wild-type UPPs and the Asp26Ala mutant are crystallized in a new trigonal unit cell with Mg^{2+}/IPP/FsPP (farnesyl thiopyrophosphate, an FPP analogue) bound to the active site. Our results here improve the understanding of the prenyltransferases reaction significantly.

Keywords: prenyltransferase, crystal structure, metal ion

P.04.02.55

Acta Cryst. (2005). A**61**, C193

Crystal Structure of Human HMG-CoA lyase

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3-Hydroxyl-3-methylglutaryl-CoA (HMG-CoA) lyase (EC 4.1.3.4) catalyzes the divalent cation dependent cleavage of HMG-CoA to form acetyl-CoA and acetoacetate. This reaction is a key step in ketogenesis and the final step in leucine catabolism. Human HMG-CoA lyase has been previously cloned and overexpressed in *Escherichia coli.* Crystals of the lyase containing a competitive inhibitor have been obtained with PEG 8K using sitting-drop vapor diffusion method. The crystals belong to the monoclinic space group C2, with unit cell parameters a=197.0Å, b=116.1Å, c=86.8Å, and β=122.5˚. The native data set has been collected which is 99.8 % complete to 2.1 Å. The calculated Vm is 2.34 $Å^3$ Da^{-1}, with a solvent content of 43.2%, which corresponds to three dimers of the enzyme in the asymmetric unit (each dimer molecular weight, 65 kDa). One heavy atom derivative (Hg) has been obtained and the search for additional derivatives is in progress.

Keywords: HMG-CoA lyase structure, fatty acid metabolism, ketogenesis

P.04.02.56

Acta Cryst. (2005). A**61**, C193

Structural Analysis of Thr342 Mutants of Soybean β-Amylase: The Role of Conformational Changes of Two Loops in the Catalytic Mechanism

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Soybean β-amylase has two mobile loops in the active site, a flexible loop (residue 96-103) and an inner loop (residues 340-346). The flexible loop moves about 10 Å from "open" to "closed" form to make interactions with substrate. Though the movement is relatively small (about 3 Å), two different conformations of the inner loop have been found in the enzyme/substrate complexes. In the "product form", the Thr 342 residue creates hydrogen bonds with the Glu 186 (catalytic acid) and with the glucose residues at subsites -1 and +1, whereas most of those interactions are lost in the "apo form". To elucidate the relationship between the structural states of inner loop and the catalytic mechanism, Thr 342 was mutated to Val, Ser, and Ala, respectively, and their crystal structures complexed with maltose were determined together with that of the apo enzyme at 1.27-1.64 Å resolutions. The k_{cat} values of the T342V, T342S, and T342A mutants decreased by 13-, 360- and 1700-fold, respectively, compared to that of the wild-type enzyme. Whereas the inner loops in the wild-type/matose and T342V/maltose complexes adopted the product form, those of the T342S/maltose and T342A/maltose complexes showed the apo form. Structural analyses suggested that the side-chain of Thr 342 in product form plays an important role in distorting the sugar ring at subsite -1, stabilizing the deprotonated form of Glu 186, and grasping the glucose residue of the remaining substrate at subsite +1.

Keywords: amylases, mutations, enzymatic reaction mechanism

P.04.02.57

Acta Cryst. (2005). A**61**, C193

Crystal Structure of the Haloalkane Dehalogenase DbjA

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Haloalkane dehalogenases are key enzymes that catalyze hydrolytic conversion of halogenated aliphatic compounds to alcohol and hydrogen halide. The enzymes have good potential for bioremediation, biosensing and biocatalysis. Recently, the haloalkane dehalogenase DbjA from *Bradyrhizobium japonicum* USDA110 was revealed to have a sufficient enantioselectivity for industry scale synthesis of optically active alcohols. This is the first example of enantioselective catalysis among the haloalkane dehalogenases. To reveal the enantioselectivity mechanism of DbjA on the basis of the structure, we determined the crystal structure of DbjA at 1.47 Å resolution by the molecular replacement method. DbjA has an alpha/beta hydrolase fold. The architecture of specificity-determining cap domain is, however, different from three dehalogenases with known crystal structure. The results of structure-function analysis will be presented.

Keywords: enantioselectivity, alpha/beta hydrolase, haloalkane dehalogenase

P.04.02.58

Acta Cryst. (2005). A**61**, C193

Structure-Function Analysis of a Novel Mitochondrial Antioxidant Protein

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Bovine mitochondrial SP-22 is a member of the peroxiredoxin family which is a novel antioxidant enzymes. It belongs to the typical 2-Cys Prxs containing three cysteines at positions 47, 66, and 168. Its co-elution with E3 of PDC indicate possible interaction between them. SP-22 can be reduced by DTT and oxidized by H_2O_2. By electronic microscopy study, SP-22 forms a stable decameric toroid consisting of five basic dimeric units. By GFC study, it is found that SP-22 stay as a decamer under different conditions (salt concentration, pH, redox statue). Preliminary crystallography study of SP-22 has been done. 3.3A data of C168S mutation SP-22 were collected.

Oxidized SP-22 needs to be reduced by thioredoxin, thioredoxin reductase system. To finish this pathway and have a better understanding of the function of SP-22, human mitochondrial thioredoxin and thioredoixn reductase were cloned, overexpressed and purified. Due to human mitochondrial thioredoxin reductase is a SeCys protein, fully active protein was under investment.

Keywords: peroxiredoxin, mitochondrial, oligomer

P.04.02.59

Acta Cryst. (2005). A**61**, C193-C194

Structural Studies on Novel Streptococcal Virulence Factors

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Streptococcus pyogenes (*S. pyogenes*) is responsible for a variety of illnesses ranging from mild sore throat to life-threatening toxic shock syndrome. The strain most strongly associated with highly invasive infections is *S. pyogenes* M protein serotype 1 (M1).

We have identified a number of putative genes from the *S. pyogenes* M1 genome which possess various sequence motifs that are often present in bacterial toxins. Three of the proteins encoded by these genes have been expressed, purified and crystallized with the aim of determining their structures by X-ray crystallography.

The protein encoded by gene *spy1492*, Spy1492, contains the GDSL-like lipase motif which is commonly found in lipolytic enzymes including some bacterial toxins such as hemolysin. Its lipolytic activity was detected in biochemical assays. Multiwavelength anomalous diffraction (MAD) data to 3.0 Å resolution have been

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

P.04.02.60

Acta Cryst. (2005). A**61**, C194

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

P.04.02.61

Acta Cryst. (2005). A**61**, C194

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

P.04.02.62

Acta Cryst. (2005). A**61**, C194

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

P.04.02.63

Acta Cryst. (2005). A**61**, C194

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