

**P04.02.150***Acta Cryst.* (2008). A64, C278**Structural and biochemical characterization of ClpP from *Bacillus subtilis***

Byung-Gil Lee, Hyun Kyu Song

Korea University, School of Lifescience &amp; Biotechnology, Anam-Dong, Seongbuk-Gu, Seoul, 136-701, Korea (S), E-mail: pkthis@korea.ac.kr

The ATP-dependent chaperone/protease complex, ClpAP or ClpXP, plays an important role in the protein degradation in most bacteria or in mitochondria and chloroplast of eukaryotes. ClpP is a proteolytic component that has 14 identical subunits organized in two stacked heptameric rings whereas ClpA and ClpX is a hexameric AAA+ ATPase that binds, denatures, and translocates protein substrates. We have solved the crystal structure of ClpP from *Bacillus subtilis* (BsClpP) at 2.4 Å resolution and tested proteolytic activity against peptide and protein substrates. The structure of BsClpP was determined by molecular replacement method using that of *E. coli* ClpP (EcClpP), which is highly characterized ClpP and has 68% homology to BsClpP. The model of BsClpP has been refined to crystallographic *R* and *R-free* factors of 24.4 and 28.8 % and shows very similar overall structure with EcClpP. Although the structural and sequential resemblance between *E. coli* and *B. subtilis* species is significantly high, ClpX from *E. coli* is not able to stimulate the proteolytic activity of BsClpP and ClpX from *B. subtilis* also is not able to stimulate that of EcClpP. It is in stark different to a similar bacterial ATP-dependent protease, HslVU which shows mutual activation through swapped combination.

Keywords: ClpP, proteolytic enzymes, *Bacillus subtilis***P04.02.151***Acta Cryst.* (2008). A64, C278**Implications for selenophosphate generation by crystal structure of selenophosphate synthetase**Shun-ichi Sekine<sup>1,2</sup>, Yuzuru Itoh<sup>1,2</sup>, Eiko Matsumoto<sup>2</sup>, Ryogo Akasaka<sup>2</sup>, Chie Takemoto<sup>2</sup>, Mikako Shirouzu<sup>2</sup>, Shigeyuki Yokoyama<sup>1,2</sup><sup>1</sup>University of Tokyo, Department of Biophysics and Biochemistry, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan, <sup>2</sup>SSBC, RIKEN Yokohama Institute, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan, E-mail: sekine@biochem.s.u-tokyo.ac.jp

Selenium is an essential micronutrient incorporated into proteins and RNAs, as selenocysteine (Sec) and 2-selenouridine residues, respectively. For both of the Sec synthesis and RNA modification reactions, selenophosphate is utilized as a reactive selenium donor. Selenophosphate synthetase (SPS) is the enzyme responsible for the generation of selenophosphate, by activating selenide with ATP. Many SPSs are themselves Sec-containing proteins, in which Sec replaces the catalytically essential Cys residue (designated Sec(Cys)). We solved the crystal structures of a bacterial SPS, and performed mutational analyses. The structure of SPS complexed with  $\alpha$ ,  $\beta$ -methylene ATP (AMPCPP) revealed the ATP-binding site, formed at the subunit interface of the homodimer. Four Asp residues coordinate four metal ions to bind the AMPCPP phosphate groups. The conformations of the N-terminal loop, including the essential Sec(Cys) and Lys residues, differ between the two subunits (open and closed). The AMPCPP  $\gamma$ -phosphate group is solvent-accessible, and a putative nucleophile could attack it to generate selenophosphate and ADP. Selenide attached to the Sec(Cys) residue, as a perselenide(selenosulfide) group, could be the nucleophile, upon closing the N-terminal loop. A well-ordered water molecule,

fixed close to the  $\beta$ -phosphate group, could be the nucleophile in the subsequent ADP hydrolysis to orthophosphate and AMP.

Keywords: SeID, selenoprotein, PurM superfamily

**P04.02.152***Acta Cryst.* (2008). A64, C278**The crystallographic study of the deubiquitinating enzyme UCH37 N-terminal domain**Kazuya Nishio<sup>1</sup>, Kawai Kentaro<sup>2</sup>, Kim Sang-Woo<sup>3</sup>, Mizushima Tsunehiro<sup>3</sup>, Yamane Takashi<sup>2</sup>, Hamazaki Jun<sup>4</sup>, Murata Shigeo<sup>5</sup>, Tanaka Keiji<sup>6</sup>, Morimoto Yukio<sup>1</sup><sup>1</sup>Kyoto University Research Reactor Institute, Kumatori-cho, Sennan-gun, Osaka, 590-0494, Japan, <sup>2</sup>Graduate School of Science, Nagoya University, <sup>3</sup>Graduate School of Pharmaceutical Sciences, Nagoya City University, <sup>4</sup>Graduate School of Science, Tokyo Metropolitan University, <sup>5</sup>Graduate School of Pharmaceutical Sciences, The University of Tokyo, <sup>6</sup>The Tokyo Metropolitan Institute of Medical Science (Rinshoken), E-mail: nishio@rri.kyoto-u.ac.jp

Ubiquitin is a highly conserved protein in eukaryotes and it is used as a label of the target protein for a proteolysis with 26S proteasome in ubiquitin-proteasome system. After degradation, the ubiquitin chain is removed from target protein by deubiquitinating enzyme and recycled. The deubiquitinating enzymes are cysteine proteases. They are classified into two groups: ubiquitin carboxyl-terminal hydrolases and ubiquitin-specific proteases. UCH37 belongs to the ubiquitin C-terminal hydrolases family. The N-terminal domain of UCH37 (1-237) is a catalytic domain, the C-terminal domain (238-329) is able to interact to hRpn13 of 26S base subunit via KEKE motif (312-329). The C-terminal domain is not conserved in ubiquitin carboxyl-terminal hydrolase family and would be expected to form a unique conformation to UCH37. To elucidate the mechanism of deubiquitin activity and interaction with 26S by based on the structure, we have tried to determine the structure of UCH37 using X-ray crystallography. At first, the UCH37 could be expressed as a GST fusion protein and purified using affinity chromatographic method. However, the purified sample was digested to N- and C-terminal domains with time. Therefore, we had tried to express the N- and C-terminal domains separately. The N-terminal domain was able to construct a useful expression and purification system, and then the crystal had been obtained using a polyethylene glycol as a precipitant. We had harvested the native dataset over the 2.9Å resolution in SPring-8. We will report these expression, crystallization and crystallographic data. Now, we are trying to improve the crystal quality and to determine the phase using molecular replacement method. We will also discuss these results in this conference.

Keywords: ubiquitin system, proteases, proteasome

**P04.02.153***Acta Cryst.* (2008). A64, C278-279**Flexibility in active site of *cis*-3-chloroacrylic acid dehalogenase revealed by multiple structures**

Marvin L Hackert, Stephen R Ernst, Youzhong Guo, Hector Serrano, William H Johnson, Christian P Whitman

Univ. of Texas at Austin, Chemistry and Biochemistry, 1 University Station A5300, Austin, Tx, 78712-0165, USA, E-mail: m.hackert@mail.utexas.edu

*Trans*- and *cis*-1,3-dichloropropenes are active ingredients in widely used fumigants such as Telone II. These environmentally toxic dichloropropenes can be degraded by a number of soil bacteria such as *Pseudomonas pavonaceae*. The bacterial degradation of the 1,3-dichloropropene depends on a hydrolytic dehalogenation reaction catalyzed by both *cis*- and *trans*-3-chloroacrylic acid dehalogenases. *cis*-3-Chloroacrylic acid dehalogenase is a member of the 4-OT family characterized by a conserved  $\alpha$ - $\beta$ - $\alpha$  motif and a catalytic N-terminal proline. Pro-1, Arg-70, Arg-73, and Glu-114 had initially been implicated as key catalytic residues in the *cis*-CaaD mechanism by amino acid sequence alignment with known members of the 4-OT family. Mutagenesis studies confirmed their importance prior to the availability of crystal structures. His-28 and Tyr-103 were identified as additional key catalytic residues based on recent crystal structures, and combined with the earlier results, led to a working hypothesis for the catalytic mechanism. Recent structures showed density for additional C-terminal residues and identified another active site residue, Arg-117. The importance of Arg-117 for activity has been confirmed by mutagenesis and kinetic studies on the R117A mutant. Furthermore, our structure shows a distinctly different conformation for the enzyme inactivated by (R)-Oxirane-2-carboxylate than that in the previously published structure, and adds to our understanding of the catalytic mechanism of *cis*-CaaD. This flexibility of the active site contributes to the diversity of substrates within the 4-OT family of enzymes. A summary of these results will be presented. This work is supported in part by grants from The Welch Foundation (F-1219 MLH; F-1334 CPW).

Keywords: 4OT superfamily, enzyme structure, mechanism

## P04.02.154

*Acta Cryst.* (2008). A64, C279

### Crystal structure of the covalent intermediate of human cytosolic $\beta$ -glucosidase

Junji Noguchi, Yasuhiro Hayashi, Yuichi Baba, Nozomu Okino, Makoto Kimura, Makoto Ito, Yoshimitsu Kakuta

Kyushu university, 6-10-1 Hakozaki Higashi-ku, Fukuoka, Fukuoka, 812-8581, Japan, E-mail: NoguchiJunji610317@yahoo.co.jp

Human cytosolic beta-glucosidase, also known as klotho-related-protein (KLRP), is an enzyme that hydrolyses various beta-D-glucosides including glucosylceramide and belongs to the glycoside hydrolase family 1 (GH1). We recently determined the crystal structure of KLRP in complex with glucose (KLRP/Glc). In this poster, we present the crystal structure of a covalent intermediate of the KLRP mutant E165Q, in which a glucose moiety in par-nitrophenyl-beta-D-glucopyranoside (pNP-Glc) was covalently bound to a nucleophile Glu373. The *Fo-Fc* electron density clearly showed the (a) covalent linkage between the C1 atom of the glucosyl moiety in pNP-Glc and the nucleophile Glu373. The glucose ring is firmly stabilized by an extensive hydrogen bond network basically identical to those of glucose in the KLRP/Glc. When the structures of the bound sugars were superimposed, the C1 atom in the covalent intermediate complex shifts its position toward the nucleophile Glu373. Concomitantly, the Glu373 carbonyl oxygen moves toward the C1 atom of glucose to form the covalent intermediate. This shortening the distance between the C1 carbon of glucose and the carbonyl oxygen of the nucleophile Glu373 made possible to form a covalent bond between them. As a result, the glucose in the covalent intermediate has rotated in the plane of its ring compared with that in the product complex. The present structure confirms a double displacement mechanism of the retaining beta-glycosidase. The structure further suggests that Asn164, Gln307, and a water molecule could be involved in the stabilization of a transition state through a

sugar 2-hydroxyl.

Keywords: beta-glucosidase, covalent intermediate, reaction mechanism

## P04.02.155

*Acta Cryst.* (2008). A64, C279

### Crystal structure analysis of the oligo-peptide binding protein OppA complexed with peptides

Naoki Sakai, Ayane Morita, Yukako Ushijima, Min Yao, Nobuhisa Watanabe, Isao Tanaka

Hokkaido University, Faculty of Advanced Life Sciences, Kita-21, Nishi-11, Kita-ku, Sapporo, Hokkaido, 001-0021, Japan, E-mail: nsakai@sci.hokudai.ac.jp

The oligopeptides permease (Opp) system of bacterium takes oligopeptides from the outside of cell membrane as nutrient of nitrogen and carbon and is a member of the ABC family of transporter. These transporters are comprised of two transmembrane subunits that form a channel, two cytoplasmic nucleotide-binding subunits and a periplasmic or cell surface associated solutes binding protein. OppA from Gram negative bacterium is located at periplasm and work as the initial receptor of oligopeptides. OppA captures various length of peptides and delivers them into the transmembrane subunits. This enzyme is not selective with the amino acids side chains of the substrates. We have determined the crystal structures of OppA from *Thermus thermophilus* complexed with tetra-, penta-, hepta- and nonapeptides. The peptides were bounded to the central cleft surrounded by two domains. The main chain of the ligand interacted to the main chain of OppA via hydrogen bonds, and consequently formed  $\beta$ -sheet with the enzyme. The carboxy termini of the ligand was recognized by Arg418 side chain and the amide of Gln14 side chain. These residues were considered as the bottom of the ligand binding cleft. OppA grasped four residues from the C-termini of the ligand. The ligand binding cleft had relatively large space toward to the N termini of the ligand, and N terminal extra residues of the ligand filled this space. This mechanism allows that OppA captures wide range of the length of oligopeptides as the ligand. The side chain binding pockets of this cleft had enough space to accept bulky residue such as Trp. And OppA bound to the small side chain such as Thr with hydrogen bonds via water molecules. Therefore OppA can bind to the oligopeptide ligands with amino acids sequence independently.

Keywords: protein-peptide interactions, protein-ligand complexes, protein structural analysis

## P04.02.156

*Acta Cryst.* (2008). A64, C279-280

### Structural basis for the enzymes in de novo pathway of Human Malaria Parasite *Plasmodium falciparum*

Saki Konishi<sup>1</sup>, Keiji Tokuoka<sup>1</sup>, Yukiko Kusakari<sup>1</sup>, Sundaratana Krungkrai<sup>2</sup>, Hiroyoshi Matsumura<sup>1</sup>, Yasushi Kai<sup>3</sup>, Jerapan Krungkrai<sup>4</sup>, Toshihiro Horii<sup>2</sup>, Tsuyoshi Inoue<sup>1</sup>

<sup>1</sup>Osaka University, Graduate school of engineering, Yamadaoka 2-1, Suita City, Osaka, 565-0871, Japan, <sup>2</sup>Department of Molecular Protozoology, Research Institute for Microbial Diseases, Osaka University, <sup>3</sup>Fukui University of Technology, <sup>4</sup>Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, E-mail: konishi@chem.eng.osaka-u.ac.jp