Induction of Flip-Flop by Helix-Forming Peptides

M. Nakano(A), M. Kaihara(A), H. Yokoyama(A), H. Endo(B)
(A)Graduate School of Pharmaceutical Sciences, Kyoto Univ., (B)Quantum Beam Science Directorate, JAEA

Most of cellular lipids are synthesized in the endoplasmic reticulum (ER), in which half of the phospholipids synthesized on the cytosolic leaflet should be translocated to luminal leaflet rapidly to maintain mass balance between two leaflets. This is presumably governed by flippases, but the detailed mechanisms remain to be clarified. Kol et al. have observed that peptides that mimic the alpha-helices of transmembrane proteins can stimulate flip-flop of fluorescence-labeled phospholipids in liposomes [1], which indicates that the ability to catalyze flip-flop in the ER is not necessarily restricted to one specific protein. We have developed a method to determine the rates of intervesicular exchange and flip-flop of phospholipids in vesicles with time-resolved small-angle neutron scattering (TR-SANS) [2, 3]. In this study, we used palmitoyloleoylphosphatidylcholine (POPC), which has been previously shown not to flip spontaneously, and constructed model systems of POPC membranes with transmembrane peptides to determine the rate of peptide-induced flip-flop by neutron scattering, based on a hypothesis that fast flip-flop in the ER is achieved by a mere presence of transmembrane helices of several membrane proteins.

LUVs consisting of deuterated (D-LUV) or hydrogenated POPC (H-LUV) were prepared by extrusion method in the presence and absence of 0.5 mol% transmembrane peptides. TR-SANS measurement was started immediately after mixing equivalent volume of D- and H-vesicles in the presence of methyl-β-cyclodextrin. Time-course of the normalized contrast was calculated from the scattering intensity.

TR-SANS showed that POPC in vesicles does not flip in the absence and presence of transmembrane helical peptides whose transmembrane region is fully consist of hydrophobic amino acids. This result suggests that a mere insertion of transmembrane helices into bilayer does not mediate the lipid flip-flop. Interestingly, however, flip-flop of POPC was induced by the peptides whose central amino acid (leucine) was substituted for lysine or glutamic acid.

References

[1] M. A. Kol, A. N. C. van Laak, D. T. S. Rijkers, J. A. Killian, A. I. P. M. de Kroon, B. de Kruijff, Biochemistry 42 (2003) 231.
[2] M. Nakano, M. Fukuda, T. Kudo, H. Endo, T. Handa, Phys. Rev. Lett. 98 (2007) 238101.
[3] M. Nakano, M. Fukuda, T. Kudo, N. Matsuzaki, T. Azuma, K. Sekine, H. Endo and T. Handa. J. Phys. Chem. B 113 (2009) 6745.