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