % H_2O; $L_{\beta'} \rightarrow P_{\beta'}$ transition = 33 °C; $P_{\beta'} \rightarrow L_\alpha$ transition = 41 °C.

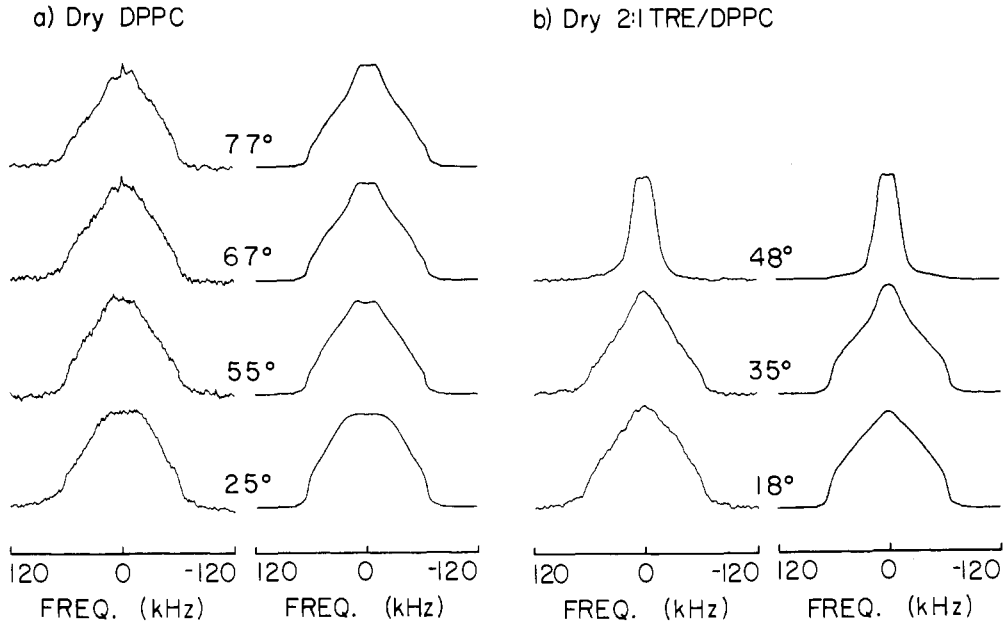

FIGURE 4: (Left) Experimental 61.047-MHz ^2H NMR spectra. (Right) Computer simulations of the experimental spectra. (a) Parameters used for simulations with dry 2[7,7-$^2H_2$]DPPC were as follows: (1) at 25 °C, two-site hop, D_1–C–D_2 = 93°, P_1:P_2 = 0.63:0.37, and k_{12} = 5×10^6 s^{-1}; (2) at 55 °C, two-site:four-site = 0.95:0.05; (two-site parameters) D_1–C–D_2 = 93°, P_1:P_2 = 0.6:0.4, and k_{12} = 1×10^7 rad s^{-1}; (four-site hop parameters) all angles = 109.5°, P_1:P_2:P_3:P_4 = 0.3:0.3:0.267:0.133, and $k_{12} = k_{13} = k_{14} = 1 \times 10^7$ s^{-1}; (3) at 67 °C, two-site:four-site = 0.9:0.1; same parameters as those at 55 °C; (4) at 77 °C, two-site:four-site = 0.87:0.13; same parameters as those at 55 °C. (b) Parameters used for simulations with dry 2:1 TRE/2[7,7-$^2H_2$]DPPC were as follows: (1) at 18 °C, two-site hop, D_1–C–D_2 = 90°, P_1:P_2 = 0.65:0.35, and $k_{12} = 1 \times 10^7$ s^{-1}; (2) at 35 °C two-site:four-site = 0.89:0.11; two-site parameters same as those at 18 °C; (four-site parameters) all angles = 109.5°, P_1:P_2:P_3:P_4 = 0.3:0.3:0.267:0.133, and $k_{12} = k_{13} = k_{14} = 1 \times 10^7$ s^{-1}; (3) 48 °C, two-site:four-site = 0.9:0.1; same parameters as those at 35 °C.

widths (Bloom et al., 1980). In modeling the motion of a polymethylene chain, the sites are usually defined with tetrahedral angles so that normally only the jump rate and population need be optimized for a best fit. In the simulations employed here we have found that better simulations are possible if we permit the hop angle to vary as well.

Although the line shapes exhibited by these dry DPPC mixtures resemble those of gel-phase lipids in the intermediate exchange regime, none of the NMR spectra in this study exhibited the intensity losses associated with intermediate exchange spectra. The characteristic intensity losses occur when the correlation time of the motion is of the order of the reciprocal of the quadrupole interaction and T_2 becomes comparable to the interpulse spacing in the quadrupole echo sequence (Spiess & Sillescu, 1981). The absence of these intensity losses suggests that rates are approaching the fast-limit regime (10^6–10^7 s^{-1}). Nevertheless, it should be noted that the absence of these characteristic intensity losses can also be attributed to a distribution of correlation times as observed in polymeric systems (D. M. Rice, B. A. Lewis, S. K. Das Gupta, J. Herzfeld, and R. G. Griffin, unpublished results; D. M. Rice, E. T. Olejniczak, S. K. Das Gupta, J. Herzfeld, and R. G. Griffin, unpublished results). A more careful study of the ^2H spectra is necessary to resolve this question.

For both dry 2[7,7-$^2H_2$]DPPC and 2:1 TRE/2[7,7-$^2H_2$]-DPPC, a two-site model was used to fit the line shapes for temperatures below the T_c. The D_1–C–D_2 angle (the two-site hop angle) was taken to be between 90° and 95° (values for individual simulations are listed in the figure caption of Figure 4), and the jump rates varied between 5×10^6 s^{-1} and 1×10^7 s^{-1}. The population of the second site becomes larger at higher temperature as the increase in thermal energy allows for greater disorder in the lipid. Since the head group in these systems is immobile, this model corresponds to a large angle librational motion.

Above the phase transition, a four-site model with tetrahedral angles and fast-limit hopping rates provided the best fit for the 2:1 TRE/DPPC spectra. For example, for the spectrum of Figure 4b populations for the four sites were 0.3, 0.3, 0.27, and 0.13, respectively. Although the phase transition for the 2:1 TRE/DPPC samples is centered at 46 °C, it extends to <40 °C. As a consequence the NMR spectra indicate that a substantial amount (~10%) of the four-site motion occurs even 10° below the actual transition. This is to be contrasted with dry 2[7,7-$^2H_2$]DPPC where there is no drastic change in the ^2H NMR spectra above the T_c of 67 °C. However, the best simulation of the spectrum is obtained when a small amount of four-site motion is included in the calculation.

Discussion

In contrast to normal $L_{\beta'} \rightarrow P_{\beta'} \rightarrow L_\alpha$ phase transitions (Janiak et al., 1976), the $\kappa \rightarrow \lambda$ phase transition of 2:1 TRE/DPPC is conditionally reversible. This is seen in the DSC experiments as a gradual disappearance of the endotherm on repeated heating scans. In the ^2H NMR experiments it is manifest by the presence of the narrow 29-kHz spectrum on cooling from the λ- to the κ-phase. Upon prolonged storage this narrow component reverts to the 120-kHz κ-phase spectrum (not shown). This sort of conditional reversibility is not a phenomenon unique to dry sugar/lipid mixtures since a variety of hydrated lipids, such as DPPC, cerebroside, and sphingomyelin, exhibit metastable phases (Ruocco & Shipley, 1982; Ruocco et al., 1981; Estep et al., 1980). In all previously known cases, prolonged incubation at low temperatures causes the hydrated gel-state lipid to form thermodynamically favored crystalline phases. This change is generally accompanied by changes in hydrocarbon chain packing and a loss of hydration in the head group. For the TRE/lipid mixtures changes in head-group interactions are unlikely since this would lead to phase separation of sugar from lipid. Thus, it would appear that the hysteresis and metastability observed upon cooling

are due to the trapping of the lipid acyl chains in a disordered state. The ^{31}P NMR spectra suggest two features of the TRE/DPPC mixture. First, the shape and breadth of the powder line shapes indicate there is no motion of the head group. This, in turn, implies that axial diffusion of the whole molecule is absent, a feature that is borne out by the ^2H spectral line shapes. Second, from the change in the size of the tensor we detect some sort of direct interaction between sugar and DPPC. The specific molecular details, i.e., which sugar OH is coupled to the phosphate, are lacking since these are broad-line powder spectra. Nonetheless, the 17% change in the spectral breadth resembles that observed previously in going from anhydrous DPPC to DPPC·H_2O or to DPPE (Griffin, 1976; Herzfeld et al., 1978). Both of these observations are consistent with other results (Crowe et al., 1984), which indicate that the sugar forms some sort of hydrogen bonding network to the PO_4.

As was noted above, the ^2H line shapes observed for dry DPPC and TRE/DPPC mixtures in the κ-phase are only superficially similar to those of hydrated DPPC. Thus motional models previously employed for line-shape analysis in hydrated lipids (Blume et al., 1982; Blume & Griffin, 1982) fail to account for the line shapes observed in this study. Instead, the best simulations are obtained with a large angle libration model where the hop angle is about 90°. This is a significant departure from the 112° angle used for the rotational isomeric model. Although this is a somewhat surprising result, it may be consistent with some features of lipid chains in dry and crystalline phases.

First, the acyl chains in crystals of phosphatidylcholine are not in a perfect all-trans conformation. Instead, as one proceeds from the carboxyl to the methyl end, there is a twist of the hydrocarbon chains (Pearson & Pascher, 1979). Such a conformation could favor librations over gauche-trans isomerization. Second, preliminary data indicate that X-ray diffraction lines in dry DPPC and in TRE/DPPC are diffuse and not very numerous (M. J. Caffrey, private communication; G. G. Shipley, private communication). This indicates that chain packing is less efficient, which in turn could lead to motion of a nontetrahedral variety. In contrast, in glycolipids, sharp X-ray diffraction lines are present in the crystalline phase (Ruocco et al., 1981), and well-defined $\eta \simeq 1$ spectra due to gauche-trans isomerization are observed in ^2H spectra of these phases (Huang et al., 1980). Despite the success of the simulations in Figure 4, a certain amount of ambiguity remains because these line shapes are relatively featureless. Thus, we caution the reader against overinterpreting these data.

The most remarkable result of this study is the ^2H NMR spectrum of 2:1 TRE/2[7,7-$^2H_2$]DPPC, which collapses from a half-width of 120 kHz to 29 kHz upon passing through the $\kappa \rightarrow \lambda$ phase transition. Since the shape of this spectrum is not axially symmetric, fast long axis diffusion of the lipid molecules in this phase can be excluded. This result is, of course, consistent with the ^{31}P data. The full width of the spectrum, at half-height (29 kHz), also places constraints on the types of motional models that can be used to simulate the spectra. For instance, if tetrahedral hops are used, then two- or three-site models would yield spectra that are much too wide. Instead, a four-site model is required, and in this particular case we have found that site populations $P_1 = P_2 = 0.3$, $P_3 = 0.27$, and $P_4 = 0.13$ yield excellent spectral simulations. Even though there may appear to be a large number of variables associated with this problem, the spectral line shape severely constrains the populations and rates that yield an acceptable spectrum. These site populations indicate that the acyl chains are highly disordered and several *gauche* isomers occur along the length of the hydrocarbon chain. In order to accommodate this disorder the bilayer must expand laterally with the addition of the sugar.

Conclusions

DSC and solid-state NMR spectra of dry DPPC and TRE/DPPC mixtures have revealed a number of interesting features of these systems. First, with both DSC and NMR we have found the phase transition in TRE/DPPC mixtures to be conditionally reversible. Apparently, after passing through the main transition, the acyl chains require an extended period to reanneal into their original state. Second, ^{31}P spectra indicate that the sugar binds to the DPPC head group and that the head group is immobile. ^2H spectra of chain-labeled lipids support these results; however, upon passing through the phase transition, the 120-kHz spectrum observed in the κ-phase undergoes a remarkable narrowing to form a new type of liquid-crystalline phase, which we call a λ-phase. This spectral width and shape can be successfully simulated with a model involving four-site tetrahedral hops and indicate that the lipid acyl chains are highly disordered. Further, the results suggest that the mechanism whereby TRE lowers the transition temperature of DPPC involves spacing the acyl chains to permit this disorder. A similar mechanism is probably involved in maintaining the integrity of dehydrated biological membranes.

Acknowledgments

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Registry No. TRE, 99-20-7; DPPC, 63-89-8.

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^{31}P NMR Spectra of Rod Outer Segment and Sarcoplasmic Reticulum Membranes Show No Evidence of Immobilized Components due to Lipid–Protein Interactions†

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ABSTRACT: ^{31}P NMR studies of rod outer segment (ROS) and sarcoplasmic reticulum (SR) membranes have been performed under conditions where broad and narrow spectral components can be clearly resolved. Control studies of an anhydrous, solid powder of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), as well as aqueous binary mixtures of 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), demonstrate clearly that broad spectral components can be detected. For the codispersions of DSPC and DOPC in the mixed-phase region at 22 °C, the ^{31}P NMR spectra consist of a superposition of a broad component and a narrow, axially symmetric component, due to coexisting solid and liquid-crystalline domains, which are in slow exchange on the ^{31}P NMR time scale. The ^{31}P NMR spectra of the native ROS and SR membranes, however, consist of only a narrow component, to within experimental error, indicating that most or all of the phospholipids are in the liquid-crystalline (L_α) phase at 22 °C. The above conclusions are in agreement with many, but not all, previous studies [see, e.g., Yeagle, P. L. (1982) *Biophys. J. 37*, 227–239]. It is estimated that at most 10% of the phospholipids in the ROS and SR membranes could give rise to broad ^{31}P NMR spectral components, similar to those seen for anhydrous or solid-phase lipids, corresponding to ~7 phospholipids/rhodopsin molecule and ~11 phospholipids/Ca^{2+}-ATPase molecule, respectively.

The effects of intrinsic membrane proteins on the structural and dynamic properties of membrane lipids have been studied by various spectroscopic techniques, including fluorescence depolarization (Wolber & Hudson, 1982), spin-label electron paramagnetic resonance (EPR) (Ellena et al., 1983; Pates et al., 1985b; Marsh, 1985),[1] and nuclear magnetic resonance (NMR) (Seelig & Seelig, 1980; Brown et al., 1982; Davis, 1983). Spin-label EPR studies of different membrane systems have shown that under the appropriate conditions two spectral components can be resolved (Jost et al., 1973a,b; Marsh, 1985). One component has been attributed to the lipids in van der Waals contact with the hydrophobic surfaces of intrinsic membrane protein molecules, and the other component to bulk lipids not in direct steric contact with membrane proteins. Most NMR studies of membrane systems have yielded somewhat different results, however, in that only a single spectral component is usually detected. One explanation for the discrepancy is that the motional rates necessary to produce averaging of the spectra are different in the two cases (Brown et al., 1977; Seelig & Seelig, 1980; Paddy et al., 1981). That is, since the NMR frequencies are less than those in EPR spectroscopy, the distinct components seen with spin-label EPR could be averaged over the much longer NMR time scale and only a single component observed (Brown et al., 1977). Several EPR spin-label studies have further suggested that there may be some selectivity at the membrane lipid-protein interface (Brotherus et al., 1981; Knowles et al., 1981; Ellena et al., 1983; Brophy et al., 1984; Esmann et al., 1985). Also, in some cases there may be a small number of lipids that are tightly bound to high-affinity sites of intrinsic membrane proteins (Robinson, 1982). Nonetheless, most current opinion favors the view that the motional rates and amplitudes of the lipids in steric contact with intrinsic membrane proteins are not greatly different from those of lipids in the bulk bilayer phase of the membrane (Sefcik et al., 1983).

Reports of additional NMR spectral components due to membrane protein associated lipids have appeared, however

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[1] Abbreviations: DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; EPR, electron paramagnetic resonance; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid; MOPS, 3-(*N*-morpholino)propanesulfonic acid; M_r, molecular weight; NMR, nuclear magnetic resonance; ROS, rod outer segment; SR, sarcoplasmic reticulum; $\Delta\sigma$, chemical shift anisotropy; τ_c, correlation time; T_M, gel to liquid-crystalline phase transition temperature; X, mole fraction.

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