crystal structure of the rice Rubisco complexed with NADPH and Mg^{2+} as well as the 1.4 Å structure complexed with Mg^{2+}. The high resolution structures indicate the detailed active site, in which Mg^{2+} and its ligand waters were stabilized by the interaction with NADPH. These observations probably suggest that the activation upon NADPH binding is induced by both increases in the accessibility of the active site and decrease in the rate of deactivation.

[1] *Ann. Rev. Plant Physiol.*, 1977, **28**, 379.

Keywords: crystal structure determination, photosynthesis, activity and mechanism of enzymes

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Crystal Structure of Sulfotransferase from *Mycobacterium tuberculosis* H37Rv

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We determined the crystal structure of *Mycobacterium tuberculosis* H37Rv sulfotransferase (STF1). STF1 was crystallized in two conditions: neutral (pH 7.1) and acidic (pH 4.6). Diffractions of these crystals were observed up to 1.5 Å of resolution with synchrotron radiation at SPring-8. The phase was determined with MIR-AS and with molecular replacement. STF1 structure has 1 beta sheet with 5 parallel strands and 22 helices. PAPS binding structural motif (5'-PSB loop and 3'-PB) was conserved. STF1 forms unique structures, one at the amino terminal and another at between the PAPS binding motifs. The amino terminal structure forms 3-helical alpha-bundle formation, and may stabilize the monomeric conformation of STF1. The inserted structures between the PAPS binding motifs covered on acceptor-binding pocket. Alignment of primal structures with STF1 and other mycobacterial ST homologues revealed high similarity all through the sequences. The conservation of key amino acid residues for sulfation (containing the PAPS binding motifs) suggests that other mycobacterial STs will also perform ST reaction, and moreover, form the characteristic structures like as STF1.

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Keywords: structures of proteins, enzyme structure function, sulfate

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Structure of a Class 4 aldo/keto Reductase

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Plants produce at least three types of class 4 aldo/keto reductases (AKR), of which two have been assigned a specific enzymatic activity: polyketide reductases (class 4A) and codeinone reductases (class 4B). The *in-vivo* substrate for class 4C AKRs is not known, but the presence of class 4C enzymes have been correlated with drought resistance, and genes and EST for class 4C AKRs are found in most plants [1]. Barley aldose reductase is a class 4C AKR sharing app. 40% sequence identity with codeinone and polyketide reductases. We here present the structure of barley aldose reductase, the first structure of a class 4 aldo/keto reductase and experimental identification of the anion binding site.

Barley aldose reductase is a 320 amino acid NADPH containing enzyme. It shares the AKR TIM barrel fold with other AKR family members, but the substrate binding site defined by the A to C loops is substantially different from previously described binding sites. Sequence identity between barley aldose reductase and codeinone and polyketide reductases indicates that the structures of the substrate binding loops are very similar amongst class 4 AKRs.

[1] Oberschall A., Deák M., Török K., Sass L., Vass I., Kovács I., Fehér A., Dudits D., Horváth G.V., *Plant J.*, 2000, **24**, 437.

Keywords: aldo/keto reductase, drought resistance, plant aldose reductase

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Crystal Structure and Catalytic Regulation of an *S*-Formylglutathione Hydrolase

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S-formylglutathione hydrolase from *Arabidopsis thaliana (At*SFGH) shows hydrolytic activity toward xenobiotic carboxyl-esters and glutathione thioesters [1]. *At*SFGH has the characteristics of a cysteine hydrolase, being sensitive to inactivation by thiol alkylating agents while insensitive to conventional serine hydrolase inhibitors. However, when the crystallographic structure of *At*SFGH was determined, although a conserved cysteine (Cys59) implicated in catalysis was identified in the active site, a serine-histidine-aspartic acid catalytic triad was also observed. The importance of the serine residue in catalysis was subsequently demonstrated using a combination of site-directed mutagenesis and covalent modification using a fluorophosphono biotinylated suicide substrate. Mutation of the Cys59 to serine had no effect on carboxyesterase activity. However, Cys59 could reversibly regulate the activity of *At*SFGH through the formation of mixed disulfides, with glutathione or a range of synthetic functionalised thiols, leading to temporary inactivation of the enzyme. We conclude that while Cys59 does not play a direct role in the catalytic mechanism, its conservation and location close to the active site serine confers a specific regulatory function in which the *At*SFGH can be reversibly inactivated by *S*-glutathionylation in the course of catalysis and under oxidative conditions.

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Keywords: hydrolase, protein crystallography, enzyme catalysis

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Crystal Structure of *m*-hydroxybenzoate Hydroxylase from *Comamonas testosteroni*

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m-Hydroxybenzoate hydroxylase (MHBH) from *C. testosteroni* is a flavin-containing monooxygenase of the glutathione reductase family. It catalyzes the conversion of *m*-hydroxybenzoate derived from lignin to 3,4-dihydroxybenzoate with requirements of NADPH and molecular oxygen. To establish a structural basis for characterizing its substrate specificity, the crystal structure of MHBH in complex with its substrate was determined to 1.8 Å resolution. The active-site architecture, including the positions of FAD- and substrate-binding sites, is similar to those of other members of the family, suggesting that flavoprotein aromatic hydroxylases share a similar catalytic mechanism for the hydroxylation of their respective phenolic substrates. Structural comparison of MHBH with the homologous enzymes, however, shows some structural differences in the substrate-transport and the recognition mechanisms. In particular, the presence of a large channel between the catalytic domains indicates a unique pathway for substrate transport. The size of the entrance and the characteristic stratified environment of the channel interior would enable the enzyme to select *m*-hydroxybenzoate on the basis of its molecular size and charge distribution. In addition, the Xe-derivative structure at 2.5 Å resolution led to identification of a putative oxygen-binding site adjacent to the substrate-binding pocket.

Keywords: crystal structure, flavoprotein, substrate recognition