2P016 カルシウムイオンを介した基質結合様式をもつ高度好熱菌由来乳酸結合タンパク質の結晶構造解析
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Crystal Structure of a Bacterial Lactate Binding Protein with a Calcium Ion Mediating Ligand-Binding Manner
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TRAP (tripartite ATP-independent periplasmic) transporters were discovered as secondary transporters using electrochemical proton gradient across inner membranes as a driving force. A wide range of sequence analysis shows that TRAP transporters are widespread in bacteria and archaea. These transporters consist of SBPs (solute-binding proteins), large integral membrane subunits with 10-12 transmembrane helices which probably form translocation pathways and small integral membrane subunits with 4 transmembrane helices whose function is unknown. While many structures of SBPs have been reported about ABC (ATP-binding cassette) transporters, only a few structures of SBPs are available about TRAP transporters.
We determined the crystal structure of TTHA0766, a SBP of a TRAP transporter from *Thermus thermophilus* HB8. The structure of TTHA0766 consists of two α / β domains (domain I and domain II). The topology of β -strands of domain I (β 2- β 1- β 3- β 10- β 4) indicates that TTHA0766 belongs to the type II SBP family. Between two domains, there is a cleft for solute-binding. TTHA0766 binds a L-lactate in this cleft and this binding is assisted by a calcium ion. Calcium ion seems to have an important role in stable binding of L-lactate. Furthermore, conformations of histidine residues around the solute-binding cleft are changed by calcium binding.

2P018 小型熱ショックタンパク質、StHsp14.0のX線結晶構造解析
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X-ray Crystal Structure Analysis of a Small Heat-Shock Protein, StHsp14.0
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Small heat shock proteins (sHsps) are the most ubiquitous molecular chaperones that bind to unfolded proteins and protect them from forming aggregations. Usually, sHsps exist as oligomers of 9-40 subunits. The crystal structures have shown that Hsp16.5 from *Methanococcus jannashii* (MJHsp16.5) assembles into a spherical 24-mer with the 432 symmetry and that Hsp16.9 from wheat (TaHsp16.9) exists as a 12-mer with the 32 symmetry. The oligomers undergo reversible temperature-dependent dissociation into smaller units as dimers, which interact with denaturing substrate proteins. However, little is known about structural and functional features of these sHsps by which substrates are protected from thermal denaturation. We have performed the crystal structure analysis of wild type StHsp14.0 from *Sulfolobus tokodaii* strain 7 and its mutant, StHsp14.0FKF, which can not form an oligomeric structure. We have obtained single crystals of the wild type StHsp14.0, which gave only weak diffraction up to the present. On the other hand, StHsp14.0FKF afforded single crystals that enabled us to collect high-resolution data up to 1.85 Å by the use of synchrotron. The crystals of StHsp14.0FKF belong to the space group *P*21212 with unit cell dimensions $a = 40.37$ Å, $b = 61.10$ Å, $c = 96.14$ Å. The crystal structure of StHsp14.0FKF shows that the protein molecule forms a dimer, which is thought to be a minimum unit of the active form of this chaperone.

2P017 アミノ酸配列を単純化したBPTI変異体の結晶構造解析
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Crystal Structures of Simplified BPTIs Solved at 1.0 and 1.4 Å Resolutions
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The information contained in natural amino acid sequences is redundant and several studies indicate that a fraction of it is sufficient for specifying native protein folds. Recently, we observed that a highly simplified Bovine Pancreatic Trypsin Inhibitor containing 21 alanines (BPTI-21) possesses all essential thermodynamic properties of small natural globular proteins including a cooperative thermal folding/unfolding process. Here, we report the crystal structures of two stabilized BPTI-21 variants, one where both A14G and A38V are substituted (-21A14GA38V) and another where A14G is substituted (-21A14G). The structures of -21A14GA38V and -21A14G were determined by molecular replacement at 1.0 and 1.4 Å resolutions, respectively, and with crystallographic R factors of 0.1567 (Rfree: 0.1764) and 0.1565 (Rfree: 0.2052). We observed that the structures of the two simplified BPTIs are very similar to that of the wild-type BPTI (5PTI) except for some local changes, especially at the C-terminal alpha helix region. This local structural perturbation might be due to the concerted effects of movement of the alpha helix (48-54) towards the beta strand (29-35) to partially compensate the packing defect created upon substitution of the [30-51] disulfide-bridge by alanines.

2P019 溶液中の多糖蛋白質複合体の構造のキャラクタリゼーション
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Physicochemical characterization of the structure of a polysaccharide-protein complex in solution
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Mucins are the glycoproteins in epithelial mucous secretions that coat the oral cavity and the respiratory, gastrointestinal and urogenital tracts of animals; their protective function is due to their solution properties that refer to the molecular structure. It is important to clarify the molecular assembly and chain conformation of the conjugates in solution for understanding the physiological functions. The macromolecules contain a large number of oligosaccharide side chains covalently bound to a protein backbone. Mucins typically contain 60-90 wt % carbohydrate. In this study, the molecular weight of bovine submaxillary mucin (BSM) was estimated by using low-angle laser light scattering photometry coupled with high performance gel chromatography. The chain conformation of the mucin was characterized by solution small-angle X-ray and neutron scattering. The synchrotron radiation X-ray and neutron scattering measurements were performed with an optics system at the beamline 10C station in the Photon Factory and the small angle scattering instrument (SWAN) in the KENS lab. of High Energy Accelerator Research Organization. Total molecular weight of BSM was 55 million and the molecular weight of the main fractionated components was about 2 million and about 0.5 million in the absence /presence of sodium dodecylsulfate. The results of solution small-angle X-ray and neutron scattering experiments indicate that the chain conformation of BSM molecules is unfolded random coiled and/or expanded structure in solution.