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Scope of Research

The ultimate goal of our research is the regulation of cellular functions using designed peptides and proteins. Current research subjects include 1) development of novel intracellular delivery systems aiming at elucidation and control of cellular functions using designed membrane-permeable peptide vectors, 2) elucidation of the DNA binding modes of zinc finger proteins and TALEs, and design of artificial transcription factors with various DNA binding specificities, 3) elucidation and control of membrane curvature, and 4) design of stimulation-responsible artificial peptides and proteins.

KEYWORDS

Membrane-Permeable Peptides
Intracellular Delivery
Peptide Design
DNA/RNA Binding Protein
Membrane Curvature

Selected Publications

Akishiba, M.; Takeuchi, T.; Kawaguchi, Y.; Sakamoto, K.; Yu, H. H.; Nakase, I.; Takatani-Nakase, T.; Madani, F.; Graslund, A.; Futaki, S., Cytosolic Antibody Delivery by Lipid-Sensitive Endosomolytic Peptide, *Nat. Chem.*, **9,** 751-761 (2017).

Murayama, T.; Masuda, T.; Afonin, S.; Kawano, K.; Takatani-Nakase, T.; Ida, H.; Takahashi, Y.; Fukuma, T.; Ulrich, A. S.; Futaki, S., Loosening of Lipid Packing Promotes Oligoarginine Entry into Cells, *Angew. Chem. Int. Ed. Engl.*, **56,** 7644-7647 (2017).

Futaki, S.; Nakase, I., Cell-Surface Interactions on Arginine-Rich Cell-Penetrating Peptides Allow for Multiplex Modes of Internalization, *Acc. Chem. Res.*, **50,** 2449-2456 (2017).

Tsuji, S.; Futaki, S.; Imanishi, M., Sequence-Specific Recognition of Methylated DNA by an Engineered Transcription Activator-Like Effector Protein, *Chem. Commun.*, **52,** 14238-14241 (2016).

Loosening of Lipid Packing Promotes Oligoarginine Entry into Cells

The mechanisms for internalization of arginine-rich cell-penetrating peptides (CPPs), including the HIV-1 TAT peptide and oligoarginines, are still under debate although many factors that promote direct translocation of CPPs across the cell plasma membrane were reported (Figure 1A). These days, amphipathic helices are found in the common motif for membrane active peptides / proteins, including curvature inducing proteins. We previously reported that the N-terminal segment of epsin-1 possesses positive membrane curvature inducibility and that this peptide promotes membrane translocation of R8, which is known as a representative CPP. We this time used differential scanning calorimetry (DSC) to assess the curvature inducibility of amphipathic peptides, which are derived from proteins to induce tabulation/fusion of liposomal membranes. As the result, cytosolic distribution of FITC-R8 was observed in the presence of EpN18 and Sar1p(1-23) with positive membrane curvature inducibility at about 80 % and 100 % of the cell population, respectively. Furthermore, the sites of R8 influx were found to have looser lipid packing than surrounding areas, revealed by using environment-sensitive probe (di-4-ANEPPDHQ). Taken together, the importance of lipid packing was demonstrated as a key factor, which underlies the various conditions to promote the direct membrane translocation of arginine-rich CPPs (Figure 1B).

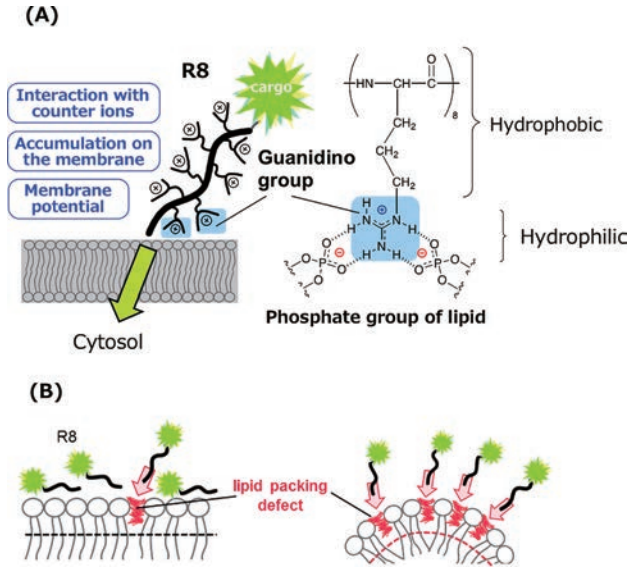

Figure 1. The mechanism underlying the membrane penetration of R8.

Cytosolic Antibody Delivery by Lipid-sensitive Endosomolytic Peptide

Antibodies have high abilities in molecular recognition and targeting. Intracellular antibody delivery could thus achieve controlling cellular events, such as protein-protein interaction and post-translational modification. This suggests the potential applicability of antibodies to attack intracellular therapeutic targets. Many approaches for intracellular delivery of biomacromolecules have been reported up to the present time. However, few of them are efficient enough to deliver high-molecular-weight proteins such as antibodies into cytosol to effectively modulate cell functions.

In order to deliver various membrane-impermeable molecules into the cytosol effectively and efficiently, we developed a novel endosome-destabilizing peptide, L17E by engineering the structure of a hemolytic peptide derived from a spider toxin. This peptide showed significant stimulation of cytosolic release of endocytosed molecules, including polydextran (10kDa), Cre recombinase and antibodies (IgG). Successful recognition of intracellular targets by the intracellularly delivered antibodies was confirmed by confocal microscopic analysis and the effect on signal transduction. These results suggested that this peptide holds the promise of modifying cell functions.

Unlike previously reported intracellular delivery peptides and polymers, the mechanism of action of L17E was found to be its preferential perturbation of negatively charged endosomal membranes. Additionally, we found that L17E also has a property to stimulate cellular uptake amount of biomacromolecues. These are unique features equipped with our peptide which have not been reported for other delivery peptides and polymers. We serendipitously found these features after we obtained this peptide.

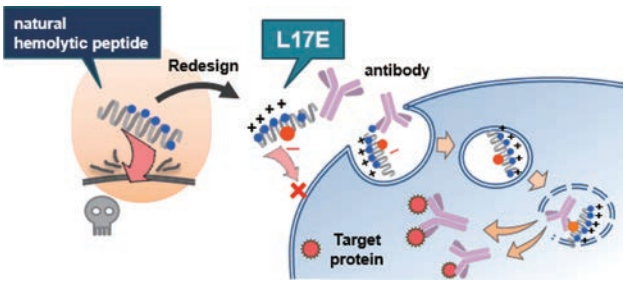

Figure 2. A simple redesign of spider venom peptide 'M-lycotoxin' into L17E enables the efficient release of antibodies from their endosome cages.