ELSEVIER

Biophysical Chemistry 110 (2004) 39–47

Biophysical Chemistry

www.elsevier.com/locate/bpc

Effects of ethanol on lipid bilayers with and without cholesterol: the distearoylphosphatidylcholine system

Rosalie Tran, Shirleen Ho, Phoebe Dea*

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

Received 6 September 2003; received in revised form 6 January 2004; accepted 6 January 2004
Available online 27 April 2004

Abstract

Differential scanning calorimetry (DSC) and fluorescence spectroscopy are useful techniques for investigating the phase transitions of phospholipid bilayers. In this study, these methods have been extended to determine the effects of ethanol on DSPC and DSPC/2 mol.% cholesterol bilayers. The biphasic effect of the main transition was observed on the DSC heating scans above 0.60 M ethanol. In addition, the concentration at which the biphasic effect occurs is not significantly changed in the presence of 2 mol.% cholesterol. For the fluorescence studies, 1,6-diphenyl-1,3,5-hexatriene (DPH) has been incorporated into the bilayer to monitor the phase transitions through the displacement of DPH. This fluorescent probe is used to directly determine the onset of interdigitation in the bilayer systems as indicated by a large decrease in the DPH fluorescence intensity. The addition of cholesterol lowered and broadened the transition temperatures of the phosphatidylcholine (PC) system. However, 2 mol.% cholesterol did not have a significant effect on the induction of the interdigitated phase in DSPC as observed from the small difference in ethanol threshold concentration for the two systems. This suggests that DSPC forms a more stable interdigitated gel phase than other PCs with shorter acyl chains.

Keywords: Phospholipid; Cholesterol; Interdigitation; DSC; Fluorescence

1. Introduction

It is now well established that saturated like-chain phosphatidylcholines (PCs) can form the fully interdigitated phase, in which the terminal methyl groups of the acyl chains effectively interpenetrate into the opposing monolayer, in the presence of ethanol and other short-chain alcohols. Previous studies by Rowe and others have shown that the induction of interdigitation by ethanol in PCs depends on alcohol concentration, lipid chain length, and temperature [1–9]. Several attempts at rationalizing the mechanism of the transition from the gel-to-interdigitated phase indicate that the binding of two ethanol molecules to the terminal methyl ends of the two hydrophobic chains of DSPC may be responsible for ethanol's favoring of the interdigitated phase [6,7,10,11]. This idea is based on the

Abbreviations: DSC, differential scanning calorimeter; Tp, pretransition temperature; Tm, main transition temperature; $L_{\beta'}$, planar gel phase; $P_{\beta'}$, ripple gel phase; L_{α}, liquid crystalline phase; $L_{\beta}I$, interdigitated gel phase; DSPC, 1,2-distearoyl-sn-glycero-3-phosphocholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; chol, cholesterol; EtOH, ethanol.

* 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 © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2004.01.005

general concept of shielding the hydrophobic terminal methyls from the aqueous interfacial environment by ethanol, blocking the unfavorable water molecules from interacting with the hydrophobic tails.

Many compounds have the ability to induce the interdigitated phase in PCs. These inducers of interdigitation are typically small amphiphilic molecules with the ability to displace water from the interfacial region and increase the headgroup surface area [11]. They do not extend too deeply into the bilayer interior; hence, long-chain fatty acids and cholesterol do not cause interdigitation in PCs. Some extrinsic elements shown to induce the interdigitated phase in like-chain PCs include glycerol, methanol, ethylene glycol, benzyl alcohol, chlorpromazine, tetracaine, and thiocyanate ion [10–14]. In addition, each of the *n*-alcohols up to heptanol and several branched alcohols are capable of inducing interdigitation in DSPC. The interdigitated phase has also been shown to be induced in dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) bilayers at increased hydrostatic pressure in the absence of additives [15,16]. The threshold concentrations for interdigitation for each of the alcohols were found to be correlated with the membrane: buffer partition coefficient [17]. Because of the apparent prevalence of the interdigitated phase, this unique phase may have some biological relevance [7].

Differential scanning calorimetry (DSC) has been used extensively in studies of the interdigitated phase. It is a powerful tool for investigating the thermotropic phase transitions of a phospholipid bilayer. Indicators of interdigitation include the disappearance of the pretransition, the biphasic effect, and reversibility shown by hysteresis [7,18].

Fluorescence spectroscopy has been used to confirm the presence of the interdigitated phase as observed in calorimetry results. Since DSPC does not fluoresce on its own, the fluorophore, 1,6-diphenyl-1,3,5-hexatriene (DPH), was incorporated into the phospholipid and used to monitor the phase transitions of DSPC. In this study, the technique developed by Nambi et al. was used for the detection of interdigitation [6]. DPH is believed to have at least two possible orientations in the bilayer: either parallel to the lipid acyl chains, or in the center of the bilayer and aligned perpendicular to the acyl chains. The preferred position for DPH is to be perpendicular to the acyl chains. However, in the case of the interdigitated phase, this orientation would not be possible due to the chain penetration across the bil - ayer center. Hence, DPH is most likely oriented parallel to the acyl chains during interdigitation. It has been shown that the $L_{\beta'}$ to $P_{\beta'}$ transition gives a small decrease in DPH fluorescence intensity, the $P_{\beta'}$ to L_{α} transition also produces a slight decrease in intensity, while the $L_{\beta}I$ to L_{α} phase transition is accompanied by a large increase in intensity [17]. The $L_{\beta'}$ to $L_{\beta}I$ transition involves a greater decrease in intensity due to DPH's closer proximity to the aqueous environment of the interfacial region [6]. The $L_{\beta'}$ to $P_{\beta'}$ and $P_{\beta'}$ to L_{α} phase transitions produce smaller changes in intensity compared to that of the $L_{\beta'}$ to $L_{\beta}I$ phase transition because there is a less drastic structural change from the gel to liquid crystalline phase.

Cholesterol is a major component of most eukaryotic cellular membranes, where it coexists with a variety of phospho- and sphingolipid species [19]. The cholesterol content, however, varies from one type of membrane to another. The average concentration of cholesterol in most biological systems is 30–50 mol.% [18]. Their influence on the physical properties of membranes and lipid bilayers has been studied by various experimental and theoretical methods [18,20–24]. Although a considerable amount of information is known about the manner in which cholesterol affects the organization and mechanical properties of phospholipid bilayers, an understanding of the molecular basis of cholesterol/phospholipid interactions still remains incomplete. Cholesterol molecules are composed of three main parts: a polar head group, a rigid planar steroid ring structure, and a flexible non-polar hydrocarbon tail. Each cholesterol molecule tends to occupy the position of an acyl chain of a phospholipid [20]. It can be thought of as a molecule with a single acyl chain. It has been shown that cholesterol lowers the main transition temperature of phosphatidylcholines, and is generally accepted that cholesterol fluidizes and disorders phospholipids below the main transition temperature (T_m), while making them more rigid at temperatures above T_m [22]. Furthermore, many experiments indicate that cholesterol is not evenly distributed throughout the cell membrane; rather, they are segregated into cholesterol-rich and cholesterol-poor domains, promoting a heterogeneous membrane [20].

Although much work has been done by Rowe, McElhaney, McIntosh and others in the last two

decades on the interactions between liposomes and either alcohol or cholesterol [1–10,17–19,21–24], few investigations have reported the effects of both compounds on these model membrane systems [20,25–27]. Furthermore, many of these studies focused on systems with high concentrations of cholesterol; only a few quantitative biophysical studies have reported the effect of low cholesterol concentrations on structural membrane dynamics, but without the addition of any alcohol [28–30]. Trandum et al. have observed by isothermal titration calorimetry (ITC) that the affinity of ethanol for unilamellar DMPC vesicles is strongly dependent on the bilayer phase behavior, which varies with cholesterol content [20]. They have suggested that ethanol association with lipid bilayers may be greatly influenced by the existence of dynamically coexisting gel and fluid phase domains in the main transition temperature region.

In addition, Rosser, Lu and Dea have shown that fluorescence spectroscopy and differential scanning calorimetry are useful methods for investigating the effects of alcohols on DPPC and DPPC/2 mol.% cholesterol bilayers [25]. The combination of these techniques have been used extensively to examine the structure and dynamics of biological membranes, and shown to be very effective in analyzing the thermotropic behavior of lipid bilayers. Since it had been well established that the stability of the interdigitated gel phase increased with longer acyl chains, we were interested in determining how cholesterol would affect alcohol-induced interdigitation in longer acyl-chained PCs. In this paper, we present our studies on the interaction of ethanol with DSPC and DSPC/2 mol.% cholesterol in continuation of our previous work with the shorter acyl-chained phospholipid, DPPC [25]. In our continued efforts to better understand these systems, we hope to elucidate some of the physical properties of cholesterol-poor membrane domains and the physiological reason for the existence of such environments in biological systems.

2. Materials and methods

2.1. Materials

1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), purity 99+%, was purchased from Avanti Polar Lipid (Alabaster, AL, USA). The fluorescence probe, 1,6-diphenyl-1,3,5-hexatriene (DPH) was obtained from Molecular Probes (Eugene, OR, USA). 5(6)-cholesten-3-ol, purity 99+%, was purchased from Sigma (St. Louis, MO, USA). All chemicals were used without further purification.

2.2. Differential scanning calorimetry (DSC)

Chloroform was used as a solvent to integrate the 2 mol.% cholesterol into the 2 mg of DSPC. The solvents were evaporated before being placed under vacuum for at least 10 h to remove any residual chloroform. Samples were hydrated with 100 μl of ethanol/phosphate buffer (25 mM, pH 7.0) by heating in a sand bath at approximately 65 °C for an hour, vortexing occasionally to ensure proper hydration. The samples were equilibrated to room temperature before putting into the Calorimetry Sciences Corp. Multi-cell DSC-HT Model 4100 differential scanning calorimeter (DSC). The samples were heated and cooled within the temperature range from 20 to 60 °C at a scan rate of 10 °C/h in the DSC. These heating and cooling scans were repeated three times to check for hysteresis and to confirm the accuracy of the data.

2.3. Fluorescence spectroscopy

Stock solutions of DSPC/DPH with and without 2 mol.% cholesterol were prepared by using appropriate volumes of chloroform containing DPH and cholesterol into DSPC using a lipid-to-probe mole ratio of 500:1. This concentration level of DPH is known not to disturb the bilayer structure. The solvents were first evaporated under the fume hood before being placed under high vacuum for at least 10 h to remove any residual chloroform. The final fluorescence samples contained 0.64 mg/ml of DSPC in varying amounts of alcohol. The samples were hydrated by heating and vortexing as previously described. Oxygen was removed from the samples by bubbling the sample with nitrogen for at least 1 min prior to fluorescence measurements.

The fluorometer used was an ISS K2 Multi-Frequency Cross-Correlation Phase and Modulation Fluorometer. A xenon arc lamp operating at 15 A was used as the light source. A Neslab RTE-111 bath was set at a scan rate of 10 °C/h from 20 to 60 °C. A

Table 1
Summary data on enthalpy values and pre- and main transition temperatures

System	Pretransition		Main transition	
	ΔH (kcal/mol)	T (°C)	ΔH (kcal/mol)	T (°C)
DSPC	1.2±0.3	51.3±0.3	9.9±0.2	55.6±0.2
DSPC/2 mol.%Chol	0.6±0.5	51.1±0.3	9.6±0.2	55.2±0.4

calibration between the bath and cuvet temperature was used to correct all temperature data. The excitation wavelength maximum ranged from 356 to 366 nm. For the emission wavelength maximum, the range was between 427 and 430 nm.

3. Results

3.1. Differential scanning calorimetry (DSC)

For pure DSPC, the transition temperatures centered at 51.3 and 55.6 °C corresponded to the lamellar gel to ripple gel pretransition (T_p) and the ripple gel-to-liquid crystalline main transition (T_m). The presence of 2 mol.% cholesterol lowered both the transition temperatures and enthalpy values, resulting in slightly broader peaks. The pretransition and main transition temperatures and enthalpies for pure DSPC with and without cholesterol are summarized in Table 1. Spink et al. [23] reported that for phospholipids with the appropriate bilayer thickness, a high concentration of cholesterol would induce order in fluid bilayers by freezing out the trans-conformers in the vicinity of the steroid ring. In our study, the pretransition peak of DSPC could still be observed with the addition of 1–3 mol.% cholesterol. However, the broadness of the DSPC's pretransition peak in the presence of 3 and higher mol.% cholesterol made it difficult to distinguish the peak temperature. Hence, 2 mol.% cholesterol was used in this study to avoid this complication.

Typical DSC scans in the pre-transition temperature range for pure DSPC in different ethanol concentrations are shown in Fig. 1. The pretransition temperatures decreased dramatically with increasing ethanol concentrations. In addition, the area of the pretransition peak decreased rapidly with increasing ethanol concentrations until it was barely detectable at 0.69 M ethanol. This peak could no longer be detected in DSPC samples containing ethanol concentrations above 0.77 M. DSC scans for the DSPC/2 mol.% cholesterol samples are shown in Fig. 2. The pretransition peaks were broader, and with increasing ethanol concentration, became impossible to detect beyond 0.69 M (Fig. 2).

The main transition temperatures for the heating scans for DSPC without and with 2 mol.% cholesterol

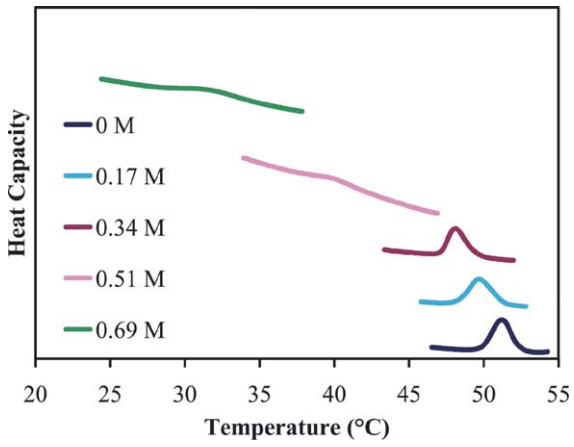

Fig. 1. A stack plot of DSC thermograms of DSPC pretransitions with varying ethanol concentrations.

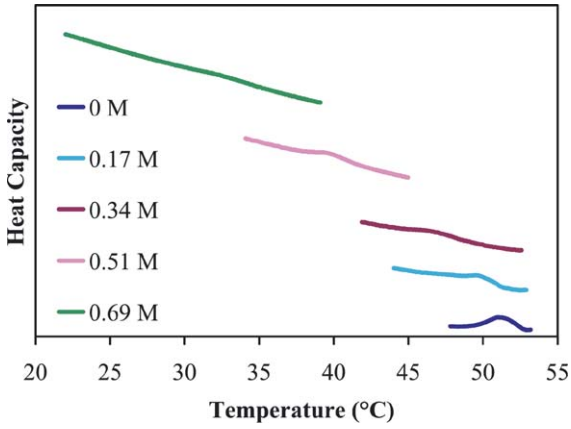

Fig. 2. A stack plot of DSC thermograms of DSPC pretransitions with varying ethanol concentrations showing the effect of 2 mol.% cholesterol on phase stability.

were lowered with increasing ethanol concentrations up to 0.58 M and 0.60 M, respectively (Fig. 3). Beyond these ethanol concentrations, the T_m increased with increasing ethanol concentration. This decrease in T_m followed by an increase upon reaching a minimum threshold ethanol concentration is referred to as the biphasic effect, and has been shown to be an indication of interdigitation [1]. This biphasic effect is the result of an initial interaction between the ethanol molecules and the fluid bilayer, followed by a stronger interaction with the gel state past a certain alcohol threshold value [8,9]. This interdigitated gel phase becomes more stable with increasing ethanol concentration; hence, the main transition temperature increases steadily after the onset of interdigitation.

Fig. 3 shows a comparison of the effect of ethanol on the main gel-to-liquid crystalline phase transition temperature during heating scans of DSPC without and with cholesterol. For the DSPC system without cholesterol, the main phase transitions occurred at 0.2–1 °C higher than the system with 2 mol.% cholesterol. The biphasic effect was clearly observed in both systems during heating scans.

A compilation of the main transition temperatures of DSPC/EtOH and DSPC/2 mol.%Chol/EtOH vs. ethanol concentration upon cooling is shown in Fig. 4. Unlike the results obtained for the heating scans, the main transition temperatures continued to decrease

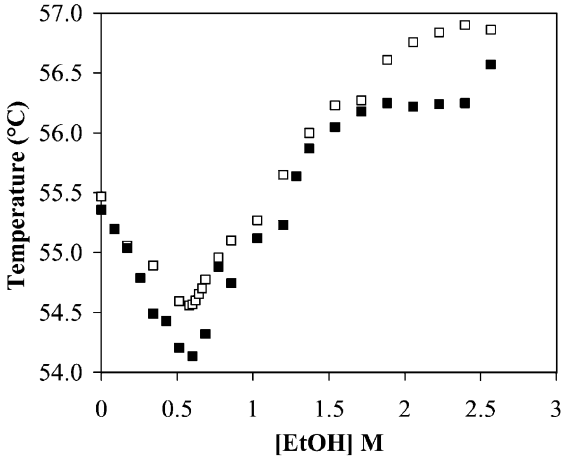

Fig. 3. Biphasic effect of ethanol on DSPC main transition temperature without (□) and with 2 mol.% cholesterol (■) upon heating.

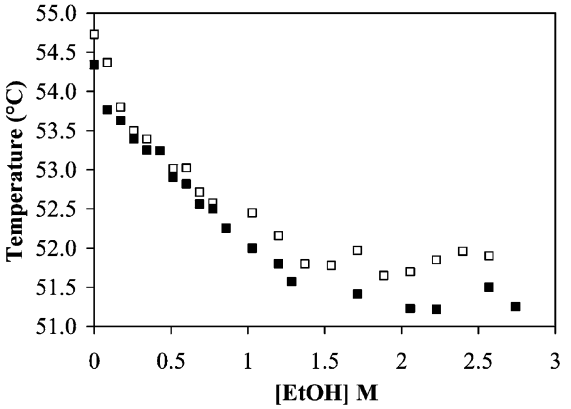

Fig. 4. Effects of ethanol on DSPC main transition temperature without (□) and with 2 mol.% cholesterol upon cooling (■).

with increasing ethanol concentration, and no biphasic effect was observed.

In the DSPC system without cholesterol, extra peaks were observed on cooling scans as shoulder peaks of the main transition at ethanol concentrations above 0.86 M. With increasing ethanol concentration, the shoulder peaks shifted farther away from the main transition peak. In the DSPC/2 mol.%Chol/EtOH system, these shoulder peaks appeared in some concentrations of ethanol both on the heating scans and the cooling scans. On the heating scans, the shoulder peak was a small peak lower in temperature than the main transition peak. On the cooling scans, the shoulder peak appeared at a correspondingly higher temperature than the main transition.

3.2. Fluorescence spectroscopy

Fig. 5 shows some typical heating scans for the DSPC system containing 0–0.86 M ethanol. At low ethanol concentrations, we observed a slight decrease in intensity around the pretransition temperature and the main transition temperature. However, with increasing ethanol concentration above 0.60 M, there was a much larger decrease in fluorescence intensity, starting at approximately 32 °C, reflecting the onset of some dramatic structural changes in the bilayer. This fluorescence intensity decrease by ~50% had been previously reported by Rosser, Lu and Dea for DPPC [25], and can be correlated to the formation of the interdigitated gel

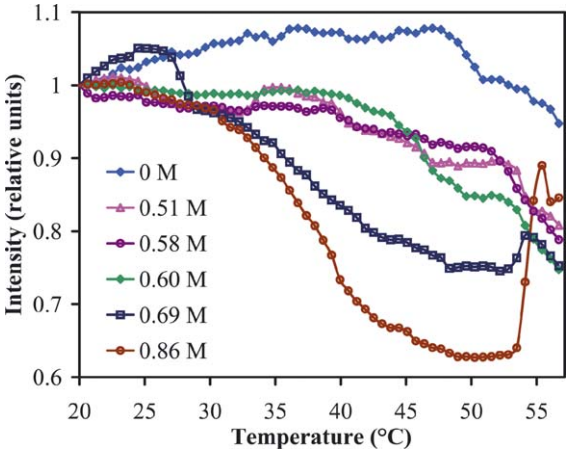

Fig. 5. Stack plot of fluorescence intensity of DPH in DSPC without cholesterol at varying ethanol concentrations (heating scan).

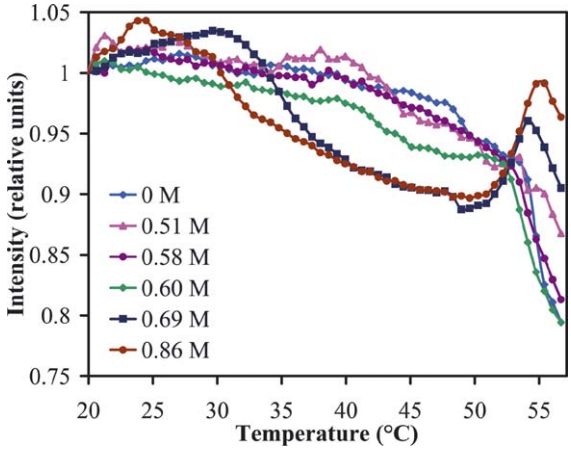

Fig. 6. Stack plot of fluorescence intensity of DPH in DSPC with 2 mol.% cholesterol at varying ethanol concentrations (heating scan).

phase. This decrease in fluorescence intensity became even more drastic for the planar gel phase-to-interdigitated gel phase transition in the DSPC sample containing higher concentrations of ethanol. The interdigitated phase transition temperatures may be obtained by taking the midpoint of the fluorescence slope resulting from the structural changes from the planar gel to the interdigitated gel phase.

Another notable feature, which reflected the presence of the interdigitated phase, was the change in fluorescence intensity of the main transition. As shown in Fig. 5, at ethanol concentrations up to 0.60 M, the main transition was accompanied by a decrease in fluorescence intensity. This was replaced by a large increase in fluorescence intensity for the interdigitated gel-to-liquid crystalline phase transition for samples containing 0.69 M and higher ethanol concentrations. These results suggest that a concentration above 0.60 M ethanol corresponded to the onset of the induction of the interdigitated gel phase in DSPC.

In the presence of 2 mol.% cholesterol, the transitions were more gradual than the system without cholesterol. These results are shown in Fig. 6. The large change in fluorescence intensity during the interdigitated transition in DSPC/2 mol.% cholesterol was not as apparent as that of DSPC, and occurred over a wider temperature range. The threshold concentration for interdigitation in the DSPC/2 mol.% cholesterol system could be determined to be also above 0.60 M ethanol by noting the reversal in fluorescence intensity changes for the main transition.

Fig. 7 shows the cooling scans obtained for the DSPC system without cholesterol. For ethanol concentrations below the threshold value, the fluorescence intensity increased slightly at the main transition upon cooling. The fluorescence intensity trends for the pretransition in the cooling scan were similar to those observed in the heating scan, although

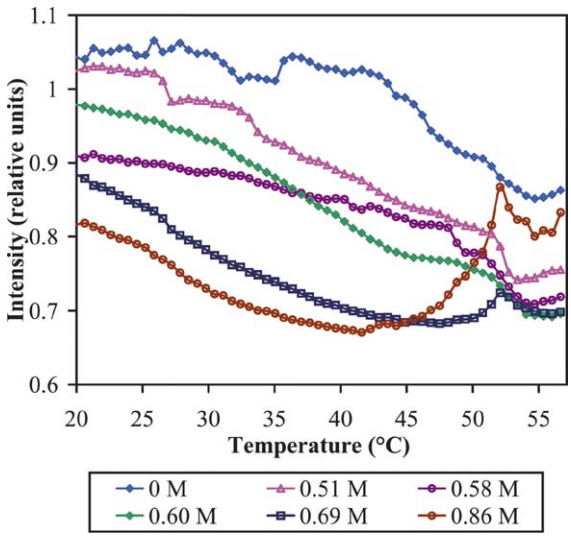

Fig. 7. Stack plot of fluorescence intensity of DPH in DSPC without cholesterol at varying ethanol concentrations (cooling scan).

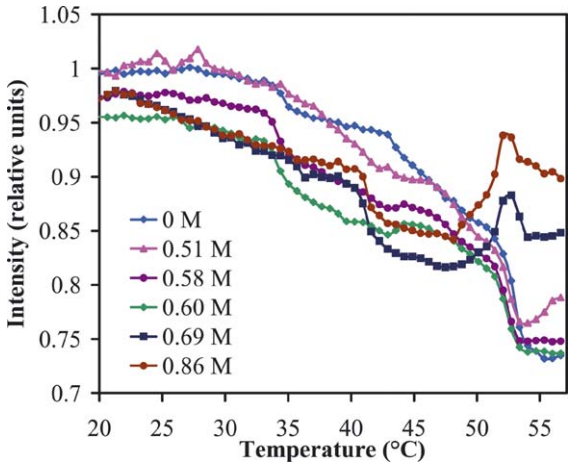

Fig. 8. Stack plot of fluorescence intensity of DPH in DSPC with 2 mol.% cholesterol at varying ethanol concentrations (cooling scan).

the changes in intensity were considerably reduced. Sharp decreases in fluorescence intensity were observed for the main transition upon cooling at concentrations higher than 0.60 M ethanol. Beyond the threshold concentration, the main transition involved a structural transformation from the liquid crystalline phase to the interdigitated gel phase. The characteristic increase in fluorescence intensity for the transition from the interdigitated phase to the gel phase beyond the 0.60 M threshold concentration was not fully reversible in the cooling scan. These fluorescence intensity changes, in addition to the hysteresis observed by DSC, indicated that the liquid crystalline-to-interdigitated gel transition might be a slower process requiring some incubation time. Even above the threshold concentrations, the interdigitated gel phase did not appear to fully develop from the liquid crystalline phase upon cooling.

Fig. 8 shows a plot of the cooling scans obtained for the DSPC/2 mol.% cholesterol system. At low concentrations of ethanol, the trend of a slight increase in intensity for the main transition followed by further increase in intensity for the pretransition was also seen in DSPC with 2 mol.% cholesterol. For higher concentrations of ethanol that induced the interdigitated phase, there was a decrease in intensity at the main transition, followed by a smaller and gradual increase during the interdigitated transition. It was difficult to see the pretransition in these samples.

4. Discussion

The threshold value for DSPC with 2 mol.% cholesterol, as indicated by calorimetric and fluorescence data, is quite similar to the threshold concentration without cholesterol. These results are summarized in Table 2 for the heating scans. Calorimetry studies have narrowed the concentration down to between 0.6 and 0.7 M ethanol. Fluorescence results confirm that the threshold concentration for DSPC is higher than 0.60 M based on the observed changes in emission intensity for the main transition and the interdigitated transition. With ethanol concentrations well above the threshold value, there are a sufficient number of ethanol molecules to perturb the bilayers and stabilize the interdigitated gel phase via hydrophobic interactions between the methyl groups. The significant changes in fluorescence intensity occurred as a result of DPH repositioning from a perpendicular orientation relative to the acyl chains in the lipid phase to an unfavorable parallel orientation that exposes the probe to the aqueous environment in the interdigitated phase [31]. This is a consequence of the considerable reduction of the interbilayer spacing in DSPC upon interdigitation.

Rosser et al. had reported the presence of an extra peak on the DSC cooling scans for the DPPC and DPPC/2 mol.% chol systems beyond the threshold concentration [25]. They reported two possibilities for these shoulder peaks. One possible explanation for the appearance of the shoulder peak is that some regions

Table 2
Summary data on the induction of the interdigitated phase during heating scans

System	Conc. at which pretransition disappears (M)	Conc. at which biphasic effect is observed (M)	Conc. at which fluorescence shows interdigitation (M)
DSPC/EtOH	0.77	0.58	>0.60
DSPC/EtOH/2 mol.%Chol	0.69	0.60	>0.60

of the bilayer underwent induction into the interdigitated phase, whereas others did not go into the interdigitated phase until a lower temperature. With the addition of cholesterol, this is probably more likely to occur since cholesterol induces the formation of different bilayer phase domains. Another possibility is that the bilayer proceeded from the L_β to $P_{\beta'}$ to $L_\beta I$. The shoulder peaks may also occur as a result of the coexistence of the L_β and $L_\beta I$ phases in which the bilayer went from L_β to L_β+$L_\beta I$ to $L_\beta I$. Other studies have provided evidence that biological membranes have coexisting fluid and gel domains under certain circumstances [32–34]. This presence of coexisting lipid phases is of biological interest since it can possibly influence the lateral distribution of membrane solutes during cell processes [23].

Our results also occasionally showed shoulder peaks on the DSC scans. These peaks may represent the existence of cholesterol-rich and cholesterol-poor regions in which the cholesterol-rich domains have a lower transition temperature than the cholesterol-poor ones. This result is expected since there is a possibility that cholesterol may not be evenly distributed in the phospholipid bilayers despite careful hydration and vortexing. In our comparison of the DSPC/EtOH system with the DSPC/2 mol.% chol/EtOH system, it was observed that the cholesterol-poor region resembled the DSPC/EtOH system. Hence, the addition of such low amounts of cholesterol to DSPC does not seem to significantly disturb the bilayer formed by phospholipids with longer acyl chains as shown by the insignificant change in ethanol concentration necessary for the induction of interdigitation.

Although 2 mol.% cholesterol may not have a significant effect on the threshold concentration of DSPC, it does lower the main transition temperature, broaden the DSC peaks, and reduce the sharpness in fluorescence changes during transitions. In general, cholesterol is known to disrupt the ordering of the planar gel phase below the main transition and rigidify the liquid-crystalline phase above the main transition temperature [21]. This disturbance in the lipid bilayer ordering results in a less drastic change for the main transition from a gel to liquid crystalline state. The addition of cholesterol also results in domains of varying cholesterol content. Because of the heterogeneity of the lipid system, a particular phase transition may occur over a wider range of temperatures. These factors may explain the broadness of the DSC peaks and the reduced change in fluorescence intensities during transitions after cholesterol is incorporated into the bilayer.

Acknowledgements

This project has been funded by grants from the National Science Foundation – Research Experience for Undergraduates (NSF-REU), the Howard Hughes Medical Institute (HHMI) through the Undergraduate Biological Sciences Education Program, the Arnold and Mabel Beckman Foundation through the Beckman Scholars Program, and Occidental College.

References

[1] E.S. Rowe, Lipid chain length and temperature dependence of ethanol–phosphatidylcholine interactions, Biochemistry 22 (1983) 3299–3305.
[2] J. Barry, K. Gawrisch, Direct NMR evidence for ethanol binding to the lipid–water interface of phospholipids bilayers, Biochemistry 33 (1994) 8082–8088.
[3] E.S. Rowe, F. Zhang, T.W. Leung, J.S. Parr, P.T. Guy, Thermodynamics of membrane partitioning for a series of n-alcohols determined by titration calorimetry: role of hydrophobic effects, Biochemistry 37 (1998) 2430–2440.
[4] J.A. Viero, P. Nambi, L.L. Herold, E.S. Rowe, Effect of *n*-alcohols and glycerol on the pretransition of dipalmitoylphosphatidylcholine, Biochim. Biophys. Acta 900 (1988) 230–238.
[5] J.A. Viero, P. Nambi, E.S. Rowe, Effects of alcohols on the phase transitions of dihexadecylphosphatidylcholine, Biochim. Biophys. Acta 943 (1988) 108–111.
[6] P. Nambi, E.S. Rowe, T.J. McIntosh, Studies of the ethanol-induced interdigitated gel phase in phosphatidylcholines using the fluorophore 1,6-diphenyl-1,3,5-hexatriene, Biochemistry 27 (1988) 9175–9182.
[7] E.S. Rowe, T.A. Cutrera, Differential scanning calorimetric studies of ethanol interactions with distearoylphosphatidylcholine: transition to the interdigitated phase, Biochemistry 29 (1990) 10 398–10 404.
[8] L. Löbbecke, G. Cevc, Effects of short-chain alcohols on the phase behavior and interdigitation of phosphatidylcholine bilayer membranes, Biochim. Biophys. Acta 1237 (1995) 59–69.
[9] S. Li, H. Lin, G. Wang, C. Huang, Effects of alcohols on the phase transition temperatures of mixed-chain phosphatidylcholines, Biophys. J. 70 (1996) 2784–2794.
[10] 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.

[11] T.J. McIntosh, R.V. McDaniel, S.A. Simon, Induction of an interdigitated gel phase in fully hydrated phosphatidylcholine bilayers, Biochim. Biophys. Acta 731 (1983) 109–114.
[12] O.P. Bondar, V.G. Pivovarenko, E.S. Rowe, Flavonols – new fluorescent membrane probes for studying the interdigitation of lipid bilayers, Biochim. Biophys. Acta 1369 (1998) 119–130.
[13] B.A. Cunningham, L.J. Lis, Thiocyanate and bromide ions influence the bilayer structural parameters of phosphatidylcholine bilayers, Biochim. Biophys. Acta 861 (1986) 237–242.
[14] J.L. Slater, C. Huang, Interdigitated bilayer membranes, Prog. Lipid Res. 27 (1988) 325–359.
[15] L.F. Braganza, D.L. Worcester, Hydrostatic pressure induces hydrocarbon chain interdigitation in single-component phospholipid bilayers, Biochemistry 25 (1986) 2591–2596.
[16] 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.
[17] E.S. Rowe, J.M. Campion, Alcohol induction of interdigitation in distearoylphosphatidylcholine: fluorescence studies of alcohol chain length requirements, Biophys. J. 67 (1994) 1888–1895.
[18] L. Miao, M. Nielson, J. Thewalt, J. Ipsen, M. Bloom, M. Zuckermann, O. Mouritsen, From lanosterol to cholesterol: structures evolution and differential effects on lipid bilayers, Biophys. J. 82 (2002) 1429–1444.
[19] D. Tang, W. Van Der Meer, S. Chen, Evidence for a regular distribution of cholesterol in phospholipid bilayers from diphenylhexatriene fluorescence, Biophys. J. 68 (1995) 1944–1951.
[20] C. Trandum, P. Westh, K. Jorgensen, O. Mouritsen, A thermodynamic study of the effects of cholesterol on the interaction between liposomes and ethanol, Biophys. J. 78 (2000) 2486–2492.
[21] T. McMullen, R.N. McElhaney, New aspects of the interaction of cholesterol with dipalmitoylphosphatidylcholine bilayers as revealed by high-sensitivity differential scanning calorimetry, Biochim. Biophys. Acta 1234 (1995) 90–98.
[22] J. Lu, Y. Hao, J. Chen, Effect of cholesterol on the formation of an interdigitated gel phase in lysophosphatidylcholine and phosphatidylcholine binary mixtures, J. Biochem. 129 (2001) 891–898.
[23] C.H. Spink, S. Manley, M. Breed, Thermodynamics of transfer of cholesterol from gel to fluid phases of phospholipid bilayers, Biochim. Biophys. Acta 1279 (1996) 190–196.
[24] T. McMullen, R.N. McElhaney, Differential scanning calorimetric studies of the interaction of cholesterol with distearoyl and dielaidoyl molecular species of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, Biochemistry 36 (1997) 4979–4986.
[25] 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.
[26] O.P. Bondar, E.S. Rowe, Role of cholesterol in the modulation of interdigitation in phosphatidylethanols, Biochim. Biophys. Acta 1370 (1998) 207–217.
[27] H. Komatsu, E.S. Rowe, Effect of cholesterol on the ethanol-induced interdigitated gel phase in phosphatidylcholine: use of fluorophore pyrene-labeled phosphatidylcholine, Biochemistry 30 (1991) 2463–2470.
[28] J. Lemmich, K. Mortensen, J.H. Ipsen, T. Hønger, R. Bauer, O.G. Mouritsen, The effect of cholesterol in small amounts on lipid-bilayer softness in the region of the main phase transition, Eur. Biophys. J. 25 (1997) 293–304.
[29] S. Raffy, J. Teissié, Control of lipid membrane stability by cholesterol content, Biophys. J. 76 (1999) 2072–2080.
[30] F. Richter, L. Finegold, G. Rapp, Sterols sense swelling in lipid bilayers, Phys. Rev. E 59 (1999) 3483–3491.
[31] B. Lentz, Use of fluorescent probes to monitor molecular order and motions within liposome bilayers, Chem. Phys. Lipids 64 (1993) 99–116.
[32] J.M. Steim, M.E. Tourtellotte, J.C. Reinert, R.N. McElhaney, R.L. Rader, Calorimetric evidence for the liquid-crystalline state of lipids in a biomembrane, Proc. Natl. Acad. Sci. USA 63 (1969) 104–109.
[33] J.C. Reinert, J.M. Steim, Calorimetric detection of a membrane-lipid phase transition in living cells, Science 168 (1970) 1580–1582.
[34] D.E. Wolf, V.M. Maynard, C.A. McKinnon, D.L. Melchior, Lipid domains in the ram sperm plasma membrane demonstrated by differential scanning calorimetry, Proc. Natl. Acad. Sci. USA 87 (1990) 6893–6896.