Small-Angle Neutron Scattering Study of *N*-Dodecyl-*N*,*N*-dimethylamine *N*-Oxide Induced Solubilization of Dioleoylphosphatidylcholine Bilayers in Liposomes

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Abstract. Mixtures of *N*-dodecyl-*N*,*N*-dimethylamine *N*-oxide (DDAO) and 1,2-dioleoylphosphatidyl choline (DOPC) in chloroform/methanol were evaporated, dried and hydrated in excess ^2H_2O. Aqueous dispersions thus prepared were extruded through polycarbonate filter with pores of diameter 500Å These samples were studied using small-angle neutron scattering. DDAO destabilizes the bilayer in unilamellar liposomes and solubilizes it into mixed micelles whose shape changes with the DDAO : DOPC molar ratio. Bilayers or/and bilayer fragments have been observed up to DDAO : DOPC = 1.5, rod-like particles (tubular, cylindric micelles) at 2.5 < DDAO : DOPC < 3.5, and transition to globular particles (spheroid micelles) at DDAO : DOPC > 4. In bilayers or/and bilayer fragments, DDAO modulates the thickness of the bilayer.

Key words: *N*-Dodecyl-*N*,*N*-dimethylamine *N*-oxide — 1,2-Dioleoylphosphatidylcholine — Unilamellar liposome — Bilayer solubilization — Small-angle neutron scattering

Introduction

Non-ionic surfactants *N*-alkyl-*N*, *N*-dimethylamine *N*-oxides were found to possess antimicrobial (Devínsky et al. 1990), antiphotosynthetic (Šeršeň et al. 1992) and immunomodulatory (Bukovský et al. 1996; Ferenčík et al. 1990; Jahnová et al. 1993; Kačáni et al. 1996) activities. These compounds modulate also the activity of transmembrane enzyme (Ca-Mg)ATPase from sarcoplasmic reticulum (Andriamainty et

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al 1997, Karlovska et al 1999b) The well known homologue with dodecyl substituent (DDAO) is widely used as a mild biological detergent in membrane studies for solubilization, purification, reconstitution and crystallization of membrane proteins

These surfactants penetrate into phospholipid bilayer and affect its fluidity (Balgavy et al 1989, Šeršeň et al 1989, Glover et al 1999), thickness (Dubničková et al 1997, Karlovská et al 1999a) and phase transitions (Gallová 1999) In the phosphatidylcholine fluid lamellar phase, they induce formation of non-lamellar phases at high concentrations (Uhríková and Stanovská 1990) Before solubilization, DDAO induces fusion of small unilamellar liposomes to larger vesicles (Kragh-Hansen et al 1998)

In the present communication, we report the results of the small-angle neutron scattering (SANS) study of the DDAO mixtures with dioleoylphosphatidylcholine (DOPC) extruded through polycarbonate filter

Materials and Methods

DOPC was purchased from Avanti Polar Lipids (Alabaster, USA), DDAO was from Fluka (Buchs, Switzerland) and heavy water (99 98% ^2H_2O) was obtained from Izotop (Moscow, Russia) The other chemicals were obtained from Mikrochem (Bratislava, Slovakia) Organic solvents were redistilled before use

Weighted amounts of DOPC and DDAO were dissolved in chloroform/methanol and mixed in solution Solvent was evaporated to dryness under a stream of pure gaseous nitrogen, followed by evacuation in a vacuum chamber at 10 Pa for 18 hours, then transferred to a glass tube and evacuated again in the chamber ^2H_2O was added, the tube was purged with pure gaseous nitrogen and sealed with Parafilm M (American National Can, Greenwich, USA) DOPC + DDAO in ^2H_2O was dispersed by hand shaking and briefly sonication in a bath sonicator (Vráble, Slovakia) This dispersion was extruded through polycarbonate filter (Nucleopore, Plesanton, USA) with pores of diameter 500 Å, using the LiposoFast Basic extruder (Avestin, Ottawa, Canada) fitted with two gas-tight Hamilton syringes (Hamilton, Reno, USA) The samples were subjected to 25 passes through the filter at room temperature The extruded samples were flushed with the pure gaseous nitrogen and sealed The maximum concentration of DOPC in the final preparation was 10 g/l, the maximum period between the sample preparation and its measurement was 3–4 hours The extrusion procedure as described above produces large unilamellar liposomes when using pure phospholipids without admixtures (MacDonald et al 1991, Dubničkova et al 1997, Balgavy et al 1998, Uhríková et al 2000, Balgavy et al 2001a)

The SANS measurements were performed at the small-angle time-of-flight axially symmetric neutron scattering spectrometer YuMO at the IBR-2 fast pulsed reactor (Ostanevich 1988, Vagov et al 1983) The samples were poured into quartz cells (Hellma, Mullheim, Germany) to provide the 2 mm sample thickness The sample temperature was set and controlled electronically at 20 0 ± 0 1 °C The

sample in quartz cell was equilibrated for 1 hour at the given temperature before measurement The scattering patterns were corrected for background effects The coherent scattering cross section was obtained by using a vanadium standard scatterer

Results and Discussion

The neutron scattering function can be written as

$$I(Q) = NP(Q)S(Q) \tag{1}$$

where N is the number of scattering particles in unit volume, $P(Q)$ is the particle structure factor, $S(Q)$ is the size- and orientation-dependent interparticle structure factor Q is the scattering vector defined as

$$Q = 4\pi \sin\theta/\lambda \tag{2}$$

where 2θ is the scattering angle and λ the wavelength of neutrons $S(Q)$ approximately equals to 1 for dilute and weakly interacting particles It has been found experimentally that for the unilamellar liposomes at the phospholipid concentration (1 wt %) as used in our experiments, $S(Q) \cong 1$ is a good approximation and that deviations occur at concentrations > 20 g/l (see Dubničkova et al (1997), Balgavy et al (1998) and Balgavy et al (2001a) for references) According to Guinier approximation for very small scattering angles, one can rewrite then eqn (1) as

$$I(Q) = AQ^{r-3} \exp(-Q^2 R_g^2/r) \tag{3}$$

where A is a constant, R_g is the object radius of gyration and $r \approx 1$, 2, and 3 hold for infinite sheet-like object, for rod-like object of infinite length and uniform cross section, and for a globular object, respectively (Hjelm et al 1990, Dubničkova et al 1997), $r \approx 1$ is a good approximation also for polydisperse hollow spheres having a constant shell thickness (Balgavý et al 1998) The equation 3 can be thus used for the evaluation of experimental SANS data to obtain an information on the geometry of scattering particles We have fitted our SANS data in the interval of 0 006 Å^{-2} $\geq Q^2 \geq$ 0 001 Å^{-2} by the least squares method to obtain r and R_g values The results of fitting are shown in Fig 1 It is seen that the value of r remains constant and equal to 1 up to about DDAO DOPC = 1 5 mol/mol The value $r \approx 1$ indicates the presence of unilamellar liposomes, eventually discoid micelles – isolated fragments of bilayers having large lateral dimensions The region 1 5 < DDAO DOPC < 2 5 is characteristic by the increase in the r value For 2 5 < DDAO DOPC < 3 5 the observed value of $r \approx 2$ indicates the presence of rod-like particles, e g tubular micelles For DDAO DOPC > 4 one can observe

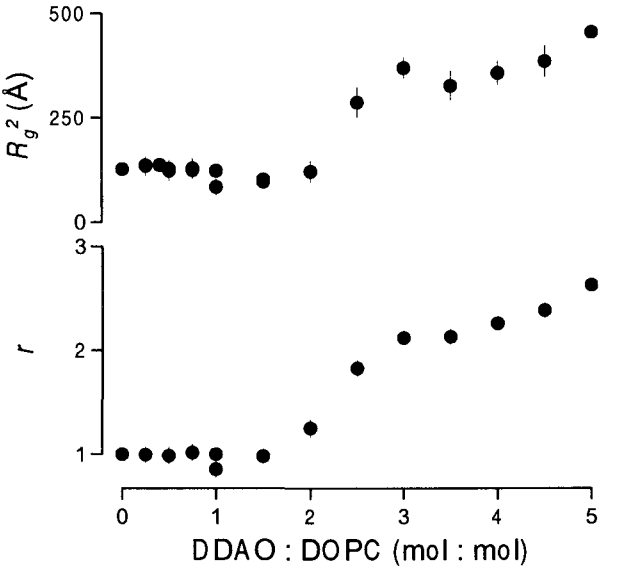

Figure 1. Dependence of the gyration radius R_g and of the shape parameter r on the DDAO : DOPC molar ratio.

transition of the rod-like particles into globular particles. These could be spheroid micelles. The values of R_g show the same tendency as r.

The thickness parameter of the bilayer (a shell in polydisperse hollow sphere – unilamellar liposome) and the thickness parameter of the planar sheet (discoid micelles) can be obtained from the gyration radius as

$$d_g = 12^{0.5} R_g \tag{4}$$

The thickness parameter d_g is equal to the steric bilayer thickness in unilamellar diacylphosphatidylcholine liposomes when supposing that there are no water molecules located in the bilayer polar region (Balgavý et al. 2001b). We have also shown in our recent paper (Balgavý et al. 2001a) that the thickness parameter d_g is a linear function of the transbilayer phosphate-phosphate distance in unilamellar diacylphosphatidylcholine liposomes. The parameter d_g is thus a good measure of the bilayer thickness in unilamellar liposomes and can be used to study its relative changes. The results of calculation of the bilayer thickness parameter are shown in Fig. 2. After a small increase at small DDAO concentration, the bilayer thickness decreases below that in the control sample without DDAO. The largest decrease is observed close to the transition region from bilayers in liposomes or in discoid micelles into tubular (cylindric) micelles.

The changes in the DOPC bilayer thickness in the presence of DDAO and the transition of the DOPC bilayer into mixed micelles closely correlate with the

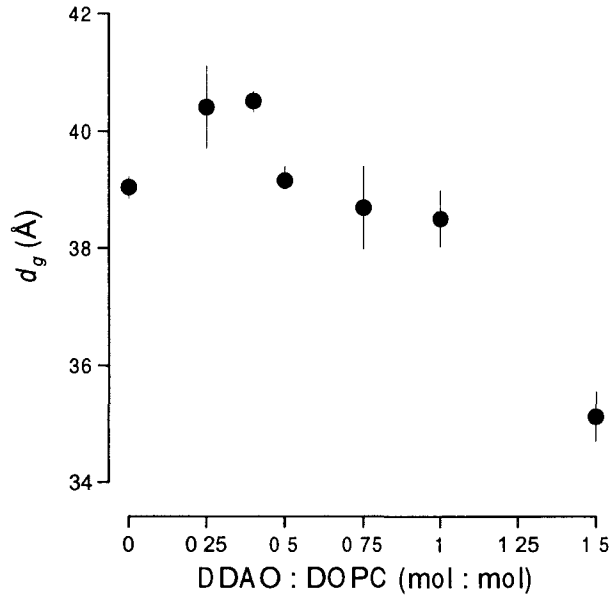

Figure 2. Dependence of the bilayer thickness parameter d_g on the DDAO DOPC molar ratio

changes in activity of the sarcoplasmic reticulum Ca^{2+}-transporting ATPase reconstituted into DOPC unilamellar liposomes (Karlovská, Devínsky, Lacko, Hammel and Balgavý, to be published).

In conclusion, we have observed that DDAO destabilizes the bilayer in unilamellar liposomes and solubilizes it into mixed micelles whose shape changes with the DDAO : DOPC molar ratio

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