remains poorly understood. The enzyme used in this study is papain, a member of the C1 family and the archetypal CP. Papain is obtained from papaya fruits; its utility in tenderizing meat has been known for thousands of years. Papain finds immunological utility in the cleavage of immunoglobulins into Fc and FAB fragments, and medical use in the treatment of stings and chronic wounds. Earlier work reported the crystallization of papain from ethanol/methanol solutions, revealing that papain comprises 2 major structural domains. Papain activation is believed to depend on the formation of a thiolate-imidazolium pair between residues Cys25 and His159 at the cleft between domains. In this study we have obtained crystals from a new aqueous condition containing PEG, buffer, and sodium thiosulfate. In this condition, papain crystallizes in a low-solvent-content unit cell. A 1.60 Å X-ray data set was collected at 300 K in 4 h on a copper-source diffractometer. Results show that a thiosulfate moiety is bound to the active site cysteine, Cys25. Efforts to optimize crystal size for ultra-high resolution X-ray diffraction and neutron diffraction data collection are ongoing. By locating the hydrogen atoms at the active site, we hope to determine the protonation state of His159 and obtain a clearer picture of papain activation and substrate hydrolysis.

Keywords: X-ray and neutron diffraction, proteases proteinases, agricultural natural products

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Crystal structures of *Streptococcus pneumoniae* penicillin-binding proteins acyl-enzyme complexes

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Penicillin-binding proteins (PBPs) are enzymes that catalyze the polymerization and cross-linking of peptidoglycan precursors during bacterial cell wall biosynthesis. β-lactam antibiotics inhibit transpeptidase and DD-carboxypeptidase activities of PBPs by acylating their active-site Ser. To envisage the biding of β-lactam antibiotics to PBPs, we determined a crystal structure of a trypsin-digested form of PBP 2X from *Streptococcus pneumoniae* strain R6 complexed with a cephalosporin antibiotic, cefditoren [1]. We also determined crystal structures of the trypsin-digested form of both PBPs 2X and 1A, each complexed with a carbapenem antibiotic, biapenem or tebipenem [2]. The structures of the acyl-enzyme complexes showed that the cephalosporin C3 side chain and the carbapenem C2 side chains form hydrophobic interactions with Trp374 and Thr526 of PBP 2X and with Trp411 and Thr543 of PBP 1A, however a conformational change of the Trp374 side chain of PBP 2X occurred only upon cefditoren binding. Although the structures studied here were products of inactivation reactions by β -lactams, these hydrophobic interactions are likely to play a role in drug binding upon acylation. There may be similar interactions in PBP 2B from *S. pneumoniae* because a crystal structure of PBP 2B showed that Trp429 and Thr605 occupy positions similar to those of the Trp and Thr residues in the active sites of PBPs 2X and 1A [3].

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Keywords: antibiotics, peptidoglycan biosynthesis, inhibitor interactions

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Crystal structure of a serine protease defines a novel family of secreted bacterial proteases

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Recently, a serine protease secreted by an alkaliphilic and moderately halophilic microbe AL20 belonging to the Nesterenkonia abyssinica family, has been purified and characterized. AL20 protease is optimally active at pH 10, 1.0 M NaCl and 343 K and it shows good stability at 323 K in the presence of EDTA and detergents. From sequence similarity search, AL20 protease has defined a novel protein family of bacterial secreted proteases. Combined with biochemical analyses and high resolution crystal structure determination, we have unambiguously characterized the AL20 protease and its sequence related family as a trypsin-like serine protease.

Keywords: Al20 protease, bacterial secreted proteases, trypsin-like

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Crystal structures of *Escherichia coli* γ -glutamyltrans -peptidase in complex with glutamine antagonists

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γ-Glutamyltranspeptidase (GGT) is a heterodimeric enzyme that catalyzes the transfer of the γ-glutamyl group in γ-glutamyl compounds such as glutathione and its S-conjugates either to water or to other amino acids and peptides. GGT is involved in a number of biological events such as drug resistance and metastasis of cancer cells by detoxification of xenobiotics. Azaserine and acivicin are classical and irreversible inhibitors of GGT, but their binding sites and the inhibition mechanisms remain to be defined. We have determined the crystal structures of GGT from *Escherichia coli* in complex with glutamine antagonists, azaserine and acivicin, at 1.65 Å resolution. Both inhibitors are bound to GGT at its substrate-binding pocket in a similar manner as observed previously with the γ-glutamyl-enzyme intermediate: they form a covalent bond with

the Oγ atom of Thr391, the catalytic residue of GGT, and their α -carboxy and α-amino groups are recognized by extensive hydrogen bonding and charge interactions with the residues that are conserved among GGT orthologs. Notably, in the azaserine complex the carbon atom that forms a covalent bond with Thr391 is sp^3-hybridized, suggesting that the carbonyl of azaserine was attacked by Thr391 to form a tetrahedral intermediate, which is stabilized by the oxyanion hole. Furthermore, when acivicin is bound to GGT, a migration of the single and double bonds occured in its dihydroisoxazole ring to form an unexpected adduct with sp^3-hybridized C3 atom attached to Thr391. The structural characteristics imply that the unprecedented binding modes of azaserine and acivicin are conserved in all GGTs from bacteria to mammals and give a new insight into the inhibition mechanism of these classical glutamine antagonists.

Keywords: acyl-enzyme intermediate, glutamine amidotransferase, glutathione

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Crystallization and preliminary crystallographic analysis of *Trypanosome alternative* oxidase

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African trypanosome is a parasite that causes African sleeping sickness in humans and Nagana in livestock. We found that the cyanide-insensitive Trypanosome alternative oxidase (TAO) is an attractive drug target, because mammalian hosts lack the enzyme. We found that ascofuranone, which specifically inhibits the quinol oxidase activity of TAO, kills the parasite very quickly. In order to analyze the relationship between structure and function of TAO and to design specific inhibitors of the enzyme based on its three-dimensional structure, crystallization conditions of TAO were screened. TAO and similar alternative oxidases (AOXs) contain diiron-binding motifs (EXXH), but little was known about their structural features, because purification in active state was difficult mainly due to their instability after solubilization from mitochondrial membrane. In the present study, highly stable recombinant TAO with high specific activity was expressed and purified from heme-deficient E. coli membrane. Two iron atoms stoichiometrically bound to the purified TAO were detected by ICP-MS and a diiron center was identified in the reduced form of the rTAO by the EPR spectra. This is the direct evidence of TAO as a diiron protein. After the screening of crystallization conditions of TAO, rod-shaped crystals were obtained. Analyses of the symmetry and systematic absences in the diffraction patterns recorded using synchrotron radiation indicated that the crystals belong to the monoclinic space group *I*222 with unit cell parameters, a = 149.44, b = 223.68, c = 62.03 Å. X-ray diffraction data were processed to 3.5 Å resolution with 96.7% completeness and an overall Rmerge of 13.3%. This is a first crystal of TAO including other membrane-bound diiron proteins.

Keywords: enzyme inhibitors, membrane protein crystallization, structure-activity relationships of drugs

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Structural and functional basis for (S)-allantoin formation in the ureide pathway

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The ureide pathway, which mediates the oxidative degradation of uric acid to (S)-allantoin, represents the late stage of purine catabolism in most organisms. The details of uric acid metabolism remained elusive until the complete pathway involving three enzymes was recently identified and characterized. However, the molecular details of the exclusive production of one enantiomer of allantoin in this pathway are still undefined. Here we report the crystal structure of 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline (OHCU) decarboxylase, which catalyzes the last reaction of the pathway, in a complex with the product, (S)-allantoin, at 2.5 Å resolution. The homodimeric helical protein represents a novel structural motif, and reveals that the active site in each monomer contains no cofactors, distinguishing this enzyme mechanistically from other cofactor-dependent decarboxylases. On the basis of structural analysis, along with site-directed mutagenesis, a mechanism for the enzyme is proposed in which a decarboxylation reaction occurs directly, and the invariant histidine residue in the OHCU decarboxylase family plays an essential role in producing (S)-allantoin through a proton transfer from the hydroxyl group at C4 to C5 at the re-face of OHCU. These results provide molecular details that address a longstanding question of how living organisms selectively produce (S)-allantoin.

Keywords: Ureide pathway, OHCU decarboxylase, (S)-allantoin

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Structural basis of the substrate recognition and hydrolysis reaction mechanisms of 8-oxo-dGDPase

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8-Oxo-7,8-dihydroguanine (8-oxoG) produced in cells by reactive oxygen species can pair with cytosine and adenine, and causes A : T to C : G transversion mutations. 8-oxo-dGTP can be directly incorporated into DNA, and 8-oxo-dGDP is readily phosphorylated to generate 8-oxo-dGTP. Therefore, it is important for cells to eliminate both 8-oxo-dGTP and 8-oxo-dGDP. In *Escherichia coli*, MutT hydrolyzes the both to 8-oxo-dGMP which cannot be used for DNA synthesis, whereas human MutT homologue 1 (hMTH1) does 8-oxo-dGTP but not 8-oxo-dGDP. Human NUDT5, originally identified as an ADP-ribose (ADPR) pyrophosphatase, hydrolyzes 8-oxo-dGDP to 8-oxo-dGMP. NUDT5 has a higher affinity for