Effect of *n*-decane on the lipid bilayer thickness in unilamellar dioleoylphosphatidylcholine liposomes prepared by the cholate dilution method: small-angle neutron scattering study

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Abstract

Equimolar dioleoylphosphatidylcholine (DOPC) and sodium cholate (NaChol) in organic solvent was evaporated to dryness. Mixed DOPC+NaChol micelles at DOPC and NaChol concentrations of 0.1 mol/l were prepared in ^2H_2O containing 0.135 mol/l NaCl. This micellar solution was diluted in 0.135 mol/l NaCl in ^2H_2O to reach the final DOPC and NaChol concentrations of 0.008 mol/l. Small-angle neutron scattering (SANS) confirmed the presence of unilamellar liposomes in this dispersion. The 30 µl of *n*-decane solution in methanol was added to 1 ml of the liposome dispersion. After methanol evaporation, SANS was conducted on the liposomes. From the Kratky-Porod plot $\ln[I(Q)Q^2]$ vs. Q^2 of SANS intensity $I(Q)$ in the range of scattering vector values Q corresponding to interval $0.1\ \mathrm{nm}^{-2} \leq Q^2 \leq 0.6\ \mathrm{nm}^{-2}$, the liposome bilayer radius of gyration R_g and the bilayer thickness parameter $d_g = 12^{0.5} R_g$ were obtained. The values of d_g indicate that the bilayer thickness is within the experimental error constant up to *n*-decane:DOPC~0.6 molar ratio, and increases then by 0.24±0.09 nm up to *n*-decane:DOPC=1.6 molar ratio.

Key-words

1,2-dioleoyl-3-phosphatidylcholine; Unilamellar liposomes; *n*-decane; Bilayer thickness; Small-angle neutron scattering

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Introduction

It has been observed that *n*-decane, 1,2-dibromodecane, 1,10-dibromodecane and 1-bromodecane modulate the activity of sarcoplasmic reticulum Ca-Mg-ATPase reconstituted in unilamellar 1,2-diacylphosphatidylcholine liposomes by the cholate dilution method [1,2]. Since the enzyme activity critically depends on the phosphatidylcholine acyl chain length [1,3], the effects of *n*-alkanes on the enzyme activity have been ascribed to their effects on the lipid bilayer thickness [1]. However, the fluorescence quenching studies [2] indicated that these effects could be rather caused by the direct alkane binding to protein hydrophobic binding sites.

We have recently studied the *n*-decane (C10) effect on the lipid bilayer thickness in unilamellar dioleoylphosphatidylcholine (DOPC) liposomes prepared by extrusion [4]. We have observed by using the small-angle neutron scattering (SANS) that the DOPC bilayer thickness is within the experimental accuracy constant up to C10:DOPC~0.5 molar ratio and increases then by 0.24±0.13 nm up to C10:DOPC=1.2 molar ratio. These changes in the bilayer thickness were relatively small in comparison to that observed by McIntosh et al. [5] in fully hydrated fluid lamellar phosphatidylcholine phases (~1 nm). We confirmed the results in [5] by the x-ray diffraction on fully hydrated fluid egg yolk phosphatidylcholine lamellar phase [6]. One possible cause of conflicting results obtained with unilamellar and multilamellar systems could be the unilamellar liposome preparation by the extrusion method. In [4], the multilamellar vesicles containing the *n*-decane were pushed through the 50 nm cylindrical pores in polycarbonate filters. The large multilamellar vesicles must deform significantly and must undergo a decrease in volume (e.g. through rupture) to enter the pores [7]. The observed small effect of *n*-decane on the bilayer thickness in extruded liposomes could be thus caused by a decrease of its bilayer content during the extrusion – the alkane could be squeezed-out from the liposomes deformed and ruptured in filter pores. Therefore, to check the results obtained in extruded unilamellar liposomes, we study the effect of *n*-decane on unilamellar liposomes prepared by the cholate dilution method. The liposome preparation method and the manner of *n*-decane addition closely mimic the experimental conditions under which the *n*-decane effects on the activity of Ca-Mg-ATPase reconstituted in phosphatidylcholine liposomes were studied in [1,2].

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Material and methods

DOPC was purchased from Avanti Polar Lipids (Alabaster, USA), *n*-decane and sodium cholate were from Sigma (St. Louis, USA) and heavy water (99.98 % ^2H_2O) from Izotop (Moscow, Russia). The organic solvents were from Microchem (Bratislava, Slovakia) and the other chemicals from Lachema (Brno, Czech Republic). DOPC and cholate were mixed in the methanol in the 1:1 molar ratio. The solvent was evaporated under a stream of nitrogen gas and the DOPC+cholate mixture was dried by an oil pump evacuation. The dried equimolar DOPC+cholate mixture was dissolved in 135 mmol/l NaCl solution in ^2H_2O to reach the 100 mmol/l concentration of DOPC. The micellar solution thus prepared was slightly sonicated in a bath sonicator. This transparent solution of DOPC+cholate mixed micelles was further dissolved in 135 mol/l NaCl in ^2H_2O, the final DOPC concentration was 8 mmol/l. After this last dilution the liposome formation was observed visually by the appearance of opalescence after about 2-3 hours and by an increase of turbidity checked by the turbidance measurements at 350-600 nm. To the 1 ml of the liposome dispersion incubated about 30 hours in a tube, 30 μl of *n*-decane solution in methanol was added at the room temperature and the content was mixed by vortexing. After 2 hours of methanol evaporation at the room temperature, the tubes were purged with pure gaseous nitrogen, sealed with Parafilm M (American National Can, Greenwich, USA) and stored for 8 hours before SANS measurements.

The SANS measurements were performed at the small-angle time-of-flight axially symmetric neutron scattering spectrometer MURN (named YuMO in honor of deceased Yu. M. Ostanevich) at the IBR-2 fast pulsed reactor of the Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna [8,9]. The YuMO was equipped with a two-detector system, the sample-1st detector distance was set to 5.28 m and the sample-2nd detector to 13.040 m (A. Kuklin, unpublished). 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 25.0±0.1°C. The sample in quartz cell was equilibrated minimally for 1 hour at this temperature before the measurements. The acquisition time for one sample was 30 minutes. The absolute calibration of coherent scattering intensity was done by using a vanadium standard scatterer. The data matching and

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the resolution function of this spectrometer are described in detail in [8]. The scattering patterns were corrected for background effects.

Results and discussion

The experimentally observed scattering intensity (Fig. 1) is for monodisperse system given by

$$I(Q) \sim N_P P(Q) S(Q) \quad (1)$$

where Q is the scattering vector value defined as $Q=4\pi\sin\theta/\lambda$ (2θ is the scattering angle and λ the wavelength of neutrons), N_P is the number of particles, $P(Q)$ is the particle form factor and $S(Q)$ is the interparticle structure factor. The particle form factor P is equal to the mean squared form factor, which is one-dimensional Fourier integral of the coherent neutron scattering length density. The interparticle structure factor $S(Q)$ is approximately equal to 1 for dilute and weakly interacting systems like diluted electrostatically uncharged liposomes. According to Guinier approximation for very small scattering angles [10,11], one rewrites then the equation (1) as

$$I(Q) \sim exp(-Q^2 R_g^2/r) Q^{r-3} \quad (2)$$

where 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 [12,13]; $r\approx 1$ is a good approximation also for polydisperse hollow spheres with radii substantially larger than the constant shell thickness, such as unilamellar liposomes [14]. The approximation (2) is valid for finite size objects when $L^{-1} \leq Q \leq R_g^{-1}$ where L is the longest

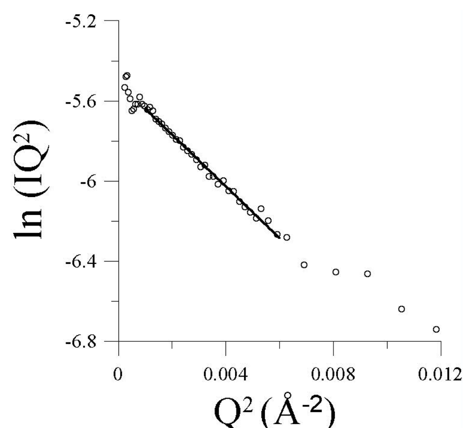

Fig. 1. Kratky-Porod plot of the neutron scattering curve for DOPC liposomes prepared by the cholate dilution method.

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size of the object. We have fitted the experimental values of $I(Q)$ in the region of small scattering vectors (0.32 nm$^{-1}\leq Q\leq$0.77 nm^{-1}) as in Fig.1 by using eqn. 2 and a nonlinear least squares program for the series of 11 samples with the molar ratios in the interval of 0≤n-decane:DOPC≤2. In the three parameter fit ($I(0)$ and R_g unconstrained, r constrained to $r\geq 1$) we have obtained the value of r in the range $1\leq r\leq 1.015$ with the maximum standard deviation of 0.080. These results indicate, that the *n*-decane+DOPC samples prepared as described above contained unilamellar liposomes or/and that the samples contained sheet-like objects, e.g. discoid micelles with very large lateral dimensions, which were stable in a broad range of *n*-decane content in the sample. The SANS method as used in the present paper cannot discriminate between these two possibilities. Noteworthy, no micelles nor other scattering objects were detected by SANS in the 8 mmol/l cholate solution in 135 mol/l NaCl in ^2H_2O in the absence of DOPC.

It is well know [10,11], that the thickness of the two -dimensional planar sheet d_g can be obtained from the radius of gyration R_g as

$$d_g^2 \cong 12 R_g^2 \qquad (3)$$

Equation (3) can be also used for the estimation of bilayer thickness in unilamellar liposomes dispersed in heavy water [15]. The values of R_g can be obtained from the Kratky-Porod plots of experimental data such as presented in Fig.1. Our computer simulations of scattering curves have shown that the value of d_g obtained from the data in the region of 0.1 nm$^{-2}\leq Q^2\leq$0.66 nm^{-2} are approximately equal to the thickness of the dry lipid bilayer in spherical unilamellar polydisperse liposomes [14,16]. Furthermore, we have found experimentally that the thickness parameter d_g is a linear function of the transbilayer phosphate-phosphate distance in unilamellar diacylphosphatidylcholine liposomes [17]. The bilayer thickness parameter d_g can be thus used as a measure of the bilayer thickness in unilamellar liposomes. Though its absolute value differs from the real bilayer thickness, its changes parallel the changes in the bilayer thickness. The dependence of d_g values obtained from the Kratky-Porod plots in the indicated Q region on the *n*-decane:DOPC molar ratio is shown in the Fig.2. It is seen that *n*-decane has no effect within experimental accuracy on the bilayer thickness up to *n*-decane:DOPC≈0.6:1 molar ratio, and increases then by 0.24±0.09 nm up to *n*-decane:DOPC=1.6 molar ratio. The change in d_g up to 1.6:1 ratio is relatively small in

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comparison to that observed by McIntosh et al. [5] and Kučerka et al. [6] in multilamellar phosphatidylcholine phases, but equal to 0.24±0.13 nm found by SANS in extruded unilamellar liposomes [4]. The difference between unilamellar liposomes and multilamellar systems could be caused by the difference in their curvature and, consequently, by different *n*-decane location in their bilayers.

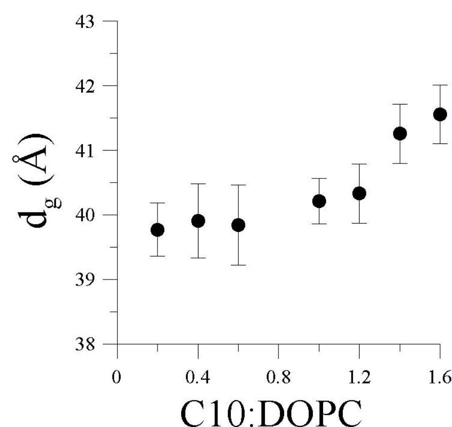

Fig. 2. Dependence of the DOPC bilayer thickness parameter d_g on the *n*-decane:DOPC molar ratio.

In conclusion, the change of the DOPC bilayer thickness in unilamellar liposomes is relatively small up to *n*-decane:DOPC=1.6:1 molar ratio. It is evident, that the activity changes of the Ca-Mg-ATPase reconstituted in unilamellar liposomes by the cholate dilution method induced by *n*-decane are not primarily caused by the changes in the bilayer thickness as originally proposed by Johansson et al. [1], but rather by the direct interaction of *n*-decane with the Ca-Mg-ATPase hydrophobic binding sites as suggested in [2].

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