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Bilayer thickness in unilamellar phosphatidylcholine vesicles: small-angle neutron scattering using contrast variation

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Abstract

The thickness of the lipid bilayer in extruded unilamellar vesicles prepared from synthetic 1,2-diacyl-*sn*-glycero-3-phosphorylcholines with monounsaturated acyl chains (diC*n*:1PC, $n=14$–22) was studied at 30°C in the small-angle neutron scattering (SANS) experiment. Several contrasts of the neutron scattering length density between the aqueous phase and phospholipid bilayer of vesicles were used. The experimental data were evaluated using the small-angle form of the Kratky–Porod approximation $\ln[I(q)q^2]$ vs. q^2 of the SANS intensity $I(q)$ in the appropriate range of scattering vector values q to obtain the bilayer radius of gyration R_g and its extrapolated value at infinite scattering contrast R_g^{inf}. The bilayer thickness parameter evaluated from a linear approximation of dependence of gyration radius on the inverse contrast was then obtained without using any bilayer structure model. The dependence of the thickness parameter $d_g \cong 12^{0.5} R_g^{inf}$ on the number n of acyl chain carbons was found to be linear with a slope of 1.8 ± 0.2 Å per one acyl chain carbon. This slope can be used in bilayer–protein interaction studies.

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Keywords: Monounsaturated 1,2-diacyl-*sn*-glycero-3-phosphorylcholine; Bilayer; Small-angle neutron scattering; Contrast variation

1. Introduction

The phospholipid bilayer of unilamellar vesicles is widely used as a convenient model of the lipid part of biomembranes because its form is topologically equivalent to the real cell. The insertion and orientation of polypeptides in this bilayer and the activity of several integral membrane proteins critically depend on the bilayer thickness [1]. This thickness can be obtained from small-angle X-ray (SAXS) or neutron (SANS) scattering experiments on unilamellar vesicles [2]. SANS can provide more information when employing the contrast variation technique [3]. This technique, together with the analysis based on the Guinier approximation, yields a method where structural information can be obtained without making any assumptions about the bilayer structure [4]. We use it to obtain the bilayer thickness in extruded unilamellar vesicles prepared from the synthetic

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1,2-diacyl-*sn*-glycero-3-phosphoryl-cholines with monounsaturated acyl chains.

2. Material and methods

diC*n*:1PCs (*n* is the number of acyl chain carbon atoms) were purchased from Avanti. Different contrasts were reached by varying the H_2O/D_2O ratio in the aqueous phase in which the diC*n*:1PCs were dispersed. Unilamellar vesicles were prepared by extrusion through polycarbonate filters with 500 Å pores according to Ref. [5]. The diC*n*:1PC concentration was $\leqslant$ 1 wt% to eliminate inter-particle correlations [6].

The SANS experiments were performed on the PAXE spectrometer located at the Orphée reactor (LLB, CEA Saclay, France) with neutrons of wavelength 6 Å and with a sample-to-detector distance of 5 m. The SANS intensity $I(q)$ as a function of the scattering vector value q was normalized per sample transmission and incoherent background was obtained from scattering from a reference blank sample and subtracted.

The experimental data were evaluated using the method of Sadler et al. [4]. It is supposed that a model of randomly oriented planar thin sheets approximates well with the vesicle bilayers in the Guinier region of q. Scattering intensity in cm^{-1} units is then expressed by

$$I(q) \propto (I_t(0)/q^2)\exp(-R_g^2 q^2), \quad R_g q < 1, \tag{1}$$

where R_g is the radius of gyration taken perpendicularly to the sheet surface, $I_t(0)$ is a normalization constant related to the planar bilayer intensity in the origin of reciprocal space and $1/q^2$ is the Lorentz factor due to averaging over all sheet orientations [7]. The radii of gyration were evaluated from the Kratky–Porod plots $\ln(Iq^2)$ vs. q^2 according to Eq. (1) in the region $0.001\text{-}Å^{-2} < q^2 < 0.006\,Å^{-2}$ where this method can be safely used for vesicles extruded through a 500 Å pore filter [8]. Typical experimental data are shown in Fig. 1.

The dependence of R_g as a function of scattering density contrast is expressed by

$$(R_g(\Delta\bar{\rho}))^2 = (R_g^{inf})^2 + \alpha/\Delta\bar{\rho}, \tag{2}$$

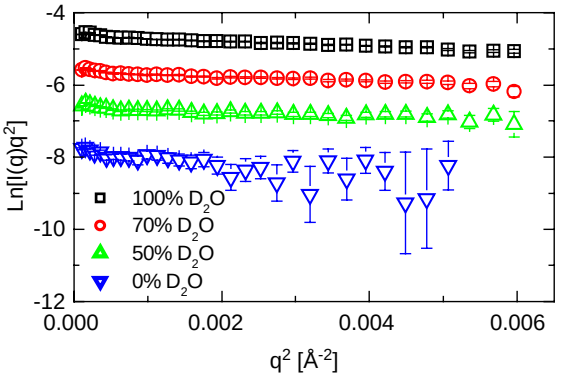

Fig. 1. Kratky–Porod plots of the experimental points of diC14:1PC vesicles in the aqueous phase with different H_2O/D_2O ratios.

where $R_g(\Delta\bar{\rho})$ is the bilayer radius of gyration measured at scattering density contrast $(\Delta\bar{\rho} = \rho_s - \bar{\rho})$ between the aqueous phase (ρ_s) and the average scattering density of the bilayer ($\bar{\rho}$), and R_g^{inf} is the gyration radius at infinite scattering density contrast; the parameter α describes deviations of the bilayer scattering density $\rho(r)$ about $\bar{\rho}$ [4]. The value of $\bar{\rho}$ was obtained from the match point k_m at which $I(q) = 0$ by plotting the square root of the scattering intensity at a particular small value of q as a function of the fraction k of D_2O in the aqueous phase [9]. After a careful inspection of the measured $I(q)$ curves we have chosen to use $q = 0.017\ Å^{-1}$ at which the $I(q)$ is not affected by the vesicle radius [8]. The parameter R_g^{inf} relates to the bilayer thickness parameter d_g following the equation $d_g \cong 12^{0.5} R_g^{inf}$.

3. Results and discussion

The match points for diC*n*:1PC bilayers where $n = 14,16,18,20$ and 22 were observed at $k_m = 17.8\%$, 16.6%, 13.8%, 13% and 9.3%, respectively. As expected, the dependence of k_m on n is almost linear. From the k_m match points, the values of $\bar{\rho}$ were calculated. The values of R_g were then obtained for each diC*n*:1PC bilayer at several contrasts $\Delta\bar{\rho}$ as illustrated in Fig. 2 for diC14:1PC vesicles.

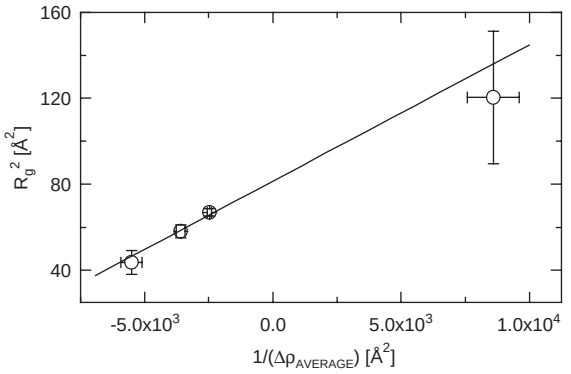

Fig. 2. Dependence of the radius of gyration on the contrast of neutron scattering length density.

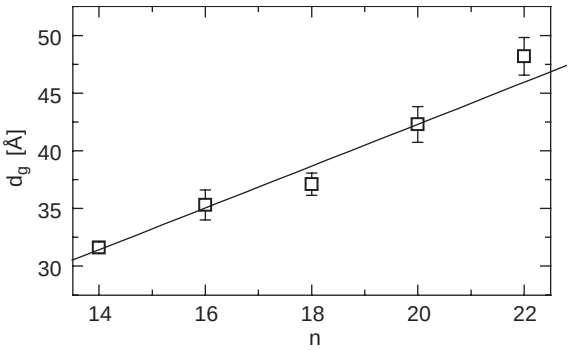

Fig. 3. The bilayer thickness parameter d_g as a function of the number of carbon atoms n in the diCn:1PC vesicles at 30°C.

The linear approximation done through the points in Fig. 2 (weighted by their errors) yields the value $(R_g^{inf})^2 = 83.1 \pm 2.8$ Å^2 for diC14:1PC vesicles. This procedure was done for all diCn:1PC vesicles and the corresponding values of the bilayer thickness parameter d_g were then calculated from R_g^{inf}. It is seen that the d_g increase with n (Fig. 3) can be approximated by a linear function $d_g \cong (6 \pm 3.2) + (1.8 \pm 0.2)$n Å.

In our recent paper [10], we have re-analyzed the SAXS data of Lewis and Engelman [13]. After corrections for Fourier truncation errors and temperature effects, we have obtained steric bilayer thicknesses $d_s = 46.7 \pm 2.2$ and 53.5 ± 2.2 Å for diC18:1PC and diC22:1PC vesicles, respectively. Comparing with the data in Fig. 3, it is seen that the d_g values are lower than the d_s values. However, the slope $\partial d_g/\partial n = 1.8 \pm 0.2$ Å found in the present paper coincides within experimental errors with the slope $\partial d_s/\partial n = 2.0 \pm 0.3$ Å found in [10] from three points (diC18:1PC, diC22:1PC and diC24:1PC).

The main advantage of the method used is the model-free analysis that does not assume any inner bilayer structure opposed to the fitting procedure. The direct fit of the data on a small q range depends highly on a bilayer model, which is, however, often supposed to be a single shell. We have compared these methods using a more realistic model of $\rho(r)$ suggested recently [11,12] in simultaneous fitting of the $I(q)$ curves measured at different contrasts over an extended range 0.01 Å$^{-1} \leqslant q \leqslant 0.2$ Å^{-1} (in preparation). We have found the bilayer thickness dependence to be $d_L \cong (15.9 \pm 1.4) + (1.5 \pm 0.2)$n Å.

The slopes $\partial d/\partial n$, which represent the increase in bilayer thickness due to an increase in acyl chain length, are frequently used in studies of bilayer–protein interactions. The values of these slopes found in the present work can be used in such studies.

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