Enhancement of Lipid Flip-Flop by Membrane Proteins and Transmembrane Peptides

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Interbilayer transport and transbilayer movement of phospholipids are crucial for cell growth, development and survival, and are controlled by lipid transfer proteins and translocase enzymes. The endoplasmic reticulum (ER), in which phospholipids are newly synthesized on the cytosolic leaflet, maintains membrane symmetry, presumably by flippase activity, which is bidirectional and energy-independent. On the other hand, the plasma membrane retains asymmetric lipid distribution via aminophospholipid translocase that mediates the unidirectional transport of phosphatidylserine and phosphatidylethan-olamine from the ectoplasmic to cytoplasmic leaflet of the bilayer. Disruption of the asymmetry in cells by the action of phospholipid scramblase is involved in apoptosis and is associated with increased binding and phagocytosis of these cells by macrophages. Understanding and control of these lipid dynamics quantitatively is therefore a key challenge in biophysics and cell biology.

Although this transbilayer movement of lipids is considered protein-mediated, it is unclear whether it involves a dedicated flippase or the mere presence of proteins in the ER bilayer. 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 previously demonstrated by SANS that shorter acyl chain lipid, dimyristoylphosphatidylcholine (DMPC) can flip-flop in large unilamellar vesicles (LUVs) [2], while longer acyl chain lipid, 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) can not. In this study, similar experiments were carried out with small unilamellar vesicles (SUVs) of DMPC, and POPC LUVs in the presence of a transmembrane peptide.

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% a transmembrane peptide KALP23. DMPC SUVs (D- and H-SUV) were prepared by sonication. TR-SANS measurement was started immediately after mixing equivalent volume of D- and H-vesicles. Time-course of the normalized contrast was calculated from the scattering intensity.

In the presence of methyl- β -cyclodextrin, the normalized contrast for POPC LUVs reached around 0.5 and became constant, which suggests that POPC does not flip-flop at all. In the presence of KALP23, however, no flip-flop was observed. This result suggests that a mere insertion of transmembrane helices into bilayer does not mediate the lipid flip-flop.

DMPC in SUVs showed faster flip-flop compared with the same lipid in LUVs. Curvature of the membranes is considered to alter the environment of lipids, such as acyl chain packing and/or headgroup hydration, and affect the lipid dynamics.

References

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