

the O γ 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 sp³-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 occurred in its dihydroisoxazole ring to form an unexpected adduct with sp³-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 R_{meas} 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-ureidoimidazole (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

8-oxo-dGDP ($K_m = 0.77 \mu\text{M}$) than for ADPR ($K_m = 32 \mu\text{M}$). Considering these facts, NUDT5 works cooperatively with hMTH1 to eliminate 8-oxoG containing nucleotides in human cells. The crystal structure of NUDT5 complexed with ADPR was reported in 2006 and revealed ADPR recognition mechanism. However, 8-oxo-dGDP recognition and hydrolysis reaction mechanisms remain unknown. In this work, we have determined the crystal structures of the ternary (NUDT5/8-oxo-dGDP/ Mn^{2+}) and binary (NUDT5/8-oxo-dGMP) complexes. Our structures show that NUDT5 adopts a unique recognition mechanism for 8-oxoG, which is quite different from those in MutT and hMTH1 crystal structures. We have also elucidated the structural insights into the hydrolysis mechanisms of MutT and hMTH1, and a comparison of active sites between NUDT5 and MutT/hMTH1 suggests that these enzymes have similar catalytic mechanisms in hydrolysis reactions. This catalytic mechanism of NUDT5 is supported by experimental data showing the site attacked by a nucleophilic water based on ^{31}P NMR analysis of the reaction products in ^{18}O -labeled water.

Keywords: DNA repair enzymes, substrate binding, catalytic mechanisms

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Crystal structure of Otubain1

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Otubain 1 is a cysteine protease that belongs to OTU family. Despite the low sequence homology to known deubiquitylating enzymes, Otubain1 have been shown to have deubiquitylating activities. Otubain1 is supposed to play important roles in the regulation of T-cell anergy by the interaction with RING-type ubiquitin ligase GRAIL. Otubain1 protein was crystallized using the hanging drop vapor diffusion method. Crystals grew when the protein was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 30% PEG 8000, 0.2 M sodium acetate, and 0.1 M sodium cacodylate at pH 6.5 in 293 K. We solved crystal structure of Otubain1 by molecular replacement at 1.7 Å resolution. The crystal structure reveals the canonical OTU fold, composed of a papain-like core (two lobes, one alpha-helical, the other beta-sheet). Like its nearest neighbor (Otubain 2), the active site includes p1 and p1' pockets, sites for two ubiquityl units of a chained substrate (hydrolysis occurs at the isopeptide bond between two units). The distorted appearance of the catalytic triad, with His267 not within hydrogen-bonding distance of the catalytic cysteine (Cys91), might suggest that the protein was crystallized in an autoinhibited state. The p1' site likely holds structural information concerning the enzyme's substrate specificity, which is in question.

Keywords: OTU, Dubs, ubiquitin

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Catalysis and electron transfer in glycerol-3-phosphate dehydrogenase

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Sn-glycerol-3-phosphate dehydrogenase (GlpD) is a key flavin-linked primary dehydrogenase of the respiratory electron transport chain. An essential membrane enzyme for aerobic growth on glycerol, it functions at the nexus of respiration, glycolysis, and phospholipid biosynthesis. GlpD catalyzes the oxidation of sn-glycerol-3-phosphate (G3P) to dihydroxyacetone phosphate (DHAP), with concurrent reduction of flavin adenine dinucleotide (FAD) to FADH₂, and passes electrons on to ubiquinone (UQ). Based on the structures of the native, substrate-analogue and product complexes, we propose two possible mechanisms, one involving Arg-317 and another His-233, to deprotonate the hydroxyl group of C2 in G3P thus initiating dehydrogenation followed by hydride transfer from C2 of G3P to N5 of FAD, resulting in the dihydroflavin anion state. We have created a series of mutants of Arg-317 and His-233, to define the role of these residues in catalysis. To more fully delineate the electron-transfer function of GlpD, we determined the structures of GlpD bound with UQ analogues. These structures identify a possible ubiquinone-binding site, approximately 12 Å from N5 of FAD. No other cofactors or metals appear to be required for GlpD activity nor metal clusters as suggested by the structures and metal-dependency experiments. This suggested that electron transfer from FADH₂ to UQ may be mediated through protein residues or that a ping-pong type mechanism may function whereby the product DHAP, first exits the cleft, permitting UQ access to FADH₂ for reduction. Mutation of the residues between the FAD and the UQ binding site as well as the structure of UQ analogues bound to FADH₂-GlpD may determine which mechanism is correct. The results from these mutational analyses will be presented.

Keywords: GlpD, electron transfer, membrane protein crystallization

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Mechanism of retaining glycosyltransferases: Structure of Kre2p/Mnt1p in complex with a donor analog

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The mechanism of retaining glycosyltransferases (rGT) remains enigmatic, as the available structures of rGT in complex with substrate donors and/or analogs often reveal no catalytically relevant group near the reactive centre. The structure of a retaining α 1,2-mannosyltransferase Kre2p/Mnt1p in complex with a donor substrate analog GDP-2-deoxy-2-fluoro-mannose (GDP-2F-Man) has been determined at 1.71 Å resolution. The intact donor analog is in an extended conformation, with the 2-fluoro-mannose moiety found in a pocket that is formed by the protein when the substrate