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 2H2O. Aqueous dispersions thus prepared were ex-
truded 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 mi-
celles) at DDAO : DOPC > 4. In bilayers or/and bilayer fragments, DDAO mod-
ulates the thickness of the bilayer.

Key words: N-Dodecyl-N,N-dimethylamine N-oxide — 1,2-Dioleoylphosphatidyl-
choline — 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;
Káčán et al. 1996) activities. These compounds modulate also the activity of trans-
membrane enzyme (Ca-Mg)ATPase from sarcoplasmic reticulum (Andriamainty et

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These surfactants penetrate into phospholipid bilayer and affect its fluidity (Balgavý 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% $\text{D}_2\text{O}$) 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. $\text{D}_2\text{O}$ was added, the tube was purged with pure gaseous nitrogen and sealed with Parafilm M (American National Can, Greenwich, USA). DOPC + DDAO in $\text{D}_2\text{O}$ was dispersed by hand shaking and briefly sonication in a bath sonicator (Vrable, 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; Dubničková et al. 1997; Balgavý et al. 1998; Uhríková et al. 2000; Balgavý 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, Müllheim, Germany) to provide the 2 mm sample thickness. The sample temperature was set and controlled electronically at 20.0 ± 0.1 °C.
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

\[ I(Q) = NP(Q)S(Q) \]  

(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

\[ Q = 4\pi \sin \theta / \lambda \]  

(2)

where \( 2\theta \) is the scattering angle and \( \lambda \) 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, \( S(Q) \equiv 1 \) is a good approximation and that deviations occur at concentrations > 20 g/l (see Dubničková et al. (1997), Balgavý et al. (1998) and Balgavý et al. (2001a) for references). According to Guinier approximation for very small scattering angles, one can rewrite then eqn. (1) as

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

(3)

where \( A \) is a constant, \( R_g \) is the object radius of gyration and \( r = 1, 2, \) 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; Dubničková 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 \( 0.006 \text{ Å}^{-2} \geq Q^2 \geq 0.001 \text{ Å}^{-2} \) 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 \( 1.5 < \text{DDAO : DOPC} < 2.5 \) is characteristic by the increase in the \( r \) value. For \( 2.5 < \text{DDAO : DOPC} < 3.5 \) 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
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

$$d_g = 10^{0.5} R_g$$

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
changes in activity of the sarcoplasmic reticulum Ca\textsuperscript{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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