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 α-β-α 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). A**64**, C279

Crystal structure of the covalent intermediate of human cytosolic β-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 hydrolys(z)es 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 para-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). A**64**, 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 β-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). A**64**, C279−280

Structual basis for the enzymes in de novo pathway of Human Malaria Parasite Plasmodium faciparum

Saki Konishi[1], Keiji Tokuoka[1], Yukiko Kusakari[1], Sundaratana Krungkrai[2], Hiroyoshi Matsumura[1], Yasushi Kai[3], Jerapan Krungkrai[4], Toshihiro Horii[2], Tsuyoshi Inoue[1]
[1]Osaka University, Gradute schhol of engineering, Yamadaoka 2-1, Suita City, Osaka, 565-0871, Japan, [2]Department of Molecular Protozoology, Research Institute for Microbial Diseases, Osaka University, [3]Fukui University of Technology, [4]Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, E-mail：konishi@chem.eng.osaka-u.ac.jp