Biophysical Chemistry 128 (2007) 13 – 18

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Effects of butanol isomers on dipalmitoylphosphatidylcholine bilayer membranes

Megan D. Reeves, Adam K. Schawel, Weidong Wang, Phoebe Dea *

Department of Chemistry, Occidental College, 1600 Campus Road, Los Angeles, CA 90041, USA

Received 2 December 2006; received in revised form 19 February 2007; accepted 19 February 2007

Abstract

Differential scanning calorimetry and ^{31}P-NMR were used to study the effects of butanol isomers on the thermotropic phase behavior of dipalmitoylphosphatidylcholine (DPPC) bilayers. The threshold concentration for the onset of interdigitation for each isomer was determined by the disappearance of the pretransition and the onset of a large hysteresis between the heating and cooling scans of the gel-to-liquid main transition. The threshold concentration was found to correlate with increased solubility of the isomers in the aqueous phase, led by *tert*-butanol. However, as the solution concentration of *tert*-butanol increased, there was an abrupt shrinking of the hysteresis, initially with well-resolved shoulder peaks indicating mixed phases. The eventual disappearance of the shoulder peaks was correlated with a breakdown of the multilamellar structure identified using ^{31}P-NMR.

Keywords: DPPC; Butanol isomers; Interdigitation; DSC; ^{31}P-NMR

1. Introduction

Biological membranes consist of separated lateral domains, each comprised of different lipid and protein compositions. In addition to their lateral heterogeneity, membranes possess vertical heterogeneity, making these systems highly complex [1]. Synthetic homogenous lipid bilayers can serve as an effective model to study the mechanisms of interactions of additives with biological membranes. Through the use of these simplified systems, McIntosh and Simon first characterized the structure of the interdigitated phase in phosphatidylcholine (PC) bilayers using X-ray diffraction [2]. In this unusual gel phase, the acyl chains of the phospholipids interpenetrate into the opposing monolayer.

Additional research has thoroughly established that the interdigitated phase can be induced in saturated, like-chain PCs in the presence of small amphiphilic molecules [1–5]. It is understood that in the induction of interdigitation these molecules displace water from the interfacial region. The hydrophilic region of the inducer molecule interacts with the phosphate in the lipid headgroup. The hydrophobic region intercalates between the hydrocarbon chains. Due to the small size of these amphiphilic molecules there is the potential of high-energy voids in the core of the bilayer. Interdigitation prevents the formation of these voids, simultaneously increasing favorable van der Waal's interactions between the acyl chains and spacing between the headgroups of the phospholipids [2,6,7]. With the induction of the interdigitated phase, the small amphiphilic molecules effectively shield the terminal methyl groups of the phospholipids from the aqueous environment.

Several different compounds have been identified as being capable of inducing interdigitation in PCs including short-chain alcohols up to heptanol, glycerol, benzyl alcohol, chlorpromazine, tetracaine, ions such as thiocyanate, and peptides including polymyxin B [1,5,6]. Interdigitation has also been shown to occur in both dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) in the absence of additives with increased hydrostatic pressure [8,9].

Rowe was the first to show that ethanol has a biphasic effect on the main transition temperatures of PC bilayers [10]. McIntosh

---

Abbreviations: DSC, differential scanning calorimeter; T_p, pretransition temperature; T_m, main transition temperature; $L_{\beta'}$, planar gel phase; $P_{\beta'}$, ripple gel phase; L_α, liquid crystalline phase; $L_\beta I$, interdigitated gel phase, DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; NMR, nuclear magnetic resonance.

* Corresponding author. Tel.: +1 323 259 2625; fax: +1 323 341 4912.
E-mail address: dea@oxy.edu (P. Dea).

0301-4622/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2007.02.004

and Simon correlated the reversal in main transition temperature (T_m) of the biphasic effect with the onset of the interdigitated phase using X-ray diffraction [2]. Differential scanning calorimetry (DSC) is a sensitive tool for monitoring the thermotropic phase behavior of lipid bilayers. Studies have established the onset of interdigitation is indicated in DSC data by the biphasic effect in addition to the disappearance of the pretransition and irreversibility of the gel-to-liquid crystalline phase transition as shown by a large hysteresis between the heating and cooling scans of the main transition [3,4,10–12].

The technique of phosphorous-31 nuclear magnetic resonance spectroscopy can be used to detect lipid polymorphism. The characteristic ^{31}P powder patterns for multilayers, micelles, and the hexagonal phase have been well documented [13]. ^{31}P-NMR also can be used to identify thermotropic phases of the lipid bilayer through the determination of chemical shift anisotropy. A chemical shift anisotropy of approximately 80 ppm corresponds to the gel phases and approximately 50 ppm corresponds to the liquid crystalline phase [13]. ^{31}P-NMR can therefore be used effectively to monitor the presence of the lipid bilayer and bilayer breakdown at high concentrations of inducer molecules.

Previous studies have thoroughly investigated the effects of *n*-alcohols up to heptanol on PC bilayers [5,6]. However, structural isomers of these alcohols have yet to be extensively studied. We have used DSC and ^{31}P-NMR to examine the varying effects of *n*-, *iso*-, *sec*-, and *tert*-butanol in the DPPC system to more fully understand the role of inducer molecule structure on PC phase behavior. The phase behavior of *n*-, *iso*-, and *sec*-butanol were explored up to their solubility limits. The miscibility of *tert*-butanol in water allowed us to investigate the effects of inducer molecules significantly beyond their threshold concentrations, and at concentrations high enough to cause breakdown of the bilayer structure.

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). Deuterium oxide, purity 99.9%, was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). 1-Butanol, purity 99.9%; 2-methyl-1-propanol (isobutanol), purity 99.5%; anhydrous 2-butanol (*sec*-butanol, purity 99.5%; and anhydrous *tert*-butanol, purity 99.5+%, were obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were used without further purification.

2.2. Differential scanning calorimetry

The lipid samples were prepared by weighing out 2 mg of DPPC into a DSC vial and adding 50 µl of alcohol solution. The samples were hydrated by heating in a sand bath at approximately 42 °C for 1 h with intermittent vortexing to ensure proper hydration. The samples were equilibrated to room temperature before being put into the DSC. Duplicate DSC scans were made using a Calorimetry Sciences Corporation Multi-cell DSC-HT Model 4100 at a set scan rate of 10 °C/h over a range of 10 to 50 °C. All transition temperatures and enthalpy values for both heating and cooling scans were calculated using the Jandel Scientific Peakfit program. The fitting of each peak was repeated three times to check for accuracy.

2.3. ^{31}P-NMR

Samples were prepared by weighing out 20 mg of DPPC into a NMR tube and adding 400 µl of a 1:1 H_2O to D_2O alcohol solution. The samples were hydrated as above. ^{31}P-NMR spectra were acquired at 161.98 MHz frequency on a Bruker DRX400 spectrometer equipped with a Windows computing system with Topspin 1.3 software and a BVT3300 temperature control unit, which maintains the temperature of the sample constant to ±0.5 °C. ^{31}P-NMR spectra were obtained using a broadband inverse probe with a spectral width of 32 kHz, a 90° pulse of 9.9 µs using a zgig30 pulse technique and a relaxation delay of 2 s.

3. Results

3.1. DSC

For pure DPPC, the transition temperatures for the planar gel to ripple gel pretransition (T_p) and the ripple gel-to-liquid crystalline main transition (T_m) were 35.8 °C and 42.1 °C respectively. The transition temperature for the liquid crystalline to ripple gel main transition upon cooling was 41.2 °C. The standard deviations for the transition temperatures were within ±0.1 °C. These temperatures are in agreement with previously reported values for DPPC [1,3,10–12].

As shown in Fig. 1, with increasing *n*-butanol concentration the temperature of the pretransition decreased significantly from 35.8 °C at 0 M to 21.1 °C at 0.14 M. Beginning at 0.15 M, the pretransition was no longer detected. The main transition temperature also decreased drastically with increasing concentration of *n*-butanol prior to the identified threshold concentration, reaching a minimum in this low concentration range of

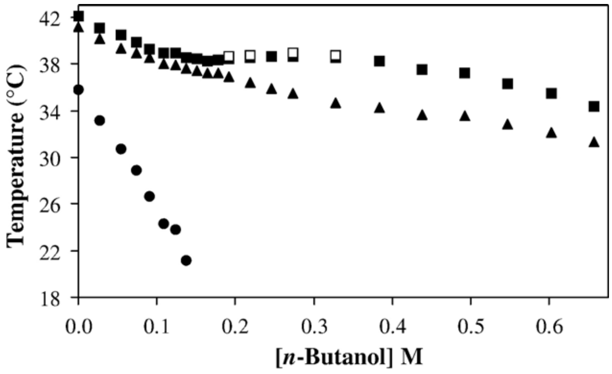

Fig. 1. Effects of *n*-butanol on DPPC phase transition temperatures (■, heating scan main peak; □, heating scan shoulder peak; ▲, cooling scan main peak; ●, pretransition peak). All transition temperatures have a standard deviation of ±0.1.

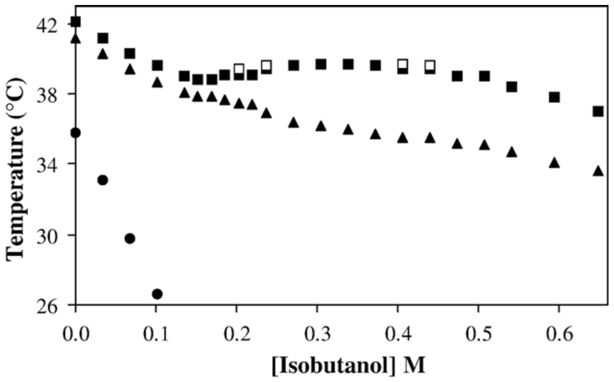

Fig. 2. Effects of isobutanol on DPPC phase transition temperatures (■, heating scan main peak; □, heating scan shoulder peak; ▲, cooling scan main peak; ●, pretransition peak).

38.2 °C at 0.16 M. The onset of a large hysteresis between the T_m of the heating and cooling scans was observed at 0.16 M. Though there was a clear inflection point at 0.16 M, as can be seen in Fig. 1, there was not a substantial reversal in the T_m of the heating scans following this concentration. The T_m increased slightly to 38.6 °C at 0.27 M but continued to decrease beyond this concentration. This continued reduction in T_m is in contrast to the biphasic effect reported for shorter-chained alcohols, in which an abrupt reversal in T_m at the threshold concentration is observed [3,4,11]. Comparatively, the *n*-butanol system exhibited "stunted" biphasic behavior. From this data it was concluded that the threshold concentration for *n*-butanol is 0.16 M, in agreement with previous studies on the DPPC system [6,14,15].

The isobutanol system exhibited very similar behavior to that reported for *n*-butanol. As shown in Fig. 2, the pretransition disappeared at 0.14 M, with the lowest detected T_p of 26.6 °C at 0.10 M. The inflection point for the heating scans and the onset of a large hysteresis were observed at 0.17 M. The decrease in T_m at lower concentrations was not as significant as observed for the *n*-butanol system, with a minimum of 38.8 °C. The behavior of DPPC in the presence of isobutanol was more biphasic. The T_m increased by 0.9 °C before continuing to decrease, compared to the 0.4 °C increase in the *n*-butanol system. The threshold concentration for the DPPC/isobutanol system was determined to be 0.17 M. In both the *n*- and isobutanol systems, sporadic, unresolved shoulder peaks of the main transition were observed after the threshold concentration on the heating scans.

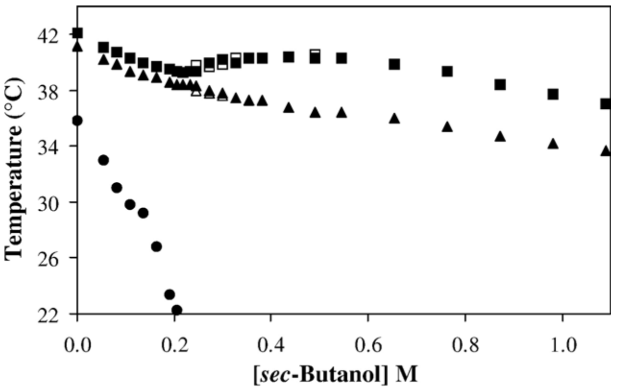

Fig. 3. Effects of *sec*-butanol on DPPC phase transition temperatures (■, heating scan main peak; □, heating scan shoulder peak; ▲, cooling scan main peak; △, cooling scan shoulder peak; ●, pretransition peak).

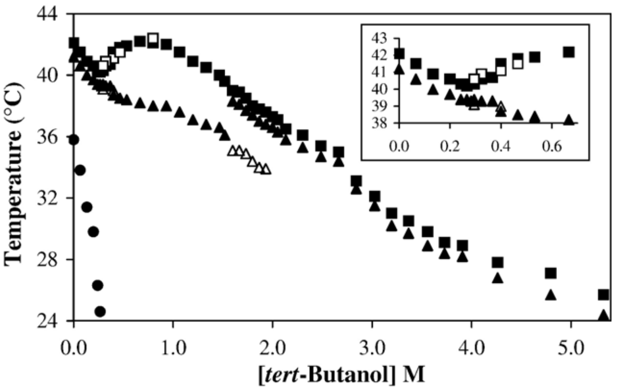

Fig. 4. Effects of *tert*-butanol on DPPC phase transition temperatures (■, heating scan main peak; □, heating scan shoulder peak; ▲, cooling scan main peak; △, cooling scan shoulder peak; ●, pretransition peak). Inset shows region around identified threshold concentration.

The thermotropic phase behavior of DPPC in the presence of *sec*-butanol is shown in Fig. 3. The pretransition reached a minimum of 22.3 °C, dropping 13.5 °C until it was no longer detectable at 0.22 M. The onset of a large hysteresis was also observed at 0.22 M. The behavior of DPPC in the presence of this isomer showed a greater degree of biphasic behavior than observed in the *n*- and isobutanol systems. The T_m of the heating scans increased by 1.1 °C following the identified threshold. The threshold concentration for the DPPC/*sec*-butanol system was determined to be 0.22 M. Unresolved shoulder peaks were observed on both the heating and cooling scans after this concentration.

The miscibility of *tert*-butanol in water allowed for the extensive study of its effects over a wide concentration range. Fig. 4 shows the clear biphasic behavior of DPPC in the presence of *tert*-butanol. The lowest detected T_p of 24.6 °C was observed at 0.27 M and the pretransition was no longer detectable at 0.28 M. The inflection point of the heating curve for this isomer was observed at 0.27 M, at which there was the onset of a large hysteresis and an abrupt reversal of the T_m, reaching a maximum of 42.2 °C at 0.67 M. Unresolved shoulder peaks of the main transition were observed primarily on the heating scans after the threshold.

With increasing concentration of *tert*-butanol beyond the threshold, the T_m of the heating scans began to decrease. At 1.60 M, there was an abrupt shrinking of the hysteresis as a result of a significant increase in the primary T_m of the cooling scans. However, the hysteresis was initially maintained by well-resolved shoulder peaks on the cooling scans. These shoulder peaks were no longer detected at 2.00 M. The heating main transition peaks past 2.00 M became increasingly broad and shallow.

In Fig. 5a–d, the enthalpies of the main transition as a function of butanol isomer concentrations are shown. For the

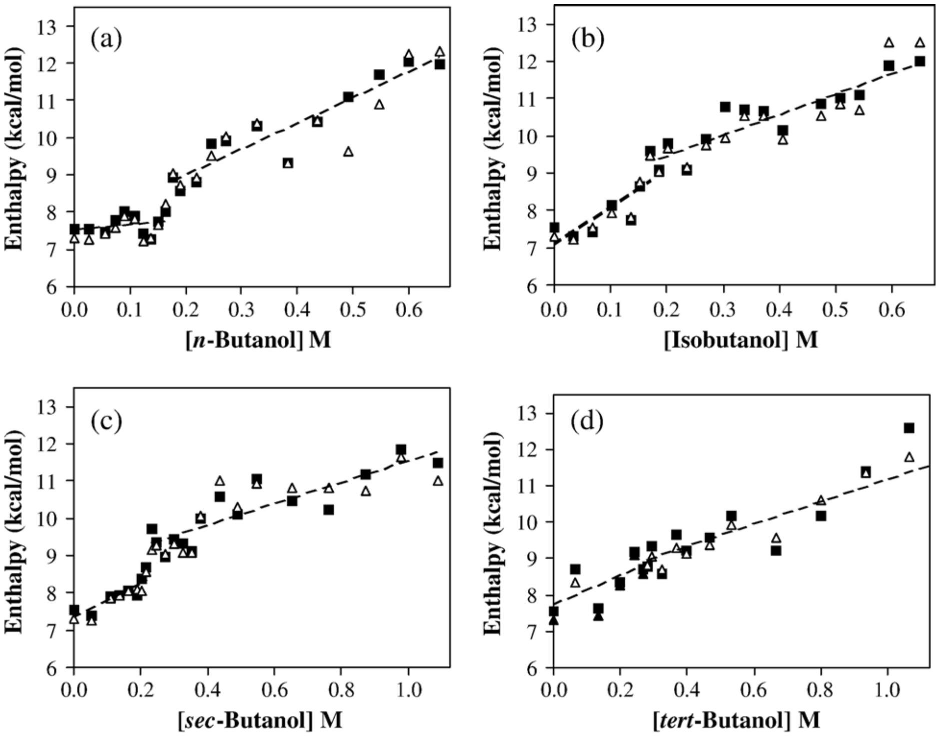

Fig. 5. Effects of (a) *n*-butanol, (b) isobutanol, (c) *sec*-butanol and (d) *tert*-butanol on DPPC main transition enthalpies (■, heating scan main transition enthalpy; △, cooling scan main transition enthalpy).

heating scans of each isomer, the enthalpies before and following the threshold concentration were separately fit to straight lines by least squares. The errors for the enthalpy values were ±0.3 to ±0.5 kcal/mol, with larger errors at higher concentrations of the isomers due to the presence of broader peaks. In all systems studied, a general increase in the main transition enthalpy with increasing concentrations of the isomers was observed. In Fig. 5a, there is a clear separation of approximately 1 kcal/mol between the two best-fit lines at the threshold concentration of the DPPC/*n*-butanol system. Fig. 5c, presenting the enthalpies of the main transition for the DPPC/*sec*-butanol system, also shows a 1 kcal/mol gap at the threshold. The DPPC/isobutanol system showed a smaller separation of approximately 0.5 kcal/mol. In the *tert*-butanol system, no significant break in the enthalpy values at the threshold concentration was observed.

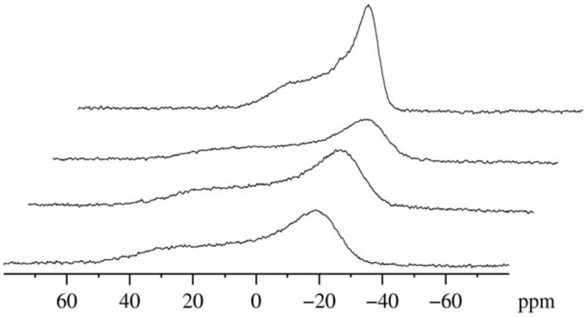

Fig. 6. ^{31}P-NMR spectra stack plot of DPPC in 1.71 M *tert*-butanol at 20 °C, 25 °C, 30 °C and 40 °C.

3.2. ^{31}P-NMR

The effects of *n*-butanol and *tert*-butanol on the DPPC multilayer system were further studied using ^{31}P-NMR. Other research groups have thoroughly established the ^{31}P-NMR spectra for the phospholipid bilayer system and other phospholipid structures where isotropic motion is observed, e.g. vesicles and micelles [13]. The ^{31}P-NMR spectra of pure DPPC showed the characteristics of a multilamellar system.

The ^{31}P-NMR spectrum for DPPC in 0.66 M *n*-butanol at 40 °C, the highest *n*-butanol concentration studied given solubility

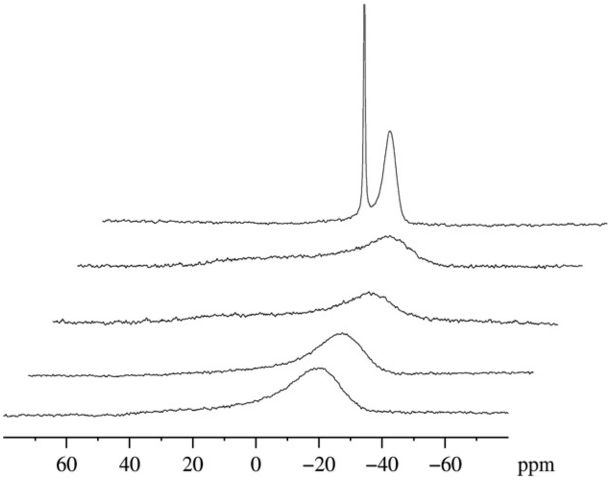

Fig. 7. ^{31}P-NMR spectra stack plot of DPPC in 2.13 M *tert*-butanol at 10 °C, 20 °C, 25 °C, 30 °C and 40°C.

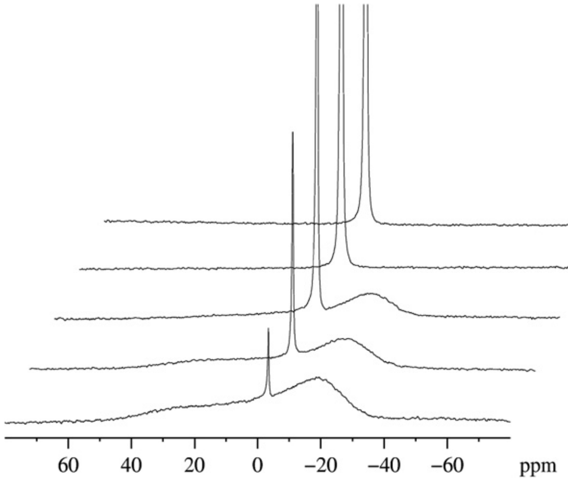

Fig. 8. ^{31}P-NMR spectra stack plot of DPPC in 5.33 M *tert*-butanol at 10 °C, 20 °C, 25 °C, 30 °C and 40 °C.

limitations, showed no evidence of an isotropic peak indicating that the DPPC continued to exist in a hydrated multilayer phase.

Fig. 6 shows the ^{31}P-NMR spectra for DPPC in 1.71 M *tert*-butanol at varying temperatures. This concentration of *tert*-butanol was within the region shown on Fig. 4 where there was an abrupt shrinking of the hysteresis with well-resolved shoulder peaks. The ^{31}P-NMR spectra showed no isotropic motion at any of the studied temperatures indicating that the multilayer structure remained fully intact. There was an obvious decrease in chemical shift anisotropy shown by narrowing of the spectrum between 30 and 40 °C corresponding to the transition from a gel-to-liquid crystalline phase.

The ^{31}P-NMR spectra of DPPC in 2.13 M *tert*-butanol at varying temperatures are shown in Fig. 7. This concentration corresponds to the region in DSC data where the well-resolved shoulder peaks were no longer detected. After hydration in a 42 °C sand bath, the sample appeared clear as opposed to the usual cloudy solution corresponding to the presence of multilamellar vesicles. From 10 to 30 °C, the ^{31}P spectra indicated the multilamellar structure remained. At 40 °C an isotropic peak was observed at 0 ppm, indicative of breakdown of the multilamellar structure.

DPPC in 5.33 M *tert*-butanol shows the presence of isotropic motion over the entire temperature range studied in Fig. 8. For this sample the solution also appeared clear after hydration. At 30 °C and higher, only an isotropic peak centered around 0 ppm was observed with no spectra characteristic of a multilamellar phase.

4. Discussion

Table 1 shows a summary of the results obtained for the DPPC and butanol systems. The threshold concentration determined for DPPC in *n*-butanol is in agreement with previously reported values [6,14,15]. Given that *n*-butanol had the lowest identified threshold concentration at 0.16 M, it is the most effective of the isomers in inducing interdigitation in DPPC. Additionally, *n*-butanol most effectively depresses the pretransition temperature and main transition temperature of both the heating and cooling scans prior to the threshold concentration. As noted before, unlike what has been reported for the effect of shorter-chained alcohols on PC bilayers, the DPPC/*n*-butanol system does not exhibit clear biphasic behavior and the T_m continues to decrease significantly following the threshold up to the solubility limit of *n*-butanol. ^{31}P-NMR data confirms the presence of the multilamellar structure past the threshold concentration, implying that the decrease in T_m occurs because *n*-butanol partitions more effectively into the L_α phase than into the $L_\beta I$ phase.

Our results show that with increasing solubility in water of the isomers, they are less effective at inducing interdigitation, as shown by the increase in threshold concentrations. Because of the greater hydrophobic nature of *n*-butanol, the least soluble of the isomers, it may engage in stronger hydrophobic interactions with the terminal methyl groups of the phospholipid tails, resulting in a lower threshold concentration. Additionally, the more soluble an isomer is in water, the less effectively it depresses both the temperature of the pretransition and the main transition prior to the threshold. *n*-Butanol most effectively lowers the temperature of the pretransition, indicating that the $P_{\beta'}$ phase has more hydrophobic character than the $L_{\beta'}$ phase because the staggered conformation of the phospholipid molecules exposes part of the acyl chain with which the alcohol can interact [12].

Increased solubility of the isomers is additionally correlated with an increase in biphasic behavior. This would indicate that the isomers that are more hydrophobic in nature partition preferentially into the L_α phase as opposed to the $L_\beta I$ phase.

The enthalpy results also exhibit differences between the four systems. The DPPC/*n*-butanol system shows a separation of 1 kcal/mol between the enthalpies for concentrations before the threshold and those after when two straight lines are used to fit the data. This separation and its magnitude are consistent with previously reported data on the enthalpies of the DPPC/*n*-butanol system from Zang and Rowe [14]. The *sec*-butanol and isobutanol systems also show separations of 1 kcal/mol and 0.5 kcal/mol respectively. These separations are consistent with the expectation that the $L_\beta I$ to L_α transition has a higher enthalpy than the $P_{\beta'}$ to L_α transition due to the greater order of the $L_\beta I$ phase when compared to the $P_{\beta'}$ phase. In contrast to the other three, the DPPC/*tert*-butanol system shows no visible

Table 1
Summary data on the induction of the interdigitated phase

System	Conc. at which pretransition disappears (M)	Conc. at which onset of hysteresis occurs (M)	Determined threshold conc. for interdigitated phase (M)
DPPC/*n*-butanol	0.15	0.16	0.16
DPPC/isobutanol	0.14	0.17	0.17
DPPC/*sec*-butanol	0.22	0.22	0.22
DPPC/*tert*-butanol	0.28	0.27	0.27

break in the enthalpy values at the threshold concentration. This may be due to a difference in chain length between the alcohols, also accounting for the smaller separation in the isobutanol system. Another possibility is that the enthalpy values in the *tert*-butanol system were high enough prior to the threshold concentration that a separation occurring at the threshold would not be visible.

Our investigation of *tert*-butanol shows previously undocumented phase behavior at high concentrations, most notably the shrinking of the hysteresis with well-resolved shoulder peaks. *tert*-Butanol has the highest threshold concentration and most biphasic behavior, resembling the effects of shorter-chained alcohols methanol, ethanol, and propanol on lipid bilayers [3,4,11]. However, with the extension of the range of alcohol concentration generally studied for lipid alcohol systems, the heating T_m eventually decreases with increasing *tert*-butanol concentration, as in the *n*-butanol system. At high concentrations, from 1.60 to 2.00 M, there is an abrupt shrinking of the hysteresis, with well-resolved shoulder peaks upon cooling. ^{31}P-NMR shows no breakdown of the bilayer within this concentration range, and we conclude that this region contains mixed phases. The presence of an extra peak on cooling scans has been reported to be due to the presence of mixed phases [3,4]. Rosser et al. suggest that part of the bilayer undergoes a transition to the interdigitated phase, and then the lower temperature peak is due to the rest of the bilayer undergoing a similar transition. It is also possible that the bilayer is following a pathway from the L_α to $P_{\beta'}$ to $L_\beta I$ phase [3]. Maruyama et al. found coexisting phases of L_α with $L_\beta I$ and $L_{\beta'}$ with $L_\beta I$ in DPPC vesicles in the presence of the anesthetic tetracaine [16]. Also, the $L_\beta I$ to $P_{\beta'}$ to L_α pathway has been observed by Ohki et al. with increasing temperature [17]. It should be noted however that this reasoning has been applied primarily to shoulder peaks seen right after the threshold concentration and not within the high concentration range we are studying. Further studies need to be conducted to determine what phases exist within this concentration range of the *tert*-butanol system. We hypothesize that between the main peak and shoulder peak of the cooling scans exists a mixture of the $L_\beta I$ and L_α phases.

^{31}P-NMR shows the presence of an isotropic peak indicative of breakdown of the bilayer when well-resolved shoulder peaks on the DSC cooling scans are no longer observed. These results are further supported by a qualitative observation of the transparent appearance of the samples at high temperatures. Within the concentration where isotropic motion is observed, the DPPC/*tert*-butanol solutions appear clear at high temperatures, in contrast to the milky white appearance of multilamellar vesicle solutions. Our data would suggest that breakdown of the bilayer structure is preceded by the presence of mixed phases.

Acknowledgements

This project has been funded by the National Science Foundation — Research Experience for Undergraduates and Research for Undergraduate Institutions, the Howard Hughes Medical Institute Undergraduate Science Education Grant, the Sherman Fairchild Foundation, and Occidental College.

References

[1] E.S. Rowe, Effects of ethanol on membrane lipids, in: Ronald Watson (Ed.), Alcohol and Neurobiology: Receptors, Membranes, and Channels, CRC Press, Boca Raton, 1992, pp. 239–267.

[2] S.A. Simon, T.J. McIntosh, Interdigitated hydrocarbon chain packing causes the biphasic transition behavior in lipid/alcohol suspensions, Biochim. Biophys. Acta 773 (1984) 169–172.

[3] M.F.N. Rosser, H.M. Lu, P. Dea, Effects of alcohols on lipid bilayers with and without cholesterol: the dipalmitoylphosphatidylcholine system, Biophys. Chem. 81 (1999) 33–44.

[4] R. Tran, S. Ho, P. Dea, Effects of ethanol on lipid bilayers with and without cholesterol: the distearoylphosphatidylcholine system, Biophys. Chem. 110 (2004) 39–47.

[5] J.L. Slater, C. Huang, Interdigitated bilayer membranes, Prog. Lipid Res. 27 (1988) 325–359.

[6] L. Löbbecke, G. Cevc, Effects of short-chain alcohols on the phase behavior of and interdigitation of phosphatidylcholine bilayer membranes, Biochim. Biophys. Acta 1237 (1995) 59–69.

[7] M. Kranenburg, M. Vlaar, B. Smit, Simulating induced interdigitation in membranes, Biophys. J. 87 (2004) 1596–1605.

[8] L.F. Braganza, D.L. Worcester, Hydrostatic pressure induces hydrocarbon chain interdigitation in single-component phospholipid bilayers, Biochemistry 25 (1986) 2591–2596.

[9] S.K. Prasad, R. Shashidar, B.P. Gaber, S.C. Chandrasekhar, Pressure studies on two hydrated phospholipids-1, 2-dimyristoyl-phosphatidylcholine and 1, 2-dipalmitoyl-phosphatidylcholine, Chem. Phys. Lipids 143 (1987) 227–235.

[10] E.S. Rowe, Lipid chain length and temperature dependence of ethanol–phosphatidylcholine interactions, Biochemistry 22 (1983) 3299–3305.

[11] E.S. Rowe, Thermodynamic reversibility of phase transitions. Specific effects of alcohols on phosphatidylcholines, Biochim. Biophys. Acta 813 (1985) 321–330.

[12] J.A. Viero, P. Nambi, L. Herold, E.S. Rowe, Effects of *n*-alcohols and glycerol on the pretransition of dipalmitoylphosphatidylcholine, Biochim. Biophy. Acta 900 (1987) 230–238.

[13] P.P. Cullis, B. de Kruijff, Lipid polymorphism and the functional roles of lipids in biological membranes, Biochim. Biophys. Acta 559 (1979) 399–420.

[14] F. Zang, E.S. Rowe, Titration calorimetric and differential scanning calorimetric studies of the interactions of *n*-butanol with several phases of dipalmitoylphosphatidylcholine, Biochemistry 31 (1992) 2005–2011.

[15] L.L. Herold, E.S. Rowe, R.G. Khalifah, ^{13}C-NMR and spectrophotometric studies of alcohol–lipid interactions, Chem. Phys. Lipids 43 (1987) 215–225.

[16] S. Maruyama, T. Hata, H. Matsuki, S. Kaneshina, Effects of pressure and local anesthetic tetracaine on dipalmitoylphosphatidylcholine bilayers, Biochim. Biophys. Acta (1997) 272–280.

[17] K. Ohki, K. Tamura, I. Hatta, Ethanol induces interdigitated gel phase ($L_\beta I$) between lamellar gel phase ($L_{\beta'}$) and ripple phase ($P_{\beta'}$) in phosphatidylcholine membranes: a scanning density meter study, Biochim. Biophys. Acta 1028 (1990) 215–222.