Lipid bilayer thickness and lipid surface area in unilamellar DPPC liposomes evaluated from small-angle neutron scattering curves measured at different contrasts

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Abstract

The lipid bilayer thickness d_L=45.1 Å, the surface area per lipid on the bilayer-aqueous phase interface A_L=62.7 Å^2 and the number of water molecules per lipid penetrated into the bilayer polar region N_L=5.9 were evaluated from the small-angle neutron scattering (SANS) curves of extruded unilamellar dipalmitoyl-phosphatidylcholine (DPPC) liposomes measured at 65 °C and two different contrasts $N_{D_2O}/(N_{D_2O}+N_{H_2O})$=1.0 and 0.4.

Introduction

Phospholipid bilayers are usually divided into two polar head group regions and one nonpolar hydrocarbon region consisting of the phospholipid hydrocarbon chains. In the bilayer, some limited number of water molecules can penetrate into the head group regions. The basic physical parameters of these models of biomembranes are the thickness of the phospholipid bilayer, d_L, the surface area per lipid on the bilayer-aqueous phase interface, A_L, and the number of water molecules per lipid penetrated into the polar region of the bilayer, N_L. In the present report we estimate the 1,2-dipalmitoylphosphatidylcholine (DPPC) bilayer thickness d_L, the surface area A_L and the number of water molecules N_L from the small-angle neutron scattering (SANS) on unilamellar DPPC liposomes.

Material and methods

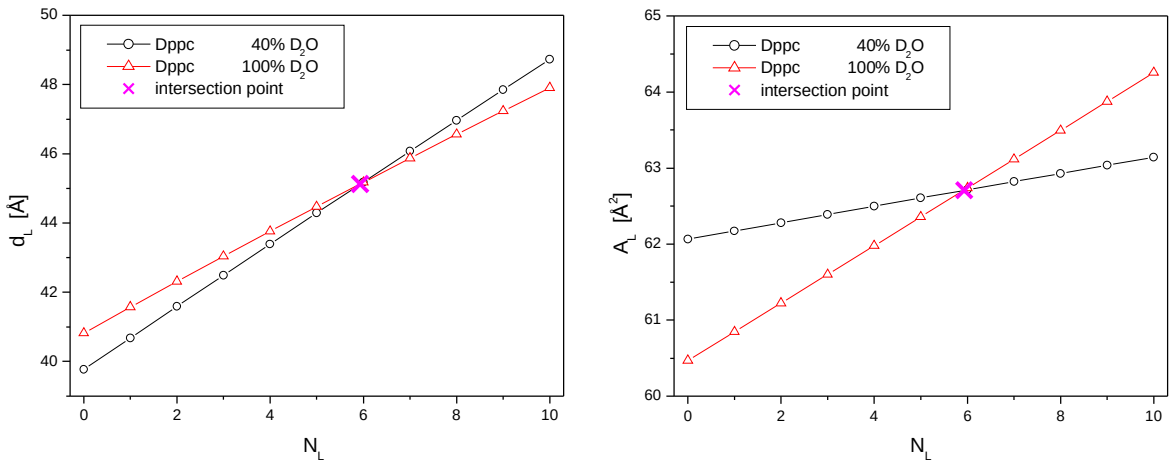
The unilamellar 1,2-dipalmitoylphosphatidylcholine (DPPC) liposomes were prepared by extrusion through two stacked polycarbonate filters with pores of 500 Å diameter.

The samples were subjected to 51 passes through the filters at about 60°C. The SANS measurements were performed at 65 °C and contrasts $N_{D_2O}/(N_{D_2O}+N_{H_2O})$=1.0 and 0.4 at the small-angle time-of-flight axially symetric neutron scattering spectrometer YuMO at the IBR-2 fast pulsed reactor of the Frank's laboratory of Neutron Physics, JINR in Dubna. The experimental methods were described in detail earlier [1-3]

Results and discussion

The experimental SANS curves in the Guinier region of scattering vectors *Q* were typical of scattering on two-dimensional infinite sheets. No indications of oligo- or multilamellarity of liposomes were observed. The experimental curves were evaluated by using a multishell model of the bilayer coherent neutron scattering length density, which divides the lipid bilayer of liposomes into the polar head group regions and the nonpolar hydrocarbon region [2, 3]. In each of these regions, the coherent neutron scattering length density is supposed to be homogeneous. The evaluation is based on obtaining of gyration radius (R_g) from the Kratky-Porod plot of SANS data in the region of scattering vector values 0.001 Å^{-2}>Q^2≥0.006 Å^{-2} [2-4]. From gyration radii obtained at different molar fractions $N_{D_2O}/(N_{D_2O}+N_{H_2O})$ in the aqueous phase (contrasts) and independent volumetric data [5], the lipid surface area A_L (or the bilayer thickness d_L) and the number of water molecules N_L penetrated into the bilayer polar region can be evaluated in the following steps [2, 3]:

Fig. 1: Dependence of the bilayer thickness d_L and surface area A_L on the number of water molecule N_L calculated from the SANS data.



The value of $R_g(exp)$ is obtained from the scattering curve by fitting the data in the region of 0.001 Å^{-2}≤Q^2≤0.006 Å^{-2}. Then, the A_L value is calculated for a given N_L value from the interval 0≤N_L≤20. This is done by fixing the N_L value, calculating the scattering curves for different A_L values using the multishell model of the liposome, and fitting them by linear functions in the region of 0.001 Å^{-2}≤Q^2≤0.006 Å^{-2} till their R_g value fulfils the condition |R_g-$R_g(exp)$|≤0.001 Å. The set of paired A_L and N_L values is obtained at given contrast and this is plotted as a continuous curve by fitting the paired A_L and N_L points by a smooth polynomial function (Fig.1). The whole procedure is repeated for another contrast. It is seen, that the continuous curves obtained at two different contrasts intersect in one point. From this point, the values of d_L, A_L and N_L are obtained. Using this method d_L=45.1 Å, A_L=62.7 Å^2 and N_L=5.9 for DPPC bilayers in unilamellar liposomes at 65 °C were obtained. These data are very close to that obtained by the synchrotron x-ray diffraction on lamellar DPPC phase [6].

Acknowledgements

This study was supported by the Slovak Ministry of Education VEGA grant 1/7704/2000 to P.B. and by the Comenius University grants UK/104100 and UK/6100 to N.K. SANS experiments were supported by the JINR project 07-4-1031-99/03. N.K. and D.U. thank the staff of FLNP for hospitality.

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