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Effects of pentanol isomers on the phase behavior of phospholipid bilayer membranes

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ABSTRACT

Differential scanning calorimetry (DSC) was used to analyze the thermotropic phase behavior of dipalmitoylphosphatidylcholine (DPPC) bilayers in the presence of pentanol isomers. The concentration of each pentanol isomer needed to induce the interdigitated phase was determined by the appearance of a biphasic effect in the main transition temperatures, the onset of a hysteresis associated with the main transition from the gel-to-liquid crystalline phase, and the disappearance of the pretransition. Lower threshold concentrations were found to correlate with isomers of greater alkyl chain length while branching of the alkyl chain was found to increase biphasic behavior. The addition of a methyl group to butanol systems drastically decreased threshold concentrations. However, as demonstrated in the DPPC/neopentanol system, branching of the alkyl chain away from the –OH group lowers the threshold concentration while maintaining a biphasic effect.

1. Introduction

The complexity of biological membranes makes it difficult to study specific mechanisms, interactions, and phase transitions [1]. Consequently, model membranes are useful because they exhibit similar behavior to natural systems such as myelin and erythrocyte membranes [2]. Therefore, synthetic homogeneous membranes can provide an effective model system to study how membrane form affects function. The saturated, 16-carbon acyl chain DPPC is an extensively studied model phospholipid, with thermotropic phase behavior that has been well elucidated [3]. At low temperatures, the bilayer is in the rigid and compact subgel phase (L_c) [4]. As the temperature increases, it transitions to the planar gel phase (L_{β}'), in which the acyl chains tilt slightly. The pretransition (T_p) occurs with a further temperature increase, resulting in a transition to the rippled gel phase (P_{β}'), where head group crowding is minimized by a rippled bilayer surface. At higher temperatures the membrane undergoes the main transition (T_m), in which the P_{β}' phase converts into the fluid liquid crystalline phase (L_{α}).

However, numerous studies have proven an alternate pathway of thermotropic phase transitions in phospholipids involving the interdigitated gel phase (L_{β}I). Rowe first reported shifts in the gel-to-liquid crystalline phase transition temperature in the presence of ethanol that were later confirmed to correlate with the formation of the L_{β}I phase [5]. McIntosh et al. showed that DPPC interdigitates in the presence of surface active, amphiphilic molecules and used X-ray diffraction to characterize the structure [6]. The L_{β}I phase, which replaces the P_{β}' phase, is characterized by the unusual interpenetration of the lipid acyl chains into the opposing monolayer. Interdigitation significantly alters membrane properties, such as drastically reducing the bilayer thickness, affecting membrane permeability, and encouraging membrane fusion [7–10].

The three main characteristics of interdigitated membranes in DSC experiments are the presence of the biphasic effect, an increase in the T_m hysteresis, and the disappearance of the pretransition [11–20]. Rowe established that ethanol induces the biphasic effect, where at low ethanol concentrations the T_m decreases, but above a certain threshold concentration, the trend reverses and the T_m increases with increasing ethanol concentrations [11]. It was also shown that the reduced reversibility of the main phase transition above the threshold concentration of ethanol results in a large hysteresis, which correlates with the induction of the interdigitated gel phase [12]. The increase in hysteresis corresponds well with the concentration of alcohol causing the disappearance of the pretransition [13].

Many chemicals have been shown to induce interdigitation in phosphatidylcholine (PC) bilayers: including glycerol, ethylene glycol, benzyl alcohol, ethanol, thiocyanate ion, and *n*-alcohols up to heptanol [5,7(and references therein),21]. The application of hydrostatic pressure can induce interdigitation as well [22,23]. Several neural active drugs, including chlorpromazine and atropine, and local anesthetics have also been found to induce interdigitation in phospholipid membranes [6,24]. In the case of short chain alcohols, the –OH moiety binds to the polar head group and increases the lateral separation between the phospholipid

Abbreviations: DSC, differential scanning calorimeter; T_m, main transition temperature; L_{β}', planar gel phase; P_{β}', ripple gel phase; L_{α}, liquid crystalline phase; L_{β}I, interdigitated gel phase; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; PC, phosphatidylcholine.

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headgroups, thereby producing energetically unfavorable voids within the hydrocarbon region [25]. The acyl chains of opposing bilayers will interdigitate to minimize these voids and to maximize the stabilizing van der Waals interactions between the acyl chains [6,7].

Long chain *n*-alcohols are also known to act as anesthetics [26,27]. Researchers have proposed that these types of anesthetics work by affecting membrane properties, such as lowering the transition temperature [28]. Since biological membranes can have melting transitions close to body temperature, a small change in the transition temperature can have a large physiological effect [29]. Furthermore, geraniol (3,7-dimethylocta-2,6-dien-1-ol), a long and branched biological alcohol, has been shown to cause PC's to form long, tubular vesicles at certain concentrations [30]. Therefore, it is of interest to see if other branched alcohols also have unusual effects on phospholipid membranes.

Additionally, the creation of homogeneous unilamellar liposomes from an interdigitated matrix may be attractive for use in drug delivery systems [10,25]. Ethanol is typically used for this process, but other alcohols are potentially viable since they also induce interdigitation.

In this study, we further examine how the induction of the L_{β}I phase is affected by alcohol chain length, branching, and location of the –OH group. The chemical structures of the isomers of interest, namely methyl-substituted butanols and isomers of pentanol, are depicted in Fig. 1.

2. Materials and methods

2.1. Materials

1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), purity 99+%, was purchased from Avanti Polar Lipids (Alabaster, AL, USA). 1-pentanol (≥99%); 2-pentanol (98%); 3-pentanol (98%); neopentanol (98%); 2-methyl-2-butanol (99%); 3-methyl-2-butanol, (≥99%); 3-methyl-1-butanol (≥99%); 2-methyl-1-butanol (≥99%), were obtained from Sigma-Aldrich (St. Louis, MO, USA).

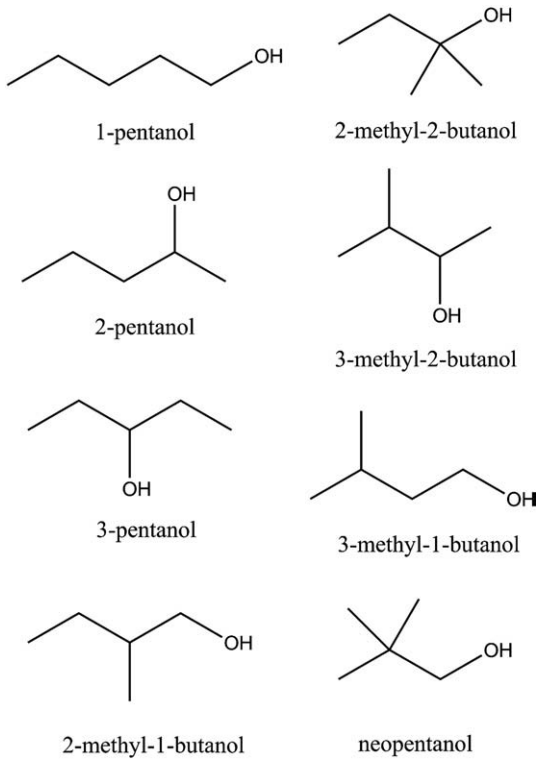

Fig. 1. Chemical structures of the alcohol isomers used in this study.

2.2. Differential scanning calorimetry

The lipid samples were prepared with 2 mg of DPPC and 50 μl of the various alcohol solutions. The samples were hermetically sealed and incubated at approximately 42 °C for 1 h with intermittent vortexing to ensure proper hydration. Duplicate DSC scans from 10 to 50 °C were carried out using a Calorimetry Sciences Corporation Multi-cell DSC-HT Model 4100 at a scan rate of 10 °C/h. These scans were reproducible and the signal-to-noise ratio for the transition peaks was excellent, which allowed for the detection of small, incremental changes in the T_m. All transition temperatures and enthalpy values were calculated using the Jandel Scientific Peakfit program and Origin Pro. The standard deviation of the transition temperatures was ±0.1 °C. The errors for the enthalpy calculations were ±0.3 to ±0.5 kcal/mol, with the greatest errors belonging to the broadest transitions.

3. Results

3.1. DPPC and DPPC/1-pentanol systems

For pure DPPC, the transition temperature for the L_{β}' to P_{β}' pretransition (T_p) was 36.1 °C and the T_m was 42.2 °C. Upon cooling, the T_m occurred at 41.2 °C. These temperatures are consistent with previously reported values for DPPC [3].

For the purpose of these experiments, we have defined the threshold concentration to be the amount of alcohol at which the main transition hysteresis (difference in T_m between the heating and cooling scans) increases. The biphasic effect refers to the change from decreasing T_m with more alcohol to an increasing T_m above the threshold concentration. In Fig. 2, typical thermograms of DPPC in water and with 1-pentanol concentrations above and below the threshold concentration are shown. All of the pentanol isomers had a similar broadening effect on the main transition.

In the DPPC/1-pentanol system, as shown in Fig. 3A, the T_p decreased from 36.1 °C to 19.1 °C as the 1-pentanol concentration increased to 0.05 M. By 0.06 M 1-pentanol, the pretransition is no longer detectable. The threshold concentration was determined to be 0.07 M. At concentrations below the threshold concentration, the T_m decreased by 6.6 °C. At concentrations above the threshold concentration, slight biphasic behavior was observed before the T_m began to decrease. After this slight increase, the T_m steadily decreased to 31.2 °C until the solubility limit was reached at 0.24 M. The T_m trend was also observed for the cooling transitions.

3.2. DPPC/2-pentanol, DPPC/3-pentanol and DPPC/3-methyl-2-butanol systems

The threshold concentrations and the degree of biphasic behavior are similar for the intermediately branched pentanol isomers. In the DPPC/2-pentanol system (Fig. 3B), the T_p decreased by 9.9 °C as the 2-pentanol concentration reached 0.07 M. At 0.08 M 2-pentanol, the pretransition was no longer detectable. In the DPPC/3-pentanol system (Fig. 3C) the T_p decreased by 17.1 °C and was no longer detectable at 0.11 M 3-pentanol. The threshold concentration of DPPC/2-pentanol was determined to be 0.10 M, and 0.11 M for the DPPC/3-pentanol system. At concentrations below the threshold concentration, the heating T_m decreased by 4.4 °C for both systems. A slight biphasic behavior was observed for both 2- and 3-pentanol systems.

The DPPC/3-methyl-2-butanol system exhibited similar results as the 2- and 3-pentanol systems. The pretransition no longer appeared above 0.10 M (Fig. 3D). The T_m shows an initial decrease of 3.9 °C before the threshold concentration (0.10 M), comparable to 2-pentanol and 3-pentanol. The heating T_m increased by 0.4 °C with increasing alcohol concentration before decreasing slightly, while the

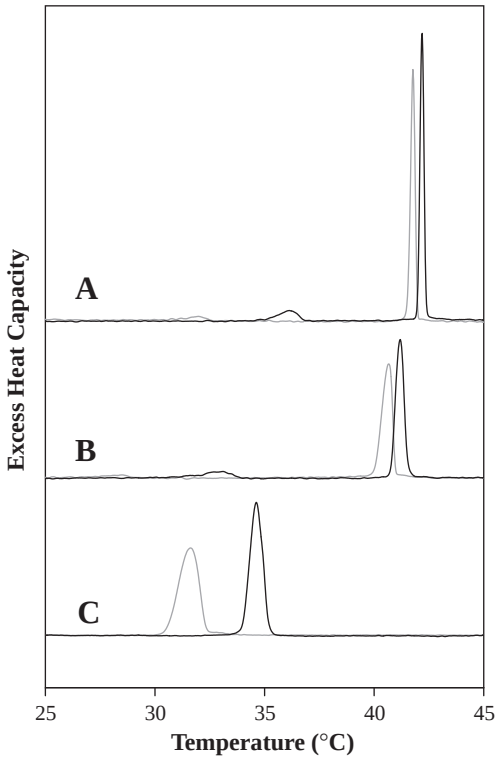

Fig. 2. Representative heating (black lines) and cooling (gray lines) DSC thermograms of the DPPC/1-pentanol system. A, DPPC in water; B, DPPC with 9.25×10^{-3} M 1-pentanol; C, DPPC with 1.48×10^{-1} M 1-pentanol. The cooling scans have been inverted to allow comparison with the heating peaks. For clarity, the thermograms are also offset vertically.

cooling scan T_m decreased linearly. This biphasic effect and the hysteresis are similar to those observed in the 2-pentanol and 3-pentanol systems.

3.3. DPPC/2-methyl-1-butanol and DPPC/3-methyl-1-butanol systems

The threshold concentrations of the structurally similar isomers, 2-methyl-1-butanol and 3-methyl-1-butanol are identical (0.08M). The pretransition dropped to 17.7 °C at a concentration of 0.07 M 2-methyl-1-butanol and was no longer detectable at 0.08 M 2-methyl-1-butanol (Fig. 4A). For the DPPC/3-methyl-1-butanol system, the T_p disappeared at 0.07 M after decreasing to 19.4 °C at 0.06 M (Fig. 4B). The T_m decreased by 4.9 °C when the concentration of 3-methyl-1-butanol increased to 0.08 M. The biphasic effect observed in the 2-methyl-1-butanol system was similar to those observed in the 2- and 3-pentanol systems. The T_m increased by 0.3 °C at 0.10 M 2-methyl-1-butanol before beginning to decrease. The T_m of the heating scan for 3-methyl-1-butanol increased 0.4 °C with increasing alcohol concentration before decreasing, while the cooling scan T_m decreased linearly. This biphasic effect and hysteresis were similar to those observed in the 1-pentanol system.

3.4. DPPC/2-methyl-2-butanol and DPPC/neopentanol systems

DPPC in the presence of the most significantly branched isomer, 2-methyl-2-butanol, had the highest threshold concentration and the greatest biphasic behavior. The pretransition disappeared at 0.13 M 2-methyl-2-butanol (Fig. 5A). The T_m decreased by 2.9 °C with increasing concentrations of 2-methyl-2-butanol. At 39.3 °C and 0.13 M 2-methyl-2-butanol, the onset of an increased hysteresis was observed. At concentrations above 0.13 M, more dramatic biphasic

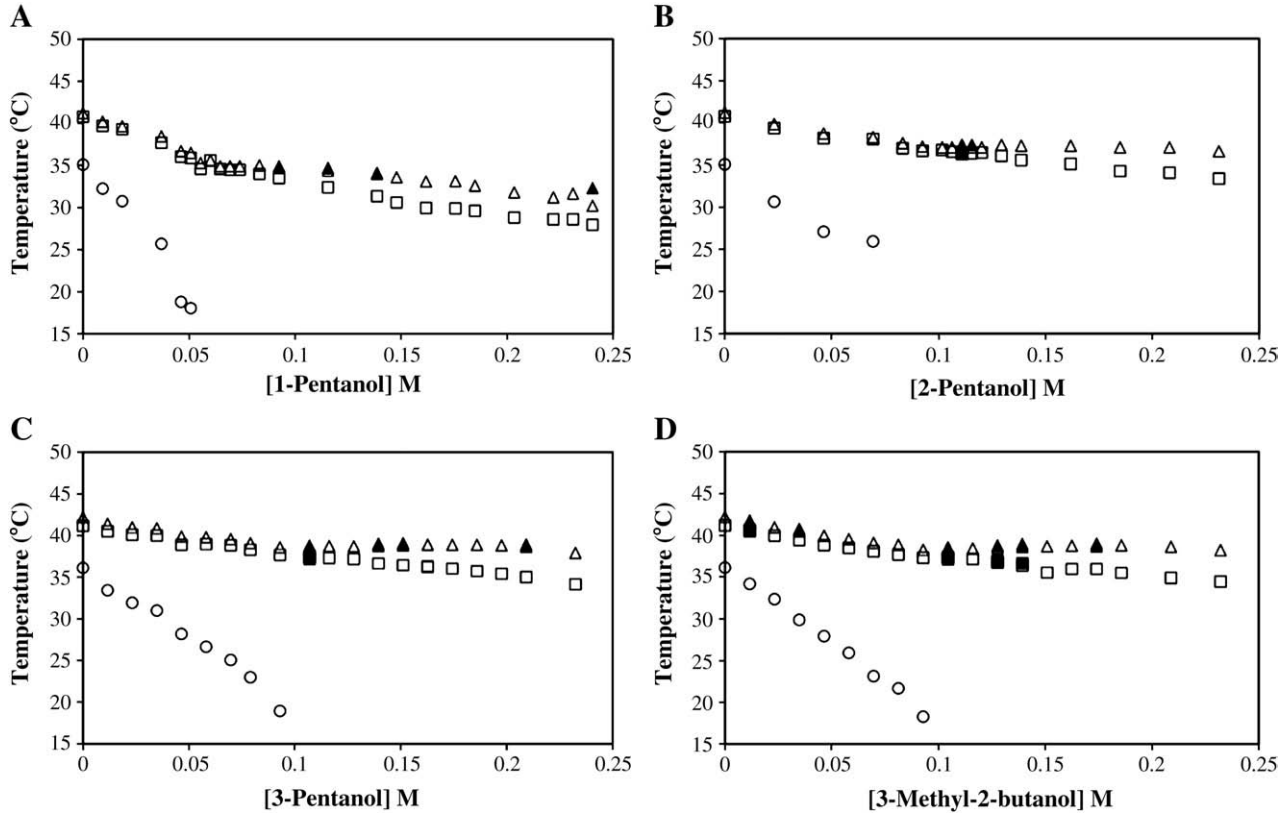

Fig. 3. A, Effects of 1-pentanol on DPPC phase transition temperatures; B, 2-pentanol; C, 3-pentanol; D, 3-methyl-2-butanol; (Δ, heating scan main peak; ▲, heating scan shoulder peak; □, cooling scan main peak; ■, cooling scan shoulder peak; ○, pretransition peak).

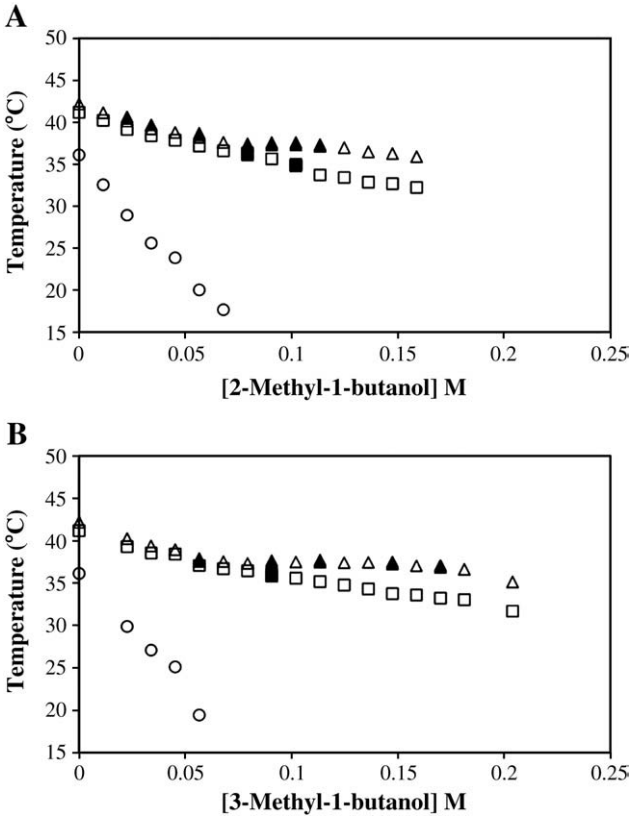

Fig. 4. A, Effects of 2-methyl-1-butanol on DPPC phase transition temperatures; B, 3-methyl-1-butanol; (Δ, heating scan main peak; ▲, heating scan shoulder peak; □, cooling scan main peak; ■, cooling scan shoulder peak; ○, pretransition peak).

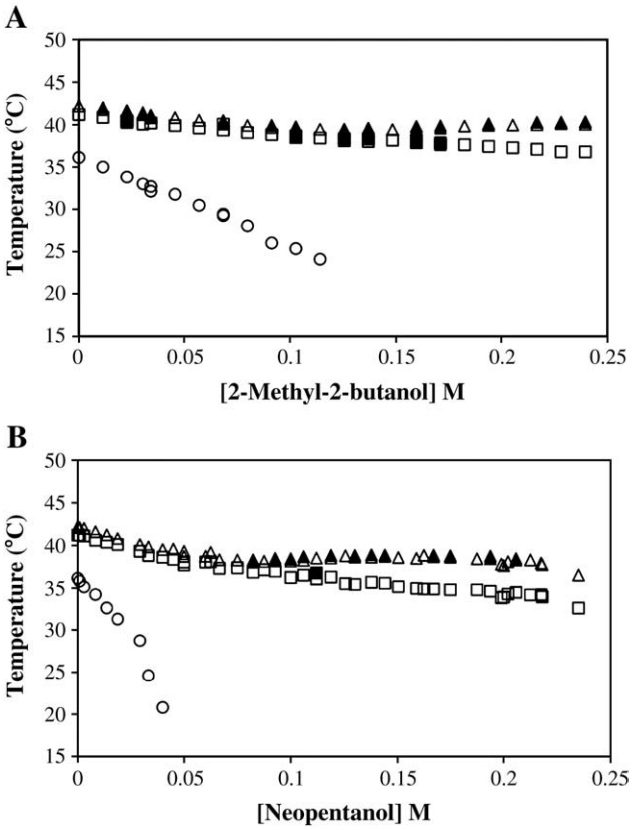

Fig. 5. A, Effects of 2-methyl-2-butanol on DPPC phase transition temperatures; B, neopentanol (Δ, heating scan main peak; ▲, heating scan shoulder peak; □, cooling scan main peak; ■, cooling scan shoulder peak; ○, pretransition peak).

behavior was observed. The T_m increased by 1.0 °C before decreasing, in comparison to an increase of 0.3 °C to 0.4 °C for the previous isomers.

Though similarly branched, neopentanol had very different effects on the phase behavior of DPPC compared to 2-methyl-2-butanol. For instance, the T_p was no longer detected at 0.05 M neopentanol (Fig. 5B). The biphasic inflection of the heating curve and the increase in hysteresis occurred at 38.0 °C and 0.08 M neopentanol. However, the severity of the biphasic effect was similar to the DPPC/2-methyl-2-butanol system. The initial decrease in T_m was 3.9 °C and, above the threshold concentration of 0.08 M, the T_m increased by 0.8 °C at a concentration of 0.16 M before decreasing.

3.5. Enthalpies

In Fig. 6A–B, the enthalpy data on the main transition of two pentanol isomers, 2-methyl-1-butanol and 3-methyl-1-butanol, are shown as representative examples. For the heating scans of each isomer, the enthalpies below and above the threshold concentration were separately fit to straight lines by least squares. In all the systems studied, a general increase in the main transition enthalpy with increasing alcohol concentration was observed. The main transition enthalpy also increased significantly above the threshold concentration for each isomer. In Fig. 6A, there is a separation of 1.8 kcal/mol between the two best-fit lines at the threshold concentration of DPPC/2-methyl-1-butanol. Fig. 6B shows a similar separation of 0.9 kcal/mol at the threshold concentration of DPPC/3-methyl-1-butanol.

Table 1 shows a summary of our data on induction of interdigitated phase in each system carried out in this study. It lists the initial decrease in T_m before the induction of interdigitated phase, the concentration of each isomer at which the pretransition disappears,

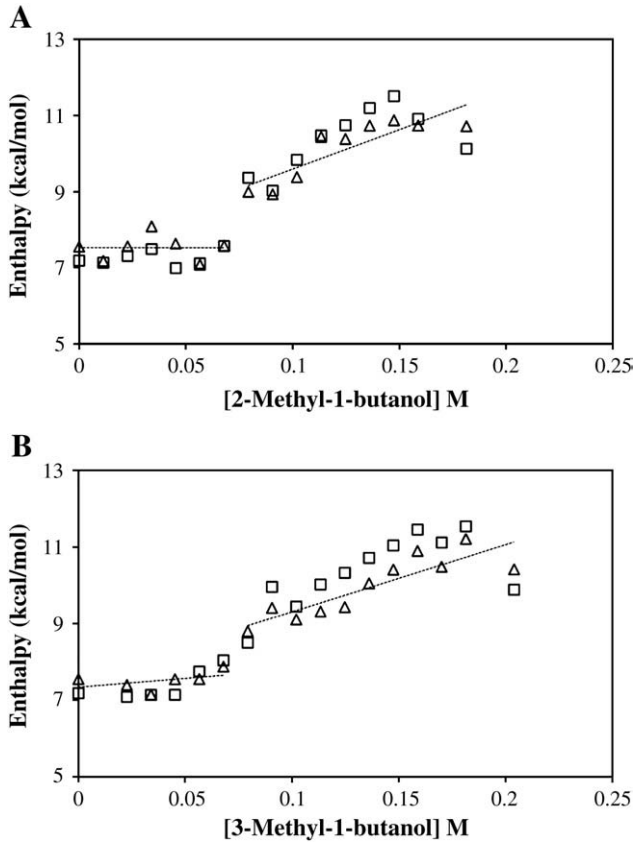

Fig. 6. A, Effects of 2-methyl-1-butanol on DPPC main transition enthalpies; B, effects of 3-methyl-1-butanol on DPPC main transition enthalpies (Δ, heating scan main transition enthalpy; □, cooling scan main transition enthalpy).

Table 1
Summary data on induction of interdigitated phase.

System	Initial T_m decrease (°C ±0.2)	Conc. at which T_p disappears (mol/L ±0.01)	Threshold concentration (mol/L ±0.01)	Enthalpy difference at threshold concentration (kcal/mol ±0.3)
DPPC/1-pentanol	6.6	0.06	0.07	0.9
DPPC/2-pentanol	4.4	0.08	0.10	0.9
DPPC/3-pentanol	4.4	0.11	0.11	1.2
DPPC/3-methyl-2-butanol	3.9	0.10	0.10	2.5
DPPC/2-methyl-1-butanol	4.9	0.08	0.08	1.8
DPPC/3-methyl-1-butanol	4.9	0.07	0.08	0.9
DPPC/2-methyl-2-butanol	2.9	0.13	0.13	1.1
DPPC/neopentanol	3.9	0.05	0.08	2.1

the threshold concentration, and the separation in enthalpy values before and after the threshold concentration.

4. Discussion

The threshold concentration observed for DPPC/1-pentanol agreed with previously reported data [21]. Since the lowest threshold concentration, 0.07 M, was observed in the 1-pentanol system, it can be concluded that 1-pentanol is the most effective pentanol isomer at inducing interdigitation in DPPC. In comparison to the other isomers, 1-pentanol most effectively depressed the T_p as well as the T_m of DPPC below the threshold concentration. The depression of T_p indicates that the alcohol interacts more strongly with the P_{β}' phase than with the L_{β}' phase, due to the staggered nature of the phospholipid acyl chains in the P_{β}' phase [7].

Studies of methanol, ethanol, *n*-propanol, and *n*-butanol with DPPC have shown that the threshold concentrations decrease from 2.7 M, 1.0 M, 0.39 M, and 0.16 M, respectively [15,18,19,21]. The drastically lower 1-pentanol threshold concentration is in agreement with the previously reported conclusion that longer alcohol chain lengths more effectively induce interdigitation because their greater hydrophobicity interacts strongly with the exposed acyl chains of the interdigitated bilayer [7,11,21].

Clear biphasic behavior has been observed with DPPC/methanol, ethanol, and *n*-propanol systems [7,21]. However, DPPC/*n*-butanol did not exhibit clear biphasic behavior. After a slight increase following the threshold concentration, the T_m began to decrease [18]. This is contradictory to the abrupt reversal in T_m reported for the shorter alcohols [12,15]. This "stunted" biphasic behavior is observed in the 1-pentanol system as well. This may be due to 1-pentanol's ability to partition more effectively into, and therefore favor the L_{α} phase over the L_{β}I phase [18].

The DPPC/2-pentanol, DPPC/3-pentanol, and DPPC/3-methyl-2-butanol systems all had threshold concentrations of about 0.1 M. The increase in threshold concentrations compared to that of 1-pentanol is due to the decrease in hydrophobicity of these isomers as compared to DPPC/1-pentanol. These alcohols also exhibited similar depressions in the T_m below the threshold concentration. This depression was less than that of DPPC/1-pentanol, indicating that increasing concentrations of DPPC/2-pentanol, 3-pentanol, and 3-methyl-2-butanol do not stabilize the L_{α} phase as effectively as DPPC/1-pentanol. The biphasic behavior induced by 2-pentanol, 3-pentanol, and 3-methyl-2-butanol was similar to that of 1-pentanol. DPPC/2-pentanol had the least increase in T_m and biphasic behavior, followed by DPPC/3-pentanol, then DPPC/3-methyl-2-butanol. This trend of increasing T_m and greater biphasic effect seems to correlate with the increase in branching of the isomers. Therefore, highly branched isomers seem to favor the L_{β}I phase.

The 3-methyl-1-butanol and 2-methyl-1-butanol systems had threshold concentrations of 0.08 M. These butanols were the second most effective isomers at inducing interdigitation, following 1-pentanol at 0.07 M. Similar to 1-pentanol, the low threshold concentrations are probably due to strong hydrophobic interactions with the lipid acyl chains [7,11,21]. However, both 3-methyl-1-butanol and 2-methyl-1-butanol have alkyl chain branching, which increases the threshold concentrations for interdigitation.

The branching of 3-methyl-1-butanol also decreases its effectiveness at partitioning into the L_{α} phase at low concentrations. The biphasic behavior induced by 3-methyl-1-butanol was similar to that of the intermediately branched isomers: 2-pentanol, 3-pentanol and 3-methyl-2-butanol. 3-methyl-1-butanol showed a similar increase in T_m above the threshold concentration. This trend of increasing T_m and greater biphasic effect seems to correlate with an increase in branching of the isomers. Furthermore, increasingly branched isomers, as indicated by the biphasic effect caused by 3-methyl-1-butanol, seem better able to stabilize the L_{β}I phase.

However, 2-methyl-1-butanol also had a threshold concentration of 0.08 M. This concentration is lower than the other intermediately branched isomers. In addition, 2-methyl-1-butanol induced very slight biphasic behavior. This indicates that 2-methyl-1-butanol does not stabilize the interdigitated phase as well as other isomers. While 2-methyl-1-butanol is very effective in inducing the L_{β}I phase, it is poor at stabilizing the phase, despite branching away from the –OH group. 2-Methyl-1-butanol's structure maintains long chain characteristics, and the branching one carbon away from the –OH increases hydrophobicity, which may account for the low threshold concentration.

Of all the pentanol isomers, 2-methyl-2-butanol was the least effective at inducing the interdigitated phase with the highest threshold concentration of 0.13 M. This increase in the threshold concentration correlates with 2-methyl-2-butanol's lesser hydrophobicity compared with the other isomers, as its –OH group is attached to the most substituted carbon. 2-Methyl-2-butanol also caused the least depression in T_m at concentrations below the threshold concentration, indicating that it does not partition effectively into the L_{α} phase. 2-Methyl-2-butanol also showed the greatest biphasic behavior of all the isomers studied. The bulky nature of the molecule may fill the gap between the headgroups of the phospholipids, thereby stabilizing the L_{β}I phase. However, *tert*-butanol shows a much greater biphasic behavior than 2-methyl-2-butanol [18]. 2-Methyl-2-butanol's decreased ability to stabilize the interdigitated phase compared to *tert*-butanol indicates that the extra carbon length present in 2-methyl-2-butanol greatly increases its relative hydrophobicity.

Previously, it was shown that an increase in effectiveness of an inducer molecule was coupled with a decrease in its ability to stabilize the interdigitated phase [15,18]. This trend correlated with a molecule's increasing hydrophobicity. In the butanol isomer study, increasingly branched isomers showed an increase in threshold concentration and a greater biphasic effect [18]. However, neopentanol, which contains a tertiary carbon, was shown to have a threshold concentration only slightly higher than that of 1-pentanol. Not only was a decrease in threshold concentration observed, but biphasic behavior was also noted. Neopentanol stabilized the interdigitated phase nearly as much as 2-methyl-2-butanol, the isomer that was least effective at inducing interdigitation. The structure of neopentanol is similar to that of 2-

methyl-2-butanol; however, one carbon separates the tertiary carbon from the –OH. In this unique case, branching increased the effectiveness of inducing interdigitation, while still maintaining the predicted biphasic behavior. This may be the result of the very high hydrophobicity of the molecule coupled with its bulky structure.

These results provide new insight into the induction of the interdigitated phase by alcohols and their effect on the T_m. In addition to the amphiphilic nature of the alcohols, it is important to take into consideration the hydrophilic and hydrophobic regions as reflected by their solubility in both the membrane and the aqueous phases, and specific interactions with the polar head group region of the lipid bilayer. The location of the –OH group on branched alcohols significantly affects the threshold concentration. Longer alcohols are more effective at inducing interdigitation, while highly branched alcohols cause increased biphasic behavior. Furthermore, in comparison to the butanol isomers, the addition of one methyl group drastically decreases the threshold concentration. The unique results of the neopentanol system highlight the importance of alcohol structure in inducing and maintaining the interdigitated phase. In addition to inducing interdigitation, branched alcohols may also act as anesthetics by lowering the melting transition temperature, similar to *n*-alcohols [26–28]. We expect that the anesthetic potency would be related to how dramatically each alcohol decreases the T_m in model systems such as the DPPC results reported here.

Although interdigitated gel phases have been observed in numerous model membrane systems composed of naturally occurring lipids, the presence of *in vivo* interdigitation is unknown at this time. However, if interdigitation is present in biological systems, then membrane functions would be expected to change because the bilayer thickness is greatly reduced and the membrane midplane is lost [7]. For example, the behavior of some proteins is altered in interdigitated membranes [31,32]. Additionally, the presence of interdigitated gel phase domains is expected to modulate membrane permeability and biological processes such as membrane fusion [8,9].

Acknowledgements

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