The Small-Angle Neutron Scattering Data Analysis of the Phospholipid Transport Nanosystem Structure

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Abstract. The small-angle neutron scattering technique (SANS) is employed for investigation of structure of the phospholipid transport nanosystem (PTNS) elaborated in the V.N. Orekhovich Institute of Biomedical Chemistry (Moscow, Russia). The SANS spectra have been measured at the YuMO small-angle spectrometer of IBR-2 reactor (Joint Institute of Nuclear Research, Dubna, Russia). Basic characteristics of polydispersed population of PTNS unilamellar vesicles (average radius of vesicles, polydispersity, thickness of membrane, etc.) have been determined in three cases of the PTNS concentrations in D$_2$O: 5%, 10%, and 25%. Numerical analysis is based on the separated form factors method (SFF). The results are discussed in comparison with the results of analysis of the small-angle X-ray scattering spectra collected at the Kurchatov Synchrotron Radiation Source of the National Research Center “Kurchatov Institute” (Moscow, Russia).

1. Introduction

The small-angle scattering of neutrons (SANS) and of X-rays (SAXS) is the well known experimental technique that uses the elastic neutron scattering and the X-ray scattering at small angles to study the structure of various substances at a scale of about 1-100 nm [1, 2]. This technique is widely utilized for investigation of unilamellar phospholipid vesicles (ULVs) with different chemical compositions in large water excess, see [3, 4, 5, 6] and references therein. Investigations of the structure and properties of multilamellar and unilamellar vesicles are of great interest because they can provide an insight to the fundamental properties of biological and artificial lipid membranes. This knowledge can be of practical use in biochemistry and pharmacology [7, 8].

An efficient tool for analysis of the SANS and SAXS spectra is the separated form factors (SFF) method developed in [9, 10]. The SFF method was successfully applied for a study of the structure of dimyristoylphosphatidylcholine (DMPC) ULVs in different phase states of the lipid bilayer [10], at different concentrations of aqueous sucrose solutions [11]. The SFF-based approach was employed to study the multicomponent ULV systems based on ceramide 6 [11, 12, 13, 14]. In [15], the structure of dipalmitoylphosphatidylcholine ULVs in heavy water in the dimethyl sulfoxide presence has been analysed on the basis of the SFF method.
In this work, on the basis of the SFF analysis of SANS experimental data, we study a structure of ULVs of the phospholipid delivery nanosystem (PTNS) obtained in the V.N.Orekhovich Research Institute of Biomedical Chemistry (Moscow, Russia). Due extremely small size of PTNS nanoparticles, the medical drugs incorporation into PTNS ULVs sufficiently improves their therapeutic effectiveness [16, 17]. Estimations of basic parameters of PTNS ULVs within the frame of different approaches are presented in [18, 19, 20]. In our recent work [21], the structure of PTNS polydispersed population of ULVs in water solvent of maltose (concentration 20%, 25%, and 30%) has been analysed on the basis of the SAXS experimental data collected at the Kurchatov Synchrotron Radiation Source of the National Research Center “Kurchatov Institute” (Moscow, Russia).

Here, we investigate the polydispersed population of PTNS ULVs in heavy water solvent of maltose on the basis of the SFF analysis of the SANS spectra collected at the YuMO facility of the IBR-2 reactor (Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna).

2. The Separated Form Factors Method

In case of monodispersed population of ULVs with radius \( R \) and the bilayer thickness \( d \), the SANS intensity is determined as follows [22]:

\[
I_{\text{mon}}(q) = n I_o A^2(q) S(q), \quad A(q) = 4\pi \int_0^\infty \rho_c(r) \cdot \frac{\sin(qr)}{qr} r^2 \, dr. \tag{1}
\]

Here \( I_o \) is the incident beam intensity, \( n \) – number of vesicles in cm\(^3\), \( A \) – the small-angle scattering amplitude, \( q \) – the scattering vector, \( \rho_c(x) = \rho - \rho_0 \) – the contrast between the bilayer scattering length density \( \rho \) and the solvent scattering length density \( \rho_0 \), \( S \) – the structural factor depending on \( n \) and on the ULV radius \( R \) [22].

In case \( R \gg d \), the factorization of the scattering amplitude formula yields the following form of intensity:

\[
I_{\text{SFF}}(q) = n I_o F_s(q; R) F_b(q; d) S(q), \tag{2}
\]

where \( F_s(q; R) \) is the form factor of the spherical surface with radius \( R \)

\[
F_s(q; R) = \left( \frac{4\pi R^2 \sin(qR)}{qR} \right)^2, \tag{3}
\]

and \( F_b(q; d) \) – the form factor of the symmetric lipid bilayer of thickness \( d \):

\[
F_b(q; \Theta_b) = \left( \int_{-d/2}^{+d/2} \rho_c(x) \cos(qx) \, dx \right)^2. \tag{4}
\]

The SFF method simplifies the numerical analysis and extends a chose of model of the scattering length density distribution across bilayer. Calculations in [21] showed that both “classical” intensity formula (1) and the SFF expression (2) provide the same results of analysis of the PTNS structure, in spite of the small radius of PTNS ULVs. Hence, the SFF approach is shown to be applicable for the further investigations of PTNS ULVs.

Polydispersity \( \sigma \) of the ULV average radius \( \bar{R} \) is accounted for by means of the Schulz distribution with parameter \( m \):

\[
G \left( \bar{R}, R \right) = \frac{\bar{R}^m}{m!} \left( \frac{m + 1}{R} \right)^{m+1} \exp \left[ -\frac{(m + 1)\bar{R}}{R} \right], \quad \sigma = \frac{1}{\sqrt{m + 1}}. \tag{5}
\]
Then the intensity $I_m$ of the polydispersed ULV system is obtained by means of the standard convolution procedure:

$$I_m(q) = \frac{R_{\text{max}}}{R_{\text{min}}} \int_{R_{\text{min}}}^{R_{\text{max}}} I_{\text{SFF}}(q,R) G(\tilde{R},R) dR.$$  

The final expression of intensity $I(q)$ has the following form:

$$I(q) = I_m(q) + \frac{1}{2} \Delta^2 \frac{d^2 I_m(q)}{dq^2} + I_B,$$

where $I_B$ is the incoherent background parameter and $\Delta^2$ – the second momentum of the resolution spectrometer function.

The average radius $\bar{R}$, the polydispersity parameter $m$, the number of vesicles per volume unit $n$, the incoherent background factor $I_B$, the bilayer thickness $d$, and the parameters characterizing the internal bilayer structure, are adjusted by minimization of the discrepancy between theoretical and experimental values of intensity:

$$\chi^2 = \frac{1}{N-k} \sum_{j=1}^{N} \left( \frac{I(q_j) - I_{\text{exper}}(q_j)}{\delta_{\text{exper}}(q_j)} \right)^2,$$

where $N$ – number of experimental points, $k$ – number of adjusted parameters, $I$ is determined by Eq. (7), $I_{\text{exper}}$ – experimental values of intensity, $\delta_{\text{exper}}$ – experimental errors. Depending on value of $k$, we employ one of two fitting procedures. The first one is based on the generalized least square method [23, 24]. The second approach is the global minimization algorithm of the asynchronous differential evolution, see [25] and references therein for details.

3. Experiment

SANS spectra were measured on a YuMO small-angle neutron spectrometer at the IBR-2 pulsed reactor (Frank Laboratory of Neutron Physics of the Joint Institute for Nuclear Research, Dubna, Russia). The measurements were performed at the room temperature of PTNS ULV sample. The samples of polydispersed populations of PTNS ULVs were prepared by means of the dissolution of the lyophilized PTNS powder in the heavy water at concentrations 5%, 10%, and 25% (w/w). The concentration of maltose after dissolution of the drug was, respectively, 4%, 8%, and 20%.

4. Results and discussion

Results of the SFF-SANS analysis of PTNS ULVs are presented in Fig. 1 and Table 1. In Fig. 1(a-c), the calculated curves $I(q)$ are plotted in comparison with experimental data. Respective values of PTNS ULVs basic parameters obtained within the frame of the SFF approach, are given in Table 1. The calculation has been done with the “step” model of the scattering length density distribution across bilayer (see Fig. 1(d)). In general, the SANS-based estimations are in agreement with the SAXS-based results obtained in [21]. At 20% concentration of maltose where we have both SANS and SAXS data, the discrepancy between SANS-based and SAXS-based values of $\bar{R}$ and $d$ is about 10%.

Figure 2 demonstrates the SANS and SAXS estimations of the average radius $\bar{R}$ and the bilayer thickness $d$ of PTNS ULVs versus the disaccharide (maltose) concentration. SANS estimations are shown by blue squares, SAXS estimations – by blue circles. For comparison, the same characteristics versus the disaccharide (sucrose) concentration are given for the “classical”
Figure 1. (a–c) The SANS spectra for the PTNS ULVs in D$_2$O. Circles – experiment, solid lines – theory. (d) The “step” model of the scattering length density distribution across bilayer. Here $\rho_{\text{CH}_2}$ – density of the hydrocarbon chains region, $\rho_{\text{PH}}$ – density of the polar head region, $\rho_0$ – the solvent density, $d$ – thickness of the ULV bilayer, $D$ – thickness of the hydrocarbon chains region in the bilayer.

Table 1. PTNS ULVs in D$_2$O: results of the SFF-fitting of SANS data at 5–25% concentration of PTNS.

| Concentration of PTNS | Concentration of maltose | $R$, Å | $\sigma$, % | $d$, Å | $D$, Å | $\chi^2$ |
|-----------------------|--------------------------|--------|------------|--------|--------|---------|
| 5%                    | 4%                       | 191.5±3.3 | 41       | 45.9±1.9 | 13.0±0.5 | 6.7     |
| 10%                   | 8%                       | 199.6±2.8 | 41       | 46.7±1.8 | 17.0±1.0 | 7.5     |
| 25%                   | 20%                      | 192.2±1.5 | 34       | 38.9±3.4 | 18.0±1.9 | 8.2     |

size DMPC ULVs (red triangle). It is seen that in case of the weak maltose concentration, the PTNS ULV average radius $R$ is about 200±5 Å that is 1.5 times smaller than the radius of “classical” vesicles of DMPC [11]. Also, one can see that the radius of both DMPC and PTNS ULVs becomes smaller as the disaccharide concentration is growing. As for the PTNS ULV bilayer thickness, its value changes from 45.9±1.9 Å in case of 4% maltose concentration to
37.0±0.2 Å at 30% concentration. Also, it is seen that the values of $d_{PTNS}$ and $d_{DMPC}$ are close to each other.

![Figure 2. SFF analysis of SAXS and SANS spectra: average radius (a) and the thickness of bilayer (b) of PTNS ULVs (blue squares – SANS estimations, blue circles – SAXS estimations) and of DMPC ULVs (red triangles) versus, respectively, maltose and sucrose concentration (%). Both the average radius and the bilayer thickness are in Å.]

5. Summary

- The SFF method has been employed for the SANS analysis of the polydispersed PTNS ULVs structure.
- Basic parameters of PTNS vesicles (average size of vesicles, thickness of bilayer, polydispersity of average radius) have been determined depending on the maltose concentration in the heavy water.
- Estimations on the basis of SANS and of SAXS data are shown to be in the reasonable agreement.
- In case of the weak maltose concentration (5–20%), the average radius of PTNS ULVs is 1.5 times smaller the standard size of the “classical” size vesicles of DMPC.

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