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 ^{15}N 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, $R_{g, \text{core}}$, of iron core. The results showed that, despite of same iron amount, there is significant difference in $R_{g, \text{core}}$ between native and reconstituted ferritin; rather, the value of $R_{g, \text{core}}$ 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

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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 $\sim 7 \text{ \AA}$. However, a higher magnification ($\times 170000$) solved this problem. We have reached $\sim 4 \text{ \AA}$ 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 $\sim 50 \text{ K}$ 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