other amino acids, and examined the effect of mutations on the drug resistance of AcrB. [Methods] We constructed AcrB mutants that had replaced the binding pocket's phenylalanine with Ala, Val, Leu, Ile, Tyr. The expression of the mutant protein was detected by Western blotting with an anti-AcrB rabbit antibody. The drug resistance was evaluated with determination of the MICs (minimum inhibitory concentration) of various drugs. [Results and conclusions] Ala, Val, Leu, and Ile mutants of AcrB caused drastic decreases of the drug resistance. In contrast, the effect of the Tyr mutation on the drug resistance was moderate. These results suggest that the aromatic side chains of AcrB play an important role in substrates recognition. AcrB probably interact with substrates by aromatic-aromatic interactions.

3P-042 高等植物によるヘム分解の特徴：ダイズヘムオキシゲナーゼ反応におけるプロトンリレー

Heme degradation by plant heme oxygenase: the proton-relay system in Soybean HO-1 reactions.

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Heme oxygenase converts heme into biliverdin, CO, and free iron in the presence of oxygen and electrons. In plants, the biliverdin is further reduced to phytychromobilin, the photoreceptive pigment. Chemistry of heme conversion by soybean HO1 (Gm HO1) was confirmed to be the same as that of known mammalian HOs though homology of the AA sequence is low. However, the verdoheme intermediate has much lower affinity to CO and converts into biliverdin very slowly. We have obtained some evidences that the AA sequence corresponding to the distal part of the heme pocket of rat HO1 causes such uniqueness, by use of mutants of H150 as well as S151 and R155. In this study, we made further analyses of heme conversion by these mutants from CO-bound heme with oxygen and from ferric heme in the presence of far excess ascorbate. EPR and ¹H NMR on the heme complexes of H150D and H150F revealed that these complexes had no axial water ligand. Under far excess ascorbate, the wild type and S151A reactions produced free biliverdin with no oxyheme accumulation, while R155A and H150A reactions indicated oxyheme, suggesting its conversion to verdoheme slowed. Both the heme complexes of H150F and D stabilized oxyheme and did not convert it further. In the CO-heme conversion, the proteins holding H150 residue all produced ferric verdoheme but the oxyheme conversion was markedly slowed by R155A. Then, H150 residue is revealed to stabilize ferric state of verdoheme and R155A to serve proton donation to the peroxyheme complex.

3P-043 3D-RISM 理論を用いたミオグロビンにおけるリガンド分子の脱着過程に関する理論的研究

Theoretical study of detaching process of ligand molecules in myoglobin by using 3D-RISM theory

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Myoglobin is a protein that deeply concerns the oxygen supply system in a variety of living bodies. The associating and dissociating processes of ligand molecules in myoglobin are important for clarifying a complex biological and physiological activity of the oxygen supply system, and have been studied by using a lot of ligand molecules such as carbon monoxide, xenon and so on. These ligand molecules are released from heme, which is the chromophore in myoglobin, to the bulk solvent via cavities in the protein. In this study, we aim to reproduce and predict this molecular detaching process with distribution functions of various ligand molecules by using 3D-RISM theory, the statistical mechanics theory of molecular liquids. It is also planned to calculate the partial molar volume and the coordination number from the distribution functions in order to discuss the detaching mechanism.

3P-044 Novel channel of glutamine amidotransferase CAB for ammonia transport revealed by molecular dynamics simulations

Novel channel of glutamine amidotransferase CAB for ammonia transport revealed by molecular dynamics simulations

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Glutamine amidotransferase CAB (GatCAB) acts on misacylated **Gln**-tRNA^{Gln}, and produces **Gln**-tRNA^{Gln} by two catalytic reactions, i.e. the glutaminase and transamidase reactions. The two catalytic centers for the reactions are markedly distant, but were proposed to be connected by a hydrophilic channel structure, for which the length is 30 Å. It was suggested that NH₃ or NH₄⁺ are transported through the channel, and thus, that the two reactions are associated. We have analyzed how such molecules are transported through the channel, by using molecular dynamics (MD) simulations coupled to free energy calculations. It has been found that the entrance of the channel previously reported (Science, 312 (2006), pp1954.) is not opened during MD simulations, and instead, that a new gate is identified, which is located close to the previously reported site. The new gate leads to a new channel, which is connected to the previous channel at the site of Arg323. Interestingly, in our MD simulations, the new gate has been opened and closed repeatedly by conformational changes of Asn81 and Phe206. Accordingly, we set up some models in which either NH₃ or NH₄⁺ is put at the gates or inside of the channels, and then, performed MD simulations and free energies calculations using potential of mean force (PMF). Thus, comparing free

energy values for passing through the gate of each channel, we have concluded that NH₃, instead of NH₄⁺, is transported through the new channel identified in the present study. Detailed mechanisms will be discussed in the session.

3P-045 分子動力学計算及びX線小角散乱を用いた、好熱菌 F₁-ATPase ε サブユニットの ATP 結合に伴う構造変化の研究

Conformational change of F₁-ATPase ε subunit upon ATP binding studied by molecular dynamics simulations and X-ray scattering

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The ε subunit of F₁-ATPase acts as an endogenous inhibitor of the ATPase activity in F₁-ATPase. Biochemical and structural studies has shown that the ε subunit from the thermophilic *Bacillus* strain PS3 (TF₁) binds ATP specifically, and the ATP binding induces the conformational change of the C-terminal domain of the ε subunit from the extended form to the folded form that allows ATP hydrolysis in F₁ motor. The mechanism of how ATP binding induces the conformational change of the ε subunit remains unclear, because the atomic detail of the conformational ensemble of the ATP-free form is still not known.

In this study, to address the above question, we used molecular dynamics (MD) simulations and small-angle X-ray scattering (SAXS) experiments. Analysis of the SAXS data measured at the RIKEN structural Biology Beamline I (BL45XU) at Spring-8 has shown that the overall structural characteristics of the ATP-bound form of the ε subunit in solution are consistent with the crystal structure, while the molecular shape determined for the ATP-free form shows a more expanded conformation. We are now performing MD simulations for both the ATP-bound and ATP-free forms to obtain conformational ensemble of these forms, and the validity of the calculated ensembles will be checked by a comparison of simulation-derived SAXS profiles with the experimentally observed profiles.

3P-046 FRET による MinC と FtsZ の相互作用解析

Analysis of interaction between MinC and FtsZ by FRET

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Escherichia coli is divided into the center of the cell, producing two daughter cells with equal size. Because division of cells at inappropriate site gives rise to chromosomeless minicells, the selection of the division site must be highly regulated. Division of cells in *E. coli* is initiated by formation of FtsZ ring at the center of the cell. The position of FtsZ ring is regulated by *minB* operon gene products (MinC, MinD, and MinE). MinC is a cell division inhibitor which prevents formation of FtsZ ring. MinD and MinE accumulate MinC near cell pole to preventing FtsZ ring formation other than midcell. It is important to reveal the molecular mechanism of MinC to understand division site selection system by Min proteins. Electron microscopic and sedimentation analysis indicated that MinC interacts to FtsZ polymer and inhibits bundling of the FtsZ polymer. At present, interaction between MinC and FtsZ has not been observed directly in the solution. To investigate interaction between MinC and FtsZ in the solution, in this study, we monitored the interaction using FRET analysis. We prepared fluorescent labeled MinC-Cy3 and FtsZ-Cy5. In the presence of GTP, FtsZ-Cy5 was polymerized, but FRET from MinC-Cy3 to FtsZ-Cy5 polymer was faintly observed. The FRET was significantly enhanced, when MinC-Cy3 was incubated with FtsZ-Cy5 monomer. Our FRET analysis indicated that MinC interacts with FtsZ monomer in the reaction solution. We will discuss about molecular mechanism of MinC regulating FtsZ polymerization.

3P-047 アミリン線維と脂質ベシクルの速度論的会合

Kinetic association of amylin fibrils and lipid vesicles

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The specific self-association of proteins to form amyloid fibrils is a characteristic of a number of pathologies such as Alzheimer's disease, Parkinson's disease, prion disease, and type II diabetes. Pancreatic amyloid deposits, composed primarily of the 37-residue islet amyloid polypeptide (amylin, IAPP), are a characteristic feature found in more than 90% of patients with type II diabetes. Despite a growing body of evidence implicating membrane interaction in amylin toxicity, the membrane-bound form of amylin oligomers or fibrils has not been well characterized. In this study, turbidity assay was used to elucidate the molecular details of the interaction of amylin fibrils with lipid vesicles of phosphatidylcholine (PC) as a model membrane. The effects of added fibrils or vesicles on the rate and extent of fibril-vesicle association were monitored via measurement of the time-dependent turbidity. The resultant data were fitted well to a double exponential function, suggesting that the association of amylin fibrils and PC vesicles is regulated by mainly two interactions: fibril-vesicle and vesicle-vesicle.

3P-048 ケミカルシャペロンとして働く抗プリオン化合物群の同定

Identification of a variety of anti-prion compounds that acts as chemical chaperons

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Transmissible spongiform encephalopathies (TSEs) are fatal, untreatable neurodegenerative disease associated with the accumulation of the disease