variants (四分子体); and 4SS (WT). The extent of monomolecular folding covered by this set of proteins ranges from global unfolding, through local unfolding, to local folding. Fibrillation reaction was carried out with the addition of sonicated WT lysozyme fibril fragments as seeds, at various incubation temperatures and periods, and was monitored with thioflavin T fluorescence, CD spectroscopy, and scanning probe microscopy. All the data showed that the presence of the disulfide bonds C64-C80 and/or C76-C94 enhanced amyloid-fibrillation when compared with the all-disulfide-deficient variant, 4SS, indicating that these native disulfide bonds have a positive role in the misfolding into regular intermolecular beta-structures. The presence of the other native disulfide bond, C6-C127 or C30-C115, had no direct effect compared with 4SS under strongly unfolding conditions, but had an indirect effect against fibrillation through protein stabilization under folding conditions.

1PT115 Structural analysis of hNck2 SH3 domain at various pH: non-native α-helix-rich monomer and native dimer
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hNck2 SH3 is a protein which takes β-structure at the native state. On the other side, Liu and Song reported1 that the protein forms non-native α-helix-rich structure below pH2 based on NMR spectroscopy. We have done X-ray crystallography study of hNck2 SH3 at various pH. hNck2 SH3 lost its tertiary structure below pH2, judging from Kratylo plots. That is, the non-native α-helix-rich intermediate, found by Liu and Song1, is not compact. We also found that monomer-dimer transition occurred from pH2 to pH4 by X-ray solution scattering. Below pH2, the protein is monomer and above pH4, the protein forms dimer, resulting from I(0) analyses. Structural calculations were done by using DAMMIN2 program. Elongated structure was obtained at pH2 and ellipsoidal structure was obtained at pH4. The volume of the structure at pH4 is found to be almost twice as large as that at pH2, which also supports the structure below pH2 is monomer and the structure above pH4 is dimer. 1) Liu & Song (2008) Biophys. J., 95, 4803-4812 2) D.Svergun (1999) Biophys. J., 76, 2879-2886

1PT116 Crystal structure of the fatty acid binding protein 3 (FABP3) with or without fatty acid
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Recently, in biological membrane, it revealed that lipids were not only the component of the membrane but also influenced the nature of membrane, and lipids were involved in various intracellular signaling affecting differentiation, regulation, of growth, and gene expression. Thereby, lipids have been proposed as a potential drug target. The structural studies of lipids are helpful to investigate the precise functions of lipids. However, so far as we know, they show no clear and well separated electron densities corresponding to individual atoms of a lipid molecule. To obtain well-defined lipids, we focused on the structure of fatty acid binding protein 3 (FABP3) previously determined at 1.4 Å resolution. In cell, long-chain fatty acids (FA) are insoluble molecules, therefore, binding with FABP is essential for intracellular trafficking. Fatty acid binding proteins (FABPs) are 14-15 kDa cytosolic proteins found in different cell types and their amino acid sequences exhibit considerable tissue specific variation. We tried to purify and crystallize apo-FABP2 by conventional methods for structure determination of FABP3 with or without FA. However, it was difficult to remove endogenous fatty acids from FABP3 and impossible to obtain clear electron densities of a lipid molecule. It was assumed that the binding affinity of FABP3 to fatty acid was higher than other FABPs. We will report a removal of endogenous fatty acids from FABP3 and structure analyses of FABP3 with or without FA at high resolution.

1PT117 結晶構造に基づく薬物設計の新しい道: 研究
Structure study on inhibitor specificity of bacterial multidrug efflux pumps
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Pseudomonas aeruginosa highly expresses intrinsic resistance against various antibiotics. Resistance in P. aeruginosa is mainly caused by two RND type multidrug efflux pumps, MexB and MexY. Complex A (CA) inhibits both MexB and AcrB of the major RND pump in Escherichia coli, but CA can’t obstruct MexY. There is no useful inhibitor of MexY, which is impeding for overcoming of P. aeruginosa infection. CA derivative has great potential to be a universal inhibitor for RND pumps. First, in order to clarify the interaction manner between CA and AcrB/MexB, we made AcrB-CA and MexB-CA co-crystals. With X-ray analyses, we find the interaction between CA and F178 residue in AcrB and in MexB. MexY homologous model was constructed from AcrB and MexB crystal structure. The corresponding position of F178 in AcrB/MexB is W177 in MexY, which suggests that the voluminous side chain of Trp may have the steric hindrance rather than interaction with CA.

To verify this hypothesis, we exchanged F178 in AcrB and the W177 in MexY each other, and checked whether CA inhibits or not. The result is remarkable, and MexY W177 is inhibited but AcrB F178W isn’t obstructed by CA. Furthermore, the crystal structure of AcrB F178W certifies that AcrB mutant has normal folding and that there is no space for interaction around the W178 residue. This result can be not only the indicator of the way to make a universal inhibitor of AcrB, MexB and MexY but also the good example for structure based drug design.

1PT118 STEM トモグラフィ法を用いた細胞および細胞内分子複合体の可視化
Structural visualization of supramolecules in bacterial and eukaryotic cells using STEM tomography
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In order to form hypotheses on the functions on protein complexes in living cells, it is essential to visualize the structures of these complexes. Electron tomography is a powerful tool for structural analyses of supramolecules inside cells. However, this technique does not work well for thick specimens, which limits the area that can be visualized. To overcome this problem, we used STEM (scanning transmission electron microscopy) for tomography analysis. Although this technique is not commonly used in the field of biology, it provides high-con- trast images of thick specimen. Using STEM we obtained high-contrast structural images of supramolecules in both bacterial and eukaryotic cells. We imaged the bacterial flagellar motor complex in Vibrio alginolyticus. The flagellar basal body and ring structures were clearly recognized in the STEM images we obtained. However, the cytoplasmic part of this motor structure was not visualized due to the method of sample processing. In eukaryotic cells we utilized STEM to show for the first time images of the thin actin network structure juxtaposed with the nucleus. Due to the increased thickness of the cell in this area, imaging was not possible using electron microscopy. By image processing filaments in silico, the spatial location of the actin network was visualized three dimensionally. We show that STEM tomography may be useful for imaging of supramolecular structures in the cell, and is particularly useful for imaging of thick specimens not amenable to conventional techniques.

1PT119 EM Data Bank & Protein Data Bankの自分解析3D電子顕微鏡構造データの解析システム: Similarity search system for low-resolution 3D-EM structure data in EM Data Bank and Protein Data Bank
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More than 1800 structure data by 3D electron microscopy (3D-EM) are deposited on the Protein Data Bank (PDB) and the EM Data Bank (EMDB). Extensive and indiscriminate comparison of such 3D-EM data was difficult for some reasons: 1) EMDB data are not atomic coordinates but 3D maps which are not able to be compared by existing tools for coordinates. 2) Various quality values of the 3D maps such as resolution and CTF-correction accuracies must be unified for each comparison. We have been developing a structure comparison system for 3D-EM data, in which dependencies of data types and