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

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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

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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.

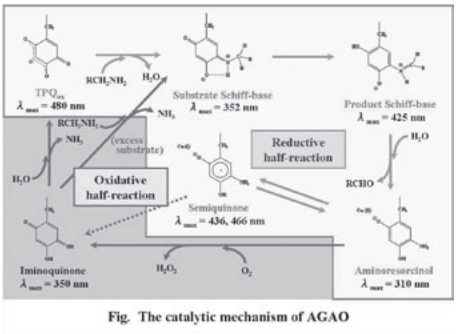

Fig. The catalytic mechanism of AGAO

[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*

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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