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Insights into the dynamics of DMSO in phosphatidylcholine bilayers

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Abstract

The solvation effects of dimethyl sulfoxide (DMSO) on the phase stability of dimyristoylphosphatidylcholine (DMPC) have been fully characterized using differential scanning calorimetry (DSC) and fluorescence spectroscopy with 1,6-diphenyl-1,3,5-hexatriene (DPH). The temperatures of the sub-, pre-, and main transitions of DMPC were found to increase linearly with increasing mole fraction of DMSO up to mole fraction $X = 0.13$ DMSO/H_2O. Beyond $X = 0.13$, the pre-transition peak started to merge with the peak representing the main transition. Simultaneously, the subtransition peak began to disappear as its transition temperature also decreased. At $X = 0.18$, with both the subtransition and pre-transition absent, the main transition between the planar gel and the liquid-crystalline phase was observed at 30.3°C. Transition enthalpy values indicated that the subgel, planar gel and rippled gel phases are most stable at $X = 0.11$, 0.16 and 0.20 DMSO/H_2O, respectively. This demonstrates that DMSO exerts distinct effects on each respective phase and corresponding transition. Temperature-dependent fluorescence emission scans show an increase in hydration as the system proceeds from the subgel phase all the way to the liquid-crystalline phase and correlated well with the effects of DMSO on the transition temperatures of DMPC observed in our calorimetry data. Initial observations for the sub- and main transition are further confirmed by fluorescence anisotropy using DPH as a probe. The results illustrate the differences in the microviscosity of each phase and how DMSO affects the phase transitions. Ultimately, our results suggest the most likely mechanism governing the biological actions of DMSO may involve the regulation of the solvation effects of water on the phospholipid bilayer.

Keywords: Dimyristoylphosphatidylcholine; Dimethyl sulfoxide (DMSO); Subtransition; Solvation effects; Planar gel phase; Fluorescence spectroscopy

Abbreviations: DMSO, dimethyl sulfoxide; DMPC, dimyristoylphosphatidylcholine; DSC, differential scanning calorimetry; DPH, 1,6-diphenyl-1,3,5-hexatriene; L_c, subgel phase; $L_{\beta'}$, planar gel phase; $P_{\beta'}$, rippled gel phase; L_α, liquid-crystalline phase; MLVs, multilamellar vesicles; *T*-jump, temperature jump

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1. Introduction

Dimethylsulfoxide (DMSO) is widely applied in organic chemistry, chemical technology, cell biology and medicine because of its many important biological properties [1]. One important fact of DMSO is that it is widely utilized in life sciences as a cryoprotectant to stabilize biological structures at low temperatures. The cryoprotective property of DMSO was discovered more than 35 years ago by Lovelock and Bishop and concentrations ranging up to 40 wt.% are now customarily used in the long-term cryopreservation of a variety of cells, tissues and organs.

Although the effects of DMSO are well known and studied, the molecular mechanisms by which DMSO exerts its cryopreservation effects are still unknown and are a question of fundamental importance in molecular pharmacology. Its interactions with natural and model membranes have been extensively investigated in order to elucidate the mechanism of physiological action of the compound at the molecular level [1–3]. It has been found that research on its modulation of membrane structure and stability may reveal insight into the molecular mechanisms of DMSO. It was found that in phospholipid bilayers, DMSO can produce new phases [2] and change their stability [3]. DMSO also has a significant effect on the repeat spacing difference [1] and modifies hydration forces [3].

Of interest to this study is the interaction of DMSO with an aqueous dispersion of DMPC, or dimyristoylphosphatidylcholine. DMPC is a saturated phosphatidylcholine that has hydrocarbon chains of 14 carbons and forms multilamellar vesicles (MLVs) in aqueous suspension. The MLVs consist locally of stacks of bilayers separated by fairly uniform layers of water, with the hydrophobic acyl chains in the interior and the hydrophilic head groups facing the aqueous environment. In addition to the hydrophobic effect, the bilayers are also stabilized by van der Waals' forces in the acyl chains and hydrogen bonding between the polar head groups and aqueous environment. DMPC is known to form a lamellar liquid-crystalline (L_α) phase at ~ 24°C, two lamellar gel phases (planar gel, $L_{\beta'}$, and rippled gel, $P_{\beta'}$) separated by a pre-transition at ~ 15°C, and the recently discovered subgel (L_c) phase with a corresponding subtransition ($L_c \rightarrow L_{\beta'}$) at ~ 8°C [4]. With insight into and clarification of the subgel dynamics of phospholipid bilayers [2,4], we are now finally able to fully characterize the solvation effects of DMSO in this system.

In this work, we present the model membrane system of DMPC MLVs and the effects of adding DMSO to these membranes as revealed by DSC and fluorescence spectroscopy via 1,6-diphenyl-1,3,5-hexatriene (DPH). For formation of the subgel phase, the *T*-jump (temperature-jump) protocol, which was first implemented by Tristram-Nagle et al. for dipalmitoylphosphatidylcholine (DPPC) [5,6], was utilized. This method was used to sort out any multiplicity of the peaks of the phase transitions that were encountered in past studies [7–9], and thus allow for consistent comparison of data, because it was discovered that the *T*-jump method results in a more perfect subgel structure, evident in the smaller volume found with dilatometry [5].

2. Materials and methods

2.1. Materials

Dimyristoylphosphatidylcholine, or 1,2,-dimyristoyl-*sn*-glycero-3-phosphocholine, was used as obtained from Avanti Polar Lipids Inc (Alabaster, AL, USA). No chromatographic tests for purity were performed; however, the narrowness of the main transition thermograms on DSC scans provided a guarantee that the lipid purity was comparable to the value claimed of $> 99\%$. The fluorescent probe DPH was purchased from Molecular Probes (Eugene, OR, USA). Stock solutions of varying concentrations of DMSO (Sigma, St. Louis, MO, USA) in double-deionized water were prepared and then used to hydrate the lipid samples. All chemicals were used without further purification.

2.2. Differential scanning calorimetry

DSC scans were carried out using a Calorime-

try Sciences Corporation (Provo, UT) multi-cell DSC-HT model 4100. Data obtained from the DSC were then converted into heat capacity (μJ/°C) and plotted against temperature (°C). Jandel Scientific Software PEAKFIT 4.0 was used to determine the peak temperatures and integrate the area of the peaks to calculate the corresponding enthalpy values.

MLVs of pure DMPC were prepared by weighing 10 mg of DMPC into ampoules designed specifically for the DSC. The sample size of 10 mg was utilized because it was known that these subtransition peaks would be easier and much more accurate to characterize with a larger sample. The lipid was then hydrated with 150 μl of respective concentrations of aqueous DMSO solutions. Samples were incubated at approximately 15°C above the main transition temperature of pure DMPC (~24.6°C) for 1 h and vortexed vigorously approximately every 10 min to ensure proper hydration.

To prevent condensation and abnormal baselines, the samples were incubated in the calorimeter that was kept dry with nitrogen gas initially at −5°C for 18 h. The second half of the *T*-jump protocol was then instituted with a growth period of 2 h at 0°C. The 2-h growth period for the *T*-jump protocol was determined to be optimal by Ruocco and Shipley [10]. Their results via X-ray diffraction have shown that the lipid rearrangement involved in subgel formation after nucleation is complete after 2 h. After incubation, heating (upscans) and cooling (downscans) scans on the samples were run twice from 0 to 40°C, at a rate of 10°C/h.

2.3. Fluorescence spectroscopy via DPH

The fluorimeter used was an ISS K2 multifrequency cross-correlation phase and modulation fluorimeter. A xenon arc lamp operating at 15 A was used as the light source. Temperature control was provided by a Neslab RTE-111 water bath with a microprocessor.

The amount of DPH incorporated so that it would not substantially interfere with the bilayer corresponded to a lipid/DPH mole ratio of 500:1 [11]. Stock solutions of DPH of appropriate concentrations for DMPC were prepared in chloroform and 50 μl of this stock solution was added to an 8-mg PC sample. The majority of the chloroform solvent was then removed by slow evaporation in a fume hood and the final traces were removed under high vacuum overnight. Finally, the samples were hydrated with 10 ml of the DMSO stock solutions. The final concentration of the fluorescence samples was 0.8 mg/ml DMPC MLVs in varying aqueous concentrations of DMSO. A low concentration of phospholipid was used in order to minimize any light scattering. The same hydration method for DSC was followed for the fluorescence samples. To avoid fluorescence quenching, all samples were purged with nitrogen gas for at least 1 min before being transferred to a quartz cuvette containing a stirring bar and into the fluorimeter. The temperature of the fluorimeter cell was taken down to −5°C by the water bath and the cell was continuously saturated with a flow of nitrogen gas to prevent condensation.

The *T*-jump protocol was also instituted for the measurement of temperature-dependent fluorescence emission spectra as the samples were incubated in an external bath at −5°C for 18 h and then jumped to 0°C for 2 h for further growth of the subgel phase in external baths. The fluorescence emission of the DMPC samples was then monitored from 0 to 40°C at a scan rate of 10°C/h. Anisotropy measurements, where the emission polarizer was adjusted both parallel and perpendicular to the excitation polarizer, were then performed from 0°C until reaching the respective liquid-crystalline phase for each sample. Excitation and emission wavelengths used for DMPC were 356 and 427 nm, respectively. Data analyses of the emission spectra were carried out using Novell QUATTRO PRO and Microsoft EXCEL.

3. Results and discussion

3.1. Calorimetric trends from the effects of DMSO on DMPC

DSC is a powerful tool in the study of phase transitions of PCs. The DSC scans in Fig. 1 illus-

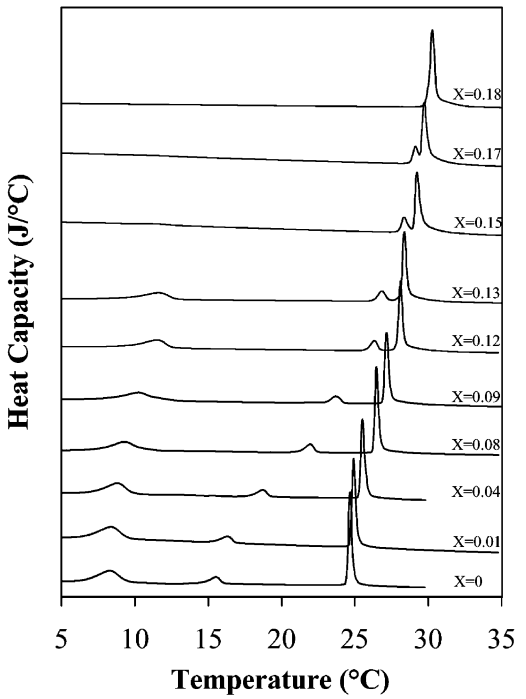

Fig. 1. Stack plot of DSC thermograms of DMPC with varying mole fractions of DMSO showing the overall solvation effects.

trate the overall solvation effects of increasing mole fractions of DMSO on the DMPC phases and their respective phase transitions. In the absence of DMSO, we observed the subgel-to-planar gel transition at 8.20°C, the pre-transition to be at 15.53°C, and the sharp transition from the rippled-gel to liquid crystalline phase at 24.67°C under the experimental conditions used. The transition temperatures of the different transitions are also plotted vs. mole fraction of $DMSO/H_2O$. In Fig. 2, it is evident that the main transition temperatures increase linearly with increasing mole fraction of DMSO, but the changes of the sub- and pre-transition temperature are more complex.

From $X = 0.13$ to 0.17, the subtransition temperature decreases until the subtransition peak is completely abolished (Fig. 2). The subgel phase seems to be stabilized by DMSO up to $X = 0.13$, where the subgel phase reaches its peak of stabilization. Beyond $X = 0.13$, the subgel phase is destabilized and eventually completely abolished after $X = 0.17$. It is also at $X = 0.13$ that the pre-transition peak begins to merge with the main transition peak and eventually disappears at $X = 0.18$. Both of these phenomena support the stabilization of the planar gel phase and lead to the direct transition between the planar gel and the liquid-crystalline phase as the main transition at 30.3°C.

Transition enthalpy values are plotted as a function of mole fraction of DMSO in Fig. 3. The enthalpy of the subtransition slowly increased until reaching a peak at $X = 0.11$, and subsequently decreased until the peak completely disappeared after $X = 0.17$. The pre-transition enthalpy, however, remained constant until $X = 0.12$. Beyond this concentration, the enthalpy for the pre-transition increased until reaching a maximum at $X = 0.16$. The enthalpy then rapidly decreased and the peak was no longer observable after $X = 0.18$. The main transition enthalpy values did not vary with increasing mole fraction of $DMSO/H_2O$ until after $X = 0.15$, when it started to increase. After reaching the highest point at $X = 0.20$, there was a drastic decreasing trend observed with the enthalpy of the main transition. We observed similar trends in the broadness of the peaks along with these trends in transition enthalpy values.

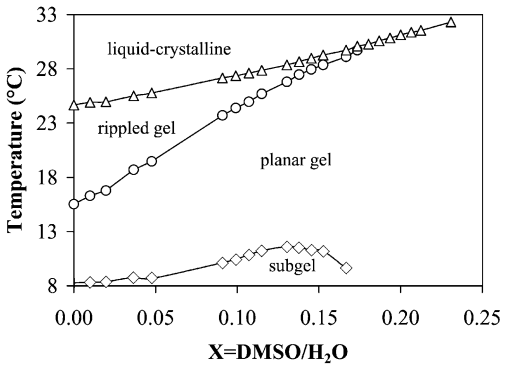

Fig. 2. Effects of DMSO on DMPC phase transition temperatures. This phase diagram illustrates the different stabilization effects, where ◇ = subtransition temperatures, ○ = pre-transition temperatures and △ = main transition temperatures.

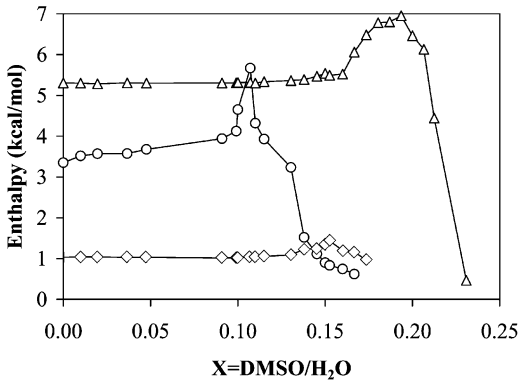

Fig. 3. Effects of DMSO on: ◇ DMPC subtransition; ○ pre-transition; and △ main transition enthalpy.

The effect of DMSO on the phase transition temperatures and transition enthalpy of DMPC vs. mole fraction of DMSO demonstrates that the planar gel phase is stabilized at higher mole fractions of DMSO at the expense of the rippled gel and subgel phases. The differences in the phase stabilization effects by DMSO result in the trends that we observed in our calorimetric data.

3.2. Temperature-dependent fluorescence emission spectroscopy

Fluorescence spectroscopy is one of the most powerful techniques utilized in the fields of biochemistry and molecular biophysics. The use of fluorescence spectroscopy in this study is based on monitoring the sensitive non-polar fluorescent probe DPH within the bilayer system to analyze the dynamics of the phase transitions that it undergoes. In the temperature-dependent emission scans, the phases exhibit distinct spectroscopic features and are qualitatively interpretable in terms of the structural organization of the bilayer polar/apolar interface and the packing of the hydrocarbon chains. In the case of DMPC, since DPH preferentially partitions into more disordered regions of the bilayer [12], it is primarily located close to the center of the bilayer and acts as an indicator of the hydration state of the phospholipid bilayer. Thus, constant intensity values would represent a specific phase, while phase transitions would correlate to changes in relative fluorescence intensity of the DPH probe.

Fig. 4 shows the temperature scans for the DMPC/DMSO samples. Since the subgel phase is dehydrated while the liquid-crystalline phase is fully hydrated, we observed a continual stepwise decrease in the fluorescence intensity with increasing temperature as the phases undergo transitions from the subgel phase all the way to the liquid-crystalline phase. Beyond the main transition, the fluorescence continued to decrease, unlike the rest of the phases, where they are represented by constant intensity values. This is due to additional hydration of the bilayer system while still in the liquid-crystalline phase. The spectral data correlated well with the DSC data, with increases in transition temperatures up to $X = 0.13$; the subtransition temperature decreases after $X = 0.13$, until ultimately disappearing after $X = 0.17$. The spectra illustrate that the subtransition initially becomes more cooperative as a result of the stabilization of the subgel phase by DMSO, which is in agreement with what was observed for the DSC data in Fig. 1. The temperature range of the subtransition decreases from $X = 0$ to 0.09, thus indicating a sharper, more cooperative phase transition. The spectra also show that the subgel phase is then destabilized, and the subtransition gradually disappeared between $X = 0.09$ and 0.13. The temperature range of the subtransition increases from $X = 0.09$ to 0.13, but later decreases at $X = 0.16$. This correlates to a broadening of the subtransition accompanying the eventual abolishment of the transition. At $X = 0.18$, the subtransition and pre-transition are no longer present in the spectrum and the only transition observed is the main transition.

3.3. Steady-state fluorescence anisotropy

Steady-state fluorescence anisotropy measurements of DPH have frequently been utilized to extrapolate the microviscosity of the phospholipid bilayer interior [12]. If DPH is placed in an environment with optimal motional freedom, its probe motion can be assumed to be that of a free, isotropic rotator and its emission anisotropy would

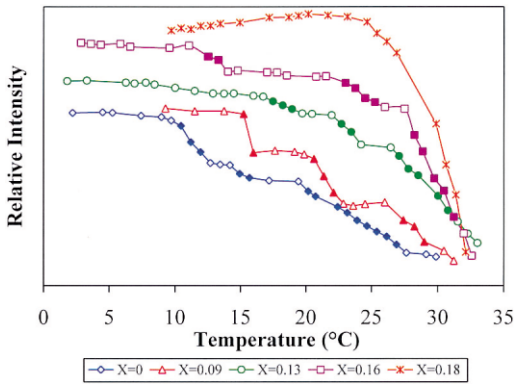

Fig. 4. Stack plot of fluorescence emission spectra of DMPC with varying mole fractions of DMSO. Open symbols represent regions of phases, while closed symbols are phase transitions.

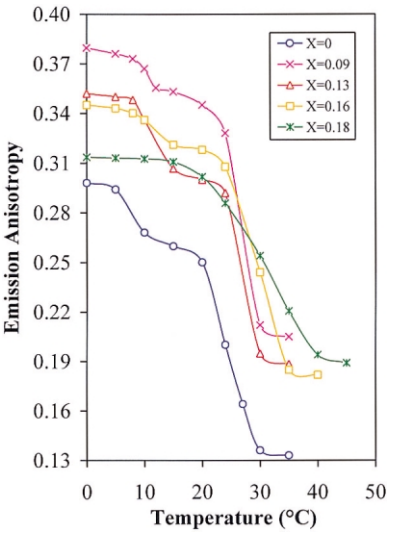

Fig. 5. Fluorescence anisotropy plots of DMPC with increasing amounts of DMSO.

approach zero. Thus, the fluorescence anisotropy of DPH in the anisotropic medium of phospholipid bilayers reflects the rotational diffusion of the DPH molecules embedded in the hydrocarbon region and indicates the microviscosity of the hydrocarbon interior of the bilayer. An increase in the fluorescence anisotropy correlates to less rotational freedom for the probe, or in other words, lower fluidity of the acyl chains.

We observed a significant difference between the anisotropy of the subgel and the planar gel phases and that the subtransition is shown as a decrease in emission anisotropy [4]. The pre-transition is not represented, due to the fact that there is little or no difference between the anisotropy of the planar gel and the rippled gel phases. The microviscosity of these two phases are similar, or even identical, because there is minimal or no change in the disorder of the acyl chains accompanying the pre-transition, only a rearrangement of polar groups and bound water of neighboring phospholipid molecules to form the 'ripple' characteristic of the rippled gel phase [13,14].

The anisotropy plots in Fig. 5 illustrate the effect of increasing amounts of DMSO on DMPC. These data show that the transition temperatures extrapolated from the decreases in microviscosity indicated the same trends as observed in our calorimetry data. The ranges of the anisotropy

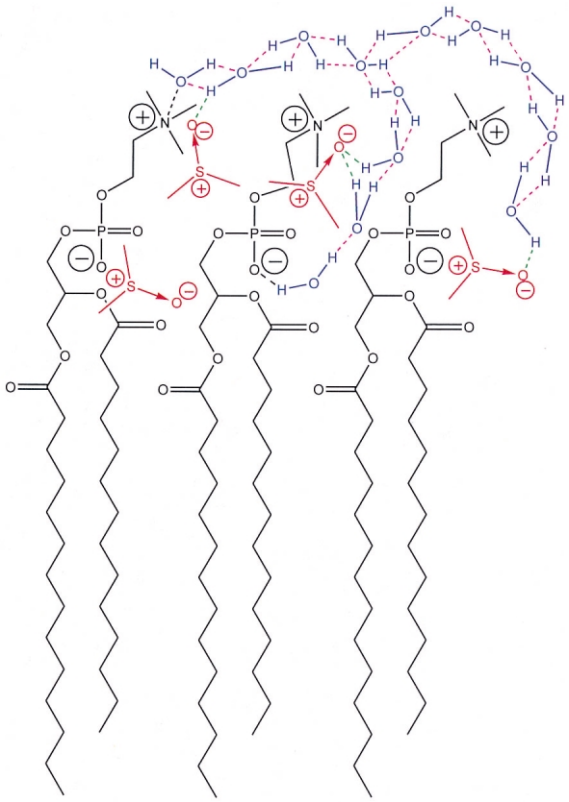

Fig. 6. Possible interactions between DMSO and DMPC.

changes for the subtransition and main transition with the varying concentrations of DMSO reflect the cooperativity of each respective phase transition as a sharper or broader transition. The anisotropy values in the subgel, gel, and liquid-crystalline phases are in no particular order with increasing mole fractions of DMSO. Thus, the plots shown in Fig. 5 reflect relative anisotropy changes at each DMSO concentration. In good agreement with our calorimetry data (Fig. 1) and emission scans (Fig. 4), the subtransition became sharper with an increase in mole fraction of $DMSO/H_2O$ from $X = 0$ to 0.09. Between $X = 0.09$ and 0.13, the subtransition started to broaden. The subtransition became further broadened at $X = 0.16$ and finally disappeared in the $X = 0.18$ anisotropy plot. The cooperativity of the main transition stayed relatively the same until $X = 0.16$, where a decrease in cooperativity is illustrated by a broader main transition. Ultimately, the main transition becomes much broader at $X = 0.18$, and thus correlates to the previous DSC and fluorescence spectroscopy data reported in this paper.

3.4. Possible interactions between DMSO and DMPC

The solvation effects of water play an important role in the properties of cell membranes. Our data suggest that the most likely mechanism governing the biological actions of DMSO involves the regulation of these solvation effects. Fig. 6 illustrates the possible interactions between DMSO and DMPC, where the magenta dashed lines represent the hydrogen bonding between water molecules, the green dashed lines are the hydrogen bonding between DMSO and water, and the black dashed lines indicate water molecules that are bound to the phospholipid head groups. It is known that DMSO forms a network of hydrogen bonds with the polar surface of the membrane that stabilizes the bilayer by preventing an increase in orientational disorder of the hydrocarbon chains and serves as an extra barrier to the membrane's transition to the liquid-crystalline phase [15]. This would account for the increase in the temperature of the main transition.

X-Ray diffraction data by Yu and Quinn [1] have shown that the thickness of the phosphatidylcholine bilayers decreases in the presence of DMSO as it penetrates between the polar head groups of the phospholipid molecules. The work of Cevc and Marsh [13] and Marek [16] showed that three conditions are required for the formation of the rippled gel phase: (1) intrinsic steric repulsion between the lipid head groups; (2) mildly weak packing on the acyl chains of lipid molecules; and (3) relatively weak interactions between the head groups. With reorientation of the polar groups and bound water in the pre-transition [14], the DMSO molecules penetrate between the phospholipid molecules at the interfacial region and electrostatic interactions between the head groups are enhanced due to the partially charged portions of DMSO interacting with the positive choline group and negative phosphate group of the head group region, as shown in Fig. 6. This leads to an intrinsic repulsion between the molecules being reduced, thus destabilizing the rippled gel phase and in turn stabilizing the planar gel phase. The drastic effect of DMSO on the pre-transition is evident in our phase diagram (Fig. 1).

Since the main driving force behind the formation of the subgel phase is the dehydration of the head group region [4,10,14], DMSO initially stabilizes the subgel phase by adsorbing onto the membrane surface and displacing water molecules to enhance the interfacial dehydration (Fig. 6). This leads to the initial increasing trend observed for the subtransition temperature. As a consequence of the interbilayer water of the MLVs having been squeezed out, the phosphate head groups in the subgel phase are packed tightly and are relatively immobile [14]. With higher mole fractions of DMSO, the head group interactions and packing are disrupted by the penetrating DMSO molecules as they cause greater head group separations. Thus, the head groups are not able to pack tightly enough to induce subgel formation. This leads to destabilization the subgel phase and the eventual abolishment of the subtransition.

These studies on the properties of the model membrane system of DMPC in the presence of

DMSO as a cosolvent showed that the solvation effects affecting the DMPC phospholipid bilayer are altered and affected by DMSO, which accounts for the observations in our data. Our present DSC and DPH fluorescence investigations have shown that DMSO does exert a direct effect on the major characteristics of model membranes. It is most possible that the cryopreservation effects of DMSO may be due to these physical membrane effects.

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References

[1] Z.W. Yu, P.J. Quinn, Solvation effects of dimethyl sulfoxide on the structure of phospholipid bilayers, Biophys. Chem. 70 (1998) 35–39.
[2] S. Tristram-Nagle, S. Moore, H.I. Petrache, J.F. Nagle, DMSO produces a new subgel phase in DPPC: DSC and X-ray diffraction study, Biochim. Biophys. Acta 1369 (1998) 19–33.
[3] Z.W. Yu, P.J. Quinn, Phase stability of phosphatidylcholines in dimethylsulfoxide solutions, Biophys. J. 69 (1995) 1458–1463.
[4] H.H. Chang, P. Dea, Subgel studies of dimyristoylphosphatidylcholine bilayers, Biochim. Biophys. Acta, submitted.
[5] S. Tristram-Nagle, M.C. Wiener, C.P. Yang, J.F. Nagle, Kinetics of the subtransition in dipalmitoylphosphatidylcholine, Biochemistry 26 (1987) 4288–4294.
[6] S. Tristram-Nagle, R.M. Suter, W.J. Sun, J.F. Nagle, Kinetics of subgel formation in DPPC: X-ray diffraction proves nucleation-growth hypothesis, Biochim. Biophys. Acta 1191 (1994) 14–20.
[7] M.A. Singer, L. Finegold, Permeability and morphology of low temperature phases in bilayers of single and mixtures of phosphatidylcholines, Biochim. Biophys. Acta 816 (1985) 303–312.
[8] W.G. Wu, L.G. Chong, C.H. Huang, Pressure effect on the rate of crystalline phase formation of L-alpha-dipalmitoylphosphatidylcholines in multilamellar dispersions, Biophys. J. 47 (1985) 237–242.
[9] J.R. Silvius, M. Lyons, P.L. Yeagle, T.J. O'Leary, Thermotropic properties of bilayers containing branched-chain phospholipids. Calorimetric, Raman, and ^{31}P NMR studies, Biochemistry 24 (1984) 5388–5395.
[10] M.J. Ruocco, G.G. Shipley, Characterization of the subtransition of hydrated dipalmitoylphosphatidylcholine bilayers: kinetic, hydration and structural study, Biochim. Biophys. Acta 691 (1982) 309–320.
[11] M.F.N. Rosser, H.M. Lu, P.K. Dea, Effects of alcohols on lipid bilayers with and without cholesterol: the dipalmitoylphosphatidylcholine system, Biophys. Chem. 81 (1999) 33–44.
[12] B.R. Lentz, Use of fluorescent probes to monitor molecular order and motions within liposome bilayers, Chem. Phys. Lipids 64 (1993) 99–116.
[13] G. Cevc, D. Marsh, Polymorphism of the bilayer membranes in the ordered phase and the molecular origin of the lipid pretransition and rippled gel lamellae, Biochim. Biophys. Acta 464 (1991) 59–69.
[14] E. Okamura, J. Umemura, T. Takenaka, Fourier transform infrared/attenuated total reflection study on the subtransition of hydrated dipalmitoylphosphatidylcholine multilayers, Vib. Spectrosc. 2 (1991) 95–100.
[15] V.I. Gordeliy, M.A. Kiselev, A.V. Pole, J. Teixeira, Lipid membrane structure and interactions in dimethyl sulfoxide/water mixtures, Biophys. J. 75 (1998) 2343–2351.
[16] J. Marek, A difference in the shapes of intermolecular potentials between phospholipid model molecules, relation to the ripple phase, J. Theor. Biol. 159 (1992) 417–429.