GS02-7 Intracellular delivery of functional proteins using endosome destabilizing peptide

○Misao AKISHIBA[1], Toshihide TAKEUCHI[1], Yoshimasa KAWAGUCHI[1], Kentarou SAKAMOTO[1], Ikuhiko NAKASE[2], Shiroh FUTAKI[1]

[1]Institute for chemical research, Kyoto Univ., [2]NanoSquare Reserch Institution, Osaka Prefecture University

Many approaches introducing bioactive proteins into the cell to control cellular events have been developed for decades. In this regard, the most potent strategy is using endocytic pathway. When this pathway is employed, proteins of interest are endocytosed, released from endosomes and function in the cytosol. The abilities of known endosomotropic agents are insufficient to release molecules. Therefore, a more effective approach for perturbing endosomal membranes is needed.

We thus developed a novel endosome-destabilizing peptide (L17E, IWLTALKFLGKHAAKHEAKQQLSKL-amide) by engineering the structure of a hemolytic peptide derived from a spider toxin. This peptide showed significant stimulation of cytosolic release of endocytosed molecules without considerable cytotoxicity, indicative of the selective perturbation of endosomal membranes. The membrane-perturbation mechanism of this peptide is the preferential disruption of negatively charged membranes over neutral membranes, and L17E has an ability to induce macropinocytosis. Here, we demonstrate successful recognition of intracellular targets by the intracellularly delivered antibodies which was confirmed by confocal microscopic analysis and the effect on signal transduction.