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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).

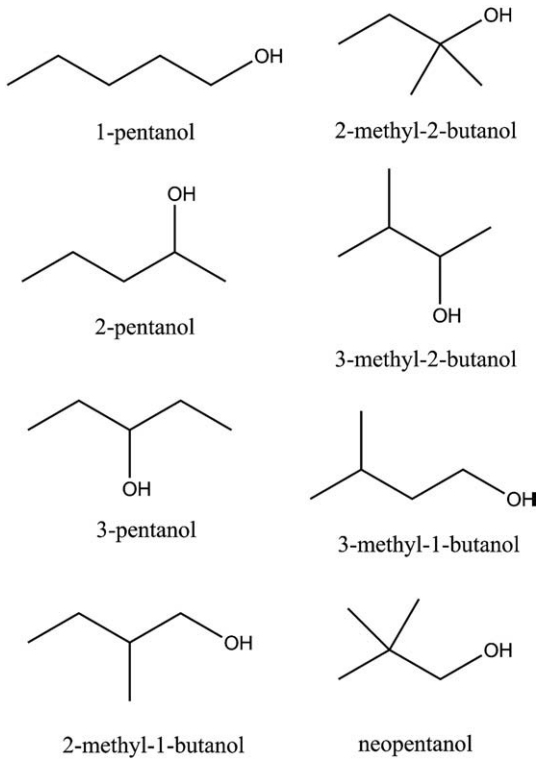

Fig. 1. Chemical structures of the alcohol isomers used in this study.

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