Simultaneous Evaluation of Lipid Exchange and Flip-Flop in Vesicles

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We recently succeeded in determining the rates of interbilayer exchange and flip-flop of dimyristoylphosphatidylcholine (DMPC) in large unilamellar vesicles (LUVs) by small-angle neutron scattering (SANS) technique [1]. This technique takes advantage of the large difference in the scattering length density between hydrogenated and deuterated lipids, and the exchange of these lipids between LUVs results in a decrease in the scattering intensity, which can be detected by time-resolved SANS (TR-SANS) measurements. In this study, effect of cholesterol (Chol) on the rate of interbilayer and transbilayer transfers of DMPC was evaluated by TR-SANS measurements.

DMPC, d54-DMPC, were obtained from Avanti Polar Lipids Inc. (Alabaster, AL). Cholesterol (Chol) was from Sigma (ST. Louis, MO). LUVs consisting of deuterated (D-LUV) or hydrogenated DMPC (H-LUV) and Chol (0, 20, and 40 mol% of total lipids) with a diameter of ca. 100 nm were prepared by extrusion method using Tris-buffered saline prepared from mixtures of D2O and H2O. LUVs consisting of 1:1 mixture of deuterated and hydrogenated lipids (D/H-LUV) were also prepared by mixing these lipids and Chol before hydration. Volume fraction of D2O in the Tris-buffer was 0.5, 0.45, and 0.4 for LUVs with 0, 20, and 40 mol% Chol, respectively, which corresponds to the contrast-matching condition between D/H-LUV and solvent. Phospholipid concentration of each LUV preparation was set to 20 mM. SANS measurements were performed at 37 °C using SANS-U with 7 angstrom of incident neutron beam. Sample-to-detector distance was set to 4 m.

First, we confirmed that D-LUV and H-LUV had almost identical scattering profile, while D/H-LUV exhibited little scattering at the contrast matching condition.

TR-SANS measurement was started immediately after mixing equivalent volume of D-LUV and H-LUV. Time-course of the normalized contrast was calculated from the scattering intensity and plotted in Figure 1. The contrast decay profiles depend on the mole fraction of Chol. In the absence of Chol, the normalized contrasts reached below 0.5, suggesting an involvement of flip-flop, and the obtained contrast decays were well reproduced by the kinetic model that takes account of both the interbilayer and transbilayer exchange. These exchange rates were reduced in the presence of Chol, and the flip-flop was vanished at 40 mol% Chol, where lipids are known to form liquid-ordered phase.

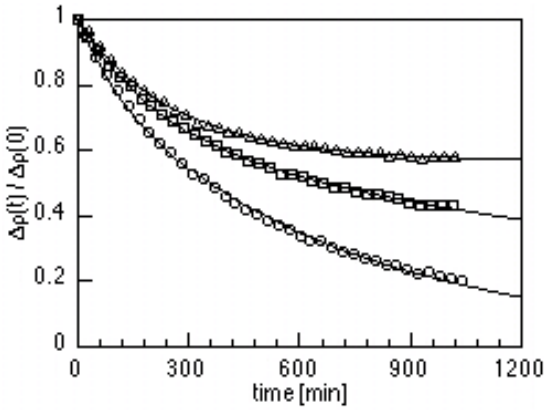

Fig. 1. Contrast decays of DMPC/Chol LUVs with 0 (circles), 20 (squares), and 40 mol% Chol (triangles) after mixing D- and H-LUV at 37 °C.