already reported, and these complex structures indicated that these inhibitors bound to the GLO1 by the hydrogen bonding of its GSH moiety. On the other hand, the corresponding moiety of M-GFN is stabilized by hydrophobic interactions.

1P-021 マルチカノニカル法を用いたタンパク質の水と極性溶媒における構造依存性と2次構造形成時におけるトリフルオロエタノールの配向性の研究

Structure variation of a 24-residue peptide on solvent, and preferential solvation by trifluoroethanol by multicanonical sampling

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It is experimentally known that 2,2,2-trifluoroethanol (TFE) promotes secondary structure formation of a polypeptide. However, the role of TFE in the secondary-structure induction has not yet understood well computationally or theoretically. To understand the role of TFE, we did conformational sampling of a 24-residue peptide (humanin) in water and in 30% TFE/water co-solvent by multicanonical molecular dynamics (McMD) simulation. The McMD simulation is a powerful sampling method to explore a variety of conformations in a wide temperature range. First we showed that various helical structures existed in the 30% TFE/water system. The most helical region corresponded to hydrophobic amino acids. Furthermore, our results showed preferential solvation by TFE around the helical regions of the peptide. We also showed that the fluorine atoms of TFE molecules were directed toward the helical region of the peptide. Analysis suggests that hydrophobic residues and TFE molecules work together to induce the helical structures. Contrarily, in water, the helix content of this peptide was negligibly small. Last, a free-energy landscape of the peptide was constructed in three-dimensional subspace by principal component analysis (PCA) at 280K for both systems: 280 K is the temperature at that the experiment was done. Both landscapes consisted of clearly distinguishable some clusters at 280K.

1P-022 ヒト由来ギャップジャンクションチャネルのX線結晶構造解析

Crystal structure of human gap junction channel

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Intercellular signaling is one of the most essential properties exhibited in multicellular organisms. Gap junctions are specialized membrane regions containing hundreds of intercellular communication channels that allow passage of small molecules such as ions, metabolites, nucleotides, and short peptides. Many cells express more than one kind of connexin and gap junction channels can be made of more than one type of connexin. The diversity of gap junction channel properties is increased by heterotypic (different connexins in opposed hemichannels) and heteromeric (different connexins in the same hemichannel) interactions. Here we determined the atomic structure of human Cx26 gap junction channel in its open state. The structure reveals a typical four-helix bundle in the trans-membrane region which was quite different from that previously proposed. Point mutations of some residues that make interactions within or between monomers to stabilize the structure are the cause of nonsyndromic hereditary deafness or skin diseases. The second extracellular loop makes specific interactions with the opposing unit and determines the compatibility of heterotypic channel interactions. And surprisingly, the N-terminal regions of six subunits are inserted into the pore and form the funnel-shaped entrance of the channel, suggesting that this region plays the key role in channel gating.

1P-023 芳香環水酸化ジオキシゲナーゼの電子伝達コンポーネント (ferredoxin reductase) のX線結晶構造解析

Crystal structure of the ferredoxin reductase component in the Rieske non-heme iron oxygenase system, carbazole 1,9a-dioxygenase

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Rieske non-heme iron oxygenase systems (ROSSs) are the initial catalysts in the degradation pathways of aromatic compounds. ROSSs catalyze the incorporation of both oxygen atoms of molecular dioxygen with tandem carbon atoms of an aromatic ring as two hydroxyl groups. Carbazole 1,9a-dioxygenase (CARDO), is a member of ROSSs, has broad substrate range and contains three components: terminal oxygenase (CARDO-O), ferredoxin (CARDO-F), and ferredoxin reductase (CARDO-R). Biochemical studies have showed that ferredoxin recognition by CARDO-O is rather specific, but CARDO-F receives electrons from not only CARDO-R but also unrelated reductases. To elucidate molecular basis in electron transfer in CARDO, we have determined the structures of CARDO-O and CARDO-F. In this study, we determined the structure of CARDO-R from *Janthinobacterium* sp. J3. Two crystal structures, with or without FAD, were obtained by SAD (SeMet) and MAD (Fe) phasing. There were no differences without several movements of loops in the FAD-binding region between two structures. Domain structures of CARDO-R were highly similar to

those of benzoate dioxygenase reductase. However, by superimposing FAD- and NAD-binding domains of two reductases, the positioning of ferredoxin domain was significantly different. This difference is probably related to the fact that their redox partner is definitely different; CARDO-R is Rieske-type ferredoxin and benzoate dioxygenase reductase is terminal oxygenase. In addition, the docking simulation between CARDO-R and CARDO-F is also reported.

1P-024 NMRによるウマβラクトグロブリンの立体構造解析

NMR solution structure of equine beta-lactoglobulin

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Beta-lactoglobulin (LG) is the major whey protein secreted in the milk of ruminants like the cow or sheep, and monogastrics like the pig, horse, dog or cat. It is absent from the milk of human and rodents. Although it binds various hydrophobic ligands, the physiological role of this protein has not yet been known. It is known that the ruminant LGs are dimeric, whereas non-ruminant LGs are monomeric. Both X-ray crystal structure and NMR solution structure are determined for bovine LG (BLG). To understand the structural difference between dimeric and monomeric LGs, the three-dimensional structure of equine LG (ELG) was determined by heteronuclear triple-resonance NMR. Like BLG, ELG assumes an eight-stranded up-and-down beta-barrel structure with a flanking alpha-helix and an off-barrel 9th strand (I strand). Large structural differences between BLG and ELG are found in the loop region between the helix and I strand so that the twist of the A and I strands is larger in ELG. This may explain why ELG does not form a dimer, because the I strand is important to form an intersubunit anti-parallel beta-sheet in BLG dimer.

1P-025 オロチジンリン酸脱炭酸酵素の構造学的研究：活性中心のリジンの働き

Structural investigation of orotidine monophosphate decarboxylase: the role of the catalytic lysine

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Orotidine 5'-monophosphate decarboxylase (ODCase) catalyzes the conversion of orotidine 5'-monophosphate (OMP) to uridine 5'-monophosphate (UMP). This enzyme is known as one of the most proficient enzymes, accelerating the reaction by 17 orders of magnitude when compared to the spontaneous reaction. Although numerous crystal structures and biochemical analyses have been reported, the reaction mechanism of this enzyme is still open to dispute.

A characteristic electrostatic network (Lys42-Asp70-Lys72-Asp75: numbering as ODCase from *Methanobacterium thermoautotrophicum*) is existent in all known ODCase. One of these residues, Lys72, is an essential residue for catalysis. Mutation of this residue generates a non-reactive mutant. The amino head of the lysine contacts to the C6 position of pyrimidine rings of the substrate analogues. In order to understand the role of this lysine in detail, we have determined the crystal structures of wild-type and Lys72Ala mutant ODCase from *M. thermoautotrophicum* with several sets of substrate analogues at 1.2-1.5 Å resolution.

Comparison of the ligand-binding positions of wild-type and Lys72Ala mutant enzymes in the determined structures demonstrates a systematic difference. The pyrimidine rings of all ligand sets in wild-type are moved approximately 0.5 Å towards the opposite side of Lys72 compared to the positions of those in Lys72Ala mutant. This observation suggests that Lys72 pushes down the pyrimidine ring at C6 of the ligands.

1P-026 F1-ATPaseの分子動力学シミュレーション

Structure and Function Analysis of F1-ATPase by Molecular Dynamics

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F1-ATPase is comprised of five different subunits (α to ϵ). $\alpha\beta\beta\beta$ hexamer contains nucleotide binding sites and γ rotates sequentially by a cooperative binding change mechanism for ATP synthesis and hydrolysis. According to single molecule studies, the sequential γ rotation has two dwell states: ATP hydrolysis and ATP binding states. Recently, several experiments suggest the snapshot in the F1-ATPase crystal structure corresponds to the hydrolysis state and in the actual ATP binding state three β subunits take opened, closed and half-closed structures. Using this information, we have constructed a structural model of F1-ATPase for the ATP binding state via targeted molecular dynamics. In the resulting structure, the γ subunit is located at a reasonable position in terms of the torque generation for the following rotation. In addition, the α/β subunit interfaces show a characteristic feature which is consistent with the chemical reaction for ATP binding dwell. We also performed standard molecular dynamics on F1-ATPase to provide insight into the chemical-physical energy exchange mechanism.