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

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Crystal structure of Otubain1

Masato Akutsu[1], John R Walker[1], Yanjun Li[1], Johan Weigelt[2], Cheryl H Arrowsmith[1], Aled M Edwards[1], Alexey Bochkarev[1], Sirano Dhe-Paganon[1]
[1]The Structural Genomics Consortium, University of Toronto Banting Building 100 College Street, Toronto, Ontario, M5G 1L5, Canada, [2]The Structural Genomics Consortium, Karolinska Institutet, Stockholm, Sweden, E-mail：akutsumasato@yahoo.co.jp

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

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Catalysis and electron transfer in glycerol-3-phosphate dehydrogenase

Shoucheng Du, Unmesh Chinte, Joanne I Yeh
University of Pittsburgh, Structural Biology, 3501 5th Ave., Pittsburgh, Pennsylvania, 15260, USA, E-mail：shd20@pitt.edu

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

Yuri D. Lobsanov[1], Luke L. Lairson[2], Pedro A. Romero[3], Barry Sleno[3], Patrick Yip[1], Stephen G. Withers[2], Annette Herscovics[3], Lynne P. Howell[1,4]
[1]Hospital for Sick Children, Molecular Structure and Function, 555 University Avenue, Toronto, Ontario, M5G 1X8, Canada, [2]Department of Chemistry, University of British Columbia, Vancouver, Canada, [3]The McGill Cancer Centre, McGill University, Montreal, Canada, [4]Department of Biochemistry, University of Toronto, Toronto, Canada, E-mail：lobsanov@sickkids.ca

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

binds. Neither the extended conformation of the donor analogue nor the induced fit it causes in the protein have been observed before in an rGT. Comparison with the previously determined binary and ternary complexes of Kre2p/Mnt1p with its GDP donor product reveals that the GDP moiety of the GDP-2F-Man is bound in a similar manner. However, its β-phosphate group is in a different position, consistent with the attached 2-fluoro-mannose moiety being buried in the pocket. A triad of charged residues, E247, R245 and D361, is involved in the formation of the pocket due to conformational changes of the R245 and D361 side chains. The carboxylate group of E247 is 3.3 Å from the reactive centre, the β-face of the C1 group of mannose, suggesting its involvement in the reaction mechanism. When the donor analogue is bound, R245 no longer interacts with E247, making E247 even more catalytically relevant. Two triad residues have been shown to be essential for catalysis by site-directed mutagenesis, and all are structurally conserved in most rGT structures, which suggests that the proposed catalytic mechanism relying on the concerted action of charged triad residues could potentially be a common mechanism for most rGTs.

Keywords: enzyme, structure, mechanism

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Solving enzyme structures by metabolic pathways

Xiao-Dong Su
Peking University, Life Sciences, National Laboratory of Protein Engineering and Plant Genetic Engineering, College of life sciences, Peking University, Beijing, Beijing, 100871, China, E-mail：xdsu@pku.edu.cn

During the era of structure genomics (SG), formidable goal for biochemists to solve all enzyme structures and to work out catalytic mechanisms becomes feasible, the resulted information will not only satisfy academic curiosity, but also serve to numerous practical fields, such as structural-based drug design. Towards achieving this goal we have been trying to solve enzyme structures by their metabolic pathways on a large-scale, high-throughput structural genomics platform at Peking University. I will present in this meeting over a dozen enzyme structures and novel mechanisms revealed by these structures in the pathways of amino acid and carbohydrate metabolism pathways we have worked over the last a few years.

Keywords: enzyme structure and mechanisms, metabolic pathway, structural genomics.

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X-ray crystal structure analysis of reaction intermediates of bacterial copper amine oxidase

Misumi Kataoka[1], Ayuko Tominaga[1], Masayuki Ohtsu[1], Toshihide Okajima[2], Katsuyuki Tanizawa[2], Hiroshi Yamaguchi[1]
[1]Kwansei Gakuin University, 2-1, Gakuen, Sanda, Hyogo, 669-1337, Japan, [2]Osaka University, 8-1, Mihogaoka, Ibaraki, Osaka, 567-0047, Japan, E-mail：m-kataoka@kwansei.ac.jp

Phenylethylamine oxidase from *Arthrobacter globiformis* (AGAO) is a copper containing amine oxidase which catalyzes the oxidative deamination of phenylethylamine to the corresponding aldehyde. AGAO contains a Cu^{2+} and a 2,4,5-trihydroxyphenylalanine quinone (TPQ) generating from tyrosine residue by post-translational modification. The catalysis consists of the initial reductive and the following oxidative half-reaction. The former, TPQ reacts with the substrates to give aminoresorcinol intermediate. The latter, aminoresorcinol turns over TPQ releasing NH_4^+, H_2O_2 and corresponding aldehyde aerobically. The some structures of the reaction intermediates have determined by using mutant (1). In order to investigate the detailed mechanism, we carried out the crystallographic studies of the intermediate in the wild-type. We soaked wild-type AGAO crystals into the substrate solution anaerobically and freeze-trapped. Reaction states in the crystals were confirmed by single-crystal microspectrometry. Diffraction data were collected on the BL38B1 at SPring-8. Some structures of the intermediates were determined at atomic resolution.

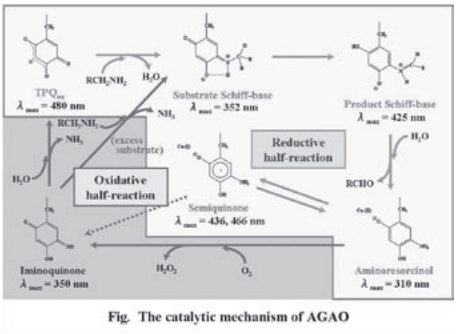

Fig. The catalytic mechanism of AGAO

[1] Chiu *et al.*, *Biochemistry*, (2006), **45**, 4105 - 4120.

Keywords: copper amine oxidase, topaquinone, post-translational modification

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Structural comparison of 5'-methylthioadenosine nucleosidases from *Arabidopsis thaliana*

Eunyoung Park[1], Woo Suk Choi[1], Seung-Ick Oh[1], Kyung-Nam Kim[2], Jeong Sheop Shin[1], Hyun Kyu Song[1]
[1]School of Life Sciences and Biotechnology, Korea University, School of Life Sciences and Biotechnology, 5-Ga, Anam-Dong, Seongbuk-Gu, Seoul, 136-701, Korea (S), [2]Department of Molecular Biology, Sejong University, Seoul 143-747, Korea, E-mail：cryoman@korea.ac.kr

5-methylthioadenosine (MTA) and S-adenosylhomocysteine (SAH) are important metabolites in all living organisms including plant. There are two similar nucleosidases for hydrolyzing MTA in Arabidopsis thaliana (AtMTAN1 and AtMTAN2), but only one of them, AtMTAN2 shows markedly broad substrate specificity for hydrolyzing SAH. To compare structural and biochemical differences of AtMTAN1 with AtMTAN2, it was over-expressed in *Escherichia coli* and purified homogeneously. Spectroscopic assay confirms that the AtMTAN2 catalyzes MTA as well as SAH hydrolysis while AtMTAN1 does MTA only. In addition to the biochemical characterization, the three-dimensional structure of AtMTAN2 enzyme in complex with a product, adenine was determined at 2.9Å resolution using X-ray crystallographic technique. Structural comparison of AtMTAN2 with previously determined structures of AtMTAN1 and *E. coli* provides a clue for the substrate specificity of MTA nucleosidases in *A. thaliana*.

Keywords: MTA, nucleosidase, SAH