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Investigation of DESO/LIPID membranes interaction by X-Ray scattering

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Abstract The influence of diethyl sulfoxide $(C_2H_5)_2SO$ (DESO) on the structure of the phospholipid DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) membranes was investigated over a wide range of DESO molar concentrations $0.0 \leq X_{DESO} \leq 1.0$. The dependences of structural parameters - the repeat distance d of the multilamellar membranes and the thickness on the molar concentration X_{DESO} in the gel and liquid-crystalline phases were determined. The main phase transition temperature T_m for DPPC multilayers was identified at different molar concentrations of DESO. A comparative analysis of the data for the system DPPC/H_2O/DESO with the data previously published for the system DPPC/H_2O/DMSO was done.

1. Introduction

Cryoprotective agents are important tools of many biological, biophysical and medical studies. The mechanism of interaction of these cryoprotectants is different. Thus, the natural sugars provide protection without penetration into the cell, polyalcohols and glycerol enter the cell and reduce the likelihood of the formation of ice crystals. The greatest interest is the cryoprotectants, such as dimethyl sulfoxide, that can replace water molecules associated with cellular elements, and affect the structure of water. DMSO is widely used in cell biology, cryobiology, pharmacology, medicine and agriculture [1, 2]. However, the rapid development of molecular and cell biology poses a challenge to find new solvents and cryoprotectants that can maintain the integrity of the cells in the process of freezing - thawing. One of relatively new interesting and efficient cryoprotectants is diethyl sulfoxide (DESO).

First results obtained with DESO are quite encouraging. First of all, DESO shows strong interaction with water, even stronger than that of DMSO [3]. The investigation of the ability of DESO to act as an effective cryoprotectant on *E. coli* survival confirms that DESO, more than DMSO, is able to penetrate living tissues without causing significant damage [4]. It was shown that the temperature of thermal denaturation of *Human Serum Albumin* decreases in the presence of DESO. The hydrophobic interaction of sulfoxides with the protein surface is one of the probable reasons for this. The electron spectroscopy investigations indicate that for DESO this effect is observed at lower concentrations if compared with DMSO [5]. The fluorescent measurements testify that the formation of hydrophobic layer protects the protein against irreversible structural changes under low temperatures.

It is well known that a lipid bilayer is the structural basis of biological membranes. Consequently, the lipid bilayer influences or/and determines many properties of the membranes, in particular, proteins embedded in it [6]. Therefore it is not surprising to see intensive studies of the cryoprotectors,

especially DMSO, on the structure and properties of lipid membranes [7 - 13]. The effect of these solvents on the structure of lipid membranes is different. For example, the main transition temperature T_m of the DPPC phospholipids dispersed in trehalose decreases from 42 to 24ºC [14]. Glycerol significantly decreases T_m with increasing concentration [15]. In contrast, DMSO increases T_m of DPPC and DMPC multilayers [10, 12, 16].

DESO is a solvent which is somehow similar to well studied DMSO. The aim of the present study is to investigate the influence and interaction of diethyl sulfoxide with phospholipid DPPC *(1,2-dipalmitoyl-sn-glycero-3-phosphocholine*) membranes. The present paper presents the first results in the investigation of the membrane structure of DPPC multilayers in an x-ray diffraction experiment at a wide range of concentrations of DESO $0.0 \leq X_{DESO} \leq 1.0$. The study was carried out for all mole fractions X_{DESO} of DESO/water in the temperature interval from 20°C to 85°C.

2. Materials and methods

2.1. Materials
DPPC (over 99% pure) was obtained from SERVA (Germany).
DESO (over 99% pure) was produced by MERK (Darmstadt, Germany).
Water (18 MV/cm) was obtained with the help of Millipor.

2.2. Sample preparation
The given amount of DESO in a DESO/water solution was mixed with the lipid in 1:1 w/w solvent/lipid ratio and placed in an x-ray borosilicate capillary with a diameter of 1.5 mm and wall thickness of 0.01 mm (W. Muller, Berlin, Germany). The mixture was spun down by centrifugation. The capillary was hermetically sealed, and the specimens were held at a higher temperature than that of the phase transition for several hours.

2.3. Experimental technique
Wide-angle measurements were performed at the X-ray diffractometer DRON - 4 adapted for membrane studies. The X-ray diffractometer has a Cu-anode tube as a radiation source. Bragg-Brentano focusing and two slits are placed at a distance of 10 mm from each other, close to the output window of the tube for x-ray beam formation. An additional slit is placed just before the sample to suppress the scattering from the boundaries of the second slit. A single detector is used to detect the scattered x-rays. A θ-2θ scan is carried out in the horizontal plane (with the rotation of the detector around the vertical axis). Measurements were made at a Ni-filtered CuKα radiation x-ray source with a wavelength of 1.54 Å. The specimen container was kept at a constant temperature by using a liquid thermostat to the precision of $\pm 0.5\,^{\circ}C$ and controlled by a thermocouple.

The repeat distance of the multilayer structure was determined from the position of diffraction peaks using the Bragg equation ($2d\sin(\theta) = \lambda$).

3. The results and discussion

3.1. The effect of DESO concentration on the lipid membranes in a gel phase
A typical diffraction pattern of the DPPC multilayers in DESO/water solution is presented in Figure 1. It corresponds to the DESO molar concentration $X_{DESO} = 0.2$ and the temperature $T = 20\,^{\circ}C$.

The dependence of the repeat distance d of DPPC membranes on the DESO molar concentration at the temperature $T = 20\,^{\circ}C$ is shown in Figure 2. The repeat period d drastically changes in the region of DESO concentrations $0 \leq X_{DESO} \leq 0.1$ in the $L_{\beta'}$ phase. The value d decreases from 64.2 Å for DPPC multilayers in pure water to 58.6 Å at $X_{DESO} = 0.1$ (see Table 1). A similar behavior in the change of the repeat distance takes place in a well investigated DPPC/DMSO/water system in the gel phase [12,

17]. An analogous pattern was observed in the study by SANS for the DMPC-d54 multilamellar membranes in the water/DMSO solution [18].

The second region corresponds to DESO concentrations $0.1 \leq X_{DESO} \leq 1.0$. In this case the repeat distance d decreases monotonously from 58.6 Å at X_{DESO} = 0.1 down to 57.1 Å at X_{DESO} = 1.0 as presented in Table 1. Thus we can assume that a further increase in the concentration of DESO does not result in structural changes in multilayer DPPC membranes in the gel $L_{\beta'}$ phase.

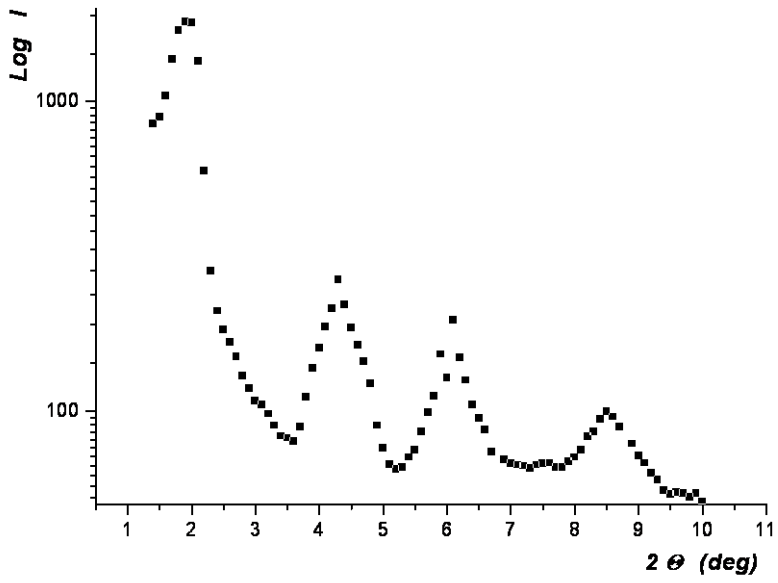

Figure1. X-ray diffraction pattern from DPPC/DESO/water mixture at the DESO concentration X_{DESO} = 0.2 and T = 20°*C*.

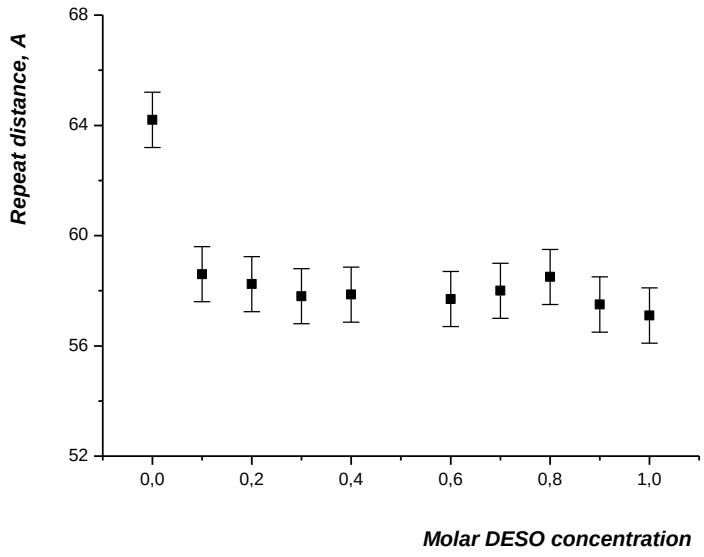

Figure2. The dependence of the repeat distance d of DPPC membranes on the DESO concentration at T = 20°*C*.

DESO as well as DMSO reduces the repeat distance of lipid membranes in contrast to other solvents (e.g. glycerol [19]). The influence of DESO on phospholipid membranes is similar to that of DMSO up to X_{DESO} = 0.9. However, in contrast to DMSO, it does not show a drastic reduction of the repeat distance (for DMSO it occurs at X_{DMSO} = 0.9) [12, 18]. A significant decrease of the repeat distance in the case of DMSO is associated with the transition of the lipid membranes to the interdigitated phase in which hydrocarbon chains of one monolayer of the bilayer penetrate to the other [20 - 22]. In case of DESO we did not observed any transition to the interdigitated $L_{\beta I}$ phase.

3.2. Effect of the DESO concentration on the lipid membranes in a liquid phase

The investigation of the DESO influence on the multilayer DPPC membranes was carried out at T= 70ºC which is above the temperature of the main phase transition T_m for all molar concentrations X_{DESO} (see Figure 4 or Table 1). The repeat distance *d* decreases abruptly with increasing DESO fraction up to X_{DESO} = 0.1 as in the gel phase and then continues to decrease slowly.

Table 1. X-Ray data relative to the heating and cooling processes of DPPC multilayers in H_2O/DESO and H_2O/DMSO mixtures with increasing amounts of sulfoxides.

Molar concentration	Repeat distance (Å)				Temperature of main phase transition (ºC)		
	Gel phase		Liquid phase				
X	d_{DESO} ±2Å	d_{DMSO}[a]	d_{DESO} ±2Å	d_{DMSO}[a]	T_{m_DESO}	T_{m_DMSO} [a]	T_{m_DMSO}[b]
0	64.2	64.0	57.5	58.0	41.0	40.7	40.7
0.1	58.6	57.8	46.0			44.0	44.0
0.2	58.2	58.0	41.1	50.0	48.0	49.0	49.0
0.3	57.8	57.6				50.0	50.0
0.4	57.9	57.0	40.8		56.0		
0.5		57.0		47.0		52.0	52.0
0.6	57.7	57.5			58.0	56.0	52.0
0.7	58.0	57.0					52.0
0.8	58.5	56.0	38.2	44.0	59.0	55.0	52.5
0.9	57.5	51.8				58.0	52.0
1	57.1	52.0		41.0	60.0	78.0	

[a] V. I. Gordeliy et al., Biophys.J. 75, 2343 (1998)

[b] M.A. Kiselev, JINR Preprint (2003)

In the first region $0 \leq X_{DESO} \leq 0.1$ the d value is changed by 11 Å. This is twice as much as the observed changes for the system DPPC/H_2O/DESO in the gel phase. With a further increase of the DESO concentration, d decreases monotonously from 46 Å at X_{DESO} = 0.1 down to 38 Å at X_{DESO} =

0.8. The data for the DESO mole fraction dependence of the repeat period in the liquid L_α phase are included in Table 1.

It is clear that the influence of DESO and DMSO solvents on the multilayer DPPC membranes in the L_α phase is similar but, nevertheless, displays important differences.

3.3. Temperature dependence of membrane structural parameters.

The temperature dependence of the DPPC membrane repeat distance at the molar concentration of DESO X_{DESO} = 0.2 is presented in Figure 3. It is clear that structural changes of the system under these conditions take place at T = 48 ºC. The repeat distance d = 58.24 Å is almost unchanged up to 45 ºC. However, a sharp drop of the repeat distance occurs at T_f = 48 ºC, which corresponds to the transition of the lipid membranes to the liquid L_α phase.

Temperature-dependent changes of the repeat period d at the DESO concentration X_{DESO} = 0, 0.2, 0.4, 0.6, 0.8, 1.0 have also been measured. In all cases we observed a similar qualitative change in the membrane structural parameters with the temperature variation.

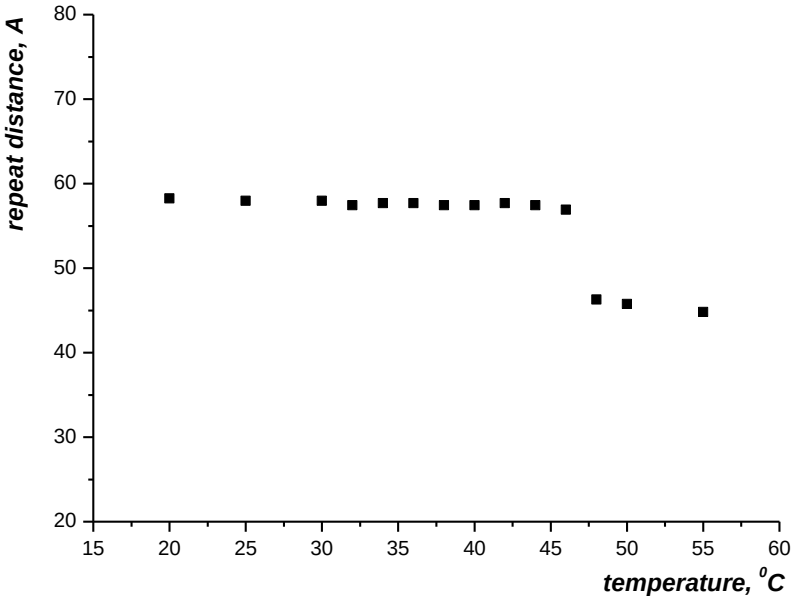

Figure 3. The temperature dependence of the DPPC membrane repeat distance at the DESO concentration X_{DESO} = 0.2.

The DESO concentration dependence of the phase transition temperature is presented in Figure 4. The temperature of the main phase transition T_{m_DESO} as T_{m_DMSO} (see Table 1) increased with increasing molar DESO concentration. The dependence of the phase transition temperature on the DESO concentration is exponential. One can see two characteristic behavior regions. In the first region at ratios $0 \le X_{DESO} \le 0.4$ one observes a fast, almost monotonic increase of the temperature of the main phase transition from 41ºC for DPPC in pure water to 56 ºC at X_{DESO} = 0.4 with increasing DESO concentration. In the second region ($0.4 \le X_{DESO} \le 1.0$) the T_{m_DESO} value changes slightly. We have not observed any transition to the interdigitated phase as shown by Gordeliy et al for the DPPC/DMSO/water system [12].

The fit of the experimental data shows that the dependence of the phase transition temperature on the DESO concentration has a non-linier (exponential) character.

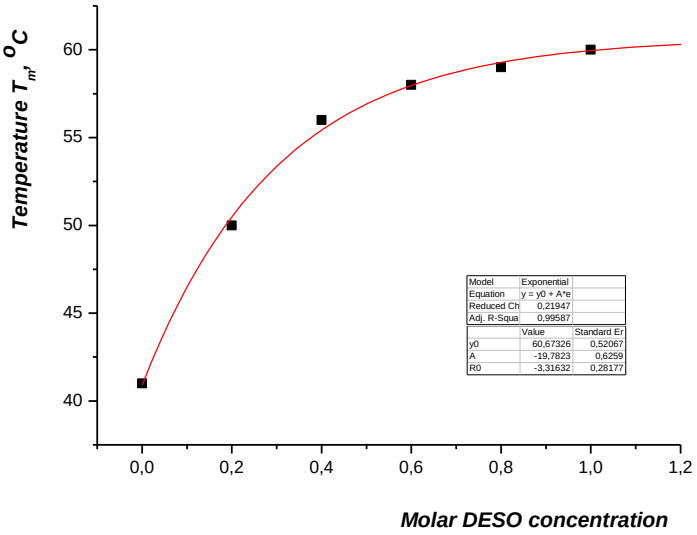

Figure 4. The dependence of the phase transition temperature vs. DESO concentration.
■ - experimental data
— - fitting line

5. Conclusions

The repeat distance d in a gel phase decreases sharply (by 6 Å) with an increase in the mole fraction of DESO from 0 up to 0.1. With a further increase in the DESO concentration, d decreases monotonously from 58.6 Å at X_{DESO} = 0.1 down to 57.1 Å at X_{DESO} = 1.0. As in the gel phase, the repeat distance d in a liquid phase decreases abruptly with increasing DESO fraction up to X_{DESO} = 0.1 and then continues to decrease slowly.

The influence of both sulfoxides DMSO and DESO on the repeat distance of the DPPC phospholipid membranes is similar for $X_{DESO} < 0.9$. However, phospholipid membranes turn into an interdigitated phase with increasing amounts of dimethyl sulfoxide [10, 12, 18]. We have not observed a transition of the lipid membranes at X_{DESO} = 1.0 to the ripple phase at T = 20°C for the DPPC/H_2O/DESO system. This fact distinguishes diethyl sulfoxide from other well-known cryoprotectants. For instance, lipid chains interdigitate in the presence of the mixture of glycerol, ethylene glycol or methanol with water [16, 19].

The phase transition temperature changes from 41°C at X_{DESO} = 0 to 56°C at X_{DESO} = 0.4. The value of T_m changes slightly for the DESO concentration $0.4 \leq X_{DESO} \leq 1.0$. This region corresponds to a smooth decrease of the repeat distance. This means that diethyl sulfoxide has significant impact on the structure of phospholipid multilayers at the concentration ratio $0 \leq X_{DESO} \leq 0.4$. The temperature of the main phase transition is higher in case of DMSO at low concentrations than in DESO. A similar situation was observed in the DSC study on the effect of DMSO and DESO on phospholipid liposomes [23]. In the presence of low amounts of both sulfoxides ($\leq$ 0.2) the main effect was an increase in the transition temperatures, which was more enhanced in the presence of DMSO. Our data support the hypothesis that diethyl sulfoxide should best behave as a cryoprotector at high concentrations. Our results show that at $X_{DESO} \geq 0.4$ the structure of the lipid multilayers does not change. This means that strong hydrophobic interactions take place between DPPC multilayers and diethyl sulfoxide. Perhaps this is the reason for the formation of a stable ice layer near the surface of the lipid surfaces and explains the effect of better protection of biological membranes during freezing as was shown earlier [4].

The comparison of the effect of the DESO and DMSO influence on the membrane structural parameters and phase transition shows that DESO changes properties of lipid bilayers similarly but in a more ‘soft’ way than DMSO. We assume that it is less stressful for biological membranes and this can be one of the reasons for higher survival of the cells when DESO is used for cryoprotection.

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