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Plants have a variety of potent defense mechanisms against pathogens, including the synthesis of secondary metabolites and antimicrobial proteins. We found that the endosperm of Ginkgo biloba seeds contains ginkbilobin-2 (Gnk2), a novel antifungal protein. Gnk2 consists of 108 amino acids and inhibits growth of plant and human pathogenic fungi. Gnk2 also belongs to DUF26 protein family in plants and has a homology (28-31% identical) to the extracellular domain of cysteine-rich receptor-like kinases (CRKs) from *Oryza sativa* and *Arabidopsis thaliana*. The CRKs are involved in the hypersensitive reaction, which is cell death reaction caused by infection of plant pathogens. Here, we present the structure and function of Gnk2 to provide the first insights into the biochemical properties of DUF26 protein family. Crystal structure showed that the conserved motif of DUF26 protein family considerably contributed to maintain the overall structure of Gnk2 by forming three disulfide bonds. In addition, NMR experiments showed that Gnk2 recognized D-mannose using a binding site surrounded by the residues, Asn11, Gly91, Ala92, Arg93 and Glu104. Their hydrophilic side-chains probably contribute to binding of D-mannose by hydrogen bonds with the free hydroxyl groups of the monosaccharide. The growth-inhibitory assay against a yeast variant lacking mannose chain on cell surface showed that the interaction with mannose chain was an essential event in antifungal mechanism of Gnk2.

2PT115 The short α -helix of the phospholipase C- δ 1 pleckstrin homology domain contributes to stable IP₃ binding

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The phospholipase C (PLC)- δ 1 pleckstrin homology (PH) domain has a characteristic short α -helix (α 2) from residues 82-87. The contributions of the α 2-helix toward the inositol 1,4,5-trisphosphate (IP₃) binding activity and thermal stability of the PLC- δ 1 PH domain were investigated. Native-PAGE analyses indicated that disruption of the α -helical conformation by replacement of Lys-86 with proline resulted in reduced affinity for IP₃ and in thermal destabilization of the IP₃-binding state. Although the mutant protein with replacement of Lys-86 with alanine showed a slight reduction in thermal stability, the IP₃-binding affinity was similar to that of the wild-type protein. Replacement of Phe-87 with alanine, but not with tyrosine, also resulted in reduced affinity for IP₃ and in thermal instability. The ¹H-¹⁵N HSQC NMR study of the selectively [α -¹⁵N]Lys-labeled PLC- δ 1 PH domain indicated that the conformation of the α 2-helix couples with the IP₃-binding activity of the PLC- δ 1 PH domain. These results indicated that the helical conformation of the α 2-helix and the phenyl ring of Phe-87 play important roles in the IP₃-binding activity and thermal stability of the PLC- δ 1 PH domain. Based on these results, the biological role of the α 2-helix of the PLC- δ 1 PH domain is discussed in terms of membrane binding.

2PT116 ヒト心臓脂肪酸結合蛋白質(FABP3)の脂肪酸結合メカニズムに関する研究

Study of the Fatty Acid Binding Mechanism to Human Heart Fatty Acid Binding Protein (FABP3)

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Understanding the interaction modes between a protein and a small molecule is essential to explain a ligand binding to a protein and to elucidate how water molecules and lipid molecules contribute to the stabilization of proteins. However, unlike water molecules and hydrophilic substrates, hydrophobic molecules interact with proteins mainly through van der Waals interactions, and it is hard to characterize clearly the ambiguous interaction mode.

We are now studying the binding modes of fatty acids to a human heart fatty acid protein (FABP3) through molecular dynamics (MD) simulation, isothermal titration calorimetry and cryogenic X-ray crystallography. We first performed MD simulations of native and mutant FABP3 binding a stearic acid (C18:0 fatty acid). When the residues lining the binding cavity are mutated to smaller amino acids *in silico*, the conformations of the fatty acids and positions of water molecule in the binding cavity are different from ones observed in the native. Then we performed MD simulations of native FABP3 binding lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids. Within the simulation runs, the palmitic and stearic acids keep the U-shape conformation in the binding cavity, but the myristic and lauric acids show L-shape conformation.

In addition to the computational study, the calorimetric experiment also suggests the interaction modes are different between these fatty acids. From these results and our high resolution crystal structure, we want to discuss the binding modes of fatty acids to FABP3.

2PT117 Actinin-4 変異体の機能解析

The prediction of three dimensional structure for mutants of actinin-4

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Actinin-4, an actin-bundling protein previously identified by our laboratory, is closely associated with cell motility and cancer metastasis. Three mutations in the *ACTN4* gene encoding the actinin-4 protein are associated with dysfunction of podocytes due to familial focal segmental glomerulosclerosis. We recently identified a novel alternative splice-variant of ACTN4 (ACTN4-sp) that was specifically expressed in high-grade neuroendocrine tumor of the lung (HGNT). The expression of ACTN4-sp, which differs from ACTN4 by three amino acids, was correlated with poor prognosis of patients with HGNT.

ACTN-4 consists of four spectrin repeats, a pair of C-terminal EF-hands, and an actin-binding domain (ABD), which itself consists of a tandem pair of calponin homology (CH) subdomains (CH1 and CH2). All of the mutations in three familial mutations and ACTN4-sp were restricted to the CH2 subdomain of the ABD. Moreover, these mutations were located on the side of the helix, facing the helix-helix interface between CH1 and CH2.

These findings suggest that the three-dimensional structures of the mutations and ACTN4-sp differ from actinin-4, and that these changes were responsible for differences in the biological characteristics of the disease.

2PT118 蛋白質濃度感受性蛍光蛋白質の開発

New fluorescent protein indicator for intracellular protein concentration

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Typical intracellular protein concentration of cell is about 350mg/mL, which makes the cell a very crowded environment. Recently, it was reported that such molecular crowding could alter enzyme activation. To observe the effects of local protein concentrations inside a cell, we developed a new protein concentration indicator by using YFP.

YFP is sensitive to protein concentration such that an increasing protein concentration causes its fluorescence intensity to decrease. To enhance this sensitivity, we inserted several glycine residues at beta-strand 7.

We next took advantage of CFP being insensitive protein concentration by combining it and a YFP with one glycine insert (hereafter called YFP1G) via a flexible linker. The ratio of the 480nm (CFP) and 525nm (YFP) fluorescent light intensity depended on the protein concentration. Increasing the protein concentration caused the fluorescence intensity of CFP to increase and that of YFP1G to decrease. These results suggest our CFP-YFP1G chimera can act as a protein concentration sensitive FRET probe.

2PT119 Interactions of SicP with SptP subdomains in SicP-binding domain

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Many of Gram-negative bacteria cause infectious diseases. Some of the bacteria construct nano-size of syringe-like structures and export virulence proteins "effector proteins" into host cells using the syringes. Each effector required the most appropriate chaperone for efficient export of the effector. *Salmonella* effector protein SptP, which is involved in cytoskeletal remodeling of the host cell, interacts with SptP-specific chaperone SicP at its N-terminal domain, and the atomic structure of the complex (SicP-SptP₁₋₁₅₈) is revealed. According to the complex structure, SptP₃₆₋₁₃₆ seems to be totally involved in SicP-interaction, and the two helices, SptP₇₇₋₈₆ and SptP₉₃₋₁₀₅, seem to interact with SicP directly. Thus, the subdomains are responsible for SicP-SptP interactions. To quantitatively reveal the effects of the subdomain on their interactions, we

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 chaperoning-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