Peptide Science 2018: Proceedings of the 10th International Peptide Symposium / The 55th Japanese Peptide Symposium
Shiroh Futaki and Katsumi Matsuzaki (Editors)
The Japanese Peptide Society, 2019

Association-Dissociation Kinetics for Dimerization of Transmembrane Helices as Detected by Single Molecule Fluorescence Spectroscopy

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Experimental system using model transmembrane helices and lipid bilayers is useful to elucidate driving forces for membrane protein folding. Here we show that real-time detection of association-dissociation dynamics for transmembrane helices is possible by single molecule FRET technique.

Keywords: FRET; single molecule; transmembrane helix

Introduction

Conformational fluctuation of helical membrane proteins among active–inactive conformations is crucial for their functions. Not only the amino acid sequence of the protein but also the composition of surrounding lipids should significantly affect the fluctuation, although the fundamental principles are mostly unknown. Experimental systems using model transmembrane helices and lipid bilayers have been proposed to be useful for direct measurements of elementary processes that determine the folding and conformational change of membrane proteins, such as insertion of the helix into lipid bilayers and helix–helix interaction in the bilayers, which are determined through the balance between helix–helix, helix–lipid, and lipid–lipid interactions. We have elucidated that membrane partitioning and self-association of the inert transmembrane helix $(AALALAA)_3$ was significantly affected by lipid compositions.[1,2] To obtain dynamic information on the helix–helix interactions, we examined real-time detection of self-association and dissociation of the transmembrane helix $(AALALAA)_3$ in lipid bilayers by single molecule FRET detection.

Results and Discussion

To detect self-association of the helices by FRET, the N-terminus of the helix was labeled with the donor Cy3B or the acceptor Cy5. The helices were incorporated into large unilamellar vesicles (LUVs, diameter ~100 nm) composed of palmytoil-oleoyl-phosphatidylcholine/cholesterol (7/3) at a peptide/lipid ratio of 2/90000 to obtain LUVs incorporating only a few number of helices. The LUVs were attached on a glass surface via biotin–avidin interaction and observed under a total internal reflection fluorescence microscope (Fig. 1). After simultaneous observation of Cy3B and Cy5 fluorescence upon excitation of Cy3B at 561 nm, LUVs that had incorporated only one Cy3B- and one Cy5- labeled helices were selected based on the number of photobleaching steps, and single-molecular FRET from Cy3B to Cy5 was analyzed.

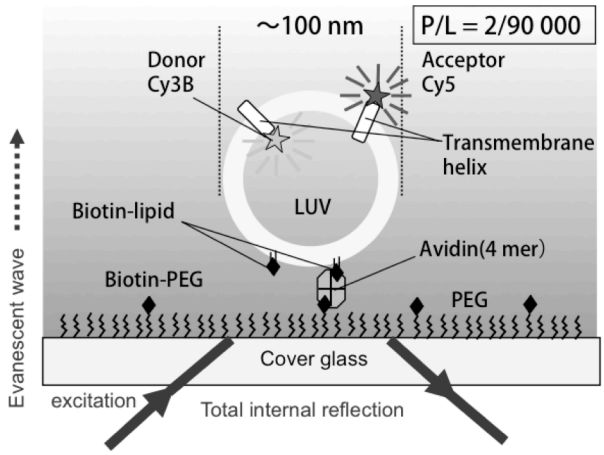

Fig. 1. Experimental setup for single molecule FRET detection of helix-helix interaction in LUVs

Stepwise increase and decrease of Cy3B fluorescence correlated with decrease and increase of Cy5 fluorescence were observed in the vesicles, indicating FRET fluctuation originating from association and dissociation of the helices, respectively. Such a fluctuation was not observed in vesicles without cholesterol, demonstrating that lipid composition dramatically modulate the self-association of transmembrane helices. Analysis of the FRET time course in cholesterol–containing vesicles revealed that the helices fluctuate between monomer and dimer with duration times of ~0.16 sec and ~0.05 sec, respectively. The obtained association constant from the kinetic constants well corresponded to that observed in conventional ensemble FRET measurements, confirming reliability of the single-molecule detection of helix–helix interaction in lipid bilayers. The above experimental system will be useful for measurement of kinetics of helix–helix interactions in membranes with various lipid compositions.

This work was financially supported in part by Grant-in-Aid for Scientific Research on Innovative Areas (Molecular Science of Fluctuations toward Biological Functons) from MEXT, Japan.

References

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2. Yano, Y., Ogura, M., and Matsuzaki, K. (2006) *Biochemistry,* **45**, 3379-3385.

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