drug discovery.

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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 Shuichi Goda (1) (1: Dept Appl Chem, Fac 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 ($> 3 \text{ cm}^{-1}$) 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 ($\text{VDOS} \propto \nu^{1.8}$). The Debye model, in which vibrational modes in a solid crystal are assumed to be harmonic, predicts that $\text{VDOS} \propto \nu^2$. 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 ($\text{RACS} \propto \nu^{1.8}$). 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)amin-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 $10^{11} \times$ 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 $\Delta\beta(4-30)$ and $\Delta\beta(4-43)$ 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 $\Delta\beta(4-43)$ 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 unclear. 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 resolution 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 hydroxysteroids such as dehydroepiandrosterone (DHEA) sulfating activity similar to SULT2 family. To elucidate the substrate recognition mechanism of the enzyme, we crystallized