[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

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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

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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

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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

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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

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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

dynamic study of colloid and macromolecular solutions. We also think that DLS is a very useful method to observe the motion of membrane protein and it with detergent in solution. DLS experiments were carried out by changing the scattering angle every 5° from 15° to 90° at room temperature. The observed samples were bR in the purple membrane and bR-triton X-100 (TX100) complex. TX100 was used as detergent. The dependence of TX100 concentration has been also observed. The bR concentration was all 5 μ M. Particle size distributions in solution were obtained from auto-correlation function of light scattering intensity. We will present more detail discussions on the basis of particle size distributions and report the dependence of concentrations of TX100 in bR-TX100 complex.

Reference: T. Sasaki, M. Sonoyama, M. Demura, S. Mitaku, *Photochem. Photobiol.*, **81**, 1131 (2005).

3P018 結び目理論を用いた遺伝的交叉による徐冷分子動力学シミュレーション A Simulated Annealing Molecular Dynamics Using Genetic Crossover with Knot Theory

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Computational simulations of biomolecular systems are performed using molecular simulation techniques such as Monte Carlo and molecular dynamics methods. However, because a protein has a large number of degrees of freedom associated with a lot of atoms, it is not yet possible to perform enough conformational searches in this extremely high dimensional space, and efficient sampling techniques are required.

We have proposed a parallel simulated annealing using genetic crossover (PSA/GAc) [1,2], in order to find the global minimum energy structures for protein systems. Especially, PSA/GAc, which incorporates the attractive features of the simulated annealing and the genetic algorithm, is useful for finding a minimum potential energy conformation of protein systems. However, after genetic crossover operations, we often find some lower energy conformations, which are obviously different from their native structures. These conformations have a deeper knot conformation than known proteins[3] with knot conformation. Therefore, we combined the knot theory with our PSA/GAc in order to avoid the conformations with knots from the conformational search space.

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3P019 Structural and thermodynamic analyses of histone H3 recognition by UHRF1

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Epigenetics, histone modifications and DNA methylation, regulate the wide range of chromosomal processes in mammals. The patterns and levels of these epigenetic traits modulate chromatin architectures, and consequently determine a gene expression pattern. The epigenetic traits are introduced by histone modifiers and DNA methyltransferases in response to particular signals, and are interpreted by various histone and/or DNA binding proteins. The protein UHRF1 (also known as Np95 and ICBP90) possesses histone binding motifs, a Tudor domain and a PHD (plant homeo domain) finger, followed by the SRA (SET and RING associated) domain responsible for a hemi-methylated DNA binding. Thus, UHRF1 is thought to be a key molecule to link histone modification and DNA methylation status. Although the UHRF1 Tudor domain has been shown to interact with methylated histone H3K9, structure and function of the two histone binding motifs remain unclear. Here we present crystal structure of the UHRF1 PHD finger in complex with the N-terminal tail of histone H3. Combined with biochemical data, the crystal structure reveals that, unlikely to other PHD fingers, the UHRF1 PHD finger strictly recognizes the modification state of Arg2 rather than Lys4 in H3. Furthermore, biochemical and thermodynamic analyses reveal that Tudor-PHD

domain (the Tudor domain linked to the PHD finger) recognizes multivalent modification status in the histone H3 tail. In this session, we'd like to discuss the read-out mechanism of the histone H3 tail modifications by UHRF1.

3P020 新規蛋白質結晶作製プラットフォームとしての B 型肝炎ウイルス様粒子蛋白質複合体の発現系構築 A novel approach for protein crystallography using VLP-based platform: *in vitro* assembly of HBV-core fusion proteins

Masaki Kikuchi, Shinichiro Iwabuchi, Yuu Matsuda, Tatsuhiko Kikkou, Matsumoto Osamu, (*Grad. Sch. of Pharmaceutical Sciences, Chiba Institute of Science*)

We describe here a novel approach for protein crystallography using virus-like particle (VLP) based platform. This concept looks back inside of hollow VLPs. The target protein of unknown structure was expressed into inside of the hollow VLP consisting of known three-dimensional structure of viral core protein by designed peptides.

The VLP formed by the core fusion proteins assembles spontaneously, target protein was regularly located inner VLP. This model applies crystallization condition of solved wild type VLP (wtVLP), maintaining same surface structure. Data collection and interpretation of diffraction pattern is simply, because VLP has a highly symmetric structure. Additionally, using conformational information of wtVLP expected to solve phase problem. We used hepatitis B virus (HBV) like particles because molecular biological and biochemical knowledge is well known. In particular, its icosahedral symmetric particle is built from multiple subunits of a single 183-aa core protein, easily expressed and purified, revealed structure from x-ray crystallography (PDBID:1QGT). In this study, we designed and prepared a DNA construct encoding a fusion core protein in which is dimerized by flexible peptide linker. Its fusion protein is replaced by the entire 238-aa green fluorescent protein (GFP) flanked by linker peptides. We report that we have confirmed good possibility of a novel approach for protein crystallography.

3P021 擬似原子モデル構築のための GUI を持った支援ツールの開発 Development of a new support tool to construct quasi-atomic model with a graphical user interface

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In processing and analyzing electron micrographs to construct an atomic model of proteins, we need to use a wide variety of software and methods. Our developed software, Eos (Extensible object-oriented system), supplies many tools to process electron micrographs and an environment for software prompt development. Eos also supplies a method to construct quasi-atomic model; we called it SRMD method. The SRMD is an extension of steered Molecular Dynamics (MD) simulation techniques, in which cryo-EM maps are used as an external potential for MD and constrain each atoms in a target protein. However, the programs are supplied as CUI. Thus, we plan to develop a new support tool with a GUI to build up a quasi-atomic model of proteins from electron micrographs by integrating current tools including a series of molecule viewers called 'molvie' under Eos. One of the objectives of Eos is prompt development but, because the main language to develop is C-language (C), Eos had limited scalability in terms of script languages: Eos was difficult to extend functions using script languages with GUI such as Ruby/Tk. Therefore, even the "molvie" were programmed by C and did not have sufficient GUI.

Thus, we built up a new platform for script languages to implement much function, which have been developed in C under Eos. After this expansion, we partly developed model viewers with GUI under Eos. Currently, we are developing more convenient tools with GUI, and so applying the tools and SRMD to construct an atomic model of heme-oxygenase complexes.

3P022 Domain-Domain interaction of GFP-GST fusion protein

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GFP is the most widely used fluorescence tracer. Using some proteins fused with PGF, transportation, localization and other interesting dynamic behaviors of protein have been visualized in living cell. However, fluorescence spectroscopic, structural and other physico-chemical properties of GFP fused protein should be more cleared to promote the validity and availability of GFP as useful fluorescent tag. In the present work, we adopted GFP-GST(Glutathione-S-transferase) fusion protein as a model protein and investigated the interaction between the GFP domain and GST domain through FRET. In order to measure FRET, the N-terminal of GST domain was stained by Cy3. The fluorescence maximum of GFP was seen at 520 nm and the absorption spectrum of Cy3 showed the maxima at 520nm and 560nm in the fusion protein. The overlap integral between the donor and acceptor was calculated to be