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Phase behavior of dipalmitoylphosphatidylethanolamine in dimethylsulfoxide–water mixture☆

Lin Chen[a], Zhi-Wu Yu[a,*], Ying Feng[a], Xin Xie[a], Peter J. Quinn[b]

[a] *Laboratory of Chemical Biology, Department of Chemistry, Tsinghua University, Beijing 100084, China*
[b] *Division of Life Sciences, King's College London, 150 Stamford Street, London SE1 8WA, UK*

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Abstract

The phase behaviors of dipalmitoylphosphatidylethanolamine (DPPE) dispersed in dimethyl sulfoxide (DMSO) aqueous solutions were studied by using differential scanning calorimetry and X-ray diffraction methods. At low concentration of DMSO (mole fraction, $x < 0.2$), phase transition between lamellar-gel and lamellar liquid-crystal phase can be observed during heating/cooling cycles at a scanning rate of $5\,^{\circ}\mathrm{C\,min^{-1}}$. The formation of the stable lamellar-crystal phase from the metastable lamellar-gel phase needs long time incubation. At moderate concentration of DMSO ($0.2 < x < 0.37$), phase transition from lamellar-gel to crystalline phase can be detected during either cooling or heating at the same scanning rate. At high concentrations ($x > 0.37$), DMSO promotes remarkably the formation of lamellar-crystal phase of the lipid, resulting in phase transitions between lamellar-crystal phase and liquid-crystal phase during heating/cooling cycling. A phase diagram has been constructed over the entire concentration range of the mixed solvent. In addition, it was found that DMSO could decrease the lamellar repeat spacing of crystalline and liquid crystalline phase.

Keywords: DSC; DMSO; Phase diagram; Phosphatidylethanolamine; X-ray diffraction

1. Introduction

The lipid bilayer, being the main structural component of membranes, is responsible for many biological functions. Knowledge of the phase behavior of phospolipids is of importance in understanding the biofunctions of lipid assemblies in living cells. Dimethyl sulfoxide (DMSO) has been widely used as a cryoprotective agent, a cell fusogen, and an enhancer to membrane permeation. Thus the mechanism of DMSO affecting the properties of biological membranes has attracted the attention of many researchers [1–5]. Among them much attention has been put on the phase stability of phosphatidylcholines, in particular, dipalmitoylphosphatidycholine (DPPC) [6–8]. It was found that the chain-melting temperature of DPPC increased with increasing in DMSO concentration.

Phosphatidylethanolamines (PEs) are another major class of phospholipids present in biological membranes. They show some new features on phase behaviors in comparison with phosphatidylcholines. At low concentrations, DMSO was found to induce an increase in the main transition temperature and a decrease in the lamellar to nonlamellar phase transition temperature [4,9,10]. On the contrary, very few publications are seen in literature at higher DMSO concentration [10] and the impact of DMSO on the phase behaviors of PE is still not fully understood. The present work aimed at investigating the effect of DMSO on the thermotropic phase behavior of dipalmitoylphosphatidylethanolamine in DMSO/water mixtures over a wide range of molar ratios and temperature by employing differential scanning calorimetry (DSC) and X-ray diffraction techniques.

2. Experimental

Dipalmitoylphosphatidylethanolamine (DPPE) used in this study was purchased from Sigma Co. (Louis, MO, USA). Dimethyl sulfoxide of AR grade was bought from

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* Corresponding author. Tel.: +86-10-6279-2492; fax: +86-10-6277-1149.
E-mail address: yuzhw@mail.tsinghua.edu.cn (Z.-W. Yu).

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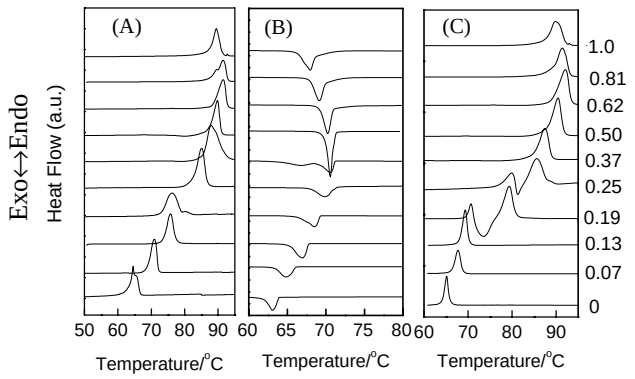

Fig. 1. DSC results of DPPE dispersed in different aqueous DMSO solutions during the first heating (A), cooling (B), and second heating (C) processes at a scanning rate of 5 °C min^{-1}.

Yili Fine Chemistry Co. (Beijing, China). Deionized water was used in all the experiments. The ratio of DPPE to mixed solvent was about 1:3 by weight for DSC and X-ray diffraction measurements to ensure the samples were fully solvated. The mole fractions of DMSO to water are 0, 0.07, 0.13, 0.19, 0.25, 0.37, 0.50, 0.62, 0.81, and 1, respectively. All the samples were prepared by several cycles of heating–cooling, interspersed with extensive vortex mixing, and then stored at −20 °C in a refrigerator before use. All samples were investigated by heating–cooling–heating process at a scan rate of 5 °C min^{-1} between 50 and 100 °C.

A Mettler-Toledo DSC821^{e} differential scanning calorimeter was used to examine phase behavior of the lipid dispersions. The signal time constant is 3 s, resolution is better than 0.7 μW, and noise (RMS) is less than 1 μW. X-ray diffraction was conducted at Station 8.2 of the Synchrotron Radiation Source of the SERC Daresbury Laboratory, using a method described previously [4]. Briefly, the lipid samples were mounted in a cell of 1 mm in thickness and sealed with thin mica windows. Measurements during first heating–cooling–second heating were performed at a rate of 5 °C min^{-1}. The small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) patterns were recorded simultaneously. Experimental data were processed using the OTOKO software package (EMBL, Hamburg, Germany).

3. Results

Illustrated in Fig. 1A–C are the DSC results of DPPE dispersed in different concentrations of DMSO during first heating, cooling, and second heating processes at a scanning rate of 5 °C min^{-1}. Fig. 1A shows that a single endothermic peak is seen in each of the samples during the first heating process. Phase transition temperatures, however, change remarkably along with the changes in the mole fraction of DMSO. This transition is assigned as lamellar-crystal (L_c) to liquid-crystal phase transition based on the previous conclusion of DPPE dispersion in pure water [11] and on the X-ray diffraction results of the lipid dispersion in DMSO (see later part of the text).

Upon cooling, most thermal traces show a singular transition except that at a mole fraction of 0.37, where a double exothermic transition was observed, which can be explained as the transitions from liquid-crystal to lamellar-gel (L_β) and then further to lamellar-crystal phase (Fig. 1B). A careful examination of the thermal curves at higher mole fractions shows obvious deviation of thermal traces from base line before the sharp transition peaks. This signals the two-step transition feature from liquid-crystal to crystal phase. At low DMSO concentrations, the phase transition sequence is the same as that in pure water, i.e. from lamellar liquid-crystal (L_α) to L_β.

During the second heating, most thermal curves show still a feature of singular transition peak. At low DMSO concentrations, it is the transition from L_β to L_α. At high DMSO mole fractions, it is the transition from crystal to liquid-crystal phase. It is interesting to note that a two endotherm–one exotherm pattern was detected at the intermediate DMSO concentrations, or mole fractions of 0.19 and 0.25, during the reheating process. The appearance of exothermic peak means obviously that more stable phase was formed during the heating process. Actually, the whole feature has been characterized as (1) partial transition from lamellar-gel to liquid-crystal phase, (2) the fast growth of crystal phase, and (3) the transformation to liquid-crystal phase [2,12].

In comparison of the DSC results during cooling and reheating, it can be concluded that lamellar-crystal phase can form much easily at high DMSO concentrations during cooling. At intermediate DMSO concentrations, crystal phase forms in such a slower manner that it can be recorded during either cooling or reheating. At low DMSO concentrations, transformation to crystal phase is too slow to be recorded during cooling/reheating cycles. Long time incubation of the L_β phase at a not-too-low temperature is needed for the crystallization of lipid assembly. These conclusions are supported by the phase transition enthalpies, which are listed in Table 1, together with the transition temperatures during cooling and heating processes. The ratios of enthalpy during cooling to that during first heating processes are shown in Fig. 2. When the DMSO mole fraction is lower than 0.30, the enthalpy ratios are almost the same, about 0.40. When the mole fraction is higher than 0.40, the ratios are greater than about 0.8 and becomes more and more close to unity along with increasing in DMSO concentrations. The smaller turning out of enthalpy during cooling suggests that the newly formed phases during cooling were metastable. It is the L_β phase at low DMSO concentrations as discussed above. At high DMSO concentrations, on the other hand, it could be an intermediate crystalline phase as will be discussed in the next paragraph.

X-ray diffraction measurement was undertaken to identify the phases of DPPE dispersed in pure DMSO. Data presented in Fig. 3 are the representative small-angle and

Table 1
Thermodynamic parameters of DPPE phase transitions at different DMSO concentrations at a scan rate of 5 °C min^{-1}

X_{DMSO}	First heating			Cooling			Heating		
	T (°C)	ΔH (J g^{-1})	Attribution	T (°C)	ΔH (J g^{-1})	Attribution	T (°C)	ΔH (J g^{-1})	Attribution
0	67.4[a]	105.2	$L_c \rightarrow L_\alpha$	64.1	−40.9	$L_\alpha \rightarrow L_\beta$	63.9	42.2	$L_\beta \rightarrow L_\alpha$
0.07	68.8[a]	110.8	$L_c \rightarrow L_\alpha$	66.2	−46.4	$L_\alpha \rightarrow L_\beta$	66.2	46.3	$L_\beta \rightarrow L_\alpha$
0.13	73.1[a]	175.2	$L_c \rightarrow L_\alpha$	67.9	−60.5	$L_\alpha \rightarrow L_\beta$	67.9	59.9	$L_\beta \rightarrow L_\alpha$
0.19	73.2[b]	140.3	$L_c \rightarrow L_\alpha$	69.2	−54.8	$L_\alpha \rightarrow L_\beta$	69.5	41.7	$L_\beta \rightarrow \alpha$
0.25	82.1[b]	169.8	$L_c \rightarrow L_\alpha$	71.4	−81.4	$\alpha \rightarrow L_\beta$	80.7	95.1	$L_\beta \rightarrow \alpha$
0.37	84.3[b]	112.3	$L_c \rightarrow L_\alpha$	71.4	−99.6	$\alpha \rightarrow L_{c1}$	84.3	109	$L_{c1} \rightarrow \alpha$
0.50	87.2[b]	125.8	$L_c \rightarrow L_\alpha$	71.4	−94.8	$\alpha \rightarrow L_{c1}$	87.7	121	$L_{c1} \rightarrow \alpha$
0.62	88.6[b]	117.4	$L_c \rightarrow L_\alpha$	71.2	−98.2	$\alpha \rightarrow L_{c1}$	88.5	119	$L_{c1} \rightarrow \alpha$
0.81	88.2[b]	110.2	$L_c \rightarrow L_\alpha$	70.3	−97.8	$\alpha \rightarrow L_{c1}$	88.9	109	$L_{c1} \rightarrow \alpha$
1	88.5[b]	105.5	$L_c \rightarrow L_\alpha$	68.9	−95.2	$\alpha \rightarrow L_{c1}$	87.6	91.1	$L_{c1} \rightarrow \alpha$

[a] Data from the initial heating.
[b] Data from the heating process after the samples were stored in refrigerator (−20 °C) for about half a year.

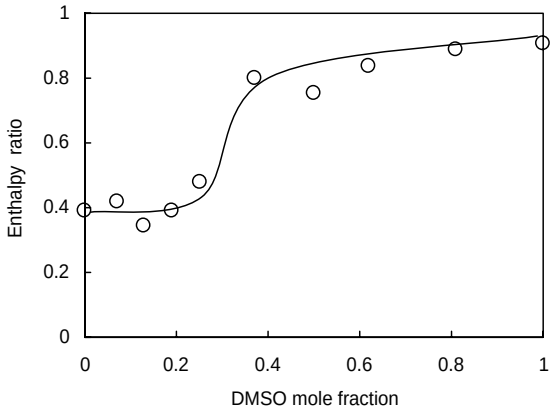

Fig. 2. The ratios of enthalpy during cooling process to that during first heating at different DMSO mole fractions.

wide-angle X-ray scattering patterns recorded at three temperatures. For the two sets of data recorded at 40 °C, during both the first heating (curve a) and second heating (curve c), two scattering maxima located at 5.10 nm ($S \approx 0.196\,\text{nm}^{-1}$)

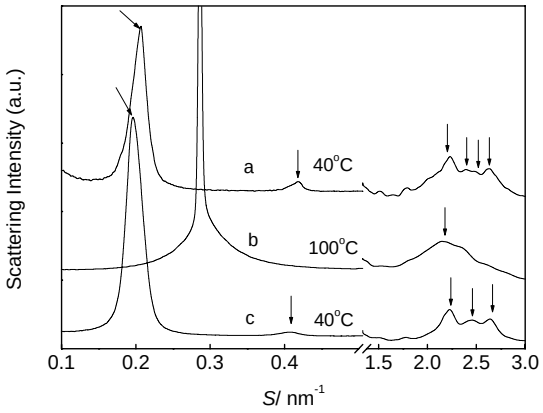

Fig. 3. Plots of SAXS (left) and WAXS (right) intensity profiles versus reciprocal spacing for a dispersion of DPPE in excess DMSO. Data were recorded at 40 °C (curve a) and 100 °C (curve b) during first heating and at 40 °C during cooling (curve c), respectively. See text for details.

and 2.45 nm ($S \approx 0.408\,\text{nm}^{-1}$) were detected in the SAXS. The S values showed a ratio of about 1:2, suggesting that the DPPE molecules were arranged in a lamellar structure at the temperature. The two WAXS curves show a little difference. The WAXS pattern at the beginning of the heating process at 40 °C is characterized by an extensive diffraction peak centered at about 0.45 nm and three additional peaks in the wide-angle pattern centered at about 0.565, 0.402, and 0.367 nm. The values are in agreement with lamellar crystalline phase (L_c) reported by Williams et al. [13]: 0.46, 0.58, 0.395, and 0.370 nm. As for the WAX pattern at 40 °C (curve c in Fig. 3) after cooling down from 100 °C, a sharp diffraction peak centered at about 0.45 nm, and two additional peaks at about 0.409 and 0.378 nm were detected. The pattern is also characteristic of a crystalline phase with slight difference from that of the normal L_c. We assign this phase as L_{c1}. At 100 °C (curve b in Fig. 3), the two scattering peaks in SAXS changed to one peak centered at about 3.51 nm. In the WAXS region, only one broad scattering peak was detected around 0.46 nm, which is typical of liquid-crystalline phase (α). The phase transition is also characterized by an abrupt decrease in the lamellar repeat spacing from 5.10 to 3.51 nm.

4. Discussion and final remarks

The effect of DMSO on the phase behavior of DPPE has been examined by the methods of DSC and X-ray diffraction. Increasing concentrations of DMSO in the solvent mixture results in a progressive increase in phase transition temperature during both first and second heating. The data have been used to construct a phase diagram as depicted in Fig. 4. For DPPE dispersed in excess water, phase behaviors have been studied in detail by time-resolved X-ray diffraction and calorimetry methods. The transition temperature from lamellar liquid-crystalline phase (L_α) to inverted hexagonal phase (H_{II}) was found to take place at about 118 °C [14] (data not shown in the figure). When the mole

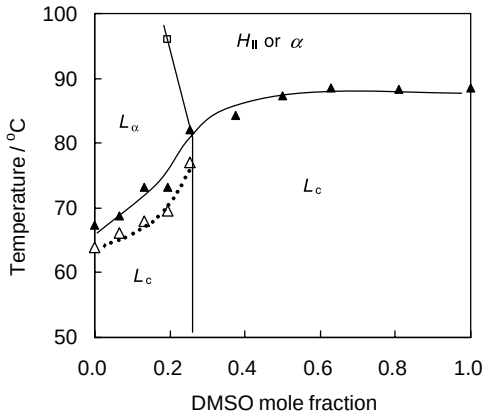

Fig. 4. Partial phase diagram of DPPE dispersed in DMSO aqueous solutions. Phase boundaries were determined from DSC results during heating scans at 5°/min. (▲) Transition from lamellar crystalline (L_c) to liquid-crystalline (α or H_{II}) phase; (△) transition from lamellar-gel (L_β) to lamellar-liquid crystalline (L_α) phase; (□) value from [3].

fraction of DMSO in the solvent mixture reaches to 0.2, the phase transition temperature decreases to 96 °C [3]. This has been incorporated in Fig. 4. One remaining question is the identification of the high temperature structure of DPPE dispersed at high concentration aqueous DMSO solutions. As demonstrated in Fig. 3, the WAXS pattern suggests that the structure is at liquid-crystal state. The SAXS pattern, however, is unable to identify if the phase is lamellar or hexagonal. Thus we denote it as α phase in the phase diagram.

As can be seen from the phase diagram, phase transition temperature from the stable phase L_c to L_α (α) increases with increasing in DMSO concentration at low mole fractions. The temperature is almost unchanged when the mole fraction is higher than 0.6. Phase transition temperature of L_β to L_α is a few degrees lower than that from L_c to L_α phase.

The molecular mechanism and kinetics of the formation of lamellar-crystalline phase is of particular interest because it may relate to phase segregation in biomembranes and freezing/chilling injury of living organisms. First, in pure water or low concentration aqueous DMSO solutions, the L_c phase cannot form from liquid-crystalline phase directly at a cooling rate of 5 °C min^{-1} or even 1 °C min^{-1}. Instead, only L_β phase can be obtained upon cooling. To allow the metastable L_β phase relax into the most stable L_c phase, long time incubation would be necessary. Second, when DMSO mole fraction is between 0.3 and 0.6, mixtures of crystalline phase and gel phase will be obtained upon cooling. The L_c phase can form relatively quickly by incubating the mixture at temperatures slightly below the L_c to α phase transition temperature. With increasing in DMSO concentration, the formation of L_c phase becomes more and more easier. When DMSO mole fraction reaches to 0.6, the lamellar-crystal phase can form directly during cooling. However, this is not the most stable L_c phase yet as evidenced by the enthalpy ratio values shown in Fig. 2. Molecular rearrangement is still going on at this stage to further fine-adjust the structure. The relative difficulty of the L_c phase formation over the entire mole fraction range could be explained by the dehydration effect of DMSO [6,8,15], which is believed to be crucial to the formation of a crystal phase. The dehydration effect is also reflected in the X-ray diffraction data. In pure DMSO, the repeat d-spacing of DPPE L_c phase is 5.10 nm. This value is much smaller than that in pure water, i.e. 5.54 nm as reported by Yao et al. [11] Similarly, the d-spacing of liquid-crystalline phase of the dispersion in DMSO, 3.51 nm, is also much smaller than that in pure water (5.22 nm) [11].

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