factor-binding (GGA1-3) proteins, Hepatocyte growth factor-regulated substrate (Hrs), Signal-transducing adaptor molecule (STAM1,2), target of myb 1 (Tom1), and Src-activating signaling molecule (Srcasm). Crystal structures of the VHS domains from human GGA1-3, Tom1 have been determined. The domain forms a super helix with eight α helices. Although the VHS domains have structural similarity, those of GGAs interact with some sorting receptors such as sortilin and mannose 6-phosphate receptors that traffic and transfer cargo between TGN and endosomal compartment. However, the VHS domains in non-GGAs don't interact with the sorting receptors. Recently, it has been revealed that the VHS domains in STAM proteins bind ubiquitin and ubiquitinated cargo proteins and participate in the sorting of cargo proteins for trafficking to the lysosome. The VHS domains have very similar three dimensional structures, but interact with distinct ligands and have different functions. Here we initiated a structural study of the VHS domain of STAM1 using NMR spectroscopy. Almost all backbone atoms (^1H , ^{15}N , ^{13}C , ^1H , ^{13}C) were assigned except T21, R39 and proline residues. The secondary structure was predicted using chemical shift index. The binding sites between the VHS domain and the ubiquitin were identified using titration study.

2P-007 極低温電子顕微鏡による MAPEG ファミリー膜タンパク質の結晶構造解析

Structures of membrane associated proteins in eicosanoid and glutathione metabolism determined by electron crystallography

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Membrane associated proteins in eicosanoid and glutathione metabolism (MAPEG) are involved in biosynthesis of arachidonic-derived mediators of pain, fever, and inflammation as well as in biotransformation and detoxification of electrophilic substances. Our structure determination of microsomal glutathione transferase 1 using electron crystallography from 2D crystals induced in the presence of phospholipids has provided the first atomic model of an MAPEG member. MAPEG protein family includes 5-lipoxygenase activating protein (FLAP), leukotriene C4 synthase (LTC4S), microsomal glutathione transferase (MGST) 1, MGST2, MGST3, and Microsomal prostaglandin E synthase 1 (MPGES1).

MPGES1 constitutes an inducible glutathione-dependent integral membrane protein that catalyzes the oxidation of cyclooxygenase derived PGH2 into PGE2. Recently, we determined the structure of MPGES1 in complex with glutathione by electron crystallography. Glutathione is found to bind in a U-shaped conformation at the interface between subunits in the protein trimer. It is exposed to a site facing the lipid bilayer, which form the specific environment for the oxidation of PGH2 to PGE2 after displacement of the cytoplasmic half of the N-terminal transmembrane helix. Hence, insight into the dynamic behavior of MPGES1 and homologous membrane proteins in inflammation and detoxification is provided.

2P-008 固体 NMR 法を用いた β 2 ミクログロブリンが形成するアミロイド線維の構造解析

Structural Analysis of Amyloid Fibrils of β 2-Microglobulin by Solid-State NMR

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Amyloid fibrils are highly ordered filamentous aggregates formed by the self-assembly of peptides or proteins.

We have investigated structure of amyloid fibrils formed by β 2-Microglobulin (β 2-m), a component of the type I major histocompatibility antigen.

Solid-state NMR spectroscopy is a powerful tool for atomic-level structural analysis of noncrystalline amyloid fibrils. In contrast to X-ray structure determination, this method does not require single crystals. Solution NMR is not applicable to fibrils having reduced mobility due to the formation of supermolecular structure.

In this study, we prepared uniformly $^{13}\text{C}/^{15}\text{N}$ -labeled β 2-m amyloid fibrils to obtain structural information about the amyloid fibrils by solid-state NMR.

To simplify NMR spectra by distinguishing between rigid fibrillar and highly flexible protein parts, we have applied two types of NMR experiments.

First, INEPT experiment revealed that amino acid residues in C- and N-terminal and loop region of β 2-m have high mobility. Therefore, such regions would contribute to core formation of amyloid fibrils.

In addition, dipolar cross-polarization experiment indicated that most of amino acid residues in rigid core of fibrils take on β sheet structure.

We will also present the structural analysis of amyloid fibrils of β 2-m based on the sequential chemical shift assignments performed by 3D inter-residue correlation experiments.

2P-009 26 残基長のタンパク質セグメントの構造変量の同定と構造クラス判別への応用

Identification of essential structural variables for medium-size protein-segment and application to structural class assignment

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It is important to classify protein structures for understanding a principle of diversity of protein folds. Classifying alpha/beta and alpha+beta proteins is,

however, sometimes a difficult issue, although several classification and also prediction methods have been proposed. Our previous study using a simple method based on principal component analysis (PCA) of intra-segment atomic distances has analyzed and compared the conformational spaces of protein-segment among the SCOP structural classes (all-alpha, all-beta, alpha/beta, and alpha+beta) with lengths from 10 to 50 residues long. In this study, we focused on medium-size segments (26 residues), and identified specific conformational axes (variables) for the four structural classes. We also applied these axes for assignment of structural classes. The eight class-specific axes (three for all-alpha, one for all-beta, two for alpha+beta, and two for alpha/beta) and some supplemental axes were obtained, and used for constructing the alternative subspaces that segregate the class-specific structural motifs. We developed a domain structural class assignment method based on frequency of segments in the subspaces using Support Vector Machines (SVMs). Using only the two variables, the accuracy to distinguish between alpha/beta and alpha+beta was 85%. In this presentation, the further detailed results will be discussed.

2P-010 X線小角散乱法と分子動力学シミュレーションを用いた分裂酵母 Swi5 タンパク質の溶液構造解析

Solution structure of Schizosaccharomyces pombe Swi5 studied by small-angle X-ray scattering and molecular dynamics simulations

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Schizosaccharomyces pombe Swi5 is an important protein in homologous recombination, and, in particular, the Swi5-Sfr1 complex plays a key role in the process. Recently, Shimizu et al. determined a crystal structure of Swi5, whose monomer forms a long- α -helix. However, the crystal structure has extensive crystal packing, and the α -helices forms a large "net" in the crystal environment. Thus, it is difficult to estimate the solution structure of Swi5 solely from the crystal structure. In addition, the structure of Swi5-Sfr1 complex has not been resolved yet. To reveal the solution structures of the Swi5 and Swi5-Sfr1 complex, we measured small-angle X-ray scattering (SAXS) of Swi5, Swi5-Sfr1, Swi5-Sfr1 Δ N177 and mouse Swi5. Sfr1 Δ N177, which is a truncated mutant of Sfr1 lacking 177 N-terminal residues, retains the binding activity to Swi5. SAXS results indicate that Swi5 forms a tetramer in solution, and has a long shape with maximum length of 150 Å. The length of Swi5-Sfr1 complex is comparable to that of a Swi5 tetramer, and the length of Swi5-Sfr1 Δ N177 complex is approximately half of the Swi5-Sfr1 complex. We also modeled low resolution structures with the dummy atom model for the Swi5 and Swi5 complexes. Now we are performing molecular dynamics simulations of a Swi5 tetramer modeled from the crystal structure. By comparison of the simulation results with SAXS data, a plausible model of the solution structure of a Swi5 tetramer will be constructed.

2P-011 EB1 の構造解析

Structural analysis of EB1

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End binding 1 (EB1) protein is a member of highly conserved +TIPs (plus end tracking proteins) that bind to microtubule plus ends and regulate MT dynamics. On growing MT plus ends, EB1 interacts with other +TIPs by its C-terminal domain. EB1 complex enhances MT polymerization and the stabilization. However, the molecular mechanisms of these processes are poorly understood. Our purpose is structure determination of EB1 and elucidation of the regulation mechanisms of MT.

In this study, we investigated EB1 structure using solution NMR spectroscopy. Currently, we have measured triple resonance experiments, and have attempted to establish the backbone resonance assignments.

2P-012 抗プリオン化合物の作用機構による分類

Categorization of a variety of anti-prion compounds by binding property to prion protein.

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Transmissible spongiform encephalopathies (TSEs) are fatal, untreatable neurodegenerative disease associated with the accumulation of the disease specific conformation for prion protein. Conversion from the cellular form (PrP^C) to the scrapie form (PrP^{Sc}) is essentially a misfolding process without changing in covalent structure. So far, it is reported several anti-prion compounds based on *ex vivo* and *in vivo* experiments. However, the working mechanism of the most compounds is not known. We have categorized these compounds using binding property to PrP^C. Surface plasmon resonance was used for determining the binding affinity and NMR spectroscopy was used for determining the binding site of the compounds. Roughly speaking, the compounds were categorized to three groups. First class compounds including GN8 bind to the native structure and act as a chemical chaperon to stabilize the native conformer [1]. The second class including congo red bind to the PrP^C to aggregate the protein. The third class including pentosan polysulfate do not bind to PrP^C and could bind to the PrP^{Sc} or other proteins. The categorization of these diverse compounds would be useful for understanding the pathogenic conversion mechanism and facilitate the anti-prion