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 (1HN, 15N, 13Cα, 1Hα, 13C) 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 ubiqutiin 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

Caroline Jegerschold (1), Nobuhiko Gyobu (2), Kaoru Mitsuoka (3), Ralf Morgenstern (1), Per-Johan Jakobsson (1) and Hans Hevert (1). (1: Karolinska Institutet; 2: Japan Biological Informatics Consortium; 3: BIRC, AIST)

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 oxidoreduction 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 oxidoreduction 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β2 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 β2-Microglobulin by Solid-State NMR

Miwako Saeki (1), Yasuto Todokoro (1,2), Ayako Egawa (1), Atsushi Kameda (1), Eri Chatani (1), Hisashi Yagi (1), Hideo Akutsu (1,2), Hironobu Naiki (3), Toshimichi Fujiwara (1), and Yuji Goto (1). (1: Inst. Protein Res., Osaka Univ., 2: OLABB, 3: Fac. Med. Sci., Univ. of Fukui)

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 β2-Microglobulin (β2-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 $^{13}C/^{15}N$-labeled β2-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 β2-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 β2-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

Kazuyoshi Ikeda (1), Junichi Higo (2) and Kentaro Tomii (3,4). (1: PharmaDesign; 2: Osaka Univ, MEI center; 3: CBRC, AIST; 4: Univ. of California, Berkeley)

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

Yuichi Kokabu (1), Tomotaka Oroguchi (1), Tsutomu Yamane (1), Hiroshi Hashimoto (1), Toshiyuki Shimizu (1), Hiroshi Iwasaki (1), Mitsunori Ikeguchi (1). (1:International Graduate School of Arts and Sciences, Yokohama City Univ)

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 longα-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-Sfr1ΔN177 and mouse Swi5. Sfr1ΔN177, 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-Sfr1ΔN177 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

Kanaba Teppei (1), Tomoyuki Mori (2), Ryoko Maesaki (1), Yutaka Ito (1),Toshio Hakoshima (2) (3) and Mishima Masaki(1).(1: Graduate School of Science and Technolgy, Tokyo Metropolitan University; 2: Structural Biology Laboratory, Nara Institute of Science and Technology; 3: CREST)

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.

Yosuke Hayano, Yuji O. Kamatari, and Kazuo Kuwata Center for Emerging Infectious Diseases, Gifu University, 1-1 Yanagi-do, Gifu, 501-1194, Japan

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 chaperion 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

drug discovery.

[1] K. Kuwata et al. (2007) PNAS 104, 11921.

2P-013 βストランドにおけるアミノ酸出現頻度のフォールド依存性

Fold dependency of the amino acid occurrence in the beta-strand

Kazuo Fujiwara, Hiromi Toda and Masamichi Ikeguchi. (Department of Bioinformatics, Faculty of Engineering, Soka University)

34 years has passed since Chou and Fasman have calculated the frequency of occurrence of each amino acid residue in the secondary structures in 15 proteins. During this period, vast amount of structures of proteins have been determined and classified by SCOP or CATH. Then, last year, we created a protein quaternary structural database, OLIGAMI, added the quaternary structural information into the SCOP classification and verified the coordinates of the biological molecule. To investigate the amino acid preferences for beta-strands in each fold, we calculated the frequency of occurrence of each amino acid residue in beta-strands by each fold hierarchy for monomeric and homo-oligomeric proteins. The results suggest that some folds have different preferences from that of Chou and Fasman. For example, the beta-strands in the lipocalins fold (beta-barrel structure) prefer lysine and glutamic acid residues, which are not generally preferred in beta-strands and are preferred in alpha-helices. We also investigated the preferences in the inside or outside of the beta-barrel structure.

2P-014 海鼠（グミ）由来溶血性レクチン CEL-III の多量化機構の X 線小角散乱による解析

Analysis of the oligomerization mechanism of hemolytic lectin CEL-III derived from sea cucumber by small-angle X-ray scattering

Hitoshi Sadakata (1), Keigo Hisamatsu (1), Yuzuru Hiragi (2), Tomomitsu Hatakeyama (1), and Shuichiro Goda (1)(1: Dept Appl Chem, Fuc of Eng, Nagasaki Univ; 2: Phys, Kansai Med Univ)

CEL-III is a Ca^{2+}-dependent, Gal/GalNAc-specific lectin purified from the sea cucumber *Cucumaria echinata*, which shows haemolytic activity by oligomerization on the cell surface. We found that the oligomerization of this protein can be induced not only in lipid membranes but also in solution under the appropriate conditions. CEL-III forms an oligomer in solution when complexed with lactose at high pH values and in the presence of high concentrations of salt. To elucidate the oligomeric structure and mechanism of oligomerization of CEL-III, small-angle x-ray scattering (SAXS) measurements were carried out.

Previous report shows that the molecular weight of the oligomer is determined as 1019 k from its forward scattering value by SAXS which is much larger than that estimated using SDS-PAGE, 270 k. To elucidate the effects of detergents, we measured SAXS in the presence of detergents. The results showed that the oligomer dissociated into hexamer (290 k) and the radius of gyration of oligomer was 62 to 75 Å, which well agreed with the result of SDS-PAGE. Kratky plot of both profiles showed that the two peaks which are typical for oligomeric proteins. α-hemolysin, one of the pore forming proteins associates into heptamer on the cell surface. These results suggest that the oligomeric structure in the presence of the detergents is a minimum unit of hemolytic activity and oligomer without detergents consists of 4 oligomers with detergents by weak interactions.

2P-015 タンパク質の低振動ダイナミクス－時間領域テラヘルツ分光によるアプローチ－

Low-frequency Dynamics of Proteins-Approach by Terahertz Time-Domain Spectroscopy-

Ohki Kambara (1), Keisuke Tominaga (1).(1: Molecular Photoscience Research Center, Kobe University)

It is reported by theoretical calculation that the structural fluctuation which is related directly with the protein function has a characteristic vibrational frequency in the low-frequency region. In this work, the terahertz time-domain spectroscopy (THz-TDS) was applied to obtain the low-frequency spectra (> 3 cm^{-1}) of proteins including lysozyme and myoglobin. In order to investigate difference of the THz spectra between the native and denature structures, the pH of each protein solution was adjusted at appropriate values for the structures. The secondary structures of the protein in each sample were confirmed by circular dichroism and FT-IR spectroscopy. A physical quantity called Reduced Absorption Cross Section (RACS) of each protein was defined and calculated from the obtained refractive index and absorption coefficient. Assuming that the IR activity does not depend on the wavenumber, RACS is proportional to the vibration density of state (VDOS). The VDOS of lysozyme was measured by inelastic neutron scattering (INS), and VDOS in the wavenumber region of 12-24 cm^{-1} shows a power-law behavior ($VDOS \propto \nu^{1.8}$). The Debye model, in which vibrational modes in a solid crystal are assumed to be harmonic, predicts that $VDOS \propto \nu^2$. The result of lysozyme by INS is close to this case. On the other hand, RACS of lysozyme obtained by the THz spectrum shows the similar wavenumber dependence ($RACS \propto \nu^{1.8}$). Therefore, it has been confirmed that RACS is proportional to VDOS. The discussion on the wavenumber dependence of RACS will be given.

2P-016 アデノシルコバラミンを補酵素とするエタノールアミンアンモニアリアーゼの構造化学的研究

Crystal structure analysis of ethanolamine ammonia-lyase.

Naoki Shibata (1), Hiroko Tamagaki (1), Hirofumi Komori (1), Yasuhito Shomura (1), Naoki Hieda (2), Keita Akita (2), Koichi Mori (2), Tetsuo Toraya (2) Noritake Yasuoka (1), and Yoshiki Higuchi (1).(1: Dept of Life Science, Graduate School of Life Science, Univ of Hyogo; 2: Graduate School of Natural Science and Technology, Okayama Univ)

Ethanolamine ammonia-lyase (EAL) catalyses the formation of acetaldehyde and ammonia from 2-aminoethanol or of ammonia and propionaldehyde from 2-amino-1-propanol. The reaction is initiated by cleavage of the cobalt-carbon bond of coenzyme B_{12} (adenosylcobalamin, AdoCbl) to form cob(II)alamin-5'-deoxyadenosyl radical pair. The 5'-deoxyadenosyl radical abstracts a hydrogen atom from the C1 carbon atom of the substrate to form a substrate radical, followed by migration of an amino group between substrate carbon atoms. Coenzyme B_{12}-dependent enzymes increase the cleavage rate of the cobalt-carbon bond by 10^{11} > compared with AdoCbl in solution. EAL is composed of six pairs of α and β-subunits, the total molecular weight reaches to ~480,000. We have crystallized Δβ(4-30) and Δβ(4-43) mutants of EAL, which have dramatically increased solubility compared with the wild type. Crystals were obtained from both mutants, and diffracted to 2.2 Å and 6.0 Å resolutions, respectively. The structures of Δβ(4-43) mutant complexed with cyanocobalamin (CN-Cbl) and 2-aminoethanol, and with CN-Cbl and L-2-aminopropanol at 2.3 Å resolution. The Co-substrate distances are 8.5 Å (Co-C1) and 9.5 Å (Co-C2). The Co-C2 distance is in good agreement with the distance estimated by EPR spectroscopy (9-10 Å), whereas the Co-C1 distance is considerably shorter than that of EPR (10-12 Å). Other details of the structure will be discussed.

2P-017 ヒト PCNA G178S 変異体の構造研究

Structural studies of G178S mutant of human PCNA

Asami HISHIKI (1), Hiroshi HASHIMOTO (2),Yuji MASUDA (2), Aya SERIZAWA (1), Kenji KAMIYA (2), Haruo OHMORI (3), Toshiyuki SHIMIZU (1), Mamoru SATO (1). (1: International Graduate School of Arts and Sciences., Yokohama City Univ; 2: Research Institute for Radiation Biology and Medicine., Hiroshima Univ; 3: Institute for Virus Research., Kyoto Univ)

DNA replication in eukaryote is a highly coordinated process, where a large variety of proteins work cooperatively to ensure the accurate and efficient replication. PCNA, which is an evolutionary conserved protein, forms a ring-shaped homo-trimer to function as sliding clamp and tethers DNA polymerase onto DNA. PCNA was also characterized as one of genes defecting UV-induced reversion in *Saccharomyces cerevisiae* . The *rev6-1* mutant strain produces a G178S substitution in PCNA and abolishes translesion DNA synthesis and damage avoidance pathways, while having little effect on the growth rate. Although the side chain of substituted serine likely causes unfavorable steric hindrance in the trimerization, because the G178 is located in a β-sheet at subunit interface of the trimer, structural and functional features of the G178S mutant are still unlcear. To clarify how the G178S substitution influences oligomerization of PCNA, we investigated the difference of structural and functional features between human PCNA and the G178S mutant by several methods, and discuss the comparisons of their behaviors in solution and crystal.

2P-018 超高分解能結晶構造解析における高電位鉄イオウタンパク質中の水分子構造

Water molecules in the ultra-high resolution crystal structure of the high-potential iron-sulfur protein (HiPIP)

Kazuki Takeda, Kouji Kusumoto, Yu Hirano, Kunio Miki.(Department of Chemistry, Graduate School of Science, Kyoto University)

Positions of hydrogen atoms and orientations of water molecules are important to functions of proteins as well as conformations of the main and side chains of polypeptides. Especially, such information is indispensable for proteins involved in the electron transfer reaction, because they significantly affect redox potentials. However, such information about hydrogen and waters is difficult to obtain with high precision in protein crystals due to their resolutonal limit and radiation damage. We could prepare high quality crystals of the high-potential iron-sulfur protein (HiPIP) from *Thermochromatium tepidum*, which is an electron carrier protein in the bacterial photosynthetic reaction [1-3]. Effects of radiation damage were taken into consideration during new data collection and incorporated into structure analysis. Consequently, we could determine the crystal structure at an ultra-high resolution (0.7 angstrom), in which 96 water molecules were found in the asymmetric unit, and hydrogen atoms can be identified in 60 water molecules. Interesting insights into the electron transfer were derived by a classification of the water molecules by temperature factors and appearances. We will report about precise structural information of water molecules as well as effects of radiation damage.

2P-019 マウス硫酸転移酵素ＳＵＬＴ５Ａ１のＸ線結晶構造解析

X-ray crystal structure of mouse sulfotransferase SULT5A1

Takamasa Teremoto(1), Rieko Fujii(1), Jyunnosuke Yamauchi(3), Tomoni Kondo(3), Makoto Kimura(2), Masahito Suiko(3), Yoichi Sakakibara(3), and Yoshimitsu Kakuta(2). (1: Laboratory of Structural Biology, Department of Systems Life Sciences and (2: Laboratory of Biochemistry, Department of Bioscience and Biotechnology, Graduate School, Faculty of Agriculture, Kyushu University. (3: Food Research Branch, Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki

The cytosolic sulfotransferase (SULT) transfer the sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to a wild variety of endogenous and exogenous phenolic substrate, such as steroid hormones, thyroid hormones, drug, and xenobiotics. Mouse SULT5A1 has a hydroxylsteroids such as dehydroepiandrosterone (DHEA) sulfating activity similar to SULT2 family. To elucidate the substrate recognition mechanism of the enzyme, we crystallized