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

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