collected from crystals of the SeMet-substituted protein and are being used for the structure determination.

We have also purified and crystallized a sortase protein, encoded by the gene *spy0129*, together with its putative substrate surface protein, Spy0128. Sortases are responsible for the covalent attachment of specific proteins to the Gram-positive bacterial cell wall. Spy0128 contains a sortase-mediated cell wall anchoring motif specific for the sortase Spy0129. We present progress in the molecular structure determination of Spy0129 and its substrate protein Spy0128.

Keywords: bacterial toxins, X-ray crystallography, structure of proteins

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Crystal Structure of L-phenylalanine Oxidase

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L-phenylalanine oxidase (PAO) from *Pseudomonas* sp. P-501 catalyzes both oxidative deamination (β-phenylpyruvic acid is product) and oxygenative decarboxylation (α-phenylacetamide is product). The enzyme contains two mol of noncovalent FAD. The enzyme is expressed as a proenzyme that has a noncatalytic polypeptide at the N-terminal end and is activated by proteolysis [1].

The proenzyme of PAO (proPAO) has been crystallized by the hanging-drop vapor-diffusion method using ammonium sulfate as a precipitant. The crystal belongs to space group $P2_12_12$, with unit-cell parameters (a = 141.8 Å, b = 145.4 Å, c = 82.2 Å) and contains two molecules in the asymmetric unit. The X-ray diffraction data were collected to a resolution of 1.9 Å at the NW-12 beamline in the Photon Factory.

We determined the crystal structure of proPAO using selenomethionine-derivative crystal and SAD method. The crystal structure was not able to solve by MAD method, because the crystal received big damage by the radiation and did not keep isomorphism between the data sets. Crystal structure of PAO is a dimmer form. The active site is like a funnel. The prosequence enters the funnel and bonds to the active site residues. It thus appears that prosequence is a substrate mimic and keeps inactive form.

[1] Haruo S., *et al*., *J. Biochem.*, 2004, **136**, 617.

Keywords: flavoprotein, SAD, enzyme activity mechanism

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Crystal Structure of a Biopolyester-hydrolyzing Enzyme PHA Depolymerase

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Polyhydroxyalkanoate (PHA) is a biodegradable polyester produced by a number of bacterial strains as an energy-storage material. Extracellular PHA depolymerases efficiently hydrolyze PHA, and are typically composed of three domains: catalytic, linker, and PHA-binding domains. Some fungal depolymerases are only composed of a single domain with an efficient affinity to PHA granules. We have determined the crystal structure of the type II PHA depolymerase composed of a single domain by a single isomorphous replacement/anomalous dispersion method. Refinement of coordinates of the model at 1.7 Å was converged to an *R*-factor of 19.6% (free-*R* 23.0%). The structure represents an α/β hydrolase fold with a circular permuted connectivity. A crevice is formed on the surface of the protein, at the bottom of which catalytic triad residues Ser-19, Asp-101, and His-135 are located. Several hydrophobic residues which may interact with polymer chains are contributed to form the crevice. The catalytic site has no obvious lid structure and is open to bulk solvent. Several surface-exposed hydrophobic residues are arranged along the mouth of the crevice, suggesting a deformation mechanism of polyester chains during degradation of PHA.

Keywords: biodegradable polymer, hydrolase, circular permutation

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Crystal Structure of PcyA-biliverdin IXα Complex

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In higher plants, algae, and cyanobacteria, phytobilins which are produced from heme, are utilized as light harvesting and photoreceptor pigments. PcyA (phycocyanobilin:ferredoxin oxido-reductase), one of ferredoxin-dependant bilin reductases, catalyzes two-step site-specific reduction of biliverdin IXα (BV) to produce phycocyanobilin, one of the phytobilins. The reduction of the vinyl group of BV D-ring precedes the reduction of BV A-ring. Thus the vinyl group should be distinguished from the methyl group by PcyA.

PcyA from *Synechocystis* sp. PCC 6803 was co-crystallized with BV. The structure of PcyA-BV complex was determined by MIRAS using gold and mercury derivatives and refined using 1.25 Å resolution data to an *R*-factor of 0.175 and a free *R*-factor of 0.198. PcyA is folded in three-layer α/β/α sandwich structure. Electron density of BV was clearly visible and its orientation and conformation were explicitly determined. Basic patch nearby BV is suitable to interact with acidic protein, ferredoxin. BV positions between β-sheet and α-helices of C-terminal side. His is hydrogen-bonded to lactam oxygen atoms of BV. Asp is very close to the center of BV. These two residues discriminate between BV and closed tetra-pyrrole compounds containing metal such as heme and chlorophyll. Most interestingly, Glu covalently bonds with the vinyl group of BV D-ring. This allows strict recognition of correct orientation of BV.

Keywords: photosynthesis-related proteins, redox enzymes, substrate binding

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Structural Studies of Rat Calucineurin B Homologous Protein 1

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Calcineurin B homologous protein 1 (CHP1), also known as p22, is a calcium binding EF-hand protein and shows substantial sequence similarity with the regulatory B subunit of the protein phosphatase calcineurin (CNB). CHP1 was involved in membrane trafficking [1] and multiple cellular functions. CHP1 associates tightly with Na^+/H^+ exchangers (NHEs) and regulates its intracellular pH sensitivity [2]. CHP1 also significantly reduces the kinase activity of death-associated protein (DAP) kinase related apoptosis inducing protein kinase 2 (DRAK2).

To clarify multiple functional mechanisms of CHP1, we have tried to determine CHP1 structure with X-ray crystallographic analysis. Crystals suitable for high-resolution X-ray analysis were obtained at 277 K by hanging drop vapor-diffusion method. Multi-wavelength anomalous dispersion method (MAD) was used for determination of phase. The polypeptide chain of CHP1 is folded into two globular domains (N-lobe and C-lobe) composed of an α-helical structure with 10 α-helices and 3_{10} helices. The target recognition mechanism will be discussed.

[1] Barroso M.R., Brend K.K, DeWitt N.D., Chang A., Mills K., Sztul E. S., *J. Biol. Chem*., 1996, **271**, 10183. [2] Pang T., Hisamitu T., Mori H., Shigekawa M., Wakabayasi, S. *Biochemistry*., 2004, **43**, 3628.

Keywords: calcium-binding proteins, EF-hand proteins, protein crystallization