prepared several truncated SptPs and SicP and examined whether the truncated SptPs interact with SicP *in vitro* by Native-PAGE and western blotting as the first step.

We constructed pET3c-based plasmids expressing SptP₁₋₁₃₆, SptP₁₋₁₀₅, and SptP₁₋₈₆, and the three kinds of the truncated SptPs were individually prepared under denaturing conditions. We also constructed pET3c-based SicP expression plasmid and prepared SicP under native conditions. Thus, we prepared the truncated SptPs and SicP individually, and then we examined whether the truncated SptPs interact with SicP. In this meeting, we will show the results and discuss further efforts.

2PT120 Purification and characterization of glycine-proline-fused type of InvC involved in *Salmonella* type III secretion system

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Some of Gram-negative bacteria export virulence proteins “effector proteins” into host cells using type III secretion systems (T3SS). *Salmonella* T3SS consists of syringe-like structures, effector-specific chaperones, T3SS-specific ATPase, and so on. InvC is the T3SS-specific ATPase in *Salmonella*, and the importance of its ATPase activity is demonstrated by mutant analyses. To reveal the detailed properties of InvC, InvC having a long extra amino acid sequence at the N-terminal end had been examined. In this study, we aimed to prepare InvC whose extra sequence was as short as possible. For this purpose, we used pET50b as an expression vector, because a target protein is expressed as a highly soluble His-Nus-tag fusion protein and the tag is removed by HRV3C protease, which has highly sequence-specific endoprotease activity at 4 °C. We cloned *invC* into the SmaI site by in-fusion method, and then transferred the plasmids into *E. coli* BL21(DE3) cells. The crude extract including His-Nus-InvC was prepared from the culture of the cells, and His-Nus-InvC was prepared using Ni-NTA agarose column. After that, His-Nus-InvC was cleaved by HRV3C protease. As the result of the sequential procedure, we prepared InvC having two amino acid residues, glycine and proline, as the extra sequence. Here, we termed the glycine-proline-fused type of InvC as GP-InvC. We applied GP-InvC to several column chromatography steps for further purification and then examined ATPase activities of GP-InvC under various conditions.

2PT121 X線1分子追跡法によるII型シャペロン協同性評価

Cooperative Motion Analysis of group II chaperonin at Single-molecule Level using Diffracted X-ray Tracking

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Group II chaperonin, found in archaea and in the eukaryotic cytosol, is an indispensable protein that captures an unfolded protein and refolds it to the correct conformation in an ATP dependent manner. ATP-induced structural changes are essential for chaperonin foldase activity; the closure and opening of the chamber are the focus of group II chaperonin studies. We had previously reported that the diffracted X-ray tracking method (DXT) could trace ATP induced twisting motion of group II chaperonin ring at single molecule level with high accuracy. In DXT, gold nanocrystal immobilized on one side of chaperonin-ring is used as tracer for structural change of chaperonin. The timing, speed and direction of twisting motion of chaperonin-ring after ATP binding could be analyzed by DXT method in detail. In this study, we checked how ATPase deficient mutant subunits modulate the speed or frequency of twisting motion. We controlled the number of ATPase deficient mutant within one chaperonin ring, constructed the ATP deficient hetero-ring using circular permuted connected mutants, and evaluated the effects of those mutants to chaperonin-ring's twisting motion. We will discuss intra- and inter-ring cooperativity of group II chaperonin from such motion analysis view at single molecule level.

2PT122 PAPの酵素活性制御因子としてのトリプトファン残基近傍のコンフォメーションとダイナミクス

The dynamics and conformation of Trp208 and Trp237 as regulating factor of enzymatic activity of Pokeweed Antiviral Protein

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Pokeweed Antiviral Protein (PAP) belongs to a family of Ribosome Inactivating Proteins (RIPs) and works as N-glycosidase to remove a specific adenine residue from ribosomal RNA. We have focused on Trp208 and Trp237 uniquely arranged in PAP as the activity regulating factor. Trp208 locates at the entrance lid of active site and Trp237 is arranged at the C-terminal domain. We have shown that enzymatic activity of PAP is dramatically affected by substitution of Trp208 and Trp237. Additionally, changes of dynamics of Trp208 and the distance between Trp237 and Tyr172 were observed when PAP bound with N-acetylglucosamine, and these changes induced the enhancement of enzymatic activity of PAP. These results demonstrate that dynamics motilities and conformations of Trp208 and Trp237 play important roles in regulating PAP activity. In the present study, we investigated in detail the correlation between the conformation and dynamics near the active site, dynamics in the C-terminal domain and the enzymatic activity of PAP based on the fluorescence spectroscopic data. To analyze the conformational changes, we measured time resolved anisotropy decay and FRET between Tyr and Trp by sub-pico second laser based TCSPC method. Results suggest that Trp208 approached closer nearest neighboring Tyr at the active site as temperature increasing. Then, the internal rotational motion of Trp208 was not observed. Other properties on the dynamics and conformation near the active site will be also discussed compared the enzymatic activity of PAP.

2PT123 フォトンファクトリーの小角散乱ビームラインの現状

Current Status of Small-Angle X-ray Scattering Beamlines at Photon Factory

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BL-6A and BL-10C at Photon Factory (PF) has been dedicated for small-angle X-ray scattering (SAXS) experiment. BL-6A is a bending magnet beamline and was moved from the previous BL-15A last summer. This beamline is optimized for measuring samples of various forms, such as a solution, a fiber, and a membrane. CCD detectors (C7300, C4880, Hamamatsu photonics) have used for the time-resolved measurement in a sub-second region. Moreover fast speed (200Hz framing rate) pixel-array detector PILATUS 300K (Dectris) will be installed in this September. BL-10C is also bending magnet beamline and is optimized for measuring a biological solution sample. The data in the broad angle region can be measured with an imaging plate detector R-AXIS VII (Rigaku). We are upgrading BL-10C especially for high-throughput measurement of Bio-SAXS experiment, and developed and installed an automatic solution mixing machine which is a part of a solution-sample changer. Construction of new BL-15A, which light source is a short-gap undulator, is planned for SAXS filed, this will be opened from the autumn of 2013. Moreover, the national project for structure life science research has been started from this April for 5 years, and the SAXS beamlines, BL-10C and new BL-15A will be opened to this project from April in 2013. In this presentation, we will introduce not only the current status of these beamlines, the construction plan of new BL-15A and the upgrade plan based on this project but our results of protein solution structure analysis.

2PT124 Legionella DotI and DotJ form a multimeric subcomplex associated with the core complex of the Dot/Icm type IVB secretion system

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Type IV secretion systems (T4SSs) play a central role in the pathogenicity of many bacterial pathogens, including *Agrobacterium tumefaciens*, *Helicobacter pylori*, and *Legionella pneumophila*. *L. pneumophila* utilizes a protein transport system categorized as a type IVB secretion system (T4BSS), which is a sophisticated supramolecular complex composed of Dot/Icm proteins, to translocate a large array of “effector proteins” into the cytosol of host cells. Due to interact with host cellular systems, effector proteins play pivotal roles for the establishment of infection. Composed of five component proteins, the core complex of the *Legionella* Dot/Icm T4BSS spans the bacterial inner and outer

membrane and is anticipated to form a protein-transport channel. However, the molecular structures of the core complex and potential peripheral subcomplexes have not yet been revealed. We report here that two inner membrane spanning proteins, DotI and DotJ, form a multimeric complex that associates with the inner-membrane components of the core complex. DotI and DotJ show a mutual dependency to maintain their protein levels in bacteria. From these results and other evidence, we hypothesize that DotI and DotJ form a ring like structure that associates with the core complex to function as a transport conduit on the inner membrane.

2PT125 基質及び阻害剤によって誘起される ABC トランスポーター ABCB1 の構造・ダイナミクス変化

Dynamics and Structural Changes of ABCB1 transporter induced by binding of substrates and inhibitors

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ATP-binding cassette (ABC) transporters form one of the largest protein families. ABC transporters translocate a number of substrates across cellular membrane by coupling with ATP hydrolysis. Several of these membrane proteins are clinically important in causing, as example, multidrug resistance to cytotoxic pharmaceuticals.

The crystal structures of some ABC transporters have recently been determined. They reveal similar arrangements of the conserved ATP-hydrolyzing nucleotide-binding domains (NBD), but various architectures of the transmembrane domains (TMD), with the exception of common coupling helices that are necessary for transmitting conformational changes. Despite the fact that a wealth of research has been conducted on these structures to resolve the mechanistic problems about how ABC transporters couple with ATP hydrolysis in cytosolic domains to translocate substrates through the transmembrane pore, the specific process about how substrates bind to the proteins and the resultant protein structural change still remains elusive.

Our present study focuses on the mechanism of protein structure changes induced by binding of ATP, substrates or inhibitors in the mouse ABCB1 transporter with an inward-facing conformation. The structure fluctuation simulated by the GROMACS package (version 4.0.7) shows the atomic-level description of the protein structural changes. The result sheds light on the mechanism about the drug translocation process of ABC transporter and the molecular design of inhibitors.

2PT126 核磁気共鳴法による DNA ミスマッチ修復蛋白質 MutL の解析 NMR study on DNA mismatch repair protein MutL

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DNA mismatch repair (MMR) works in the last process of gene repair system, enhancing the DNA replication fidelity up to 100 - 1000 folds. Familial non-polyposis colon cancers called Lynch-Syndrome (LS) caused by mutations of genes coding MMR proteins occupies 2 - 5 % of total colon cancers. About 80 % of those who hold such genetic mutations suffered from colon cancers in their life. I am currently engaged in studying one of the MMR proteins called MutL in terms of structural biology by the method of NMR and other biophysical techniques. MutL homologs can be divided into two groups: E coli. and Eukaryotic MutL. The former does not possess endonuclease activity (MutH does instead) and the latter does. In this research, I deal with the latter. MutL is comprised of three parts: dimerized and endonuclease C-terminal domain (CTD), ATPase N-terminal domain transiently dimerized through ATP-binding and hydrolytic cleavage and the linker region connecting CTD and NTD. Inter-domain configuration between CTD and NTD is thought to be regulated through ATP hydrolysis cycle. Moreover, this configuration dynamics regulates endonuclease activity of MutL and interaction with other MMR proteins. No NMR study has been officially published on both E coli. and eukaryotic MutL so far. The purpose of this research is to elucidate the regulatory mechanisms of MutL endonuclease activity through analyzing the interaction between CTD and NTD at the residue level by means of NMR.

2PT127 選択的スプライシングによる新規タンパク質アイソフォームの機能・構造予測情報を得るためのツールの開発と公開

Development of a functional and structural annotation tool of novel protein isoforms produced by alternative splicing

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Alternative splicing (AS) is thought to be a major mechanism for diversifying transcriptome and proteome in metazoans. Especially, more than 90% of multi-exon genes in human genome are shown to undergo AS using a next-generation sequencing method. Although the accumulation of novel transcript data produced by AS are accelerated by new experimental technologies, a large portion of proteins encoded by the transcripts (termed "AS isoforms") is not known in their 3D structures and functions. Therefore, information whether AS events affect 3D structures and/or functional sites is expected to be useful for studies of novel AS isoforms. For this purpose, we have developed a pipeline that automatically annotates users' uploaded transcript sequences. Given a transcript sequence, the pipeline identifies regions altered by AS events (termed "AS regions") in a putative protein sequence encoded by the transcript, and assigns functional information such as domains and inter-molecular interaction sites obtained by the 3D structure information. Furthermore, the pipeline predicts the possible 3D structure of the AS isoform using structural constraints of hydrophobic core residues mainly. Based on the pipeline, we have been developing a web tool, named AS-EAST (Alternative Splicing Effects ASessment Tools). Using our web tool, users can inquire whether their own transcripts encode a novel AS isoform and infer the impact of AS on its molecular function. AS-EAST is a freely available at <http://as-alps.nagahama-i-bio.ac.jp/ASEAST/>.

2PT128 EGFR 分子 C-末端の天然変性ドメインの 1 分子 FRET 時系列計測 Single-molecule FRET measurement of a intrinsically disordered C-tail domain of an epidermal growth factor receptor

Kenji Okamoto, Yasushi Sako (*RIKEN*)

Structural dynamics of biomolecules, especially proteins, has been known to have indispensable roles in living systems. While conventional X-ray crystallography or NMR is powerful tools to investigate a molecular structure, single-molecule FRET (smFRET) measurement has advantages in observing its dynamics. smFRET can investigate structures of, for example, the intrinsically disordered proteins (IDP) and its time resolution enables realtime observation of structural changes. Epidermal growth factor receptor (EGFR), which is a membrane protein and triggers cellular signal transduction pathways, has the intrinsically disordered (ID) C-tail domain in its intracellular part. The C-tail domain has several phosphorylation sites, one of which binds a protein Grb2 after phosphorylation. Years ago, Grb2-binding kinetics was found to be nonlinear system and it has been suggested that structural dynamics of this ID domain plays an important role. We introduce smFRET measurement of EGFR C-tail domain using recently developed apparatus and statistical data analysis, which is based on the hidden Markov model and the variational Bayes. Time series of 2ch fluorescence signals are obtained from single EGFR C-tail molecules immobilized *in vitro*. Obtained data are analyzed to reconstruct the state transition trajectory (STT) while the number of states is also estimated from data. Further analysis on those STTs gives us details of dynamics, such as possible paths or rates of state transitions.

2PT129 タンパク質の変性及びクロスベータ転移に対する浸透圧効果 Effect of osmotic pressure on protein unfolding and cross-beta transition

Kazuki Takeuchi, Mitsuhiro Hirai (*Grad. Sch. Eng., Gunma Univ.*)

[Introduction]

Proteins are designed to function in crowded environments. However, how a protein folds into its native structure under crowded macromolecular condition is still ambiguous. To clarify the crowding effect on the thermal stability of proteins, we carried out synchrotron radiation wide-angle and small-angle X-ray scattering (WAXS, SAXS) experiments. We employed so-called "osmotic stress" method by using a high molecular weight neutral polymer (osmolyte) in solutions.

[Experimental]

Polyvinylpyrrolidone (PVP) with Mt. 40,000 was used as an osmolyte. The proteins were myoglobin (MY) from horse skeletal muscle and hemoglobin (HM) from bovine blood. The proteins were dissolved in 10 mM HEPES buffers at pH 7.4 with different PVP concentration (0 % to 25 % (w/v)). WAXS and SAXS measurements were performed both at SPring-8 and PF. The temperature of the samples were controlled from 25°C to 85°C.

[Results]

The radius of gyration of the protein at 25 °C showed a minimum at a low PVP