[Introduction]

Staphylococcal γ -hemolysin (Hlg) consists of two separate proteins, Hlg1 (34kDa) and Hlg2 (32kDa), which lyses cooperatively mammalian erythrocytes. We have been showed Hlg1 and Hlg2 assemble alternately to form funnel-shaped heteroheptameric transmembrane pores with subunit stoichiometries of 3:4 and 4:3. In this study, quantitative molecular arrangements analysis were performed based on high-resolution TEM images. This study revealed mismatch arrangements of several subunits from regular heptagon, and proposed a new characteristic structure of bacterial two-component heteroheptameric pore.

[Materials and Methods]

Hlg pores were stained negatively to examine under the TEM. The lengths between centers of subunits placed side by side were measured with image analysis software. The most distant part was defined as L1 and other parts numbered in the counterclockwise direction from L1 to L7. The normalized mismatch lengths (NML) were verified with the ratio of the L1-L7 against the averaged length.

[Results and Discussion]

The NML of L1 is significantly bigger than others. The two subunits placed side by side on the L1 moved away from each other with the length of approximately 1nm. In the Hlg heteroheptameric pore, there are six linkages consisting of differential subunits and one linkage consisting of identical subunits. Therefore, the mismatched length will be due to the difference of linkage forces between the different and the identical subunits and L1 would be the site identical subunits placed side by side.

3P013 単粒子解析とSEMによるタンパク質複合体解析

3F1235 Protein complex analysis using single particle reconstruction and SEM

Chikara Sato¹, Kazuhiro Mio¹, Toshihiko Ogura¹, Yuusuke Maruyama¹, Masaaki Kawata¹, Hidetoshi Nishiyama², Mitsuo Suga², (¹National Institute of Advanced Industrial Science and Technology; ²JEOL)

Protein complexes are essential for various kinds of physiological functions in our body, but they are frequently difficult to crystallize. We determined the 3D structure of the Keap1 protein using Single Particle Reconstruction (SPR) from EM images. Keap1, a substrate adaptor of Cul3-based E3 ligase, is a xenobiotic/oxidative stress sensor that recruits the transcriptional factor of cytoprotective genes, Nrf2, to the proteasome for degradation. Reactive cysteines in the BTB or IVR domain of Keap1 are modified by electrophiles or carcinogens, preventing NRF2 from degradation. Keap1 was reconstructed using SPR, in collaboration with Dr. Masayuki Yamamoto (Tohoku Univ). Although stable protein complexes can be reconstructed using SPR, unstable complexes are difficult to visualize after the protein purification step. Conventional electron microscopes view samples in vacuum, and are thus unable to image wet samples or samples in solution. The Atmospheric Scanning Electron Microscope (ASEM) has been developed to observe aqueous samples and cells in solution at atmospheric pressures. For this new microscope, a special sample dish with a pressure-resistant window made of thin film (ASEM Dish) was developed to allow the electron beam to be projected from underneath the sample. The top of the dish is open, and can contain an aqueous sample of several ml. The ASEM system can also be used as an immuno-EM in an aqueous solution, which protects antigens.

3P014 Staphylococcal nuclease における構造変化の FRET 解析 FRET analysis of structural changes in Staphylococcal nuclease

Yusuke Kishi¹, Mariko Yamaguchi¹, Issei Iijima², Yoichi Yamazaki¹, Hironari Kamikubo¹, Takahiro Hoshaka², Mikio Kataoka¹, (¹Nara Institute of Science and Technology; ²Japan Advanced Institute of Science and Technology)

Staphylococcal nuclease (SNase) keeps the native structure by non-local interaction between the N-terminal domain (ND) and the C-terminal domain (CD). At the CD there is a cluster formed by the 140th tryptophan (Trp140), which is crucial to the non-local interaction. A SNase mutant with the substitution of Trp140 to alanine (W140A) is unfolded under a physiological condition, because the CD fails to form the cluster to interact with the ND. The W140A mutant compensates the interaction between the subdomains by a ligand binding and exhibits the so-called induced folding. Kinetic studies suggest that the W140A mutant possesses the residual structure that makes it possible to bind a ligand even in the unfolded state. We try to use the fluorescence resonance energy transfer (FRET) to investigate the residual structure of the W140A mutant in the unfolded state and the structural change on the ligand binding. For the purpose, we introduced two non-natural amino acids with fluorophores into W140A and wild type. Lysine28 in the ND was chosen as a donor site and Lys5 or Lys48 in the ND or Ser141 in the CD was chosen as an acceptor site.

Prior to the FRET studies, we confirmed that the fluorophores do not affect the SNase structure by the substitution of these residues to F. Double-labeled SNase derivatives were successfully synthesized. The FRET analysis for the W140A mutant will be discussed in comparison to the wild type

3P015 N-メチル化ペプチドによるアミロイド β ペプチドの線維化阻害 Inhibition of the fibrillization of amyloid β -peptide by its fragments with amide N-methylation

HIRONORI OCHIAI, TOMOYUKI KOMURO, HIROTSUGU HIRAMATSU, HIDEO TAKEUCHI, (Graduate School of Pharmaceutical Sciences, Tohoku University)

Alzheimer's disease (AD) is the major cause of dementia. Its hallmark is deposition of senile plaques in brain. The main component of senile plaques is β -sheet-rich aggregates of amyloid β peptide ($A\beta_{1-40}$) called amyloid fibrils. Inhibitors of the fibril growth are expected to be a drug for this disease. It is reported that methylation of the amide nitrogen in the peptide main chain (N-methylation) reduces the β -sheet formation and fragments of $A\beta_{1-40}$ retain high affinity to $A\beta_{1-40}$ fibrils. In this study, we prepared the $A\beta_{30-40}$ [³⁰AIIGLMVGGVV⁴⁰] fragments with N-methylated amides at one (L34, V36, G37, G38), two (L34V36, L34G38, V36G38), or three positions (L34V36G38) and studied their inhibitory activities. The fibrillization reaction was monitored for 50 μ M $A\beta_{1-40}$ at pH 7.4 (50 mM Tris, 150 mM NaCl) by the ThT fluorescence assay. Addition of N-methylated peptides largely reduced the fibrillization, showing that they are effective inhibitors. The inhibitory effect didn't correlate with the number of N-methylated amides in $A\beta_{30-40}$. The highest inhibitory effect was observed for the peptide N-methylated at G38, which was shown by circular dichroism to have high structural flexibility compared to most other N-methylated peptides. The G37 N-methylated peptide showed structural and inhibitory properties similar to the G38 N-methylated peptide. N-methylation appears to be most effective in inhibiting $A\beta_{1-40}$ fibrillization when it is made at sites where the flexibility of the peptide is retained at a high level.

3P016 タンパク質-タンパク質ドッキングへの分子内フレキシビリティの導入 Introducing intramolecular flexibility into protein-protein docking

Kazuya Sumikoshi, Shugo Nakamura, Kentaro Shimizu, (The University of Tokyo)

Protein-protein docking is the process of predicting the bound structure of a protein complex from known structures of monomers. One of the hardest problems in protein-protein docking is to incorporate intramolecular flexibility into the process of searching the structural space because it is the major source of the huge number of degrees of freedom in the system. Even though it is difficult to solve, it is crucial for obtaining finer results, since molecules sometimes change their shape significantly upon binding.

To deal with the problem, we tested an approach of refining candidate structures of a rigid-body docking by coarse-to-fine processes of energy minimization and molecular dynamics, which gradually introduces the flexibility into the system. The refining process begins with a completely rigid model and gradually increases the size of the set of atoms and covalent bonds around which the motion is allowed. Since the number of candidates to refine is very large (usually from a few hundred to a few tens of thousands), we have included a filtering mechanism to narrow down the set of candidates as we increased the molecular flexibility by periodically evaluating the scoring functions of the refined structures. The candidates to refine for the tests were generated by using a docking program we have developed, which uses series expansion in spherical polar coordinates. In this presentation, we will report the effects of each process and the knowledge we have found in these tests.

3P017 動的光散乱法によるバクテリオロドプシン・TritonX-100 複合体の溶液中のダイナミクス Dynamic study of bacteriorhodopsin-triton X-100 complex in solution by dynamic light scattering

Akiko Yanagawa¹, Itaru Tsukushi¹, Masashi Sonoyama², (¹Chiba Institute of Technology; ²Gunma University)

The complex of membrane protein and detergent is one of the most important topics in both membrane protein science and biophysics. Membrane proteins often undergo denaturation in solubilization process with detergents that is a very important step to observe membrane proteins in the functional state. Thus, it is important to know structure and physical properties of membrane protein in not only lipid bilayers but also protein in detergent micelles. In this study, we have observed dynamics of bacteriorhodopsin (bR) suspension by dynamic light scattering (DLS) technique. DLS is commonly used for