Available online at www.sciencedirect.com

SCIENCE DIRECT®

Biochimica et Biophysica Acta 1611 (2003) 31–34

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

www.bba-direct.com

Small-angle neutron scattering study of the lipid bilayer thickness in unilamellar dioleoylphosphatidylcholine vesicles prepared by the cholate dilution method: *n*-decane effect

Daniela Uhríková[a], Norbert Kučerka[a], Akhmed Islamov[b], Alexander Kuklin[b], Valentin Gordeliy[b,c,d], Pavol Balgavý[a,*]

[a] *Department of Physical Chemistry, Faculty of Pharmacy, J.A. Comenius University, Odbojárov 10, 83232 Bratislava, Slovakia*
[b] *Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, 141980 Dubna, Russia*
[c] *Institute of Structural Biology, Research Centre Juelich, D-52425 Juelich, Germany*
[d] *Centre of Biophysics and Physical Chemistry of Supramolecular Structures, Moscow Institute for Physics and Technology, 141700 Dolgoprudny, Russia*

Received 4 July 2002; received in revised form 16 December 2002; accepted 17 December 2002

Abstract

Previous X-ray diffraction studies on fully hydrated fluid lamellar egg phosphatidylcholine phases indicated a ~ 10 Å increase of bilayer thickness in the presence of excess *n*-decane [Biochim. Biophys. Acta 597 (1980) 455], while the small-angle neutron scattering (SANS) on unilamellar extruded dioleoylphosphatidylcholine (DOPC) vesicles detected substantially smaller 2.4 ± 1.3 Å bilayer thickness increase at *n*-decane/DOPC molar ratio of 1.2 [Biophys. Chem. 88 (2000) 165]. The purpose of the present study is to investigate the *n*-decane effect on the bilayer thickness in unilamellar DOPC vesicles prepared by the sodium cholate (NaChol) dilution method. 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. Thirty microliters of *n*-decane solution in methanol was added to 1 ml of this dispersion. After methanol evaporation, SANS was conducted on the dispersions. 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 0.001 $Å^{-2} \le Q^2 \le 0.006$ $Å^{-2}$, the 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 increases by 2.4 ± 0.9 Å up to *n*-decane/DOPC molar ratio of 1.6. This result confirms the previous SANS findings obtained with extruded vesicles.

Keywords: 1,2-Dioleoyl-3-phosphatidylcholine; Unilamellar vesicle; *n*-Decane; Bilayer thickness; Small-angle neutron scattering

1. 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 vesicles 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 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) vesicles prepared by extrusion [4]. We have observed by using small-angle neutron scattering (SANS) that the DOPC bilayer thickness is within the experimental accuracy constant up to C10/DOPC molar ratio of ~ 0.5 and increases then by 2.4 ± 1.3 Å up to C10/DOPC molar ratio of 1.2. 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 in the presence of excess C10 (~ 10 Å). We confirmed the results in Ref. [5] by the X-ray diffraction on fully hydrated fluid egg yolk

* Corresponding author. Fax: +421-2-5262065.
E-mail address: pavol.balgavy@fpharm.uniba.sk (P. Balgavý).

0005-2736/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0005-2736(02)00705-8

phosphatidylcholine lamellar phase [6]. One possible cause of conflicting results obtained with unilamellar and multilamellar systems could be the unilamellar vesicle preparation by the extrusion method. In Ref. [4], the multilamellar vesicles containing the *n*-decane were pushed through the 500 Å cylindrical pores in polycarbonate filters. The large multilamellar vesicles must significantly deform 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 vesicles could thus be caused by a decrease of its bilayer content during the extrusion—the alkane could be squeezed out from the deformed and ruptured vesicles in filter pores. Therefore, to check the results obtained in extruded unilamellar vesicles, we study the effect of *n*-decane on unilamellar vesicles prepared by the cholate dilution method. The vesicle preparation method and the manner of *n*-decane addition mimic as closely as possible the experimental conditions under which *n*-decane effects on the activity of Ca–Mg–ATPase reconstituted in phosphatidylcholine vesicles were studied in Refs. [1,2].

2. Materials and methods

DOPC was purchased from Avanti Polar Lipids (Alabaster, USA), *n*-decane and sodium cholate (NaChol) were from Sigma (St. Louis, USA) and heavy water (99.98% ^2H_2O) was from Izotop (Moscow, Russia). The organic solvents were from Microchem (Bratislava, Slovakia) and the other chemicals are from Lachema (Brno, Czech Republic). DOPC and NaChol were mixed in methanol in a 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 + NaChol 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 + NaChol mixed micelles was further dissolved in 135 mmol/l NaCl in ^2H_2O, the final DOPC concentration was 8 mmol/l. After this last dilution, the vesicle formation was visually observed by the appearance of opalescence after about 2–3 h and by an increase of turbidity checked by the turbidimetric measurements at 3500–6000 Å. To 1 ml of the vesicle dispersion incubated about 30 h in a tube, 30 μl of *n*-decane solution in methanol was added at room temperature and the content was mixed by vortexing. After 2 h 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 h 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 distance to 13.04 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 minimally equilibrated for 1 h at this temperature before the measurements. The acquisition time for one sample was 30 min. The absolute calibration of coherent scattering intensity was performed by using a vanadium standard scatterer. The data matching and the resolution function of this spectrometer are described in detail in Ref. [8]. The scattering patterns were corrected for background effects and incoherent scattering.

3. Results and discussion

The experimentally observed scattering intensity for a monodisperse system is 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 is 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 a 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 vesicles. According to the Guinier approximation for very small scattering angles [10,11], one then rewrites Eq. (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 also a good approximation for polydisperse hollow spheres with radii substantially larger than the constant shell thickness, such as unilamellar vesicles [14]. The approximation (2) is valid for finite size objects when $L^{-1} \leq Q \leq R_g^{-1}$, where L is the longest size of the object. We have fitted the experimental values of $I(Q)$ in the region of small scattering vectors ($0.001\ \text{Å}^{-2} \leq Q^2 \leq 0.006\ \text{Å}^{-2}$) as in Fig. 1 by using Eq. (2) and a nonlinear least-squares program for the series of samples with 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

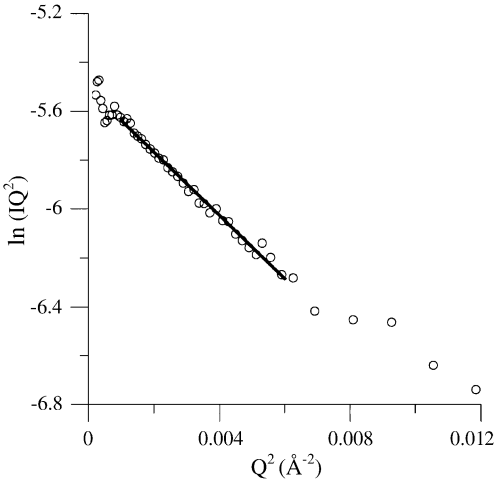

Fig. 1. Kratky-Porod plot of the neutron scattering curve for DOPC vesicles prepared by the cholate dilution method.

described above contained unilamellar vesicles and/or randomly oriented 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. However, results obtained by other authors with similar phosphatidylcholine + bile salt dispersions suggest the presence of unilamellar vesicles in our systems. Hjelm et al. [12] observed, by using SANS, that in the mixed sodium glycocholate (NaGChol) + egg yolk phosphatidylcholine (EYPC) in 150 mol/l NaCI in ^2H_2O at EYPC/NaGChol molar ratio of 0.9, globular particles were present in the dispersion at 64.1 mmol/l EYPC concentration (supposing that the molecular weight of EYPC is 780 [15]). When this dispersion was diluted to 18.8 mmol/l of EYPC, long rod-like mixed micelles were present in the dispersion. Unilamellar spherical vesicles were formed when the serial dilution was made to 2.56 mmol/l of EYPC. The transition between mixed sodium taurochenodeoxycholate (NaTChol) + EYPC micelles and vesicles in 150 mmol/l NaCl in ^2H_2O was studied by using SANS in two samples prepared by dilution [16]. At a EYPC/NaTChol molar ratio of 0.9 and 2.2 mmol/l of EYPC, the SANS curve could be fitted well by model of long flexible rod-like micelles; at the same EYPC/NaTChol molar ratio and EYPC concentration of 1.6 mmol/l, these rod-like micelles coexisted with polydisperse unilamellar vesicles. On dilution, the shape transformation in mixed phosphatidylcholine + bile salt systems can thus be schematically described as "from globules to cylinders to vesicles". Our results with DOPC + NaChol system at 1:1 molar ratio and 8 mmol/l of DOPC in 150 mmol/l NaCl in ^2H_2O prepared by dilution fit well into this scheme. At a slightly higher lipid/bile salt molar ratio and NaCl concentration, no indications of rod-like mixed micelles were observed so the scattering particles in aqueous dispersion were most probably unilamellar spherical liposomes rather than discoid micelles with large lateral dimensions. This conclusion is further supported by results of Tauskela et al. [17] and Korgel et al. [18] who observed by light scattering methods that the vesicles were present in mixed EYPC + NaChol with 0.65–0.70 molar ratio and 12.8–57.7 and 25.6 mmol/l EYPC concentration, respectively.

It is well known [10,11,19] that the thickness d_g of the two-dimensional planar sheet as well as of the shell thickness in polydisperse hollow spheres can be obtained from the radius of gyration R_g as

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

The values of R_g can be obtained from the Kratky–Porod plots of experimental data such as that presented in Fig. 1. Our computer simulations of scattering curves have shown that the values of d_g obtained from the data in the region of $0.001\ \text{Å}^{-2} \le Q^2 \le 0.006\ \text{Å}^{-2}$ correlate with the steric thickness and changes in steric thickness of lipid bilayers in polydisperse spherical unilamellar vesicles dispersed in heavy water [14,20]. Furthermore, we have found experimentally that the parameter d_g is a linear function of the transbilayer phosphate–phosphate distance in unilamellar diacylphosphatidylcholine vesicles [21]. The bilayer thickness d_g can thus be used as a measure of the steric bilayer thickness in unilamellar vesicles. Although its absolute value differs from the steric bilayer thickness, its changes parallel the changes in the steric 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 Fig. 2. It is seen that *n*-decane maximally increases the bilayer thickness by 2.4 ± 0.9 Å up to *n*-decane/DOPC molar ratio of 1.6. The change in d_g up to 1.6:1 ratio is relatively small in comparison to that observed by McIntosh et al. [5] and Kučerka et al. [6] in multilamellar phosphatidylcholine phases, but equal to 2.4 ± 1.3 Å found by SANS in extruded unilamellar vesicles [4]. The difference between unilamellar vesicles and multilamellar sys-

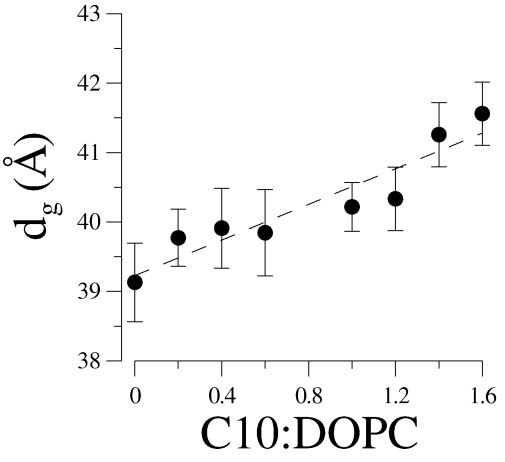

Fig. 2. Dependence of the DOPC bilayer thickness parameter d_g on the *n*-decane; DOPC molar ratio.

tems could be caused by the difference in their curvature and, consequently, by *n*-decane location in their bilayers.

In conclusion, the change in the DOPC bilayer thickness in unilamellar vesicles is relatively small up to *n*-decane/DOPC molar ratio of 1.6:1. It is evident that the activity changes of the Ca–Mg–ATPase reconstituted in unilamellar vesicles by the cholate dilution method induced by additions of *n*-decane in methanol solution 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 Ref. [2].

Acknowledgements

D. Uhríková and N. Kučerka thank the staff of the Condensed Matter Division, Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna, for the hospitality. This study was supported by the Slovak Ministry of Education VEGA grants 1/7704/2000 and 1/0123/03 to P. Balgavý and by the Comenius University grant 13/2002 to N. Kučerka V. Gordeliy thanks G. Büldt for the support of this work. The experiments in JINR Dubna were supported within the JINR project 07-4-1031-99/03.

References

[1] A. Johannsson, C.A. Keightley, G.A. Smith, C.D. Richards, T.R. Hesketh, J.C. Metcalfe, J. Biol. Chem. 256 (1981) 1643–1650.
[2] A.G. Lee, J.M. East, P. Balgavý, Pestic. Sci. 32 (1991) 317–327.
[3] A.G. Lee, Biochim. Biophys. Acta 1376 (1998) 381–390.
[4] D. Uhríková, P. Balgavý, N. Kučerka, A. Islamov, V. Gordeliy, A. Kurlin, Biophys. Chem. 88 (2000) 165–170.
[5] T.J. McIntosh, S.A. Simon, R.C. MacDonald, Biochim. Biophys. Acta 597 (1980) 445–463.
[6] N. Kučerka, D. Uhríková, J. Karlovská, P. Balgavý, Farm. Obz. 71 (2002) 182–187.
[7] D.G. Hunter, B.J. Frisken, Biophys. J. 74 (1998) 2996–3002.
[8] Yu.M. Ostanevich, Macromol. Chem. Macromol. Symp. 15 (1998) 91–103.
[9] V.A. Vagov, A.B. Kunchenko, Yu.M. Ostanevich, I.M. Salamatin, JINR Communication P14-83-898, 1983.
[10] L.A. Feigin, D.I. Svergun, Structure Analysis by Small-Angle X-Ray and Neutron Scattering, Plenum, New York, 1987.
[11] O. Glatter, O. Kratky, Small Angle X-Ray Scattering, Academic Press, New York, 1983.
[12] R.P. Hjelm Jr., P. Thiyagarajan, D.S. Sivia, P. Lindner, H. Alkan, D. Schwahn, Prog. Colloid & Polym. Sci. 81 (1990) 225–231.
[13] R.P. Hjelm Jr., P. Thiyagarajan, H. Alkan, J. Appl. Crystallogr. 21 (1988) 858–863.
[14] P. Balgavý, M. Dubničkova, D. Uhríková, S. Yaradaikin, M. Kiselev, V. Gordeliy, Acta Phys. Slovaca 48 (1998) 509–533.
[15] J. Filípek, K. Gelienová, P. Kovács, P. Balgavý, Gen. Physiol. Biophys. 12 (1993) 55–68.
[16] J.S. Pedersen, S.U. Egelhaaf, P. Schurtenberger, J. Phys. Chem. 99 (1995) 1299–1305.
[17] J.S. Tauskela, M. Akler, M. Thomson, Anal. Biochem. 201 (1992) 282–287.
[18] B.A. Korgel, J.H. van Zanten, H.G. Monbouquette, Biophys. J. 74 (1998) 3264–3272.
[19] V.I. Gordeliy, L.V. Golubchikova, A. Kuklin, A.G. Syrykh, A. Watts, Prog. Colloid & Polym. Sci. 93 (1993) 252–257.
[20] P. Balgavý, N. Kučerka, V.I. Gordeliy, V.G. Cherezov, Acta Phys. Slovaca 51 (2001) 53–68.
[21] P. Balgavý, M. Dubničkova, N. Kučerka, M.A. Kiselev, Biochim. Biophys. Acta 1512 (2001) 40–52.