obtained by 3D-EM deposited on? The answer is PDB. Structural data by 3D-EM are deposited in EMDB and/or PDB, depending on their data type. As hybrid structure analysis methods have been paid attention recently, repository of their data are also hybrid and in a rather complicated situation. On new EM Navigator, you can search and browse 3D-EM data on PDB using the same interfaces as those of EMDB data. On the detail pages for PDB entries, molecular viewers (jV and Jmol) are embedded to browse their 3D structures interactively. And also, links for related EMDB/PDB entries are shown graphically to clear the relationship among the entries. To make it easy to search, browse and, enjoy 3D-EM data, we are developing EM Navigator.

1P-004 In-cell NMR による細胞内蛋白質の分子動態解析

Structural and dynamical studies of proteins in living cells by in-cell NMR spectroscopy

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In-cell NMR has been used to detect various intracellular events such as conformational changes, dynamics and binding events in bacterial cells. In eukaryotic cells, in-cell NMR studies have been performed by injecting proteins into *Xenopus laevis* oocytes or eggs, and, most recently, cell-penetrating peptides have been used to deliver proteins that can be observed in living human cells. However, the low sensitivity and short life time of the samples had so far prevented the acquisition of sufficient structural information to determine protein structures by in-cell NMR. Very recently, by optimizing sample preparation and NMR experimental methods, we could solve the high resolution 3D structure of a putative heavy metal binding protein TTHA1718 from *Thermus Thermophilus* HB8 overexpressed in *E. coli* cells.

In this presentation, we report our recent studies of TTHA1718 focused on identifying the "in-cell" effects. By comparing the 3D structures between *in vivo* and *in vitro*, slight structural changes have been observed in the putative heavy metal binding region. NMR studies on the mutants that lack metal-binding activity suggested that interactions with metal ions in the *E. coli* cytosol may affect the conformation of the region. Furthermore, we report ¹⁵N relaxation experiments performed both *in vivo* and *in vitro*, and discuss the effect of intracellular environment to protein dynamics.

1P-005 In-cell NMR 法を用いた生細胞内におけるプロテイン G B1 ドメインの高次構造解析

Structure determination of protein G B1 domain in living *E. coli* cells by in-cell NMR spectroscopy

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Recently, we reported the first 3D protein structure calculated exclusively on the basis of information obtained in living cells. The high resolution structure of the putative heavy metal-binding protein TTHA1718 from *Thermus thermophilus* HB8 overexpressed in *E. coli* cells was solved by in-cell NMR. In this presentation, as another demonstration of our methodologies for protein structural analyses *in vivo*, we report in-cell NMR studies of *Streptococcus* protein G B1 domain overexpressed in *E. coli* cells.

For backbone and side-chain resonance assignments, we measured and analysed the six 3D triple-resonance NMR spectra, HNCA, CBCA(CO)NH, HBHA(CBCACO)NH, CC(CO)NH, HCC(CO)NH and HCCH-TOCSY. Rapid data collection using nonlinear sampling with maximum entropy reconstruction, which provided 3D NMR spectra of much higher quality than conventional spectra recorded in the same short measurement time, was the key for the success of resonance assignments of G B1. All backbone resonances and almost all side-chain resonances of G B1 were assigned exclusively from the data obtained in living *E. coli* cells. Three dimensional structure determination of G B1 in *E. coli* cells is in progress.

1P-006 異常X線小角散乱法によるネイティブおよび再構成ウマ脾臓フェリチン内の鉄イオンコアの構造研究

Evaluation of iron core size in native and reconstituted horse spleen ferritin molecules using anomalous small-angle X-ray scattering technique

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Mammalian ferritin is an intracellular iron storage protein assembled from 24 subunits forming an approximately spherical shell. Its central cavity has a capacity for up to 4500 atoms of ferric iron. The iron loading by ferritin *in vitro* has been shown to be dependent upon the presence of a Good's buffer and to fail in the presence of phosphate. On the other hand, phosphate is also a major component of core, though in variable amounts. Horse spleen ferritin has a Fe:P ratio of ~10 and bacterial ferritin has contains a much higher ratio of Fe:P up to 1:1. The significance of phosphate in the core is not well-understood. To examine the effect of phosphate on the structure of iron core, we evaluated the core size of

native and reconstituted horse spleen ferritin using anomalous small-angle X-ray scattering technique. Measurements were performed with dilute solutions of both ferritin near the K-absorption edge of iron on a solution X-ray scattering camera at BL-10C of Photon Factory. The scattering profiles of anomalous part in the total scattering were derived by mutual subtraction among the scattering intensity profiles measured at different wavelengths and analyzed in term of the radius of gyration, $R_{g,core}$, of iron core. The results showed that, despite of same iron amount, there is significant difference in $R_{g,core}$ between native and reconstituted ferritin; rather, the value of $R_{g,core}$ of native ferritin is close to that of the core in iron-rich reconstituted ferritin (2000Fe atoms). This result was in consistent with that previously obtained by contrast variation technique.

1P-007 細菌べん毛フック繊維連結部の極低温電子顕微鏡による構造解析

Structural analysis of the flagellar hook-filament junction by electron cryomicroscopy

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The bacterial flagellum is a biological nanomachine for the locomotion of bacteria. The flagellum consists of three functional parts: the basal body, which acts as a rotary motor; the filament, which acts as a helical propeller; and the hook, which acts as a universal joint. The hook and filament, which consist of FlgE and FlgC, respectively, have mechanically distinct characteristics, as the former is flexible and highly curved while the latter is rather rigid with a large radius of curvature. The hook-filament junction is made of two proteins, FlgK and FlgL, which are responsible for connecting these two mechanically distinct structures. In general, the hook-filament junction works as a mechanical adapter, the mechanism of which is still unknown. To reveal this mechanism, we analyzed the structure of the junction by using electron cryomicroscopy (cryoEM). We used a mutant strain in which the expression level of *flgC* can be controlled by tetracycline to make the filament length relatively short. This made image data collection highly efficient. Images had a strong tendency to misalign since the projection patterns of the helical subunit arrays in the hook and filament portion occupy the major fraction. We therefore tested various methods and eventually obtained a 3D density map that shows the shape and array of subunits in the junction structure. However, the resolution is still insufficient to elucidate the connection mechanism. Further improvements in cryoEM imaging and image analysis are under way.

1P-008 らせん対称性を持つ生体超分子の極低温電子顕微鏡による高速・高分解能構造解析：原子分解能を目指して

High-throughput, high-resolution cryoEM structural analysis of helical assemblies of biological macromolecules toward atomic resolution

Takashi Fujii (1), Takayuki Kato (1), Keiichi Namba (1) ((1) Graduate School of Frontier Biosciences, Osaka University)

We report structures of helical assemblies of biological macromolecules at near atomic resolution obtained by electron cryomicroscopy. These structural analyses were completed within two weeks. One of the factors that enabled such high-throughput, high-resolution analyses is the use of a CCD detector instead of film. Since the modulation transfer functions of CCD detectors are significantly worse than those of films, high-resolution image data are too poor to attain atomic resolution if conventional magnifications are used. The resolution of 3D image reconstructions from data collected at a magnification of $\times 88000$ was limited to ~ 7 Å. However, a higher magnification ($\times 170000$) solved this problem. We have reached ~ 4 Å resolution for the stacked disk aggregate of TMV coat protein. The density map clearly shows the main chain and large side chains. The only disadvantage with high-magnification imaging is a small image area of CCD, making the data collection efficiency lower. However, CCD imaging is still fast enough to allow high-throughput analysis. The other factors include the thickness of vitreous ice film embedding specimen particles and the specimen temperature, both of which affect image contrast. We improved the quick-freezing method to optimize ice thickness. We also found that images recorded at ~ 50 K have ~ 1.6 times higher contrast than those at 4 K. By improving these factors in further technical development, we believe we should be able to achieve atomic resolution within a week from data collection to image analysis.

1P-009 構造情報を含めた蛋白質ファミリーを表現するプロファイル条件付確率場モデルの開発

Development of Profile Conditional Random Fields for Modeling Protein Families with Structural Information

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A statistical model of protein families, called profile conditional random fields (CRFs), is proposed. This model may be regarded as an integration of the profile hidden Markov model (HMM) and the Finkelstein-Reva (FR) theory of protein folding. While the model structure of the profile CRF is almost identical to the profile HMM, it can incorporate arbitrary correlations in the sequences to be aligned to the model. In addition, like in the FR theory, the profile CRF can

incorporate long-range pairwise interactions between model states via mean-field-like approximations. We give the detailed formulation of the model, self-consistent approximations for treating long-range interactions, and algorithms for computing partition functions and marginal probabilities. We also outline the methods for the global optimization of model parameters as well as a Bayesian framework for parameter learning and selection of optimal alignments.

- 1P-010 ギャップジャンクションヘテロチャネル形成の適合性に関する構造的考察
Structural insight into docking compatibility of heterotypic gap junction channel

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One type of communication between cells is mediated via intercellular channels that cluster in specialized regions of the plasma membrane to form gap junctions consisting of connexins. Multiple connexins are coexpressed in a single cell type. Heterotypic gap junction channels show various properties by their combinations. We have determined the structure of human gap junction channel composed of connexin26 (Cx26) at 3.5 Å resolution. According to this structure, two connexons form a full gap junction channel by several interactions between each two extracellular loops.

In this research, docking selectivity for each pair of different connexons was inspected by homology modeling using the structure of Cx26 gap junction channel as the template. We selected 10 connexins of human origin as object of this experiment. In every combination of different connexons, E1 of one connexon binds to E1 of the other connexon with hydrogen bonds and doesn't have any steric hindrances as in Cx26 gap junction channel. In E2, however, replacement of the position 176th with His or Tyr seemed to cause the incompatibility with Lys or Arg at the position of 168th. This result is in good agreement with electrophysiological or cell biological experiments. These combinations would be unfavorable and fail to form a gap junction channel. Our study suggests that E2, especially 168th and 176th residues of Cx26, could have a key role in formation of "functional" intercellular channel.

- 1P-011 X線小角散乱法によるCEL-IIIの多量体化機構及びその構造解析
ANALYSIS OF THE OLIGOMERIZATION MECHANISM AND STRUCTURE OF CEL-III BY SMALL-ANGLE X-RAY SCATTERING

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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, a-hemolysine, 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.

- 1P-012 ヒト MTH1 活性部位の二つの Asp 残基のプロトネーション状態が異なる分子動力学シミュレーション
Molecular dynamics simulations of human MTH1 under different protonation states for two Asp residues in its active site

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Human MTH1 hydrolyzes oxidized purine nucleotide triphosphate molecules such as 8-oxo-dGTP and 2-OH-dATP. Mechanisms by which MTH1 distinguishes damaged purine nucleotides from the others are not fully elucidated. In the crystal structures of 8-oxo-dGMP-bound MTH1, two aspartate residues, Asp119 and Asp120, were in the vicinity of the guanine base of the ligand, although the oxidized location was on the opposite side of the base. Because hydrogen bonds are critical in understanding specific interactions, we studied the influence of the protonation states of the two residues on MTH1-ligand interactions, using molecular dynamics (MD) simulations. A 50 ns MD run was

carried out using AMBER 8 for each combination of four protonation states and with or without a bound 8-oxo-dGMP molecule. 2–50ns of each trajectory was divided into eight 6ns segments and structures were averaged over every time segments, yielding 64 structures. The structures were subject to cluster analysis, using C- α RMSD as a distance measure. As a result, it was found that the structures could be roughly divided into two classes, i.e., ligand-bound and ligand-unbound structures. Generally, the ligand-bound structures have more inflated binding-pocket, produced by outward shifts of three adjacent loops. An example of exception was the ligand-unbound and both deprotonated structure, which adopted a conformation similar to that of ligand-bound and singly protonated structures.

- 1P-013 複数のサポートベクタマシンの組み合わせによるタンパク質局所構造予測法の開発

Development of protein local structure prediction method based on combination of multiple support vector machines

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Recently, many researches about residual local structures in protein unfolding state and statistical analyses of protein structure database to find structural motif of proteins suggest that many parts of proteins are to some extent restricted by local amino acid sequences. It is very useful to obtain such local structural tendencies from their sequences for *de novo* protein structure prediction and understanding of protein folding mechanism.

We have developed a novel method for protein local structure prediction from sequence information based on combination of multiple support vector machines (SVMs). Prediction procedure is as follows: First, PSI-BLAST (Altschul 1997) is applied to query sequence with up to five iterations to make position specific scoring matrix (PSSM). PSIPRED secondary structure prediction (McGuffin 2000) is also applied and scores for secondary structure types (helix / strand / others) are obtained for each residue. Then PSSM and PSIPRED scores are divided into fragments with overlap. These fragments are submitted to multiple support vector machines. Each SVM predicts whether end-to-end distance of the input fragment is longer than the assigned distance or not, and outputs a decision value for the prediction. Finally, probability density function of end-to-end distance of the fragment is estimated as the distribution of end-to-end distances of fragments in a database of known structures which have similar decision value pattern. We applied our method to a set of fold representatives of SCOP 1.73 (Murzin 1995) and validated its effectiveness.

- 1P-014 アミノ酸側鎖のペプチド立体構造に及ぼす影響

The side chain effects on the conformation of the peptides

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Protein is a sequence of amino acids. To elucidate the interaction between amino acids is important as the first step to clear the principle of protein folding. The energy expressions in classical molecular dynamics simulation are limited since it use predefined potential energy function to calculate energy and forces of the system. On the other hand, the energy calculated by *ab initio* molecular orbital theory which treats the electronic structure of the system is more accurate than the one calculated by using classical force field. In this point of view, we applied multicanonical *ab initio* molecular dynamics simulation to tetrapeptide, Ace-Ala-X-Ala-Nme (X=Ala, Gly, Ser). Quantum chemical calculations were performed at the HF/6-31G** level of theory. We will report the results, the comparison with that from AMBER ff99SB simulation and the influence of side chain for the conformation of peptides.

- 1P-015 バイオロジカルユニットを考慮したリガンド結合タンパク質データベースの構築

Development of protein-ligand complex's database considering biological units

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It is pointed out that ligand binding sites sometimes exist not only in the pocket of a protein domain but also between the surfaces of two or more protein domains. When we analyze ligand binding to proteins, we need to take into account quaternary structure biological unit, the state of the proteins within the living organisms. So, we develop a database of protein-ligand complex considering biological units of proteins. We target ligands which are mainly low molecular, not all the HETATMs. We do not target DNA, RNA, proteins and low molecular ions. The initial dataset of ligand binding conformations is obtained from four databases, PDBbind, BindingDB, Protein Ligand Database and AffinDB. All the possible conformations are subjected to a clustering procedure based on the sequence identity between clusters to remove redundancy. At last, the most representative conformation from each cluster is selected and is manually checked