Bioorganic Chemistry
- Bioactive Chemistry -

http://www.scl.kyoto-u.ac.jp/~sugiura/index2.html

Prof
SUGIURA, Yukio
(D Pharm Sc)

Assoc Prof
FUTAKI, Shiroh
(D Pharm Sc)

Instr
IMANISHI, Miki
(D Pharm Sc)

Guest Res Assoc
PEI, Renjun
(D Sc)

PD
NOMURA, Akiko
(D Pharm Sc)

PD
NINOMIYA, Keiko
(D Sc)

Students

HORI, Yuichiro (D3)
NAKASE, Ikuhiko (D2)
SHIRAISHI, Yasuhisa (D1)
WAKAKO, Naoya (M2)
ITAZU, Masako (M1)
TAKEUCHI, Toshihide (M1)
NAKATSUKASA, Takako (M1)
INOUE, Daisuke (UG)
SONOMURA, Kazuhiro (UG)
KIWADA, Tatsuto (D2)
NOMURA, Wataru (D2)
NIWA, Miki (M2)
HAJI, Akiko (M2)
EN, Bi (M1)
HIRATA, Tsuyoshi (M1)
MASUI, Yumi (M1)

Scope of Research

The major goal of our laboratory is to elucidate the molecular basis of the activity of various bioactive substances by biochemical, physicochemical, and synthetic approaches. These include studies on the mechanism of sequence-specific DNA cleavage by antitumor or carcinogenic molecules, studies on the DNA recognition of zinc-finger proteins, and model studies on the action of ion channels. In addition, artificial designed peptides have also been developed as useful tools in molecular biology and potentially in human medicine.

Research Activities (Year 2003)

Presentations

Artificial zinc finger proteins: Design strategy and gene action, Sugiura Y, Annual meeting, Pharm. Soc. Jpn., Nagasaki, 28 March.

Nine-zinc finger protein: influence of TFIIIA-type linker at N- or C-terminal on DNA binding site, Nomura W, Sugiura Y, 11th International Conference on Biological Inorganic Chemistry, Cairns (Australia), 23 July.

Intercellular delivery of histidine-tagged protein, Futaki S, Niwa M, Wakako N, Nakase I, Shiraishi Y, Sugiura Y, Fifth AFMC International Medicinal Chemistry Symposium, Kyoto, 14 October.

Arginine-rich peptides: The aspects of membrane translocation, Futaki S, Cellular Drug Transport Mechanisms Workshop, Molndal (Sweden), 14 November.

Grants

Sugiura Y, Regulation of cellular gene function by novel DNA bending finger, Grant-in-Aid for Scientific Research (B) (2), 1 April 2000 - 31 March 2004.

Sugiura Y, Role of multi-zinc fingers in gene expression and creation of their architectures, Grant-in-Aid for Scientific Research (B) (2), 1 April 2002 - 31 March 2005.

Futaki S, Development of new cellular modifier using membrane transmission peptides and analysis of protein-protein interaction, Grant-in-Aid for Scientific Research (B) (2), 1 April 2002 - 31 March 2004.

Effects of Length and Position of Extended-Linker on Sequence-Selective DNA Recognition of Zinc Finger Peptides

The new zinc finger peptides with extended-linker seuqnece for recognition of noncontiguous DNA targets have been created (Figure 1). Sp1GG series are linker-extended mutants for the wild-type Sp1(zf123) and two to four amino acids are inserted into their linker sequences between finger units. From the analyses of the DNA binding affinity and specificity by gel mobility shift assay, it is clearly indicated that Sp1GG series bind to noncontiguous DNA targets with sequence-selectivity according to the position of the extended-linker (Figure 2). Moreover, the N-terminal finger substitution revealed that the linker-extended mutants bind to DNA selectively in synergy of three finger units. This is the first method for the recognition of the noncontiguous DNA target by zinc finger peptide. Consequently, our design method is widely applicable to creating zinc finger peptides with novel DNA sequence-selectivity.

W. Nomura, Y. Sugiura, Biochemistry, 42, 14805-14813 (2003).

Figure 1.

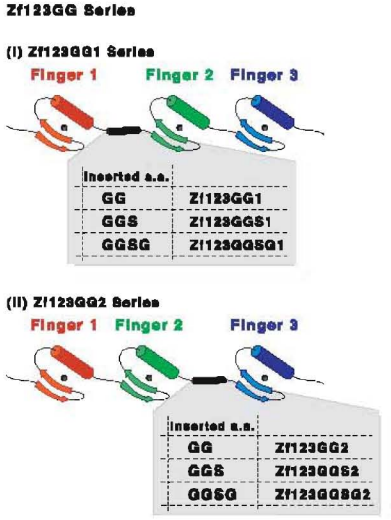
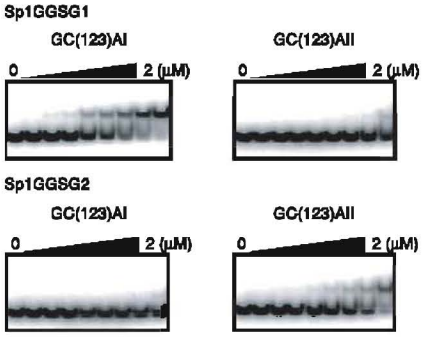


Figure 2.



Basic-Peptide Mediated Protein Delivery into Living Cells

Basic-peptide mediated protein delivery into living cells is becoming recognized as a potent approach for the understanding of cellular mechanisms and drug delivery. We have demonstrated that these features were observable among many arginine-rich peptides including octaarginine (Arg)8. The presence of a ubiquitous internalization mechanism for arginine-rich oligopeptides has also been suggested. On the other hand, there are many non-covalent protein complexes or supramolecular assemblies that have great potential for the research in chemical and cellular biology. It has never been demonstrated whether a protein assembly with a dissociation constant in the range of 10^{-5}~10^{-6} M is able to cross the membrane with retention of its structure.

We have prepared the conjugates of the S-peptide (1 - 15) derived from RNase S with membrane-permeable basic peptides such as the human immunodeficient virus (HIV)-1 Rev (34 - 50), HIV-1 Tat (48 - 60) and octaarginine. The RNase S complexes, formed among these S-peptide (1 - 15)/basic peptide conjugates, efficiently penetrated into the HeLa cells. Moreover, these RNase S complexes exerted an anti-HIV replication activity. The present study exemplified the applicability of the arginine-rich peptides to the intracellular targeting of non-covalent assemblies for intracellular applications.

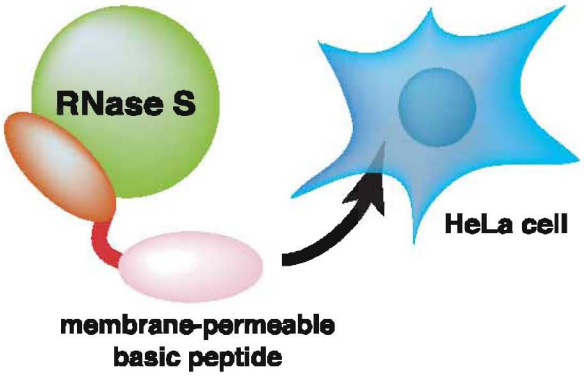


Figure 3. Intracellular delivery of non-covalent protein complexes