Interaction of 1-Propanol and 2-Propanol with Dipalmitoylphosphatidylcholine Bilayer: A Calorimetric Study†

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Differential scanning calorimetry (DSC) was employed to study the effects of 1-propanol and 2-propanol on the thermotropic behavior of dipalmitoylphosphatidylcholine (DPPC) bilayer membranes. The biphasic effect was observed in both systems based on an abrupt reversal in trend of the gel-to-liquid crystalline main transition temperatures upon heating. At this inflection point, a large hysteresis also occurred between the heating and cooling main transition temperatures, and the pretransition peaks disappeared. Such evidence indicated the formation of the interdigitated gel phase at this critical concentration, which was determined to be 0.39 mol·L^{-1} and 0.52 mol·L^{-1} for 1-propanol and 2-propanol, respectively. This threshold concentration was found to be higher for 2-propanol than for 1-propanol, suggesting that the shorter chain length and branching in 2-propanol caused it to be a less effective inducer for the interdigitated phase in DPPC. On some heating and cooling scans, an extra peak is observed around and beyond the critical concentration, indicating the presence of mixed phases as the interdigitated phase forms.

Introduction

Lipids are the basic components of highly complex biological membranes. Homogeneous synthetic lipid bilayers consisting of phosphatidylcholines (PCs), the most abundant lipid in biological systems, have undergone intensive studies.[1] The thermotropic phase behavior of such pure lipid bilayers has been well established. They transition from the most compact and highly ordered subgel (L_c) phase to the planar gel ($L_{\beta'}$) phase, then the rippled gel ($P_{\beta'}$) phase, and finally into the most fluid-like liquid crystalline (L_α) phase, as temperature increases.[1] Studies have also shown that with the addition of small amphiphilic molecules the interdigitated gel ($L_\beta I$) phase can be induced to replace the $P_{\beta'}$ phase in the phase transition pathway.[1−5] In the $L_\beta I$ phase, the opposing monolayers of the lipid bilayer interpenetrate into each other to decrease the bilayer thickness.

A variety of molecules have been shown to be good additives to induce the $L_\beta I$ phase in saturated, like-chain PCs. Such inducers include glycerol, ethylene glycol, benzyl alcohol, thiocyanate ion, chlorpromazine, tetracaine, choline, buffer molecules, myelin basic protein, bioactive labdanes, and 1-alcohols up to 1-heptanol as well as some branched alcohols.[1,5−7] Those molecules are capable of inserting between the head groups of the adjacent PCs. The hydrophilic portion of the inducer molecules interacts with the phosphate head groups of the PCs, toward hydrogen bonding in some cases, while the hydrophobic region interacts with the portion of the lipid acyl chain that is adjacent to the phosphate group.[8,9] The PC molecules interdigitate to fill in the high energy voids created by such insertions, which simultaneously increases the preferable van der Waals interactions between the PC acyl chains.[4,10] The inducer molecules block out water from interacting with the terminal methyl groups of the PCs to further stabilize the $L_\beta I$ structure. Further evidence suggests that interdigitation also relieves the headgroup crowding in the lipid bilayer to a greater extent than the $P_{\beta'}$ phase, which is the primary reason why the $L_\beta I$ phase replaces the $P_{\beta'}$ phase when inducer molecules are present.[11]

The $L_\beta I$ phase can also be induced without any additives. Dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine are able to transition into the $L_\beta I$ phase under increased hydrostatic pressure.[12−14] Dihexadecylphosphatidylcholine and 1,3-DPPC are shown to exist in the $L_\beta I$ phase just under normal pressure without any additives.[15−17] Mixed chain PCs also interdigitate if one acyl chain is approximately half the length of the other.[18,19]

DSC has been used extensively to study the $L_\beta I$ phase in different lipid systems and has been shown to be a powerful tool. Indicators of interdigitation include the disappearance of pretransitions, the biphasic effect shown on heating scans, hysteresis manifested by the irreversibility on cooling scans, and the presence of an extra peak next to the main transitions.[2,3,20−22]

Various studies have been carried out to understand the $L_\beta I$ phase. It has been established that even small changes in temperature or concentration of small inducer molecules can alter the balance between phases due to their relatively small differences in free energies.[23] The low enthalpy of transitions and the sensitivity to inducer molecules suggest that the interdigitated phase could be important in biological membranes.[5] The $L_\beta I$ phase has very different features from any other phases, as the interpenetration of the two acyl chains significantly reduces the membrane thickness. It also reduces surface charge density, which could be crucial in membrane fusion.[1] The loss of the bilayer midplane, a particularly hydrophobic region of the bilayer, could cause membrane proteins to undergo conformational changes.[1] Moreover, the $L_\beta I$ phase is of pharmacological interest since many anesthetics possess the properties of the inducer molecules. An anesthetics-induced interdigitation would affect the membrane permeability as well as the function of various membrane-bound proteins.[24] Recent studies

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also suggest that the $L_\beta I$ phase has promising potential applications in drug delivery, as it can form nanocompartments to enclose vesicles, colloids, and macromolecules upon its transition to the L_α phase.[25]

A previous study by Rowe reported that 1-alcohols up to 1-heptanol induced interdigitation on the PC bilayers.[20] However, the structural isomers of the 1-alcohols have not been studied until recently when Reeves et al. from our laboratory examined the effects of the 1-, *iso*-, *sec*-, and *tert*-butanol isomers on the thermotropic behavior of DPPC.[26] In conjunction to their work, we thoroughly investigated and compared the interactions between 1- and 2-propanol with the DPPC bilayer via DSC to gain a more complete and deeper understanding of the structural effects of the inducer molecules on the PC thermotropic behavior.

Materials and Methods

1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), purity 99+ %, was purchased from Avanti Polar Lipids (Alabaster, Al, USA). Anhydrous 1-propanol with purity 99.7 % and anhydrous 2-propanol with purity 99.5 % were obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were used without further purification.

DSC has been shown to be an effective tool to study the thermotropic behavior of lipid bilayers. DSC scans were obtained using a multicell DSC-HT model 4100 from Calorimetry Science Corporation. The DSC samples were prepared by weighing out 2 mg of DPPC into a DSC ampule followed by hydrating with 50 μL of 1-propanol or 2-propanol solutions. The DSC samples were then incubated at about 45 °C for one hour and mixed periodically using a Vortex mixer. The samples were equilibrated to room temperature before loading into the DSC. The samples were heated and cooled within the temperature range from (10 to 50) °C at a scan rate of 10 °C·h^{-1}. The samples were scanned twice in each run to ensure reproducibility. All peaks on thermograms were analyzed using the Jandel Scientific Peakfit Program.

Results

For pure DPPC, the planar gel-to-ripple gel pretransition temperature (t_p) and the ripple gel-to-liquid crystalline main transition temperature (t_m) upon heating centered at (33.1 and 41.2) °C, respectively, with standard deviations of ± 0.1 °C. These transition temperatures are in agreement with previously reported values for DPPC.[3,11,26]

Figure 1 shows a representative DSC thermogram. The top thermogram is obtained for DPPC in the presence of 0.13 mol·L^{-1} 1-propanol, where the two peaks correspond to the broad pretransition peak and the main transition peak. In the presence of a higher concentration of 0.94 1-propanol (bottom thermogram), the pretransition disappeared, and a shoulder peak appeared next to the main transition peak.

Figure 2 shows the effects of 1-propanol on the DPPC phase transition temperatures. The heating t_p decreased dramatically from (33.1 to 17.0) °C monotonically but not linearly as the 1-propanol concentration increased to 0.44 mol·L^{-1}. Beyond 0.44 mol·L^{-1} 1-propanol, the heating pretransition peak is no longer detectable. The t_p on cooling scans exhibited a trend similar to the heating scans as the t_p decreased from (31.1 to 13.5) °C and finally disappeared in the presence of 0.40 mol·L^{-1} 1-propanol. The heating t_m was also lowered from (41.2 to 38.7) °C, a 2.5 °C decrease, with increasing 1-propanol concentration up to 0.39 mol·L^{-1}. The trend in t_m reversed beyond this concentration and started to increase with increasing 1-propanol

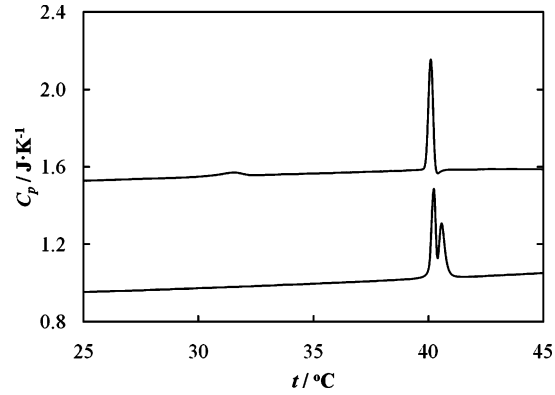

Figure 1. Representative DSC thermograms with heat capacity C_p plotted against temperature t in the temperature range (25 to 45) °C. The top thermogram represents DPPC in the presence of 0.13 mol·L^{-1} 1-propanol, a concentration before the threshold concentration. The broad peak is the pretransition peak, and the sharp peak is the main transition peak. The bottom DPPC thermogram is obtained in the presence of 0.94 mol·L^{-1} 1-propanol, a concentration after the onset of threshold. The tallest peak is the main transition peak, and the subsequent smaller peak is classified as the shoulder peak.

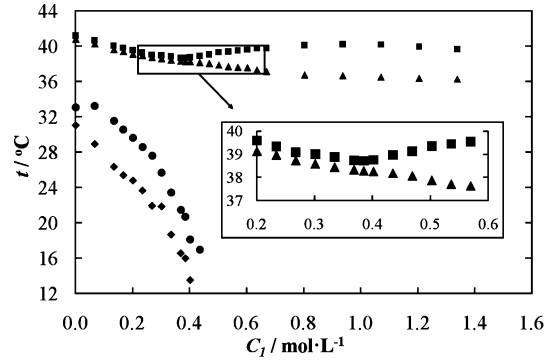

Figure 2. Effects of 1-propanol concentration C_1 on the DPPC phase transition temperatures (■, heating scan main transition peak; ▲, cooling scan main transition peak; ●, heating scan pretransition peak; ◆, cooling scan pretransition peak). Shoulder peaks are not included. Inset shows region around the identified threshold concentration. The temperature values have a standard deviation of ± 0.01 °C.

concentration. Such an abrupt reversal in trend is classified as the biphasic effect by Rowe[20] and has been reported by other studies.[2,3,11] The inflection point at which the reversal occurs is referred to as the threshold concentration. The t_m beyond the threshold concentration continued to increase by another 1.5 °C before gradually decreasing again. A large hysteresis between the heating and cooling t_m was also observed beyond the threshold concentration of 0.39 mol·L^{-1}.

The t_p and t_m data on the DPPC + 2-propanol system revealed a trend similar to the DPPC + 1-propanol system. This is shown in Figure 3. Upon heating, the t_p in this system decreased from (33.1 to 19.1) °C and was no longer detectable beyond 0.59 mol·L^{-1} 2-propanol. Upon cooling, the pretransition peak disappeared at 0.52 mol·L^{-1} as it reached the lowest temperature of 17.2 °C. The t_m in this system also underwent the biphasic effect with the inflection point occurring at 0.52 mol·L^{-1} at a transition temperature of 39.4 °C, representing a 1.8 °C decrease in t_m. The DPPC + 2-propanol system exhibited a stronger biphasic behavior compared to the DPPC + 1-propanol system as the t_m increased by another 2.3 °C beyond the threshold concentration before decreasing, compared to the 1.5 °C increase in the 1-propanol system. This observation is in agreement with the butanol system that was reported by Reeves et al., as a

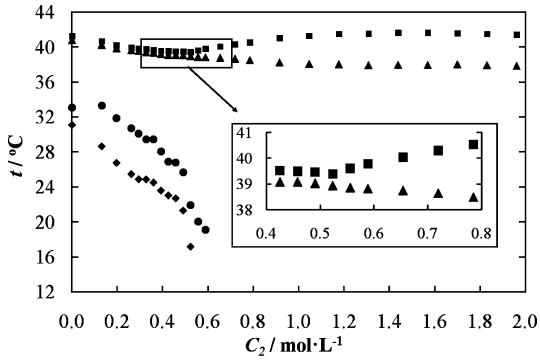

Figure 3. Effects of 2-propanol concentration C_2 on the DPPC phase transition temperatures (■, heating scan main transition peak; ▲, cooling scan main transition peak; ●, heating scan pretransition peak; ◆, cooling scan pretransition peak). Shoulder peaks are not included. Inset shows region around the identified threshold concentration. The temperature values have a standard deviation of ± 0.01 °C.

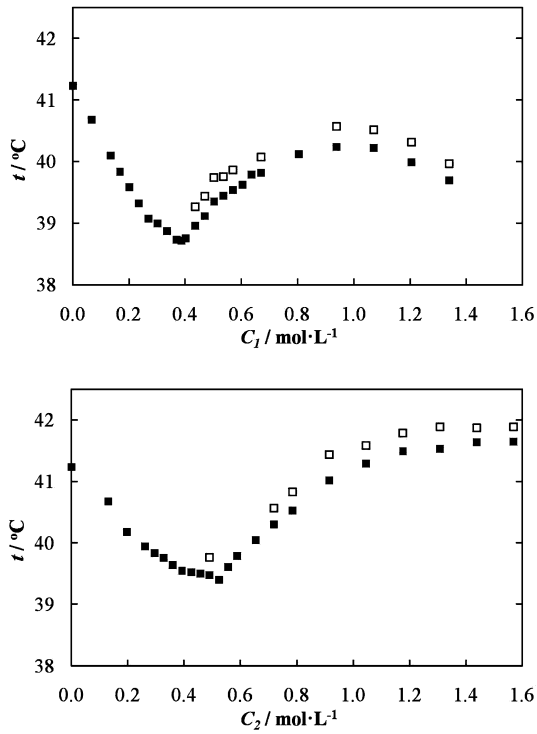

Figure 4. Effects of (a) 1-propanol concentration C_1 and (b) 2-propanol concentration C_2 on the DPPC heating scan phase transition temperatures (■, main transition peak; □, shoulder peak). The temperature values have a standard deviation of ± 0.01 °C.

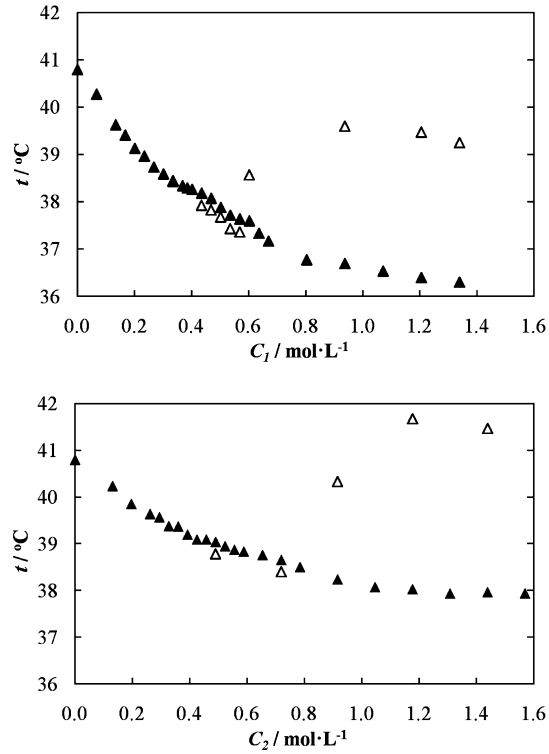

Figure 5. Effects of (a) 1-propanol concentration C_1 and (b) 2-propanol concentration C_2 on the DPPC cooling scan phase transition temperatures (▲, main transition peak; Δ, shoulder peak). The temperature values have a standard deviation of ± 0.01 °C.

greater degree of biphasic behavior was observed for *tert*-butanol, followed by *sec*-butanol, isobutanol, and 1-butanol. A large hysteresis between heating and cooling t_m was also observed beyond 0.52 mol·L^{-1}.

From the thermograms, some heating and cooling scans have an additional smaller peak next to the main transition peak around and after the threshold concentration for both DPPC + 1-propanol and DPPC + 2-propanol systems. Such peaks are referred to as shoulder peaks. The shoulder peaks do not appear consistently on all scans but on most samples with alcohol concentrations above the threshold. The shoulder peaks were not shown in Figures 2 and 3 for clarity purposes but are included in Figure 4a and 4b. In both systems, the shoulder peaks on heating scans were observed at a higher temperature than the corresponding t_m. However, the shoulder peaks on cooling scans that existed below and around the threshold concentration appeared at lower temperatures than their t_m. Shortly beyond the threshold concentration, the shoulder peaks suddenly shifted above the corresponding t_m. In fact, these shoulder peaks are reminiscent of the higher t_m values observed in the heating scans in both DPPC + 1-propanol and DPPC + 2-propanol systems.

The enthalpies of the main transitions as a function of 1-propanol and 2-propanol concentrations are shown in Figure 6a and 6b, respectively. For the heating scans of each isomer, the enthalpies below and above the threshold concentrations were separately fitted to straight lines using least-squares. The enthalpy values generally increased with increasing concentrations of the isomers, and there is a break in the trend line at the threshold concentrations. These effects are much stronger in the 1-propanol system.

Discussion

Table 1 shows a summary of the important thermotropic events for the DPPC + propanol isomer systems. The observation of the biphasic effect upon heating, the formation of the large hysteresis between heating and cooling t_m beyond the threshold concentration, and the disappearance of the pretransitions all indicated the formation of the interdigitated phase at the threshold concentration. The threshold concentration was determined to be 0.39 mol·L^{-1} and 0.52 mol·L^{-1} for 1-propanol and 2-propanol, respectively. This indicates that 1-propanol is a more effective inducer than 2-propanol. In addition, the t_m of the 1-propanol system decreased by 2.5 °C as 1-propanol concentration increased up to the threshold concentration, while that of the 2-propanol system only decreased by 1.8 °C. The larger decrease in t_m for the 1-propanol system suggests that 1-propanol molecules suppress the t_m to a greater extent, causing the $P_{\beta'}$ phase to be less stable and hence more effectively induces

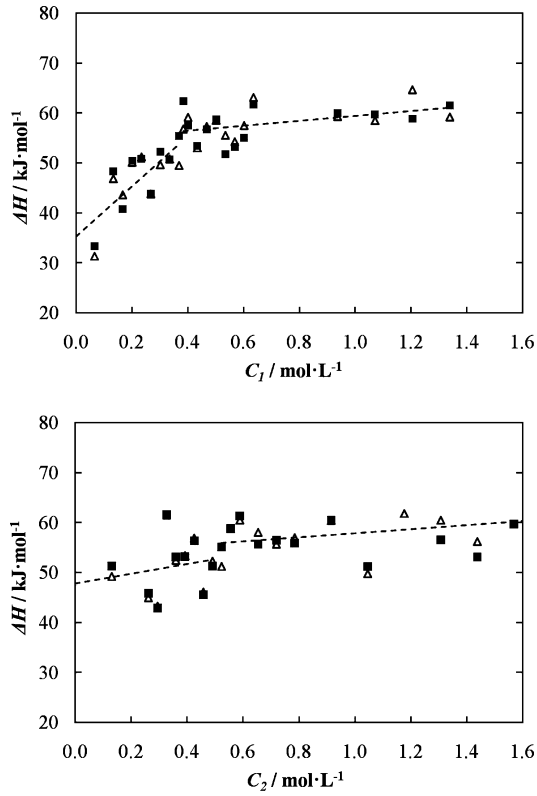

Figure 6. Effect of (a) 1-propanol concentration C_1 and (b) 2-propanol concentration C_2 on the DPPC main transition enthalpies (■, heating scan main transition enthalpy; Δ, cooling scan main transition enthalpy). The enthalpy values have a standard deviation of 0.5 kJ·mol^{-1}.

Table 1. Summary Data for the Induction of the Interdigitated Gel ($L_\beta I$) Phase in the Temperature Range of (10 to 50) °C[a]

system	C_a/mol·L^{-1}	C_b/mol·L^{-1}	C_c/mol·L^{-1}
DPPC + 1-Propanol	0.44	0.39	0.39
DPPC + 2-Propanol	0.59	0.52	0.52

[a] C_a is the concentration at which the pretransition disappears; C_b is the concentration at which the biphasic effect is observed; and C_c is the determined threshold concentration for the interdigitated phase. The uncertainty for C_a, C_b, and C_c is 0.01 mol·L^{-1}.

the $L_\beta I$ phase. This observation provides further evidence for the relative effectiveness in inducing the $L_\beta I$ phase between the propanol isomers. In previous studies, the threshold concentrations of methanol, ethanol, and 1-butanol are determined to be 2.5 mol·L^{-1}, 1.0 mol·L^{-1}, and 0.16 mol·L^{-1}.[11,26] The threshold value for 1-propanol, 0.39 mol·L^{-1}, falls between those of ethanol and 1-butanol. The descending trend of the threshold concentrations as the alcohol chain length increases leads to the conclusion that between 1- and 4-carbon carbon chains alcohols with longer carbon chains have a higher tendency to induce the $L_\beta I$ phase. This accounts for the greater effectiveness of 1-propanol in inducing the $L_\beta I$ phase compared to 2-propanol, as the former has three carbons in its main carbon chain, whereas the latter only has two. Additionally, the branching in 2-propanol allows the molecule to occupy a larger volume when it inserts between the DPPC head groups. The steric hindrance between the 2-propanol branching and the DPPC head groups makes it more difficult to integrate into the bilayer compared to 1-propanol and hence serves as a less effective inducer for the $L_\beta I$ phase. Compared to the butanol system, an identical trend was observed as 1-butanol served as the most effective inducer for the interdigitated phase, while *tert*-butanol was the worst.[26] Their study proposed that the effectiveness is related to the solubility of the isomers in water. 1-Butanol has the lowest water solubility because it is most hydrophobic. This factor enhances its hydrophobic interactions with the phospholipid hydrocarbon tails and hence serves as the most effective inducer among the isomers.[26] For the propanol systems, there are no solubility concerns since both 1-propanol and 2-propanol are readily miscible with water. However, the 1-propanol molecule is more hydrophobic than 2-propanol due to its longer hydrocarbon chain. It is possible here that the more hydrophobic nature of 1-propanol also contributed to its greater effectiveness in inducing the $L_\beta I$ phase. In most cases studied, the alcohol concentrations are much higher than the lipid concentrations; e.g., at the threshold concentration, the molar ratios of alcohol to DPPC are 7.16 and 9.54 for 1-propanol and 2-propanol, respectively.

The presence of a shoulder peak on some heating and cooling scans was observed in both propanol systems. It has been proposed that the shoulder peaks represent a mixture of phases.[3] Upon heating, previous study has suggested a phospholipid phase transition pathway going from L_c to $L_{\beta'}$ to $P_{\beta'}$ and to L_α at low alcohol concentration or in pure lipid.[6−9] However, the transition becomes L_c to $L_{\beta'}$ with $L_\beta I$, to $P_{\beta'}$ with $L_\beta I$, and finally to L_α as alcohol concentration increases.[6,27−29] Nagel et al. also observed the coexistence of the $L_{\beta'}$ and $L_\beta I$ phases in their DPPC + ethanol system experimentally.[30] In addition, Mou et al. proposed that the coexistence of phases is due to an inhomogeneous distribution of alcohol molecules in the lipid bilayer,[22] which was verified by Kranenburg et al.'s computer simulations.[21] Their computer-simulated model demonstrated that at a constant temperature, in the coexistence region, the mole fraction of alcohol in the interdigitated gel phase is constant. As the number of alcohol molecules increases, the mole fraction in the interdigitated gel phase region remains constant, and the mole fraction in the noninterdigitated region increases, resulting in an increase in the size of the interdigitated part of the bilayer and a decrease in the size of the noninterdigitated part.[21] For the DPPC + 1-propanol and DPPC + 2-propanol systems in our study, the shoulder peaks all appear slightly higher and alongside the corresponding main transition peaks. It is possible that due to the inhomogeneous distribution of propanol molecules the propanol-rich domains in both systems exist in the interdigitated phase, while the propanol-poor domains remain in the $L_{\beta'}$ phase. The $L_{\beta'}$ domain goes into the L_α phase at a lower temperature, which shows up as t_m. This transition creates a coexistence of L_α and $L_\beta I$ phases in the region between the heating shoulder peaks and the heating t_m values. The $L_\beta I$ portion of the bilayer undergoes the transition into the L_α phase at the correspondingly higher temperature, resulting in the shoulder peaks.

In addition, the data summarized in Table 1 elucidate that for both systems the pretransition peaks did not disappear right at the threshold but slightly after the onset of the $L_\beta I$ phase. This suggests that immediately after the propanol threshold concentration is reached, part of the membrane may still undergo the pretransition, with a coexistence of $P_{\beta'}$ and $L_\beta I$ phases in the region around and right after the onset of interdigitation. This $L_{\beta'}$ to $L_\beta I$ to $P_{\beta'}$ and eventually to L_α pathway agrees with Ohki et al.'s conclusion in their DPPC + ethanol system that was confirmed by differential scanning densitometry.[28]

Shoulder peaks are also detected on the cooling scans for both DPPC + 1-propanol and DPPC + 2-propanol systems. As shown in Figure 5, the shoulder peaks initially appeared below the t_m but later appeared (3 to 4) °C above the t_m at higher propanol concentrations. This clearly demonstrates the biphasic

behavior, indicating the presence of the $L_\beta I$ phase. Therefore, the cooling shoulder peaks could represent a small portion of the lipid bilayer undergoing the L_α to $L_\beta I$ phase transition at the higher temperature induced by the 1-propanol or 2-propanol molecules. Studies have shown that the transition from the $L_\beta I$ phase to the L_α phase is not fully reversible;[11] hence, it may be difficult for the bilayer to form the $L_\beta I$ phase from the L_α phase during the cooling scans. This would account for the small size of the shoulder peaks and leads to the conclusion that beyond the threshold concentration there is a coexistence of the $L_\beta I$ and L_α phases present in temperatures between the t_m and the shoulder peaks of the cooling scans. The remaining L_α portion, which is the majority of the bilayer, goes back into the $L_{\beta'}$ phase, and the transition appears as the main transition peak. This transition leaves the area below the cooling t_m curve to the coexistence of $L_\beta I$ and $L_{\beta'}$ phases. Another possible explanation is that the bilayer is going from the L_α phase to the $L_{\beta'}$ phase and then going into the $L_\beta I$ phase due to the presence of inducer molecules. The DPPC + propanol system is similar to the DPPC + methanol and DPPC + ethanol systems reported by Rosser et al.[3] and some of the DPPC + butanol systems studied by Reeves et al.[26] in our laboratory. This study completes the effects of these short-chain alcohols (from methanol to butanol) and most of their structural isomers on the structure of hydrated DPPC systems.

Acknowledgment

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Supporting Information Available:

Experimental data for all the transition temperatures used in the figures for the DPPC + 1-propanol system and the DPPC + 2-propanol system are summarized in two tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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