8-oxo-dGDP (K_m = 0.77 μM) than for ADPR (K_m = 32 μM). 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

P04.02.143

Acta Cryst. (2008). A**64**, C276

Crystal structure of Otubain1

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Otubain 1 is a cystaine 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

P04.02.145

Acta Cryst. (2008). A**64**, C276

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

P04.02.146

Acta Cryst. (2008). A**64**, C276–277

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 α1,2-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