environmental contaminants. Here we present the crystal structure of the terminal oxygenase component of the biphenyl dioxygenase (BphA1A2) derived from *Rhodococcus* strain sp. RHA1. This is the first crystal structure of the biphenyl dioxygenase. We also determined the crystal structure of the BphA1A2-biphenyl complex. Structural comparison between the substrate free and complex forms revealed that the substrate-binding pocket makes significant conformational changes upon substrate binding to accommodate the substrate into the pocket. The analysis with the two crystal structures suggested that the residues in the substrate-binding pocket can be classified into three groups, which respectively seem to be responsible for the catalytic reaction, the orientation/conformation of the substrate, and the conformational changes of the substrate-binding pocket.

Keywords: oxygenase, substrate binding, conformational change

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Crystal Structures of Serine Racemase from *S. pombe*

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Free D-serine has been found to be confined predominantly to the forebrain structure of mammals and persists at high levels throughout embryonic and postnatal life. Several reports strongly suggest that serine racemase exits in mammalian brain and produces D-serine, an endogenous ligand for *N*-Methyl-D-Aspartate receptor. Serine racemase requires pyridoxal 5'-phosphate and catalyzes racemization from L-serine to D-serine. Surprisingly, the distribution of putative serine racemase is not limited to higher animals. Mouse serine racemase might fall on a new class of pyridoxal amino acid racemases, which is distinct from both bacterial and fungal alanine racemases, but is similar to bacterial threonine dehydratase in primary structure.

S. pombe gene homologous with mouse serine racemase is overexpressed in *E. coli*. The enzyme is purified and crystallized. Three-demensional structures of serine racemase in its unliganded form and complexed with the AMP-PCP of activator have been determined at 1.7 and 1.9 Å resolution, respectively. The enzyme is a homodimer, and each subunit is divided into small and large domains. Based on its folding, the enzyme belongs to fold type II. Interestingly, these structures show that an activator is bound at the subunit interface away from the active site PLP.

Keywords: three-dimensional protein structure, enzyme activity mechanism, racemases

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Crystal Structures of Formaldehyde Dehydrogenase Complexed with Inhibitors

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Formaldehyde dehydrogenase from *Pseudomonas putida* (PFDH) is a member of the zinc-containing medium-chain alcohol dehydrogenase family. The pyridine nucleotide NAD(H) in PFDH, which is distinct from the coenzyme (as cosubstrate) in typical alcohol dehydrogenases (ADHs), is tightly but not covalently bound to the protein and acts as a cofactor. PFDH can catalyze aldehyde dismutations without an external addition of NAD(H). The crystal structure of PFDH was solved by the multiwavelength anomalous diffraction method using intrinsic zinc ions and was refined at a 1.65Å resolution [1].The 170-kDa homotetrameric PFDH molecule showed 222-point group symmetry. Although the secondary structure arrangement and binding mode of catalytic and structural zinc ions in PFDH were similar to those of typical ADH, a number of loop structures that differ between PFDH and ADHs in their lengths and conformations were observed.

Here we have determined the crystal structures of PFDH complexed with pyrazole inhibitors.

[1] Tanaka N., Kusakabe Y., Ito K., Yoshimoto T., Nakamura K.T., *J. Mol. Biol.*, 2002, **324**, 519.

Keywords: crystal structure, formaldehyde dehydrogenase, alchol dehydrogenase

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Molecular Basis for Tay-Sachs Revealed by the Crystal Structure of Human β-HexosaminidaseA

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β-hexosaminidase-A (HexA) is essential for the degradation of G_{M2} glycolipids in the central and peripheral nervous system. Accumulation of G_{M2} leads to neurodegeneration associated with Tay-Sachs and Sandoff disease. Here we present the X-ray crystallographic structure of human lysosomal HexA to 3.0Å resolution. The structure reveals a heterodimer consisting of an α- and a β-subunit. The dimer interface creates two distinct active sites: and α(β) and β(α); only the α(β) site can hydrolyse G_{M2} whereas both sites can hydrolyse oligomers of NAG. We also present a second structure of HexA with a NAG-thiazoline, a transition state substrate analog, bound in the active site. Hex A, a member of Family 20 of the glycosyl hydrolases, makes use of substrate-assisted catalysis in the removal of the non-reducing sugar from an oligosaccharide. The intermediate in the reaction pathway of G_{M2} is a cyclic oxazolinium formed by the acetyl group on C2' of the non-reducing β(1,4)-GalNAc of the substrate. In both active sites, the general acid is a Glu residue and the positive charge of the oxazolinium ring is stabilized by the negative charge on an Asp residue. In the α(β) site, G_{M2} binding is promoted by Arg424 and Asn425 residues. The β(α)-site lacks these key residues but has Leu453 and Asp452 in their place that would repel the negatively charged sialic acid of G_{M2}.

Keywords: enzyme mechanisms, glycosyl hydrolases, neurobiology

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Structural Basis for the Induced Fit, Substrate Recognition, and Mechanism of Threonine Synthase

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Threonine synthase, which is a pyridoxal 5'-phosphate dependent enzyme, catalyzes the β, γ-replacement reaction of an *O*-phospho-L-homoserine to give a threonine and an inorganic phosphate. The three-dimensional structures of the enzyme from *Thermus thermophilus* HB8 in its native form and complexed with the substrate analogue (2-amino-5-phosphonopetanoic acid) have been determined at 2.15 and 2.0 Å resolution, respectively. The enzyme is a homo dimer, with the polypeptide chain of the subunit folded into large, small, and swap domains. The complexed form of the enzyme assigned as an enamine uncovered the interactions of the cofactor-analogue conjugate with the active-site residues. The binding of the substrate analogue induces the large conformational change at the domain level to close the active-site. The small domain rotates by about 25° and approaches the large domain to shield the substrate analogue from the solvent region. The complicated catalytic process of the enzyme has been elucidated based on the complex structure to reveal the stereochemistry of the reaction and present the released inorganic phosphate as the possible catalyst to

carry a proton to Cγ atom of the substrate.

[1] Omi R., Goto M., Miyahara I., Mizuguchi H., Hayashi H., Kagamiyama H., Hirotsu K., *J Biol Chem*., 2003, **14**, 278(46), 46035.

Keywords: three-dimensional protein structure, enzyme catalysis, conformational change

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Structure and Mutational Analysis of *Trypanosoma brucei* Prostaglandin $F_{2\alpha}$ Synthase

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Trypanosoma brucei prostaglandin (PG) $F_{2\alpha}$ synthase (TbPGFS), an aldo-keto reductase, catalyzes the NADPH-dependent reduction of the endoperoxide moiety of PGH_2 to $PGF_{2\alpha}$**.** The overproduction of $PGF_{2\alpha}$ during trypanosomasis causes miscarriage in infected subjects. Here we report the crystal structures of TbPGFS-$NADP^+$ bounds citrate or sulfate at 2.1 Å and 2.6 Å resolution respectively. TbPGFS adopts a parallel $(\alpha/\beta)_8$- barrel folds lacking the protrudent loops. The core active site structure is hydrophobic to bind hydrophobic substrates and contains tyrosine, lysine, histidine and aspartate known as a catalytic tetrad which is preserved in most of other aldo-keto reductases. These four residues are said to be indispensable for the reduction of PGH_2, but mutagenesis shows that Tyr52 and Asp47 are not involved in the enzyme reaction and identifies His110 and Lys77 work as catalytic dyad. His 110 acts as a general acid catalyst, while Lys 77 facilitates proton donation by His 110 through a water molecule and forms a salt-bridge to stabilize the Asp 47 that binds NADPH. By comparing the citrate and sulfate complex structure, we detected that Trp187 holds the nicotinamide ring of NADPH from tilting on the access of PGH_2. These findings reveal a novel catalytic mechanism for the biological reduction of the endoperoxide PGH_2 by an aldo-keto reductase. The structure should allow for rational design of specific inhibitors useful to investigate the physiological roles of TbPGFS in trypanosomes.

Keywords: aldo-keto reductase, prostaglandins, mutational analysis

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Crystal Structure of Prostaglandin $F_{2\alpha}$ Synthase from *Leshmania major*

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Leishmania major is the causative agent of leishmaniases. A gene encoding prostaglandin $F_{2\alpha}$ synthase, catalyzing NADPH-dependent reduction of 9,11-endoperoxide PGH_2 to $PGF_{2\alpha}$was identified from *L.major* (*Lm*PGFS)[1]. In the previous study, we have crystallized prostaglandin $F_{2\alpha}$ synthase from *Trypanosoma brucei* (*Tb*PGFS) using citrate as a precipitant and successfully solved the ternary structure of $NADP^+$/citrate/*Tb*PGFS[2]. We also attempted to solve the inhibitor complex of *Tb*PGFS, but a citrate molecule strongly binded to the active site.

In this study we have successfully crystallized *Lm*PGFS using PEG instead of citrate and determined the structure of *Lm*PGFS in the unliganded form by the molecular replacement method at 1.8 Å resolution. Comparing the structure of *Lm*PGFS with *Tb*PGFS, we found that the overall structure and the catalytic residues are almost the same without the extra α-helix between β4 and α4. The structural analysis of inhibitor complex is in progress to develop drug preventing a miscarriage

[1] Kabututu Z., *et al.*, *Int. J. Parasitol.*, 2003, **33**. [2] Kilunga B. K., *et al.*, *J. Biol. Chem.*, *in press*.

Keywords: prostaglandins, X-ray crystal structure determination, parasites

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Structual Evidence of pH-induced Changes of the Reduction Potential of Mavicyanin from Zucchini

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Mavicyanin, a glycosylated protein isolated from *Cucurbita pepo medullosa* (zucchini) is a member of the phytocyanin subfamily containing one polypeptide chain of 109 amino residues and an unusual type-I Cu site in which the copper ligands are His^{45}, Cys^{86}, His^{91}, and Gln^{96}. The crystal structures of oxidized and reduced mavicyanin were determined at 1.6 and 1.9 Å resolution, respectively. Mavicyanin has a core structure of seven polypeptide β-strands arranged as a β-sandwich organized from two β-sheets, and the structure considerably resembles that with stellacyanin from cucumber (CST) or cucumber basic protein (CBP). A flexible region was not observed from superimposition of the oxidized and reduced mavicyanin structures. However, the residue of Thr^{15} rotated 60.0 degrees and O-γ 1-Thr^{15} moved from a distance of 4.78 to 2.58 Å toward the ligand Gln^{96} forming a new hydrogen bond between O-γ 1-Thr^{15} and ε-O-Gln^{96} upon reduction and changing, significantly altered the coordination structure containing Gln^{96}. It has been proposed that the reorganization of copper coordination geometry above pH 8 aroused reduction potential decreased [Battistuzzi *et al*. (2001) *J. Inorg. Biochem*.83, 223-227]. The rotation of Thr^{15} and the hydrogen bonding with the ligand Gln^{96} may provide structural evidence for the decrease of the reduction potential at high pH.

Keywords: crystal structure determination, blue copper proteins, structure comparison

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Crystal Structure of Rice Rubisco Complexed with NADPH

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Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the carboxylation of ribulose 1,5-bisphosphate (RuBP) in the initial step of the photosynthesis. It can also catalyzes the oxygenation of RuBP in photorespiration. To exhibit carboxylation and oxygenation, Rubisco must be activated via the covalent carbamylation of a specific lysine residue on the active site and subsequent stabilization of the carbamate by Mg^{2+} coordination. This process is known to be relatively slow, and be modulating the activity of Rubisco. Evidence has been obtained for the role of several sugar phosphates as important regulators of the carbamylation of Rubisco. Both RuBP and 2-carboxyarabinitol 1-phosphate (CA1P) are reported to be potent inhibitors, whereas NADPH and 6-phosphogluconate accelerate the carbamylation of Rubisco [1]. To investigate the NADPH-accelerated carbamylation mechanism of Rubisco, we have crystallized the Rubisco from rice (Oryza sativa L.) under the condition similar to that in the stroma. Here, we report the 1.8 Å