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

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Crystal structure of the covalent intermediate of human cytosolic β-glucosidase

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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

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

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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

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

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There are an estimated 300-500 million cases of malaria and up to 3 million people die from this disease annually. Plasmodium falciparum is the causative agent of the most lethal and sever form of human malaria. Chemotherapy of malaria is available, but is complicated by both adverse effects and widespread resistance to most of the currently available anti-malaria drugs. The malaria parasite depends on de novo synthesis of pyrimidine nucleotides, whereas the human host has the ability to synthesize them by both de novo and salvage pathways. The de novo pathway contains six reaction steps. In the final two steps, uridine 5'-monophosphate (UMP) requires the addition of a ribose phosphate moiety from 5-phosphoribosyl-1-pyrophosphate to orotate by orotate phosphoribosyltransferase (OPRT) to form orotidine 5'-monophosphate (OMP) and pyrophosphate (PPi), and the subsequently decarboxylation of OMP to form UMP, by OMP decarboxylase (OMPDC). Here, we report the X-ray analysis of OMP or UMP-complex forms of OMPDC from Plasmodium falciparum (PfOMPDC) at 2.65 Å resolution. The structural analysis provides the substrate recognition mechanism with dynamic structural changes. And anti-malaria drugs design by using the structure of OMPDC is in progress.

Keywords: malaria, X-ray analysis, structural analysis

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Structural study of enzyme inhibitor complexes of eukaryotic glutamine synthetase from *Zea mays*

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Plants provide nourishment for animals and other heterotrophs as the sole primary producer in the food chain. Glutamine synthetase (GS), one of the essential enzymes for plant autotrophy catalyzes the incorporation of ammonia into glutamate to produce glutamine with concomitant hydrolysis of ATP, and plays a crucial role in the assimilation and re-assimilation of ammonia derived from a wide variety of metabolic processes during plant growth and development. We have determined the crystal structures of maize glutamine synthetase in complexes with three kinds of substrate analogues (J. Biol. Chem., 281, 29287-29296(2006)). From these structures we found a unique decameric structure of the enzyme which is significantly different from the bacterial glutamine synthetase and proposed a phosphate transfer reaction mechanism of ATP. In this study, we aims at gaining insights how the enzyme recognizes the substrate glutamic acid by the methods of mutagenesis and X-ray crystal structure analysis and we prepared several mutant enzymes. We determined new two crystal structures. One structure (WT/PPT/AMPPNP) is a wild type (WT) enzyme in complex with phosphinothricin (PPT) and AMPPNP (ATP analog). The other (H249A/MetSox-P/ADP) is an H249A mutant enzyme in complex with methionine sulfoximine phosphate (MetSox-P) and ADP. Crystal structures of the two complexes were determined at 3.06 and 2.97Å resolutions, respectively. In comparison to the previously reported structures, PPT in the WT/PPT/AMPPNP complex is closer to the γ-phosphate group than MetSox in the WT/MetSox/AMPPNP complex. It is expected that the phosphotransfer energy to PPT is smaller than that of MetSox. It was confirmed that the H249A/MetSox-P/ADP complex contains three manganese ions.

Keywords: plants, enzyme mechanics, ATP dependent reactions

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H/D-exchange and water structure in diisopropyl -fluorophosphatase as reveald by neutron diffraction

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The calcium-dependent phosphotriesterase Diisopropylfluorophosphatase (DFPase) from the squid Loligo vulgaris is an enzyme capable to detoxify a range of highly toxic organophosphorus compounds including DFP ad the nerve agents Tabun (GA), Sarin (GB), Soman (GD) and Cyclosarin (GF). In addition to an already existing atomic-resolution X-ray structure (0.85 Å, PDB: 1PJX) neutron diffraction was employed to reveal the protonation states and indentity of a catalytically important water molecule in the DFPase active site. Additional information was gained on the extend and distribution of H/D-exchange in the protein leading a detailed picture of structural rigidity in the hightly symetrical β-propeller structure of DFPase. Also we were able to determine the positions and oritentations of water molecules in the central tunnel of DFPase with high accuracy. Based on these finding we employed Molecular Dynamics (MD) simulation to investigate the dynamics of these internal water molecules that form an extended network connecting both metall ions in the protein. The results of several simulation runs of 30 ns each obtained using the OPLS all-atom force field and both TIP4P and SPC water models suggest a highly odered and correlated movement of water molecules in the tunnel. As DFPase is currently the protein structure with the largest extended network of internal water molecules characterized by neutron difraction, it might serve as a valuable model for other water filled narrow tunnel and channel like structural moieties. We show that information obtained from neutron diffraction and computational simulations is complementary to other currently employed exterimental methods for investigating internal water dynamics like NMR-spectroscopy.

Keywords: neutron crystallography, molecular dynamics simulations, protein dynamics

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Structural study of putative aminotransferase from *Thermus thermophilus* HB8

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Amiontransferase (AT) is one of pyridoxal 5'-phosphate (PLP) -dependent enzyme and plays an important role in amino acid