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Ripple Phase Stability in Lipid Systems that Form Interdigitated Bilayers

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Interdigitated bilayer phases have been unambiguously shown to occur in phospholipid systems. The presence of the interdigitated bilayer phase is influenced by the character of the lipids present in the bilayer and the structure of the solvent at the bilayer-solvent interface. Phase structures and transitions involving interdigitated phases are examined using real time x-ray diffraction. The synthetic lipids 1,2 O-dihexadecylphosphatidylcholine (DHPC) in water and dipalmitoylphosphatidylcholine (DPPC) in ethanol/water or 1 M KSCN have been previously shown to form an interdigitated gel state bilayer phase. The dynamic phase sequence observed for DHPC in water was found to be:

$$L_c(\text{inter}) \rightarrow L_\beta(\text{inter}) \rightarrow P_\beta \rightarrow L_\alpha.$$

The dynamic phase sequence observed for DPPC in ethanol/water or 1 M KSCN was found to not contain a ripple (P_β) phase. It can be concluded that changes in the ordering of the solvent structure at the bilayer-solvent interface can prevent the dynamic formation of the rippled bilayer phase.

Keywords: interdigitated phases, phospholipid bilayers, solvent structure, real time x-ray diffraction, ripple phase

INTRODUCTION

Hydrated phospholipids at relatively low temperatures typically form densely packed bilayer structures (L_c) in which the hydrocarbon chains form orthorhombic hybrid chain subcell lattices.[1–4] Increasing the temperature of the L_c phase causes the higher order subcellular packing to be less energetically favored due to thermally induced chain excitations (i.e. rotations). At the subtransition temperature, the L_c phase acyl chain packing transforms to a more expanded state in which the hydrocarbon chains pack in an hexagonal two dimensional lattice or gel phase. The acyl chains can be slightly tilted ($L_{\beta'}$) or untilted (L_β) relative to the bilayer plane.[3,5–7]

A further increase of the temperature in this system causes an increase in the oscillations within the hydrocarbon chains resulting in unhindered long-axis chain rotations at the pretransition temperature.[8,9] In addition, rotations about the lipid headgroup phosphate bond relative to the lipid glycerol backbone occur resulting in an increase in the surface area per lipid molecule.[10-12] The formation of ripple phases at the pretransition is probably due to individual acyl chains mutually shifting along the long axes in order to stay in close contact. This results in the bilayer surface breaking up into a series of periodic, asymmetric quasi-lamellar bilayer segments ($P_{\beta'}$ or P_{β}).[13] A further increase in the sample's temperature causes a loss of the hexagonally packed chain order and, in particular, at the main phase transition temperature, a loss of acyl chain order and a re-establishment of an unrippled bilayer phase (L_{α}) occurs.[5] Cevc[14] has recently presented arguments that the subgel and main lipid phase transitions are driven primarily by changes in the acyl chain interactions while the transition to the ripple phase is primarily driven by intra-lamellar headgroup interactions at the bilayer-water interface and is stabilized by both headgroup and acyl chain interactions. The strength of the headgroup interactions thus determines the relative position of the pretransition between the subgel and main transition temperatures. It is proposed that strong interactions between lipid molecules can eliminate the presence of an intermediate L_{β} or $L_{\beta'}$ phase between the L_c and $P_{\beta'}$ phases while weaker interactions may lead to an elimination of the $P_{\beta'}$ phase.

Interdigitated bilayer phases have been unambiguously shown to occur in phospholipid systems. Although the presence of the interdigitated bilayer phase is influenced by the character of the lipids in the bilayer[15-17] and the structure of the solvent at the bilayer-solvent interface,[18-25] it is apparent from the close packed nature of the acyl chains that acyl chain interactions have a stabilizing influence on the interdigitated phase. Recent reports have shown that both the subgel and gel phases can be formed in interdigitated bilayers.[16,26-28] Furthermore, the phase transition sequence differs depending upon whether the lipid spontaneously forms an interdigitated phase in water[26] or the solvent structure induces this phase.[27,29] It is interesting to speculate how the differences in the character of the lipid-solvent system influence interdigitated and ripple phase formation and the relationships between transitions involving these lipid phases in the context of the theory proposed by Cevc.[14] In particular, real time measurements would be required for classification of these transitions since they involve dynamic processes.

In this report, we examine the relationships between phase formation, lipid character, solvent structure, and mechanical stresses within a phospholipid model membrane. Particular attention is paid to the dynamic production and stability of gel state ripple phases as indicated during the real time x-ray diffraction experiment.[28]

MATERIALS AND METHODS

Dipalmitoylphosphatidylcholine was obtained from Avanti Polar Lipids (Birmingham, AL) and dihexadecylphosphatidylcholine from Sigma Chemical Co. (St. Louis, MO). The salt and ethanol were reagent grade, and the water was distilled.

Lipids were hydrated and then equilibrated at ~60°C until no unhydrated powder

was observed. Samples for x-ray examination were cooled to at least −50°C and then raised to the desired initial temperature while in the x-ray sample holder.

X-ray experiments were performed using a monochromatic (0.15 nm) focused x-ray beam at station 8.2 of The Daresbury (UK) Synchrotron Laboratory. A purpose-built camera allowed clear resolution of reflections corresponding to Bragg spacings from 10 nm down to 0.35 nm. The sample holder was a cryo-stage (Linkam) to which mica windows were fitted and the sample size was about 30 μl with an x-ray path length of 1 mm. The stage was cooled to the temperature of liquid nitrogen and heated to the required temperature by embedded heating elements in the stage. Temperature programming over the range −50° to 200°C at rates between 0.001°/min to 2.3°/sec was possible. Sample temperature was monitored by thermocouples embedded in the stage adjacent to the sample.

X-ray data were collected on a single wire linear detector fabricated at the Daresbury Laboratory. The acquired data was stored in a VAX-11/750 computer and corrected for detector response by comparison with a pattern recorded using a fixed source and averaged over several hours. Data were analyzed using the OTOKO program provided by the Daresbury Laboratory.

RESULTS AND DISCUSSION

It has been previously shown[16] using thermally equilibrated samples that fully hydrated DHPC can undergo transformations from the subgel state interdigitated

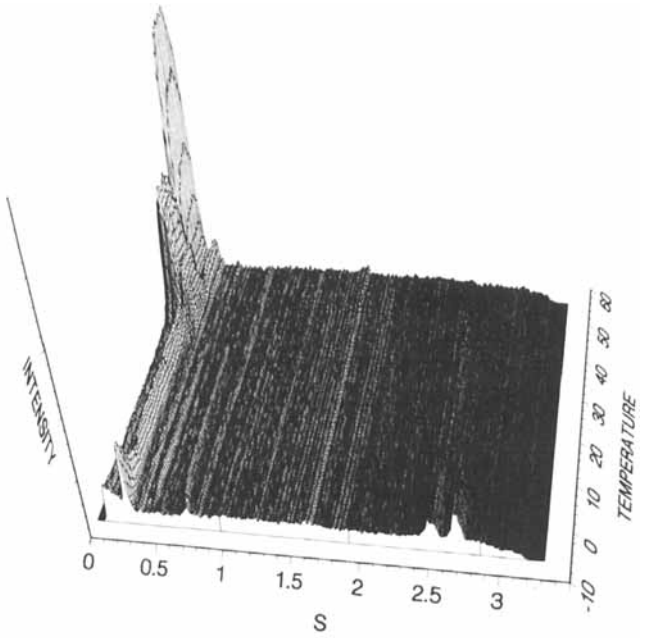

FIGURE 1 Three dimensional plot of scattering intensity versus reciprocal spacing for fully hydrated DHPC undergoing a 5°C/min heating scan. Every third frame of 3s duration for the total data set of 255 diffraction patterns is shown. Temperature units are in °C and reciprocal space units are in nm^{-1}, with intensity in arbitrary units.

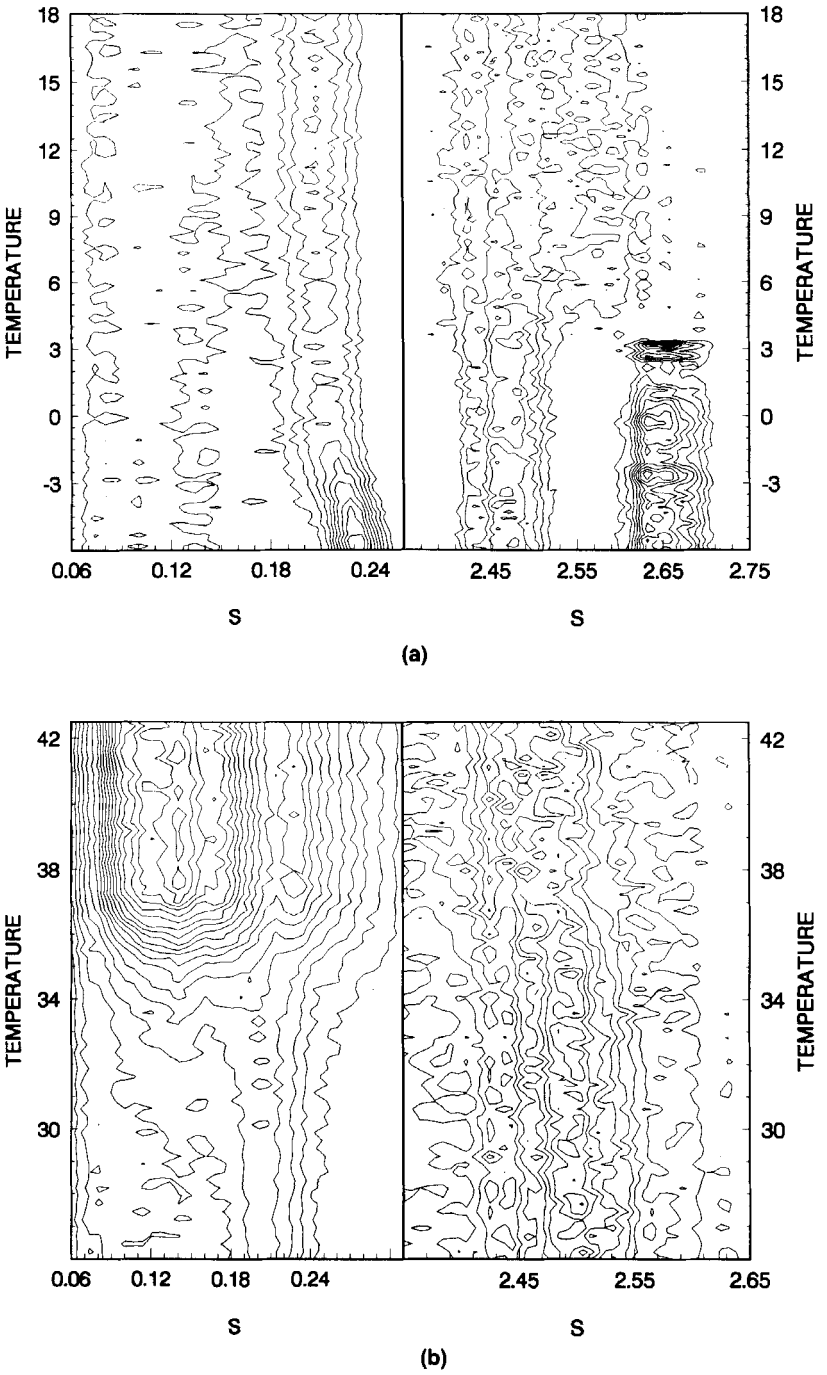

FIGURE 2 Two dimensional projections of the appropriate diffraction patterns from Figure 1 representing the a) L_c(inter) → L_β(inter), b) L_β(inter) → P_β, and c) P_β → L_α phase transitions in fully hydrated DHPC with transition temperatures occurring at approximately 5.6°C, 35.5°C, and 45.0°C, respectively. Temperature units are in °C and reciprocal space units are in nm^{-1}.

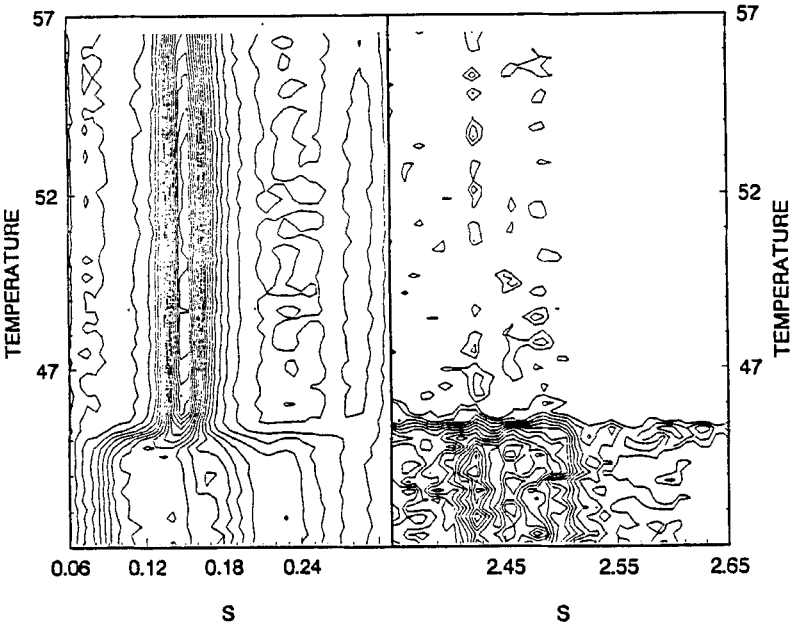

FIGURE 2c

bilayer phase, L_c(inter), to the gel state interdigitated bilayer phase, L_β(inter), to the ripple phase, P_β, and finally to the disordered bilayer phase, L_α, with increasing temperature. In Figure 1, we show x-ray diffraction patterns for the dynamic phase transition sequence for fully hydrated DHPC undergoing a heating scan starting from approximately -6°C. The initial phase in this system produced a wide angle x-ray scattering (WAXS) profile containing two diffraction peaks which is consistent with an interpretation of the presence of a crystalline bilayer phase.[16] A comparison of the bilayer repeat for this phase is consistent with the presence of an interdigitated crystalline bilayer phase, L_c(inter), as first reported by Laggner *et al.*[16] The fully hydrated DHPC dynamic phase sequence with increasing temperature can thus be inferred to be: $L_c(\text{inter}) \rightarrow L_\beta(\text{inter}) \rightarrow P_\beta \rightarrow L_\alpha$. The transition temperatures and repeat spacings for the phases involved in the sub, pre, and main DHPC phase transitions are consistent with those from the previous report utilizing thermally equilibrated samples.[16] Figure 2a–c shows a collection of x-ray diffraction patterns which describe the $L_c(\text{inter}) \rightarrow L_\beta(\text{inter})$, $L_\beta(\text{inter}) \rightarrow P_\beta$ and $P_\beta \rightarrow L_\alpha$ phase transitions, respectively, using the data shown in Figure 1. The resolution of these patterns are equivalent to those for temperature equilibrated samples. The subgel transition proceeded via the production of intermediate states while the pre and main phase transitions proceeded via two state mechanisms. Higher order processes have been inferred for the transition from the subgel to gel phases in noninterdigitated bilayers.[4]

The phase transition sequences for DPPC in 150 mg/ml ethanol/water[27] or 1 M KSCN[24,29] have been previously determined to be:

$$L_c(\text{inter}) \rightarrow L_\beta(\text{inter}) \rightarrow L_\alpha.$$

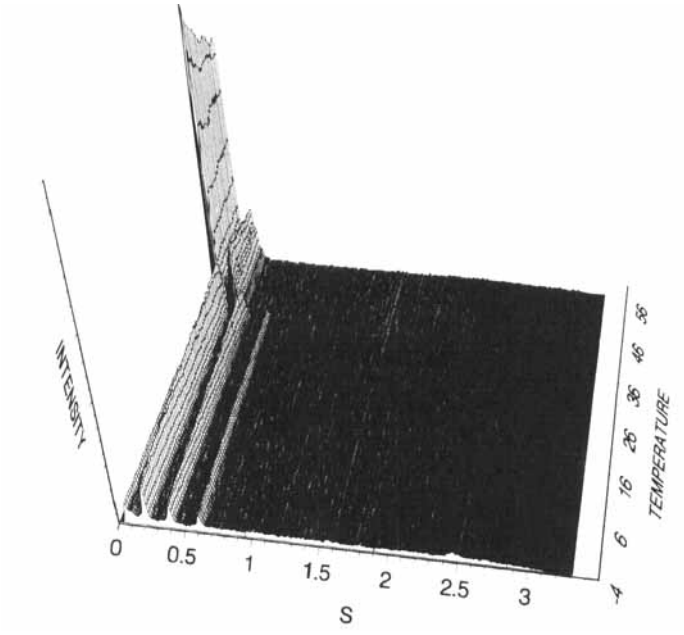

FIGURE 3 Three dimensional plot of scattering intensity versus reciprocal spacing for DPPC in 150 mg/ml ethanol/water undergoing a 5°C/min heating scan. Every third frame of 3s duration from the total data set of 255 diffraction patterns is shown. Temperature units are in °C and reciprocal space units are in nm^{-1}, with intensity in abitrary units.

In Figure 3, we show representative diffraction patterns for DPPC in 150 mg/ml ethanol/water undergoing a heating scan starting from about −4°C. It can be determined from the appropriate real time x-ray diffraction data that the L_c(inter) → L_β(inter) phase transition proceeded via the continuous change in structure (Figure 4a) while the L_β(inter) → L_α phase transition proceeded via a two state process (Figure 4b).

A number of general conclusions can be drawn from these data. Subgel or crystalline interdigitated bilayer acyl chain packing typically transformed into more disordered bilayer states via higher order or continuous changes in lattice structure. It can be speculated that the interactions within the acyl chain lattice structure in the subgel phase are sufficiently strong as to preclude even the formation of small localized regions of a second independent phase, thus requiring a series of small conformational changes to take place cooperatively over relatively large areas. Such small changes in state would also reduce the entropy change in the transformation as opposed to the direct transformation from a closely packed lattice to a less close-packed lattice. Even the presence of interdigitated bilayer phases as the initial and final transformation states does not provide a sufficient condition for a two state transition process to occur.

The general interactions between lipid moieties can be inferred by correlation to Cevc's theory[14] for ripple phase stability. The presence of the ripple structure in the DHPC phase sequence is an indication that the relative interactions between the DHPC headgroups are "strong" while the presence of interdigitated subgel

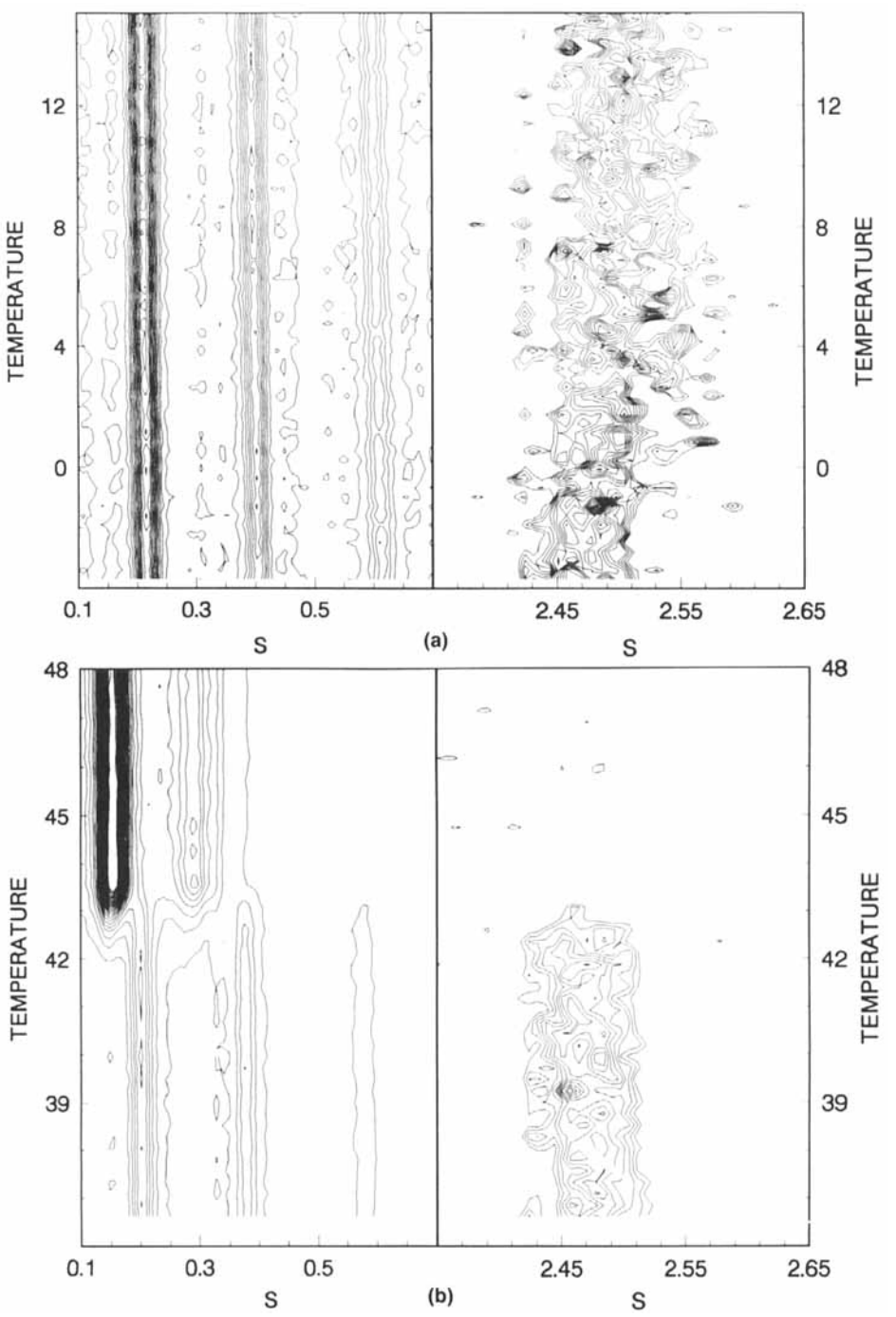

FIGURE 4 Two dimensional projection of the appropriate diffraction patterns from Figure 3 representing the a) L_c(inter) → L_β(inter), and b) L_β(inter) → L_α phase transitions for DPPC in 150 mg/ml ethanol/water with transition temperatures at approximately −1.3°C and 43.1°C, respectively. Temperature units are in °C and reciprocal space units are in nm^{-1}.

and gel state bilayers is an indication that the relative acyl chain interactions are also strong. It is recognized that both headgroup and acyl chain interactions are important in phase stability but one interaction can dominate over the other in describing the driving force of phase production. The DHPC headgroup interactions are dominant over that of the acyl chains since the ripple phase is stable at higher temperatures. The absence of a ripple phase for DPPC in either ethanol or KSCN is indicative of a loosening of the DPPC headgroup interactions as a function of solvent concentration. The presence of a solvent containing chaotropic reagents with a lesser order than water, as modelled by 1 M KSCN for example, would be expected to reduce the ordering at the lipid-solvent interface. This effect would be opposite to the effect of kosmotropic reagents such as sugars[30] which would stabilize the structure of the bulk water. It is thus apparent that either changes in the solvent structure[31] and/or possible changes in solvent binding between headgroups are sufficient to change interactions between DPPC headgroups and possibly the orientation of the molecule with the bilayer. This was also observed[31] for noninterdigitated DPPC bilayers in the presence of 10 mM $CaCl_2$.

CONCLUSION

The real time x-ray diffraction data presented are consistent with Cevc's phenomenological description for the driving forces for various lipid phase transitions. Specifically, this data confirms that the transitions between subgel and gel interdigitated bilayers and gel (interdigitated or ripple phase) to disordered bilayer are driven by the thermally induced change in molecular motion of the acyl chains as reflected in changes in the wide angle x-ray scattering patterns during the transition process. However, the driving force for the formation of ripple phases clearly resides in the solvent-bilayer interfacial region. DHPC interdigitated bilayers can transform to a ripple phase since both the acyl chain and headgroup motions are temperature dependent. The requirement for relaxation of the thermally induced stresses within a bilayer caused by the increased acyl chain molecular motion via a gross deformation/undulation of the bilayer interface results in the formation of a ripple phase. The thermally induced increase in headgroup motion, in this system, also releases the acyl chains from occupying close positions within the interdigitated bilayer structure. The solvent structure within the bilayer interfacial region for DPPC in ethanol/water or 1 M KSCN, however, is not temperature dependent within the thermal range studied. Thus, thermally induced increases in the molecular energy are dissipated within the coupled solvent-bilayer system until the acyl chain energy is so high as to induce chain melting or the transition to a disordered bilayer phase. We cannot ascertain whether these solvents act by changing the solvent structure/order,[30] per se, or by allowing solute molecules to reside between lipid headgroups thereby restricting lipid headgroup motional or orientational changes. The latter is consistent with our previous observation that the presence of bound Ca^{2+} preserves the gel bilayer structure at the expense of ripple phase formation.[31]

Acknowledgments

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