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

Aya Watanabe, Fumio Hayashi, Kenji Oosawa (*Department of Chemistry and Chemical Biology, Graduate School of Engineering, Gunma University*)

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

Hiroshi Sekiguchi^{1,2}, Ayumi Nakagawa⁴, Kazuki Moriya⁴, Mayuno Arita⁴, Yohei Yamamoto⁴, Kouhei Ichihyanagi^{2,3}, Masafumi Yohda⁴, Naoto Yagi¹, Yuji Sasaki³ (¹Research Utilization Div., JASRI, ²JST CREST Sasaki Team, ³Grad. School Frontier Sci., Univ. Tokyo, ⁴Dept. Biotech. Life Sci., Tokyo Univ. Agricult. Tech.)

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

Hiromichi Nakashima¹, Yukihiro Fukunaga², Keiichi Watanabe³, Shoji Yamashita⁴, Etsuko Nishimoto⁴ (¹Institute of Biophysics, Faculty of Agriculture, Graduate School of Kyushu University, ²Interdisciplinary Graduate School of Engineering Sciences Kyushu University, ³Department of Applied Biological Sciences, Saga University, ⁴Molecular Biosciences, Bioscience and Biotechnology, Kyushu University)

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

Nobutaka Shimizu¹, Noriyuki Igarashi¹, Takeharu Mori¹, Ohta Hiromasa², Yasuko Nagatani¹, Takashi Kosuge¹, Kenji Ito¹ (¹KEK, Photon Factory, ²Mitsubishi Electric SC)

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

Tomoko Kubori¹, Takuya Kuroda², Katsumi Imada², Hiroki Nagai¹ (¹RIMD, Osaka Univ., ²Grad. Sch. Sci., Osaka Univ.)

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