Division of Biochemistry
- Biofunctional Design-Chemistry -

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Visitors

Ms PUJALS, Silvia R — University of Barcelona, Spain, 1 July–28 November 2007
Ms WATKINS, Catherine L — Cardiff University, UK, 16 November–10 December 2007

Scope of Research

The ultimate goal of our research is the regulation of cellular functions by 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 and recognition modes of C2H2-type zinc finger proteins and design of artificial transcription factors with various DNA binding specificities, and (3) design of stimulation-responsible artificial peptides and proteins.

Research Activities (Year 2007)

Presentations

"Creation of Zinc-Finger-Type Transcription Factors toward Gene Regulation and Analysis", Imanishi M, 127th Annual Meeting, The Pharmaceutical Society of Japan, Toyama, 29 March 2007.

"Cell Penetrating Peptides and Their Internalization Mechanisms", Futaki S, Special Lecture at Welsh School of Pharmacy, Cardiff, UK, 8 May 2007.

"Arginine-Rich Peptides and Their Internalization Mechanisms", Futaki S, Biochemical Society Focused Meeting Cell Penetrating Peptides, Telford, UK, 10 May 2007.

"Membrane Interaction and Internalization of Arginine-Rich Peptides", Futaki S, Stockholm University Neurochemistry Seminar, Stockholm, Sweden, 23 May 2007.

"Controlling Channel Peptide Assembly and Gating by Extramembrane Conformational Switch", Futaki S, Japanese-Swiss Symposium on Chemical Biology (JSCB), Lausanne, Switzerland, 25 June 2007.

"Efficient Cellular Uptake of Arginine-Rich Peptides", Nakase I, The Mini Peptide Symposium for Young Researchers, Toyama, 6 November 2007.

Grants

Futaki S, Chemical Biology in Translocation of Membrane Permeable Peptides into Cells, Grant-in-Aid for Scientific Research (A), 1 April 2007–31 March 2010.

Futaki S, Developing Methodologies of Efficient Intracellular Delivery for Cell Imaging and High-throughput Analysis, Grant-in-Aid for Scientific Research on Priority Areas, 1 April 2007–31 March 2009.

Futaki S, Cell Targeting Delivery Peptides: Functional Elucidation and Delivery Control, SORST Program, Japan Science and Technology Agency, 1 April 2006–31 March 2008.

Interaction of Arginine-rich Peptides with Membrane-Associated Proteoglycans Is Crucial for Induction of Actin Organization and Macropinocytosis

Arginine-rich peptides, including HIV-1 Tat (48-60), HIV-1 Rev (34-50), and octaarginine (R8), belong to one of the major classes of cell-permeable peptides that can deliver various membrane-impermeable molecules into 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 the peptides internalization, and that organization of F-actin accompanies this process. Using proteoglycan deficient Chinese hamster ovary cells, we have demonstrated that membrane-associated proteoglycans are indispensable for induction of the actin organization and the macropinocytic uptake of arginine-rich peptides. We have also shown that cellular uptake of Tat peptide is highly dependent on heparan sulfate proteoglycan (HSPG), whereas R8 peptide uptake is less dependent on HSPG. Additionally, activation of Rac protein and the actin organization has been observed a few minutes after the arginine-rich peptides treatment. These data strongly suggest the possibility that interaction of arginine-rich peptides with membrane-associated proteoglycans quickly activates intracellular signals and induces actin organization and macropinocytosis.

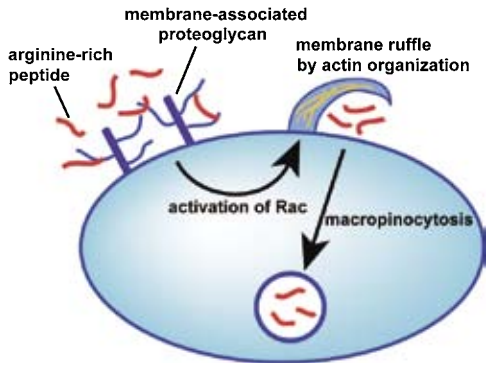

Figure 1. A possible pathway for cellular uptake of arginine-rich peptides. The interaction of the peptides with membrane-associated proteoglycans induces actin organization and macropinocytosis.

Alpha-Helical Linker of an Artificial 6-Zinc Finger Peptide Contributes to Selective DNA Binding to a Discontinuous Recognition Sequence

Although many zinc finger motifs have been developed to recognize specific DNA triplets, a rational way to selectively skip a particular non-recognized gap in the DNA sequence has never been established. We have now created a 6-zinc finger peptide with an alpha-helix linker, Sp1ZF6(EAAAR)$_4$, which selectively binds to the discontinuous recognition sites in the same phase (10 bp-gap) against the opposite phase (5 bp-gap) of the DNA helix. The linker peptide forms a helix structure stabilized by salt bridges, and the helical length is estimated to be about 30 Å, corresponding to that of 10 bp DNA. The gel shift assays demonstrate that Sp1ZF6(EAAAR)$_4$ preferably binds to the 10 bp-gapped target rather than the 5 bp-gapped target. The CD spectra show that the alpha-helical content of the linker is higher in the complex with the 10 bp-gapped target than with the 5 bp-gapped target. The present results indicate that the helical linker is suitable for binding to the recognition sites in the same phase, and that the linker induces the loss of binding affinity to the opposite phased recognition sites. The engineering of a helix-structured linker in the 6-zinc finger peptides should be one of the most promising approaches for selectively targeting discontinuous recognition sites depending on their phase situations.

Figure 2. Estimated DNA binding modes of an artificial 6-zinc finger peptides with (EAAAR)4 linker targeting discontinuous GC box sequences.

Imanishi M, Screening and Evaluation of Novel Clock-related Proteins Using Zinc-finger Technology, PRESTO program, Japan Science and Technology Agency, 1 October 2005–31 March 2009.

Imanishi M, Creation of Transcription Activation Peptides Based on Protein-protein Interaction between DNA Binding Zinc Finger Domains, Grant-in-Aid for Young Scientists (B), 1 April 2006–31 March 2008.

Nakase I, Development of New Cell-Targeting Peptides Having Functional Activities for Recognition of Various Proteoglycans on Cell Membrane, Grant-in-Aid for Young Scientist (B), 1 April 2007–31 March 2009.