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SURFACE, THIN FILMS

Dimethyl Sulfoxide-Induced Dehydration of the Intermembrane Space of Dipalmitoylphosphatidylcholine Multilamellar Vesicles: Neutron and Synchrotron Diffraction Study

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Abstract—Small-angle neutron scattering spectra of a polydispersed population of dipalmitoylphosphatidylcholine (DPPC) unilamellar vesicles in heavy water in the presence of dimethyl sulfoxide (DMSO) are analyzed by means of the separated form-factor method. An increase in the mole fraction of DMSO in water from 0 to 15% was shown to lead to an increase in the thickness of the bilayer to the characteristics repeat distances of DPPC multilamellar membranes. This fact is indicative of dehydration of the intermembrane space and a steric contact between adjacent DPPC bilayers at 15% mole fraction of DMSO.

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INTRODUCTION

Investigations of the structures and properties of phospholipid membranes as multilamellar (Fig. 1) and unilamellar (Fig. 2) vesicles in water excess are of great interest because they can provide an understanding of the fundamental principles of organization and functioning of biological and artificial lipid membranes. This knowledge can be of practical use in biochemistry and pharmacology.

Small-angle neutron scattering (SANS) using wavelengths in the range from 1 to 10 Å is the main experimental technique for investigations of unilamellar vesicle systems in large water excess. The X-ray dif-

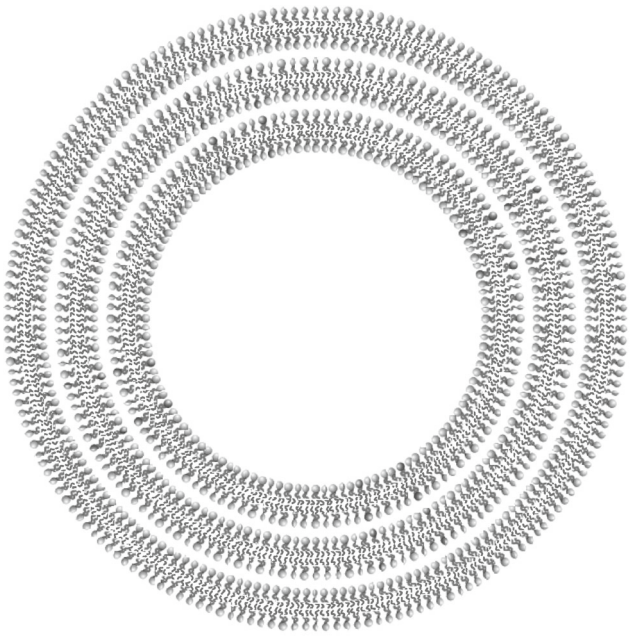

Fig. 1. Multilamellar lipid vesicle in water excess.

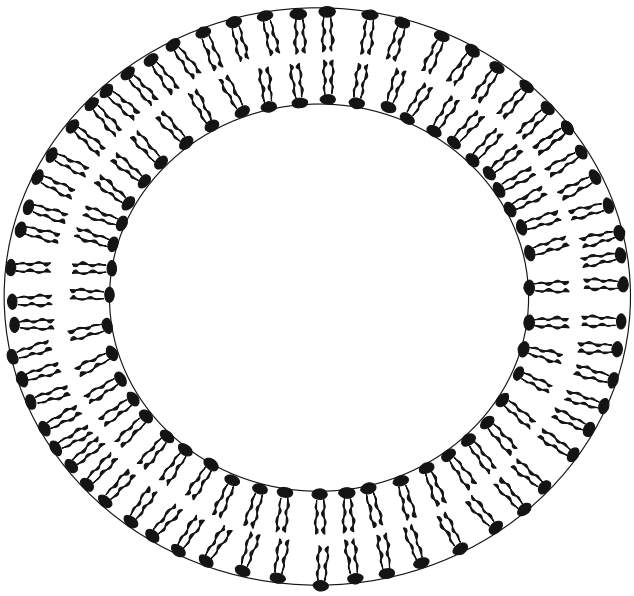

Fig. 2. Unilamellar lipid vesicle in water excess.

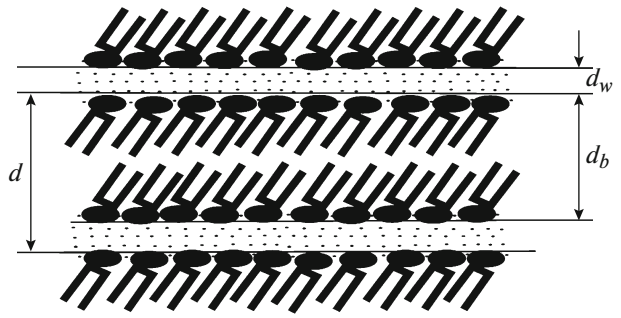

Fig. 3. Schematic representation of the gel phase of the DPPC multilamellar membrane. The multilamellar structure is composed of a bilayer of DPPC molecules with the membrane thickness d_b and the solvent (water) interlayer with the thickness d_w. The DPPC membrane repeat distance is $d = d_b + d_w$. In the gel phase, hydrocarbon tails are inclined at an angle of 28° to the membrane surface.

fraction using synchrotron radiation sources is widely applied to study the structures of multilamellar vesicles in water excess. Extensive studies of vesicle systems with different chemical compositions are being performed by Russian and foreign researchers [1, 2].

The separated form-factor (SFF) method developed in [3, 4] is an efficient tool for analysis of SANS spectra that provides the data on the structures and properties of unilamellar vesicle systems in large water excess. The SFF method was successfully applied to analyze the structure of dimyristoylphosphatidylcholine unilamellar vesicles in different phase states of the lipid bilayer [4] at different concentrations of aqueous sucrose solutions [5] and to study multicomponent vesicle systems based on ceramide 6, which model the outermost layer of mammalian skin (*Stratum Corneum*) [6–8]. In [5] the SFF method was adapted to the analysis of small-angle X-ray scattering data. The description of the SFF method is included in *Advances in Planar Lipid Bilayers and Liposomes* [9].

In this work, we studied the structure of dipalmitoylphosphatidylcholine (DPPC) unilamellar vesicles in heavy water (D_2O) in the presence of deuterated dimethyl sulfoxide (DMSO) by means of SANS.

Aqueous DMSO solutions (10–50%) are widely used in medicine as a local anti-inflammatory drug and a local anesthetic. Dimethyl sulfoxide is added to foam compositions in order to enhance transdermal drug delivery. This compound penetrates the skin within seconds. Another important field of application of DMSO is its use as a cryoprotectant. Dimethyl sulfoxide is added to cell media to prevent cell damage during freezing. A 10% aqueous solution of DMSO can be used for the safe freezing of cells and their storage at liquid nitrogen temperature.

It was shown [10] that the amount of free water in the intermembrane space of DPPC multilamellar vesicles decreases with an increase in the DMSO concentration (Fig. 3). However, the question about the influence of DMSO on the thickness of the DPPC lipid bilayer d_b, which was calculated from the SANS data using the Guinier approximation, remained open. As demonstrated in [4], the analysis of small-angle scattering data using the Guinier approximation gave an underestimated value of the thickness of the lipid bilayer.

The goal of this work is to study in more detail the DMSO-induced dehydration of the intermembrane space of DPPC based on the analysis of small-angle neutron scattering spectra using the SFF method.

We analyze the SANS data for DPPC unilamellar vesicles and compare these data with the results of investigation of DPPC multilamellar vesicles in aqueous DMSO solutions by synchrotron-based small-angle X-ray scattering.

EXPERIMENT AND SAMPLE PREPARATION

Small-angle neutron scattering spectra of DPPC unilamellar vesicles were measured on a YuMO small-angle neutron spectrometer at the IBR-2 pulsed reactor (Laboratory of Neutron Physics of the Joint Institute for Nuclear Research, Dubna) at two positions of the detector corresponding to sample-to-detector distances of 13.17 and 4.38 m. The measurements were performed at a sample temperature of 20°C corresponding to gel-phase DPPC.

Dipalmitoylphosphatidylcholine multilamellar vesicles were prepared by dissolution of 1 wt % DPPC in an aqueous (D_2O) solution of deuterated DMSO. The mole fractions of DMSO in mixtures of DMSO and D_2O were 0, 5, 10, and 15%. Dipalmitoylphosphatidylcholine unilamellar vesicles were prepared by repeated extrusion of a solution of multilamellar vesicles through polycarbonate filters with a pore diameter of 500 Å using a LiposoFast Basic extruder (Avestin, Ottawa, Canada) [11].

SEPARATED FORM-FACTOR MODEL

As mentioned above, the structure of a vesicle system was studied by the SFF method developed for the analysis of the SANS data for unilamellar vesicles [3, 4]. As opposed to other known approaches (e.g., the shell model [12], the Guinier approximation [13]), the SFF method enables the employment of every appropriate function for the description of the neutron scattering length density distribution along an axis normal to the membrane plane $\rho(x)$, thus extending the possibility of modeling of the bilayer structure.

The SFF method is described in detail in [3–5]. This approach is based on the factorization of the expression for the scattering amplitude giving the equation for a macroscopic cross-section of a monodispersed vesicle population

$$\frac{d\Sigma}{d\Omega_{\text{mon}}}(q) = nF_s(q,R)F_b(q,\rho)S(q,R), \quad (1)$$

where F_s and F_b are the form-factors of the infinitely thin spherical shell with radius R and the lipid bilayer, respectively:

$$F_s(q,R) = \left(4\pi\frac{R^2}{qR}\sin(qR)\right)^2,$$
$$F_b(q,\rho) = \left(\int_{-d_b/2}^{d_b/2}\Delta\rho(x)\cos(qx)dx\right)^2. \quad (2)$$

Here, n is the number of vesicles per unit volume, d_b is the thickness of the lipid bilayer, $\Delta\rho(x) = \rho(x) - \rho_{D2O}$ is the neutron scattering length density difference between the lipid bilayer $\rho(x)$ and heavy water ρ_{D2O} (contrast), and $S(q, R)$ is the Debye structure factor [14]:

$$S(q,R) = 1 - \frac{8V_\nu}{\nu}\left(\frac{\sin(2qR)}{2qR}\right), \quad (3)$$

where V_ν is the vesicle volume, $\nu = 1/n$.

Taking into account the polydispersity of the vesicle system described by the Schulz distribution

$$G(R,\langle R\rangle) = \frac{R^m}{m!}\left(\frac{m+1}{\langle R\rangle}\right)^{m+1}\exp\left[-\frac{(m+1)R}{\langle R\rangle}\right], \quad (4)$$

where $\langle R\rangle$ is the average vesicle radius and m is the polydispersity coefficient, the macroscopic cross-section $d\Sigma(q)/d\Omega$ of a polydispersed vesicle population takes the form

$$\frac{d\Sigma}{d\Omega}(q) = n\frac{\int_{R\min}^{R\max}\frac{d\Sigma}{d\Omega_{\text{mon}}}(q,R,\langle R\rangle)G(R,\langle R\rangle)dR}{\int_{R\min}^{R\max}G(R,\langle R\rangle)dR}, \quad (5)$$

where the integration limits $R_{\min}$ and $R_{\max}$ were assumed to be equal to 100 and 1000 Å, respectively. Taking into account the incoherent background I_B and the spectrometer resolution [15], the macroscopic cross-section takes the final form

$$I(q) = \frac{d\Sigma}{d\Omega}(q) + \frac{1}{2}\Delta^2\frac{d^2}{dq^2}\left[\frac{d\Sigma}{d\Omega}(q)\right] + I_B. \quad (6)$$

Here Δ is the second moment of the resolution function of the spectrometer calculated as proposed in [16].

In the calculations, the neutron scattering length density of the lipid bilayer $\rho(x)$ was approximated using a model shown in Fig. 4 (the step approximation model), which takes into account the neutron scattering length density difference between the hydrophilic region ρ_H and the hydrocarbon chain region of the bilayer ρ_{CH}. As it was shown in [4], the step approximation $\rho(x)$ is suitable for the analysis of vesicle systems in the gel phase. It provides an explicit formula for the form-factor F_B in Eq. (2), thus simplifying calculations. In this case, the lipid bilayer is divided into two parts: the hydrophilic part, which includes the polar head region, and the hydrophobic part consisting of nonhydrated hydrocarbon chains. In the calculations, the thickness of the hydrophobic region D was kept equal to 20 Å. The results of calculations are summarized in Table 1.

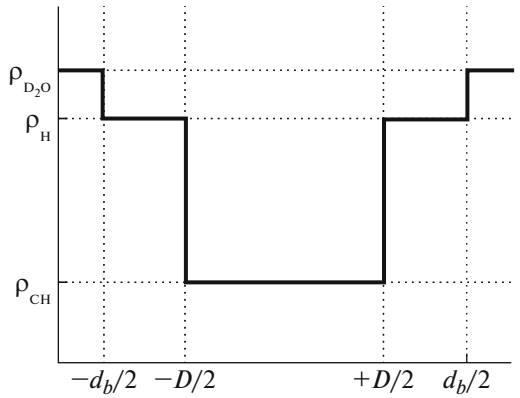

Fig. 4. Step approximation of the neutron scattering length density distribution (cm^{-2}) across the lipid bilayer: ρ_{D2O}, in heavy water; ρ_H, in the hydrophilic region; ρ_{CH}, in the hydrocarbon tail region; d_b is the thickness of the lipid bilayer (Å); D is the thickness of the hydrophobic region of the lipid bilayer (Å).

We used the following fitting parameters: the average vesicle radius $\langle R\rangle$, the polydispersity coefficient m, the number of vesicles per unit volume n, the incoherent background I_B, and parameters determining the structure of the bilayer, such as the bilayer thickness d_b and the neutron scattering length density in the hydrophilic polar head region ρ_H. The scattering length density of hydrocarbon chains was kept fixed at $\rho_{CH} = -0.36 \times 10^{10}$ cm^{-2}. The neutron scattering length density in aqueous DMSO solutions was assumed to be equal to $\rho_{D2O} = 6.37 \times 10^{10}$ cm^{-2}. The rms deviation of the vesicle radius (polydispersity) from the average radius $\langle R\rangle$ was calculated by the equation $\sigma = \sqrt{\frac{1}{(m+1)}}$.

The calculations were performed with the DFUMIL program from the JINRLIB library (http://www.jinr.ru/programs/jinrlib), which implements the generalized least-squares method. The χ^2 values given in Table 1 were calculated by the equaiton

$$\chi^2 = \frac{1}{N-k}\sum_{i=1}^{N}\left(\frac{\frac{d\Sigma}{d\Omega}(q_i) - \frac{d\Sigma}{d\Omega_{\text{exp}}}(q_i)}{\delta(q_i)}\right)^2, \quad (7)$$

Table 1. Parameters of a 1 wt % population of DPPC vesicles in aqueous DMSO solutions at concentrations of 0–15%

Mole fraction of DMSO, %	$\langle R \rangle$, Å	m	σ, %	d_b, Å	χ^2	ρ_H, 10^{10} cm^{-2}
0	280 ± 2	17.5 ± 1.3	23.3	52.7 ± 0.4	3.7	2.8 ± 0.5
5	257 ± 2	17.7 ± 1.1	23.2	55.9 ± 0.5	3.6	3.2 ± 0.5
10	257 ± 2	18.0 ± 1.0	22.9	56.9 ± 0.6	3.8	3.1 ± 0.5
15	262 ± 2	20.3 ± 1.2	21.7	57.2 ± 0.5	5.5	3.1 ± 0.5

$\langle R \rangle$ is the average vesicle radius, m is the polydispersity coefficient in the Schulz distribution (4), σ is the polydispersity of the vesicle population, d_b is the thickness of the lipid bilayer, χ^2 is the discrepancy between calculated and experimental curves, and ρ_H is the neutron scattering length density in the hydrophilic region of the lipid bilayer.

where $\delta(q_i)$ are errors in experimental data, N is the number of experimental data points, and k is the number of fitting parameters.

RESULTS AND DISCUSSION

The results of fitting of the calculated SANS spectra to experimental curves using the SFF method are presented in Fig. 5. The corresponding values of the fitting parameters are given in Table 1. These data demonstrate that the SFF model provides a good agreement with experimental data.

At the minimum concentration (5%) of DMSO in water, the vesicle radius is 257 Å, which is 23 Å smaller than the radius of DPPC vesicles in D_2O (280 Å). A further increase in the DMSO concentration leads to an increase in the vesicle radius up to 262 Å at 15% mole fraction of DMSO. This value is 18 Å smaller than the vesicle radius in D_2O (280 Å). Dimethyl sulfoxide has almost no effect on vesicle polydispersity. The neutron scattering length density in the hydrophilic region ρ_H is not changed, within experimental error, depending on DMSO. The neutron scattering length density in heavy water is similar to that in deuterated DMSO. Therefore, the replacement of heavy water molecules in the polar head region by DMSO molecules should not lead to a change in ρ_H.

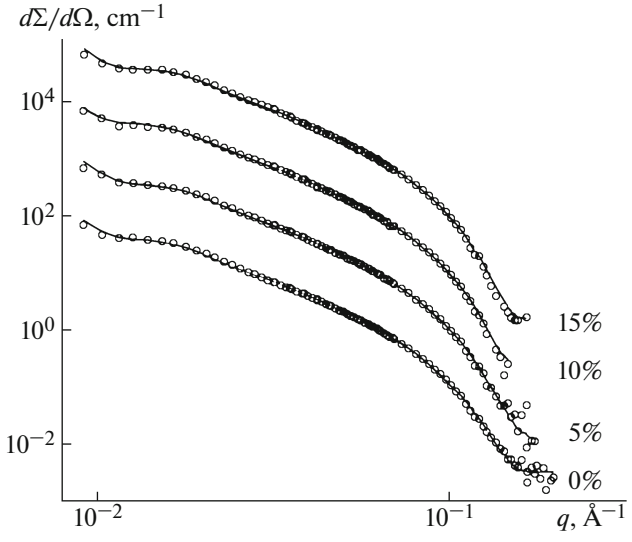

Fig. 5. Fitting of the SANS spectra of a polydispersed population of DPPC unilamellar vesicles in a solution in D_2O in the presence of DMSO at a concentration of 0–15%, which were measured on a YuMO spectrometer, to the calculated data using the SFF model; $d\Sigma(q)/d\Omega$ is the macroscopic cross-section of the polydispersed vesicle population and q is the scattering vector. The fitting parameters are given in Table 1. For the sake of clarity, the experimental and calculated cross-sections at concentrations of 5, 10, and 15% are multiplied by 10, 100, and 1000, respectively.

The membrane thickness d_b increases with an increase in the DMSO concentration from 52.7 ± 0.4 Å at a zero DMSO concentration to 57.2 ± 0.5 Å at 15% mole fraction of DMSO. The observed DMSO-induced increase in the membrane thickness by 4.5 ± 0.8 Å is the key result, which makes it possible to draw more accurate quantitative conclusions about the DMSO-induced dehydration of the intermembrane space.

Table 2 summarizes the data on the influence of DMSO on the repeat distance of DPPC multilamellar vesicles in water excess obtained by synchrotron-based X-ray diffraction [10].

In this study, the thickness of the DPPC lipid bilayer in aqueous DMSO solutions was estimated with higher accuracy compared to the data reported in [10], which allowed us to more accurately calculate the thickness of the intermembrane space (water interlayer) $d_w = d - d_b$. The d_w values are given in Table 2 for DMSO concentrations of 0, 5, 10, and 15%.

In pure water the thickness of the intermembrane space is $d_w = d - d_b = 11.3 \pm 1.2$ Å. The d_w values decrease with an increase in the DMSO concentration. In [10] the thickness of the lipid bilayer was calculated using the Guinier approximation, and $d_w = 7.8 \pm 1.8$ Å was obtained for 10% mole faction of DMSO. The calculation of the thickness of the lipid bilayer using the step function (Fig. 4) gave $d_w = 1.8 \pm 1.4$ Å at 10% mole faction of DMSO and $d_w = 0.9 \pm 1.3$ Å at 15% mole faction of DMSO. Therefore, the intermembrane space disappears and adjacent bilayers are in steric contact with each other at 15% mole fac-

Table 2. Dependence of the DPPC membrane repeat distance d [10] and the thickness of the water interlayer d_w on the mole fraction of DMSO in water

Mole fraction of DMSO, %	0	5	10	15
d, Å	64 ± 0.8	60 ± 0.8	58.7 ± 0.8	58.1 ± 0.8
d_w, Å	11.3 ± 1.2	4.1 ± 1.3	1.8 ± 1.4	0.9 ± 1.3

tion of DMSO. This is in agreement with the differential scanning calorimetry data [10], which were indicative of the DMSO-induced dehydration of the intermembrane space. The number of free water molecules per DPPC molecule decreases from 4 in pure water to 1.1 ± 0.4 at 15% mole fraction of DMSO [10].

CONCLUSIONS

The combined analysis of the small-angle neutron scattering and X-ray diffraction data demonstrates that the intermembrane space of DPPC multilamellar vesicles decreases with an increase in the DMSO concentration in the ternary DPPC–water–DMSO system from 11.3 ± 1.2 Å at a zero DMSO concentration to 0.9 ± 1.3 Å at 15% mole faction of DMSO. At 15% mole faction of DMSO, adjacent bilayers are in steric contact with each other, which is in agreement with DMSO-induced dehydration of the intermembrane space [10].

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