2P589

Structure and functional regulation of DNA complex formed with Cell-Signal responsive Gene Carrier

○Yuko T. Sato^{1,2}, Kenji Kawamura^{1,2}, Jun Oishi^{1,2}, Takeshi Mori^{1,2,3,4}, Takuro Niidome^{1,2,3,4}, Yoshiki katayama^{1,2,3,4}

¹Department of Applied Chemistry, Faculty of Engineering, Kyushu University,

²JST-CREST, ³Center for Future Chemistry, ⁴21st COE

Recently, there are many researches for the development of non-viral gene vector. But, it isn't easy to deliver the transgene only into cancer cells. We aim to create gene delivery system responding to cellular signal using polymer possessing a substrate peptide for the target signal enzyme i.e. exogenous DNAs are expressed only in target disease cells (D-RECS: Drug Delivery Responding to Cellular Signal). We previously reported that the system can successfully worked rising Caspase-31) or protein kinase-A2) as a target signal.

In this study, we characterized the DNA/D-RECS carrier complex: high order structure of the DNA/carrier complex and the ability of the carrier to control transcription/ translation, and compared those properties with DNA/substrate peptide complex. First, we synthesized polymer that grafted Caspase-3 substrate peptide as gene carrier. DNA and Carrier electrostatic interact through the mediation of substrate peptide cation (+5). And we confirmed that the DNA was released from the complex with by the cleavage reaction of Caspase-3 by electrophoresis and cell-free protein expression experiment.

In this meeting, we will report on the result of comparing between substrate peptide/DNA complex and carrier/DNA complex; the higher-order structure, the difference of the cleavage reaction by Caspase-3, and control ability of gene expression.

1)K. Kawamura, et al., *Biomacromolecules*. 6, 908-913, (2005). 2)J. Oishi, et al., *J. Control. Release* 110, 431-436, (2006).

2P591

Kinetics of membrane fusion between HVJ-envelope/DNA complexes and cells

○Nobuaki Yonekura, Kana Yamamoto, Takeshi Ohshio, Masayuki Tadano
University of the Ryukyus

For reliable gene therapy, it is necessary to obtain quantitative data on the kinetics and dynamics of internalization into target cells and intercellular trafficking of vector-DNA complexes. Despite that such data can be measured by biophysical techniques that have been applied to studies of virus infection, they have been limited. HVJ (hemagglutinating virus of Japan)-envelope is a unique gene vector that introduces DNA into mammalian cells through membrane fusion. In the present work, we have studied the kinetics of membrane fusion of the complex of plasmid DNA and HVJ-envelope vector with C6 glioma and NIH 3T3 cells by R18 (octadecyl rhodamine B) fluorescence dequenching assay using a single-photon counting microscope. The dequenching curves have been analyzed using a mass action kinetic model to obtain the adhesion and fusion rate constants of the complex. In addition, we have found that adding the receptors (ganglioside GD1a) of the hemagglutinin-neuraminidase (HN) protein of HVJ-envelope vector to cell surface and activating the fusion (F) protein by mild trypsin treatment enhance significantly the adhesion and fusion rates.

2P590

A novel mechanism for gene delivery to nucleus by non-viral vectors containing biosurfactant MEL-A

○Yoshikazu Inoh¹, Yoshinobu Ueno², Furuno Tadahide¹, Hirashima Naohide², Kitamoto Dai³, Nakanishi Mamoru¹

¹School of Pharmacy, Aichi Gakuin University, ²Graduate School of Pharmaceutical Sciences, Nagoya City University, ³National Institute of Advanced Industrial Science and Technology

Gene transfection into the cells is a fundamental technology for not only molecular and cell biology but also a clinical gene therapy. In recent years, several methods for gene transfection have been investigated, but more efficient and safe systems are needed. Biosurfactant mannosylerythritol lipid A (MEL-A) is produced by yeast, and exhibits not only surface-active properties but also cell differentiation activities against several cells. We found that MEL-A remarkably promoted gene transfection efficiency mediated by cationic liposomes with a cationic cholesterol derivative. We previously found that liposome-DNA complex were taken up slowly by endocytosis of the target cells and then the internalized DNA was released from endosomes by their acidification. However, in the case of MEL-A-containing-liposome-DNA, liposome and DNA distributed rapidly in the plasma membrane and the nucleus of target cells, respectively. When we examined the fusogenic activities of MEL-A-containing-liposome to induce membrane fusion between cationic and anionic liposomes by FRET assay, MEL-A accelerated the membrane fusion between them. These results suggested that MEL-A in cationic liposomes achieved high gene transfection efficiency via membrane fusion between liposomes and the plasma membrane of target cells. This finding will break through in the experiments of gene transfection using non-viral vectors like cationic liposomes.

2P592

Maturation and Mechanism Based Inhibitor Design of SARS-CoV 3CL Protease: Implication in Antiviral 3C(L) Protease Drug Design

○Min-Feng Hsu^{1,2}, Syaulan Yang³, Yao-Chen Teui^{1,4}, Chih-Jung Kuo¹, Kai-Ti Chang^{1,4}, Shu-Jen Chen³, Hui-Lin Shr^{1,2}, Tzu-Ping Ko^{1,2}, Po-Huang Liang^{1,2,4}, Ming-Chu Hsu³, Andrew H.-J. Wang^{1,2,4}

¹Institute of Biological Chemistry, Academia Sinica, ²Core Facility for Protein X-ray Crystallography, Academia Sinica, ³TaiGen Biotechnology, ⁴Institute of Biochemical Sciences, National Taiwan University

Severe acute respiratory syndrome (SARS) is an emerging infectious disease caused by a novel human coronavirus. Viral maturation requires a main protease (3CL protease) to cleave the virus-encoded polypeptides. The dimeric 3-D structure of the C145A mutant protease shows that the active site of one protomer binds with the C-terminal six amino acids of the protomer from another asymmetric unit, mimicking the product-bound form and suggesting a possible mechanism for maturation. Mechanism based designed inhibitors with substrate-like peptidyl-binding elements specific for SARS 3CL protease undergo a Michael reaction mediated by nucleophilic addition of Cys-145, resulting in covalent-bond formation and irreversible inactivation of the viral protease. For structure-based drug design, four high resolution co-crystal structures of the protease-inhibitor complexes allow us to search more effective inhibitors. Coxsackie virus B3 (CVB3) 3C protease cleaves the substrates between Gln-Gly (Q-G) pairs which is similar to the substrate specificity of SARS 3C-like protease. Based on the sequence specificity of the substrates, we analyzed the inhibition activity of CVB3 3C protease with several SARS 3C-like protease inhibitors and solved the structures of protease-inhibitor complexes. These structures show the active site environment of the 3C protease and provide a basis for antiviral drug design. This structure-based inhibitor design strategy will be discussed.