Determination of protein-mediated intervesicular phospholipid transfer by time-resolved measurements

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1. Introduction

Bilayer structure of biomembranes consists of several thousand of lipids, and the composition differs at each organelle. Since most lipids are synthesized in the endoplasmic reticulum (ER), the subsequent distribution to each organelle determines the composition and function of the biomembranes (*J Lipid Res 1981, 22, 391*), and lipid transfer proteins are members that take the role. Among yeast proteins, Sec14 is known to transfer phosphatidylcholine (PC) and phosphatidylinositol (PI) (*Nature 1990, 347, 56*). However, the mechanism by which this protein transfers lipids between membranes has not been discussed in detail based on quantitative analysis.

We have utilized time-resolved small-angle neutron scattering (TR-SANS) for determination of the spontaneous phospholipid transfer between vesicles (*Phys. Rev. Lett. 2007, 98, 238101*). This technique takes advantage of the large difference in the scattering length density between hydrogenated and deuterated phospholipids. Exchange of these lipids between vesicles brings about a decrease in the scattering intensity. By this method, we have reported that 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), represents extremely slow intervesicular exchange dynamics ($t_{1/2}$ ~ 90 h), and that methyl-β-cyclodextrin (MβCD) can mediate and accelerate the lipid exchange (*J. Phys. Chem. B 2009, 113, 6745*). Based on these findings, we have got an idea that TR-SANS can be used to determine phospholipid transfer activity of proteins using the proteins instead of MβCD. Hence, in this study we carried out the TR-SANS experiments for quantitative analysis of Sec14-mediated transfer of phospholipids.

2. Experiment

Large unilamellar vesicles (diameter ~120 nm) consisting of 90% POPC and 10% 1-palmitoyl-2-oleoylphosphatidylserine (POPS) or 90% d_{31}-POPC and 10% POPS (denoted as H-LUV and D-LUV, respectively) were prepared. TR-SANS experiments were performed at J-PARC BL15 TAIKAN. Measurements were started immediately after mixing several concentrations of Sec14 to mixtures of D-LUV (15 mM) and H-LUV (15 mM) in Tris buffer containing 30% D_2O at 37 °C. The D_2O volume fraction of 30% was settled on so that the solvent has the intermediate value of the scattering length density between that of D-LUV and H-LUV. Scattering data collected for every one minute were processed including conversion to I-Q data, subtraction for the solvent scattering, and integration of $I(Q)$ between $0.007 < Q < 0.011$ Å^{-1} to obtain the total scattering intensity. The normalized contrast, $\Delta\rho(t)/\Delta\rho(0)$, is calculated by $\Delta\rho(t)/\Delta\rho(0) = \{I(t)/I(0)\}^{0.5}$, where $I(0)$ and $I(t)$ are the total scattering intensity at time 0 and t, respectively, after the protein's mixing. Because POPC does not flip spontaneously (*J. Phys. Chem. B 2009, 113, 6745*), reduction in the scattering intensity is solely ascribed to the protein-mediated intervesicular exchange of POPC. Because only POPC (but not POPS) in D-LUV is deuterated, transfer of POPS, even if it occurs, does not change the scattering intensity and, thus, only the POPC transfer is detected in this study.

3. Results

The normalized contrasts, calculated from the SANS intensities, were plotted against time after the

addition of Sec14 into the mixture of D- and H-LUVs (Figure 1). Decay in the normalized contrast was observed depending on the Sec14 concentration, suggesting the protein-mediated POPC transfer. The decay curves could not be fitted by a single-exponential function, although the reason is unknown. Thus, we used a double-exponential function with a constant term of 0.6, which is the value obtained for the mixture of D- and H-LUVs incubated with MβCD for 6 h. Initial rates of the transfer were calculated by the differential of the fitting curve at t = 0, and were plotted against the protein concentration in Figure 2. The transfer rate of POPC in the presence of 10% POPS was significantly faster than that in the absence of POPS, which was previously measured at SANS-U using 100% POPC vesicles. Since lipid transfer is caused by the proteins that are on the membranes, the initial rate (y-axis of Figure 2) should be proportional to the amount of Sec14 binding to the membranes. Thus, the dissociation constant (K_D) could be figured out to be 262 μg/mL (7.5 μM) and 116 μg/mL (3.3 μM) for POPC membranes with and without 10% POPS, respectively. In addition, from the initial slope of the curves in Figure 2 the maximum transfer rate per protein could be calculated, which was 0.88 or 3.79 PC·protein^{-1}·s^{-1} in the absence or presence of POPS, respectively.

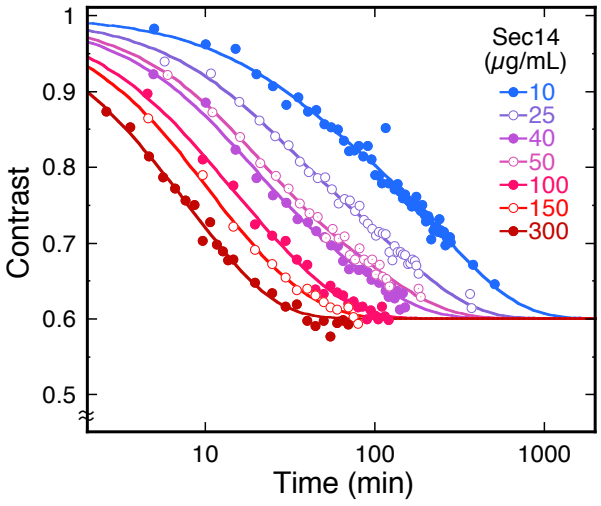

Figure 1. Temporal changes in the normalized contrasts of POPC/POPS (9:1) vesicles with different concentrations of Sec14.

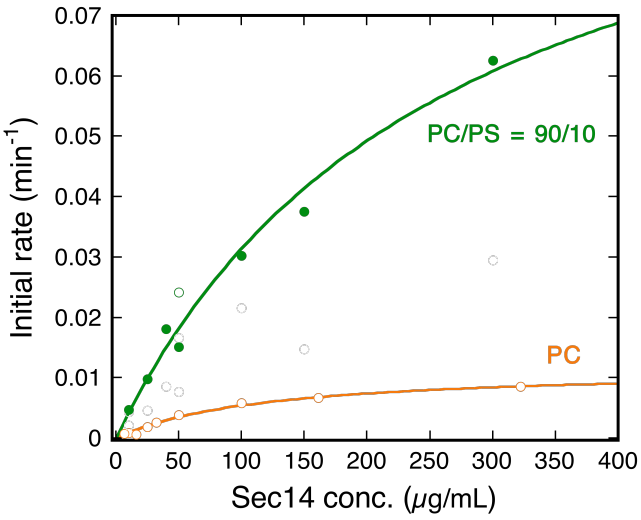

Figure 2. Initial POPC exchange rates for POPC and POPC/POPS (9:1) vesicles with different concentrations of Sec14. Open circles are the data previously obtained at SANS-U.

4. Conclusion

PC transfer activity of Sec14 could be detected by TR-SANS. The PC transfer rate per protein was increased 4.3-fold (from 0.88 to 3.79 PC·protein^{-1}·s^{-1}) by the incorporation of 10% of POPS into POPC vesicles. Because the binding affinity ($1/K_D$) was not increased by the incorporation of POPS, this acidic lipid would raise the PC transfer activity not by increasing membrane-binding rate but by enhancing lipid-exchanging process of Sec14 on membranes.