

Department of Biofunctional Chemistry

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Research Projects:

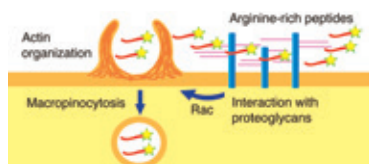
The ultimate goal of our research is the regulation of cellular functions by designed peptides and proteins, aiming at elucidation of biological functions as well as development of novel functional bio-devices having therapeutic potentials.

1) Development of membrane-permeable peptide vectors: Arginine-rich peptides, including octa-arginine (R8), HIV-1 Tat, and branched-chain arginine-rich peptides, belong to one of the major classes of cell-permeable peptides which deliver various proteins and macromolecules to cells. The importance of the endocytic pathways has recently been demonstrated in the cellular uptake of these peptides. We have previously shown that macropinocytosis is one of the major pathways for cellular uptake and that organization of the F-actin accompanies this process. In this study, using proteoglycan-deficient CHO cells, we have demonstrated that the membrane-associated proteoglycans are indispensable for the induction of the actin organization and the macropinocytotic uptake of the arginine-rich peptides. We have also demonstrated that the cellular uptake of the Tat peptide is highly dependent on heparan sulfate proteoglycan (HSPG), whereas the R8 peptide uptake is less dependent on HSPG. This suggests that the structure of the peptides may determine the specificity for HSPG, and that HSPG is not the sole receptor for macropinocytosis. Comparison of the HSPG specificity of the branched-chain arginine-rich peptides in cellular uptake has suggested that the charge density of the peptides may determine the specificity. The activation of the Rac protein and the actin organization was observed within a few minutes after the peptide treatment. These results strongly suggest the possibility that the interaction of the arginine-rich peptides with the membrane-associated proteoglycans quickly activates the intracellular signals and induces actin organization and macropinocytosis.

2) Creation of artificial transcription factors manipulating circadian rhythm: Regulation of a target gene at will is one of the most prospective themes in the post-genomic era. An artificial transcription factor with desired DNA binding specificity could work as a powerful tool to control target

genes. The C₂H₂-type zinc finger motif is one of the most typical DNA binding motifs. We have developed artificial zinc finger transcription factors with novel DNA binding specificity and succeeded in inducing resetting of circadian clock or regulating gene expression patterns to be circadian rhythmic. In addition, we are developing TALE proteins that have new DNA binding specificities. Our approach using artificial DNA binding proteins would be useful for elucidating the mechanisms of biological phenomena such as circadian rhythm.

3) Design of artificial receptor channel proteins: Ion channels and receptors are among the most biologically important classes of membrane proteins that transmit outside stimuli into cells. The creation of artificial proteins with these functions is a challenge in peptide/protein engineering in view of the creation of novel functional nano-devices as well as understanding the biological machinery. We have developed a novel Fe(III)-gated ion channel system that is comprised of assemblies of a channel forming peptide alamethicin bearing an extramembrane segment. The extramembrane segment contains a pair of diiminoacetic acid derivatives of lysine (Lda) residues. Addition of Fe(III) lead to the conformational switch in the extramembrane and the eventual increase in the channel current. This strongly suggests the possibility of establishing novel channel and sensor systems by transmitting an extramembrane conformational switch to the channel current levels. In addition, there are many reports on the creation of artificial ion channels that have a sensing function of the external ligands. However, most of them have been designed so that the interaction with ligands leads a decreased channel current, and very few of them have a function that can detect the ligand with the increased membrane current as are usually seen in natural ligand-gated ion channels. The system established by us is rather simple and may need further sophistication. However, we believe that this concept can extensively be applicable for the creation of various ligand-gated ion channels with novel receptor functions.



Interaction of arginine-rich peptides with membrane-associated proteoglycans leads activation of Rac protein followed by actin organization and induction of macropinocytosis. Thus, arginine-rich peptides are efficiently taken up by the cells with cargo molecules.

Recent publications

- Nakase et al. Molecular interplays involved in the cellular uptake of octaarginine on cell surfaces and the importance of syndecan-4 cytoplasmic V domain for the activation of protein kinase Ca. *Biochem Biophys Res Commun* **446**, 857, 2014.
- Tsuji et al. Creating a TALE protein with unbiased 5'-T binding. *Biochem Biophys Res Commun* **441**, 262, 2013.
- Noshiro et al. Construction of a Ca²⁺-gated artificial channel by fusing alamethicin with a calmodulin-derived extramembrane segment. *Bioconjug Chem* **24**, 188, 2013.