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 hemin into biliverdin, CO, and free iron in the presence of oxygen and electrons. In plants, the biliverdin is further reduced to phytochromobilin, 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 **Glu**-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 NH_3 or NH_4^+ 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 NH_3 or NH_4^+ 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 NH_3, instead of NH_4^+, 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 F_1-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 F_1-ATPase ε subunit upon ATP binding studied by molecular dynamics simulations and X-ray scattering

Tomotaka Oroguchi (1), Yasuyuki Kato-Yamada (2), Hiroshi Hashimoto (1), Satoru Unzai (1) and Mitsunori Ikeguchi (1). (1: International Graduate School of Arts and Sciences, Yokohama City Univ; 2: Dept of Life Science, Rikkyo Univ)

The ε subunit of F_1-ATPase acts as an endogenous inhibitor of the ATPase activity in F_1-ATPase. Biochemical and structural studies has shown that the ε subunit from the thermophilic *Bacillus* strain PS3 (TF_1) 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 F_1 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

Takashi Okuno, Mayumi Maruyama, Machiko Ogoh, Hiromitsu Tanina, Noriaki Funasaki, Kentaro Kogure

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 phosphatidylcholin (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

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. Thus it may be possible to intervene in this process by introducing a small compound which can act as a chemical chaperion capable of stabilizaing the PrP^C conformation [1]. Initially we selected the more than 200 compounds from *in silico* screening based on the PrP^C structure and finally discovered 24 efficient anti-prion compounds with diverse chemical structures from *ex vivo* experiment [2]. Surface plasmon resonance studies reveals that the binding affinities of compounds roughly correlated with their anti-prion activities. This result indicates that identification of compounds that acts as chemical chaperons is one of the efficient strategy for anti-prion drug discovery. Existence of the exception indicating the involvement of some modulation factors other than the direct binding with PrP^C. The categorization of these diverse compounds would facilitate the anti-prion drug discovery and the elucidation of the pathogenic conversion mechanism. [1] K. Kuwata et al. (2007) PNAS 104, 11921. [2] Hosokawa-Muto, Kamatari, Nakamura, Kuwata (in preparation).

3P-049 QM/MM 法によるアデニル酸キナーゼの反応機構に関する研究

Theoretical study on the reaction mechanism of adenylate kinase by QM/MM method

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Adenylate kinase catalyzes the reaction: ATP + AMP + MG^{2+} -> ADP + ADP + MG^{2+}. In order to elucidate the nature of the reaction, we have investigated the reaction mechanism of the system using molecular mechanics, molecular dinamics and quantum chemical methods. We constructed the complex structure of enzyme and substrates, ATP and AMP with Mg ion based on the PDB data 2AKY. After the full optimization of the whole molecule in vacuo, we put water molecules arround catalytic center Mg ion within radius of 12 A with spherical boundary condition centered on Mg ion, and we put harmonic constraint potentials on the surrounding atoms in the boundary region. Some model structures were sampled from 900K MD simulation followed by structural energy minimizations. In order to obtain the reasonable model structure of the transiton state of the reaction, we performed molecular mutation calculation, in which values of molecular mechanics parameters of the reactant gradually turned into those of the product during MD simulation. As the result we were able to obtain the reasonable model structure of the transition state of the reaction. We truncated surrounding atoms father than about 10 A from the reaction center, then we optimized the transition strucuture with ONIOM (QM/MM) method as well as the reactant and the product states. The reaction path of SN2 type inversion of phosphate group were retained by hydrogen bondings with surrounding atoms.

3P-050 単粒子解析における最尤法に基づく粒子拾い上げ

Maximum likelihood approach for picking-up process in single particle analysis

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We present a new method for generating a set of high quality particles in single particle analysis (SPA) from electron microscope images. The method uses maximum-likelihood (ML) approach which determines positions of particles by applying ML estimations using reference particle data. ML approach has some advantages over the conventional methods that have been used in the picking-up process in SPA; ML approach can minimize statistical errors in the determination of particle positions and excise the most reliable particle data set under some assumed conditions. However, there are some difficulties to implement the ML calculations; 1) Gaussian distributions of some parameters are required for the fast calculations, 2) complete survey of the parameter space is almost impossible due to the limited computational resources. This method was examined for its picking-up efficiency, i.e. accuracy in recognizing particles and noise tolerance, using model image sets with various signal-to-noise ratios and with electron microscope images of the negatively stained Transient receptor potential melastatin type 2. The method yielded high performance in particle recognitions and produced a set of particles accurate enough for processing data in the following steps in SPA. These results showed that ML-based picking-up method is promising even for image data with extremely low SNR or contrast images in SPA.

3P-051 3 重共鳴 4 次元 NMR への非線形サンプリングの応用

Applications of nonlinear sampling scheme to four dimensional triple resonance NMR spectroscopy.

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Despite its potential advantages in analysis, long duration of measurement and limited digital resolution in indirectly observed dimensions prevent 4D NMR experiments from being used in protein NMR projects routinely. Applications of recently proposed new acquisition schemes for speeding up multidimensional NMR experiments are therefore expected to be extremely beneficial for 4D experiments.Nonlinear sampling for indirectly acquired dimensions in combination with maximum entropy (MaxEnt) reconstruction has been shown to provide significant time savings in the measurement of multidimensional NMR experiments. In this presentation, we demonstrate the benefits of this nonlinear sampling and 3D MaxEnt processing in 4D triple-resonance experiments.4D HCC(CO)NH and 4D $^{13}C/^{15}N$ separated NOESY data were acquired with 512 ($^1H^N$) x 32 (^1H) x 24 (^{13}C) x 8 (^{15}N) linearly sampled complex points on *T. thermophilus* HB8 TTHA1718 protein (66a.a.). For both 4D experiments we succeeded in reconstructing spectra of equivalent quality to the reference spectra from eminently reduced sampling points. The time gained from this scheme can be used to increase the number of indirect data points. Resolution enhancement of 4D spectra was consequently achieved by measuring data with extended acquisition times for indirect dimensions with nonlinear sampling scheme. We also evaluated the expected artefacts in 4D spectra arising from nonlinear sampling and MaxEnt processing, such as the mis-calibration of intensities and the emergence or disappearance of cross peaks.

3P-052 タンパク質の主鎖トポロジーとフォールディングキネティクスとの関係—3 次元格子タンパク質の円順列変異体に統計力学モデルを適用して—

Relationship between folding topology and kinetics using a statistical mechanical model for 3D lattice proteins to circular permutants

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In order to understand protein folding mechanisms, we analyzed the relationship between the contact order (*CO*) and folding rates (k_f) in circular permutants of 48-mer 3D lattice proteins. At first, we examined how the interaction energies between amino acid residues in 48 circular permutants are distributed with respect to their distances along the chain. The distances between the amino acid residues along the chain, i.e., the distributions of short-, medium-, and long-range interactions are a key point of *CO*. Then, we calculated k_f using a statistical mechanical model, which we referred to as A-W model[1,2,3]. Finally, we analyzed the correlation between *CO* and ln k_f. We have grouped the 48 circular permutants into several types according to their core structures to analyze the relationship between *CO* and ln k_f. We observed an excellent correlation between *CO* and ln k_f in each type of circular permutants and found that, although the contact order contributes to folding rate essentially, the interaction energies between amino acid residues distant along the chain also play an important role. We will discuss them in detail in the meeting. 1)H.Abe & H.Wako, J. Phys. Soc. Jpn. 73(2004)1143. 2)H.Abe & H.Wako, Phys. Rev. E 74(2006) 011913. 3) H.Wako & H.Abe, J. Phys. Soc. Jpn. 76(2007)104801.

3P-053 単粒子解析で分子構造の違う画像を分離する方法

Separating images in different conformations of molecules on single particle analysis

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Using electron microscopy projection images of biological molecules, a computational method was developed to separate different conformations of the same molecule or different particles. Purified protein molecules may exhibit different conformations in solution which affects the resolution of the 3D reconstruction in which all views are merged. Resolution can only improve if the different conformations are sorted out. Although fundamental limitations exist on heterogeneity determination when only projection images are analyzed, a statistical method to build reasonable 3D models by sorting images into different models would be helpful. Furthermore, pioneering studies on ribosome complexes showed that different conformational states can be extracted from a heterogeneous data set and contribute to explain its molecular mechanism. After particle images are classified into classes, a score is introduced to measure how well two given class averages fit to the same 3-D model. In our method, the similarity between common lines of the projections, known as the cross sinogram correlation was used. Particle images of the classes are placed on a virtual map where similar images are connected to form a graph. Then, a modern algorithm for the graph-cut problem is applicable to separate images. Using a test data set containing mixed projection images of simple phantoms, a protocol to obtain correct separation was investigated. The method was also applied to an image data set of RNA polymerase whose conformational flexibility was reported previously.

3P-054 マイクロビーズとマイクロ流路を組み合わせた新規プロテインアレイの開発

A novel protein array using microbeads aligned in a microfluidic chip

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Protein array is a powerful means to investigate protein-protein interactions. Yet current protein arrays are not versatile due to their low sensitivity (1ng/ml) and cost-ineffectiveness.

In this study, we have developed a sensitive and cost-effective protein array using a commercial fluorescence microscope. The protein array has aligned antibody-immobilized microbeads (5μm in diameter) inside a polydimethylsiloxane (PDMS) microfluidic chip. The minimum concentration required for fluorescence detection was determined to be several tenths of pM (about 1pg/ml) using fluorescently-labeled glutathione-S-transferase (GST) to the protein array having α-GST antibody immobilized microbeads.

Firstly, we tried detecting a recombinant protein expressed in cultured cells. We extracted cytoplasmic components of PC12 cell expressing green fluoresent protein (GFP) and labeled them with amino group reactive fluorescent dye. The labeled product was applied to the protein array having α-GFP antibody microbeads, α-β-actin antibody microbeads as positive control and α-IgE antibody microbeads as negative control. Only α-GFP and α-β-actin antibody