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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