Effect of ethanol on the main phase transition of distearoylphosphatidylcholine

S. Cinelli, G. Onori *, A. Santucci

Istituto per la Fisica della Materia, Unità di Perugia and Dipartimento di Fisica, Università di Perugia, Via Pascoli, I-06100 Perugia, Italy

Received 16 January 2001; received in revised form 25 September 2001; accepted 5 November 2001

Abstract

A highly sensitive scanning microcalorimeter and a low scanning rate (0.1 K/min) has been used to determine the thermodynamic properties of the main phase transition of distearoylphosphatidylcholine (DSPC) in presence of ethanol in the 0–0.20 ethanol mole fraction ($x_2$) range. These measurements revealed previously unreported features of DSPC/water/ethanol phase behavior and visualized distinct changes in the main phase transition mechanism induced by addition of ethanol. It is found that the well known interdigitated phase in DSPC induced by ethanol above $x_2 = 0.01$ abruptly disappears at $x_2 = 0.12$. The abrupt change in the DSPC phase behavior at $x_2 = 0.12$ shows some analogies with the observed one relative to the conformation response of DNA molecule to solvent condition altered by varying amounts of ethanol. As inferred from previous adiabatic compressibility and IR data in water/ethanol mixtures, a correlation between changes in properties of the solvent, and the effect of ethanol on both the conformational properties of DNA and the main phase transition of DSPC clearly appears from the data. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Distearoylphosphatidylcholine; Microcalorimetry; Thermodynamic properties

1. Introduction

In recent years, our group has been interested in studying the role of the solvent in maintenance of macromolecular native state and in self-assembly processes [1,2]. The approach is to alter the composition of the solvent by adding small quantities of monohydric alcohols and to study the induced changes in the process under examination as a function of alcohol concentration. The aim is to establish correlations between the effects of alcohols on the conformational and dynamical properties of biomolecules and changes of properties of the solvent.

In this paper, in continuation of our previous work, we studied the thermal properties of distearoylphosphatidylcholine (DSPC) dispersions and the effect of ethanol on them. It is well established that the short chain alcohols such as methanol, ethanol and $n$-propanol induce interdigitation ($L_{pl}$ phase) in saturated phosphatidyl-
choline with identical chains, such as DSPC [3–11]. In the L$_{\beta}$ phase the acyl chains from opposing monolayers fully interpenetrate. Recently, there has been considerable interest in interdigitated phospholipid bilayers as models for biological membranes [12] and in conjuction with the application of liposomes in biomedicine [13]. Ethanol-induced lipid interdigitation in DSPC multilamellar vesicles is well documented [10]. DSPC is converted from a non-interdigitated gel state when the ethanol concentration is elevated from 0 to 25 mg/ml, corresponding to an ethanol mole fraction of $x_2 = 0.01$.

So far, ethanol effects on DSPC multilamellar vesicles have mainly been studied in a little range of ethanol concentration centered around the threshold concentration for interdigitation ($0 < x_2 < 0.025$). Our recent studies on properties of water/ethanol mixtures [1,14] indicate that for $x_2 \leq 0.06$ the alcohol molecules are essentially monodispersed. At higher alcohol concentration interactions between hydrophobic groups become increasing favorable and progressively replace the interactions of these groups with water molecules. In the water/DSPC/ethanol systems a modification in the DSPC/water and DSPC/ethanol interactions is also expected. To confirm this, here we extend the study of ethanol effect on phase behavior of DSPC to high ethanol concentrations where a clustering of ethanol molecules is observed.

Calorimetry is widely used in studying the phase behavior of lipid membrane since the phase transition is accompanied by excess heat capacity [15]. Recently, we used this technique to investigate the effect of replacing H$_2$O by D$_2$O on the phase transition properties of DSPC [16]. It has been shown that the use of a highly sensitive differential scanning calorimeter and a very low scanning rate was essential to making precise measurements and detecting small heat changes [16].

In this paper, a highly sensitive scanning microcalorimeter (DSC) and a low scanning rate (0.1 K/min) has been used to characterize the phase behavior and to determine the thermodynamic properties of the main phase transition of DSPC in presence of ethanol in the 0–0.20 ethanol mole fraction range. These measurements revealed pre-viously unreported features of DSPC/water/ethanol phase behavior and visualized distinct changes in the main phase transition mechanism induced by addition of ethanol.

2. Experimental section

The phosphatidylcholine (DSPC) was obtained from Sigma Chemical Co. (99% purity). Solutions containing 0.5 wt.% were prepared by adding DSPC to water/ethanol mixtures. The resulting suspension was incubated in the liquid crystalline phase above the main transition temperature for about 1 h with vortexing and then cooling to the gel phase. Subsequent sample aging at room temperature for 1 day ensured a good sample homogeneity.

DSC measurements were performed by means of a micro-DSC III (Setaram, France) on a sample (0.85 g) and scan rate of 0.1 K/min. The weights of the sample and reference cells were always matched. An excess power vs. temperature scan for the DSPC transitions was obtained by subtracting the power input of a thermal scan of water vs. water from the power input scan of the DSPC aqueous solutions vs. water. To correct for any dynamic effect of the response time of the calorimeter ($\sim 30$ s) on the shape of the excess heat capacity transition profile, the observed heat capacity signals were corrected by the Tian equation [16,17]. The transition temperature, $T_m$, is the temperature of the peak maximum and has been determined by the average temperature values of the three maximum heat capacity points of the transition. Areas under the transition peak divided by the number of moles of lipid in the sample yielded transition enthalpies, $\Delta H$, in kJ/mol.

3. Results and discussion

Upon heating, DSPC goes from the bilayer gel phase (L$_{\beta}$) to the rippled P$_\beta$ phase in a ‘pretransition’, and then through the main melting transition to the liquid–crystalline phase (P$_\beta$ to L$_\alpha$). Differential scanning calorimetry (DSC) has been
used in this work to determine the thermodynamic characteristics of the main melting transition of DSPC in the presence of ethanol. Both heating and cooling scans were employed for the studies of the main transition, and the scan rates were 0.1 K/min for both heating and cooling scans. Measurements were made at low ($x_2 < 0.025$) and high ($0.025 < x_2 < 0.20$) ethanol concentrations.

3.1. Low ethanol concentration ($x_2 < 0.025$)

Heating and cooling scans through the main transition of DSPC in water and in water/ethanol mixtures at selected values of $x_2$ are shown in Fig. 1. The DSC heating and cooling scans for DSPC in water for the main transition between the $P_{β}$ and $L_{α}$ phases are very narrow and there is only a little difference ($\sim 0.5$ K) between the transition temperature for heating and cooling scans. At ethanol mole fractions lower than the threshold concentration for interdigitation, $x_2 \sim 0.01$, both heating and cooling transitions are broader and more asymmetric respect to the corresponding ones in water and the difference between the transitions temperatures for heating and cooling scans is about 0.5 K (see Fig. 1, $x_2 = 0.008$).

In Fig. 1, results are also shown for heating and cooling scans at 0.015 ethanol mole fraction, a concentration at which the lipid is interdigitated prior to melting, so that the observed transitions are between the $L_{βI}$ and $L_{α}$ phases. There is a large hysteresis in this transition. The difference $ΔT$ between the transition temperature for heating and cooling was 2.1 K at this ethanol concentration. This hysteresis is characteristic of the induction of the $L_{βI}$ phase in saturated phosphatidylcholines by alcohols [4].

The main transition temperatures for heating and cooling for the $P_{β}$ to $L_{α}$ and the $L_{βI}$ to $L_{α}$ transition are plotted in Fig. 2a as a function of ethanol mole fraction. This figure shows that the main transition temperature decreased as a function of ethanol concentration for the $P_{β}$ to $L_{α}$ transition. Above the threshold concentration for interdigitation ($x_2 \sim 0.01$) the main transition temperature for heating scan increases while that for cooling scans decreases with increasing ethanol concentration. Fig. 2b shows the enthalpy of the main transition as a function of ethanol concentration. The $L_{βI}$ to $L_{α}$ transition has a higher enthalpy of $\sim 4$ kJ than the $P_{β}$ to $L_{α}$ transition. This result points out the greater stability of the interdigitated phase $L_{βI}$ respect to the gel phase $L_{β}$. 

![Fig. 1. DSC heating (upper trace) and cooling scans of main phase transition of DSPC in water and in water/ethanol mixtures at the indicated values of $x_2$.](image-url)
The present results are similar to those reported by Rowe and Cutrera [6] and corroborate all the principal conclusions reached in previous work.

### 3.2. High ethanol concentration

(0.025 < \(x_2\) < 0.20)

Fig. 3 shows the DSC heating and cooling scans at 0.06 and 0.20 ethanol mole fraction. The main transition temperature for heating and cooling scans are plotted in Fig. 4a as a function of ethanol concentration in the 0–0.20 \(x_2\) range. The main transition temperature for heating scans gradually increase with increasing ethanol concentration beyond \(x_2 = 0.025\) reaching a maximum at \(x_2 \sim 0.06\) (Figs. 3 and 4a) and then decreases with increasing \(x_2\). The main transition temperature for cooling scans gradually decreases down to a minimum at \(x_2 \sim 0.034\) and then increases with increasing \(x_2\). This behavior changes abruptly at \(x_2 = 0.12\) where the hysteresis characteristic of the induction of \(L_{\beta}\) phase disappears and the temperature for cooling scans goes to values slightly lower (\(\Delta T \sim 0.5\) K) respect to those of heating scans (Figs. 3 and 4a). At the same mole fraction an abrupt change in the shape of cooling transition from a broader profile to a very sharp, nearly isothermal one (Fig. 3) is also observed.

Fig. 4b shows the enthalpy of the main transitions as a function of ethanol concentration. A small gradual increase is observed from \(\Delta H = 43.5\) kJ/mol at \(x_2 = 0\) to \(\Delta H = 54\) kJ/mol at \(x_2 = 0.1\). Beyond this \(x_2\) value, on increasing \(x_2\) no significant variations in enthalpy values are detected.
where a transition in $\Delta T$ and in $[\theta]_{275}$ is observed, an anomalous behavior in several properties of water/alcohol mixtures themselves is also observed; in this region of composition a maximum is found in sound absorption [18], X-ray scattering [19] and light scattering [20,21]. Our recent studies [14] on properties of water/alcohol mixtures show that this anomalous behavior can be associated to some kind of ‘hydrophobic clustering’ of alcohol molecules in the water rich region of composition beyond a threshold value of alcohol concentration. In this concentration range a qualitative change in the nature of the interactions between solvent components occurs. Interactions between hydrophobic groups become increasingly favorable and progressively replace the interac-

![Fig. 4](image)

Fig. 4. (a) Main transition temperatures, $T_m$, and (b) enthalpy, $\Delta H$, of DSPC as a function of ethanol mole fraction. (●): heating scans. (○): cooling scans.

The abrupt change in the transition hysteresis at $x_2 = 0.12$ (Fig. 5a) shows some analogies with the recently observed one relative to the conformation response of DNA molecule to solvent conditions altered by varying amounts of ethanol [2]. These measurements reveal above a critical ethanol mole fraction a condensed form of macromolecule with unusually large ellipticity in the 250–300 nm range. A sharp transition is observed in $[\theta]_{275}$ (Fig. 5b) centered around $x_2 = 0.13$, almost the same values of ethanol concentration where an abruptly change in the transition hysteresis $\Delta T$ occurs (Fig. 5a).

The close similarity between the behavior of these systems and processes is striking. It is relevant that the only common feature is the same solvent system. Moreover, it is noteworthy that in the same concentration range,
tions of these groups with water molecules. In the water/DSPC/ethanol systems a modification in the water/DSPC and ethanol/DSPC interactions in this concentration range is also expected. In the L_{βII} phase the ethanol molecules anchor to the interface of bilayers by virtue of their polar moiety with the non-polar part of the molecule intercalating between the lipid acyl chains [11]. It can be supposed that on increasing $x_2$ the interactions between the hydrophobic groups of alcohol molecules progressively replace the interactions of these groups with the lipid acyl chains, thereby destabilizing the interdigitated phase of lipid bilayers.

The influence of solute molecules on the phase behavior of lipids is a means by which the membranes can be controlled and this fact gives biological interest to our results. Our observations are consistent with previous works [1,2,14] and indicate that the total mixing scheme of the alcohol water mixture, including the possibility of clustering effects, can have a dramatic influence on the phase behavior of phospholipid bilayers.

Acknowledgements

This work was supported in part by contributions from the Ministero dell’Università e della Ricerca Scientifica e Tecnologica.

References