Determination of peptide-induced phospholipid flip-flop by time-resolved measurements
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1. Introduction

Phospholipid transbilayer movement (flip-flop) plays an essential role in the proper functions of biological membranes in eukaryotes (Nat. Rev. Mol. Cell Biol. 2008, 9, 112). Several kinds of membrane proteins control flip-flop in the plasma membrane. An asymmetric composition of phospholipids in the plasma membrane is maintained through an energy-dependent process by the activities of amino phospholipid translocases and ATP-binding cassette transporters, and disruption of the asymmetry by phospholipid scramblases is involved in apoptotic cell death or platelet-dependent co-aggregation (Annu. Rev. Physiol. 2003, 65, 701). A rapid rate of phospholipid flip-flop is observed in the endoplasmic reticulum (ER), with the half time of the flip-flop ranging from seconds to minutes (Biophys. J. 2000, 78, 2628). Phospholipids in eukaryotic cells are mainly synthesized at the cytoplasmic leaflet of the ER membrane. Therefore, newly synthesized phospholipids must be transferred to the luminal leaflet of the membrane in order to maintain membrane integrity in the ER. In this way, protein-mediated control of the phospholipid flip-flop is crucial.

We have previously demonstrated that transmembrane model peptides with a hydrophilic residue located within the center of a hydrophobic sequence enhanced the flip-flop of phospholipids (Chem. Phys. 2013, 419, 78, Biophys. J. 2016, 110, 2689). This result implies the possibility that cellular functions can be manipulated by synthetic peptides. To achieve this, it is important to construct a model system in which the addition of peptides triggers the phospholipid flip-flop and a method to detect it. Hence, in this study we carried out time-resolved SANS experiments for analysis of the peptide-induced phospholipid flip-flop.

2. Experiment

Transmembrane peptides (4XQ peptide; Acetyl-XXXXALALALALALQWLALALALA-amide (X = K or R) and its negative-control 4XL peptide; Acetyl-XXXXALALALALALWLALALALA-amide) were synthesized using Fmoc-based chemistry and purified by reverse-phase HPLC equipped with a C18-column. The peptides obtained were dissolved in ethanol. Large unilamellar vesicles (diameter ~120 nm) consisting of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) or 1-palmitoyl-31-2-oleoylphosphatidylcholine (d31-POPC) (denoted as H-LUV and D-LUV, respectively) were prepared and the ethanol solution of the peptides was mixed with each vesicle suspension at a final peptide concentration of 0.03 mol% of total lipids. These peptides would be incorporated into vesicles immediately after the addition to the suspension. All SANS measurements were performed using buffer containing 30% D2O, which has an intermediate scattering length density between H-LUV and D-LUV. SANS experiments were performed at J-PARC BL15 TAIKAN. Time-resolved measurements were started immediately after mixing an equivalent volume of D-LUV and H-LUV (final concentration of 15 mM each) in the presence of 1 mM methyl-β-cyclodextrin (MβCD), which is known to accelerate the intervesicular exchange of POPC, and the scattering intensities were recorded. Scattering data collected were processed including conversion to I-Q data, subtraction for the solvent scattering, and integration of \( I(Q) \) between 0.007 < \( Q < 0.11 \) Å\(^{-1}\) to obtain the total scattering intensity. The normalized contrast, \( \Delta \rho(t)/\Delta \rho(0) \), was 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 mixing of MβCD.
3. Results

The normalized contrasts were calculated from the SANS intensities and plotted against time after the addition of MβCD into the mixture of D- and H-LUVs (Figure 1). In the absence of peptides, the contrast decreased and reached a plateau of ~0.6, clearly suggesting that MβCD exchanges POPC and d31-POPC in the outer leaflet of the vesicles and that these lipids do not flip-flop between outer and inner leaflets, which agrees with the previous report (J Phys Chem B 2009, 113, 6745). Incorporation of 0.03 mol% 4KL or 4RL into vesicles did not change the contrast decay profile. On the other hand, incorporation of 0.03 mol% 4KQ or 4RQ, which contain a hydrophilic Glu residue in the center of their transmembrane sequence, decreased the contrast to the values well below 0.5, suggesting these peptides induce the flip-flop of the lipids.

![Figure 1. Temporal changes in the normalized contrasts of POPC vesicles (30 mM) in the absence and presence of various peptides (0.03 mol%) after addition of 1 mM MβCD.](image)

4. Conclusion

The time-resolved data clearly detected the ability of 4RQ and 4KQ peptides, which were added to POPC vesicles, to scramble phospholipids between inner and outer leaflets of bilayers. Because 4XQ and 4XL peptides differ in that the former substitutes Glu for central Leu residue in the latter, it is obvious that the Glu residue localized in the middle of bilayers plays an important role for the scramblase activity of the peptides. The data imply the possibility that 4RQ and 4KQ peptides can induce flip-flop of the plasma membrane by adding them to cultured cells. Experiments to prove this is under progress.