Division of Biochemistry
- Biofunctional Design-Chemistry -

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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 2009)

Publications

Inomata K, Ohno A, Tochio H, Isogai S, Tenno T, Nakase I, Takeuchi T, Futaki S, Ito Y, Hiroaki H, Shirakawa M: High-Resolution Multi-Dimensional NMR Spectroscopy of Proteins in Human Cells, *Nature*, **458**, 106-110 (2009).

Kobayashi S, Nakase I, Kawabata N, Yu H, Pujals S, Imanishi M, Giralt E, Futaki S: Cytosolic Targeting of Macromolecules Using a pH-dependent Fusogenic Peptide in Combination with Cationic Liposomes, *Bioconjug. Chem.*, **20**, 953-959 (2009)

Azuma Y, Imanishi M, Yoshimura T, Kawabata T, Futaki S: Cobalt(II)-Responsive DNA Binding of a GCN4-bZIP Protein Containing Cysteine Residues Functionalized with Iminodiacetic Acid, *Angew. Chem. Int. Ed.*, **48**, 6853-6856 (2009)

Presentations

"Chemical and Biological Factors that Affect the Internalization of Arginine-Rich Cell-Penetrating Peptides", Futaki S, PepVec2009 Meeting on "Intracellular Delivery of Therapeutic Molecules: From Bench to Bedside" Montpellier, France, 1 November 2009.

"Intracellular Delivery of Macromolecules Using Cell-Penetrating Peptides", Nakase I, Kinki Bio-Industry Development Organization Follow-Up Seminar, Osaka, 11 November 2009.

"Creation of Zinc Finger-Based Artificial Transcription Factors", Imanishi M, Department Seminar, School of Parmacy, University of Maryland, Baltimore, USA, 21 November 2009.

"Physiological and Non-Physiological Factors Involved in the Internalization of Arginine-Rich Peptides", Futaki S, 5th Peptide Engineering Meeting (PEM5), Barcelona, Spain, 27 October 2009.

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.

Imanishi M, Creation of Artificial Transcription Factors towards Construction of Artificial Genetic Circuit, Grant-

Cytosolic Targeting of Macromolecules Using a pH-Dependent Fusogenic Peptide in Combination with Cationic Liposomes

pH-Sensitive peptides and polymers have been employed as additives to enhance the cytosolic delivery of drugs and genes by facilitating their endosomal escape. However, little attention has been paid to the intracellular fate of these peptides and polymers. In this study, we explored the possibility of utilizing GALA, a pH-sensitive fusogenic peptide, as a cytosol-targeting vehicle. In combination with cationic liposomes, Lipofectamine 2000 (LF2000), the feasibility of this approach for the cytosolic targeting of proteins and nanoparticles was exemplified through the delivery of avidin (68 kDa) and streptavidin-coated quantum dots (15-20 nm) in serum-containing medium. The use of cationic liposomes is critical to enhance the cell-surface adhesion of the GALA conjugates and eventual endosomal uptake. Circular dichroism studies suggests that the GALA can be liberated from cationic liposomes at a reducing pH to form a helical structure and this may eventually lead to disruption of the endosomal membrane to achieve an efficient leakage of the GALA conjugates into the cytosol.

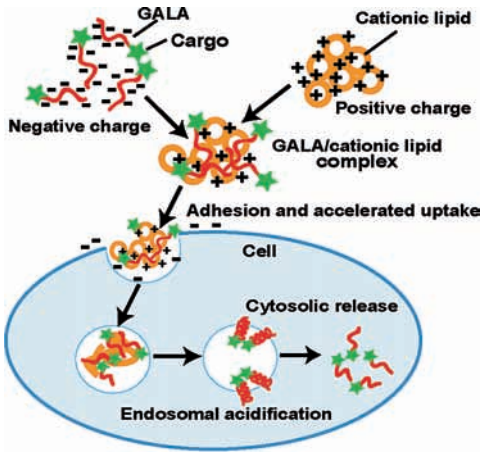

Figure 1. Concept of cytosolic targeting using GALA as an addressing vehicle in combination with cationic liposomes.

Cobalt(II)-Responsive DNA Binding of a GCN4-bZIP Protein Containing Cysteine Residues Functionalized with Iminodiacetic Acid

Endowment of novel functions inducing that of metal switch can be attainable through structural design of peptides and proteins. We previously reported that helical peptides having a pair of iminodiacetic acid (Ida) derivatives of lysine at positions *i* and *i+2* induce critical helix destabilization in the presence of metals to lead functional switch of peptides. However, due to the lack of the methodology to effectively introduce the Ida moieties at specific positions in proteins, the application of this concept has been limited to synthetic peptides.

We present a new method for introducing the Ida moieties into proteins. This employs specific modification of cysteines by treatment with a new functionalization agent, *N*-(2-tosylthioethyl) iminodiacetic acid (Ts-S-IDA). The practicability of this approach was exemplified through the metal-responding switching of the DNA binding of the yeast transcription factor GCN4-derived proteins bearing Ida moieties. Two pairs of Ida moieties were incorporated in the leucine zipper segment of the GCN4-bZIP protein in such a way that the Ida moieties of each pair were in *i* and *i+2* positions. Complex formation of the Ida groups with Co(II) led to destabilization of the helical structure and thus enabled reversible switching of the binding of the protein to the target DNA.

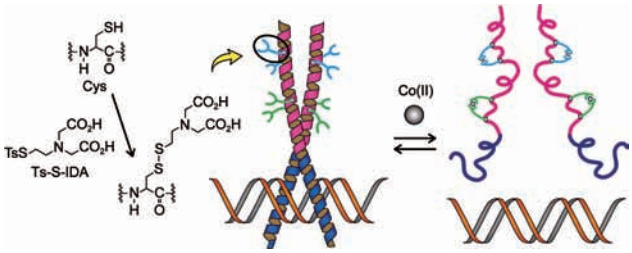

Figure 2. Preparation of Ida-modified cysteine in GCN4-bZIP protein mutant and conceptual scheme of metal-assisted DNA binding switch of GCN4-bZIP protein modified with Ida.

in-Aid for Young Scientists (B), 1 April 2008–31 March 2010.

Nakase I, Receptor Target and Efficient Internalization of Therapeutic Molecules into Cells Using Membrane Permeable Peptides, Grant-in-Aid for Young Scientists (B), 1 April 2009–31 March 2011.

Awards

Azuma Y, Best Poster Award, "Metal-Induced DNA-Binding Switch of bZIP Proteins Modified with Iminodiacetic Acid (Ida)" The 19th Symposium on Role of Metals in Biological Reactions, Biology and Medicine (SRM2009), Suita, 12 June 2009.

Nakamura A, Best Poster Award, "Creation of Artificial Zinc Finger-Type Transcription Factors towards Promoter Analysis of Clock Genes" The 16th Annual Meeting of Japanese Society for Chronobiology, Osaka, 27 October 2009.