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Crystal structure to functional correlation of WhiE aromatase/cyclase from *Streptomyces coelicolor*

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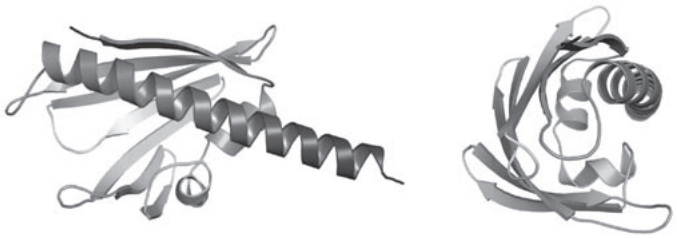
Many aromatic polyketide natural products have important pharmacological activity, including the antibiotic activity of the tetracyclines, and the anticancer activity of the anthracyclines. Aromatic polyketides are produced by the type II polyketide synthases (PKSs). Critical to the formation of aromatic rings during biosynthesis are the PKS aromatase/cyclases (ARO/CYCs). Here we present the crystal structure of WhiE ARO/CYC, solved by molecular replacement (MR) to 1.9Å resolution. WhiE ARO/CYC is involved in the biosynthesis of the polyketide spore pigment of *Streptomyces coelicolor*, catalyzing the regiospecific cyclization and subsequent aromatization of the nascent polyketide chain. The structure of WhiE ARO/CYC reveals that the enzyme possesses a helix-grip fold and contains a large interior cavity consisting of conserved hydrophobic, polar, and charged residues.



Keywords: polyketide, spore pigment, aromatase-cyclase

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Structural study of laminaripentaose-producing β-1,3-glucanase from *Streptomyces matensis* DIC-108

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Chitin and β-1,3-glucan are crucial components in the cell wall of fungas but absent in mammalian cellular membranes. *Streptomyces matensis* DIC-108 laminaripentaose-producing β-1,3-glucanase (LPHase) catalyzes the cleavage of the glycosidic bond of β-1,3-glucans into the pentasaccharide laminaripentaose and may be an attractive target for the development of new antifungal agents. LPHase is assigned to a member of the glycoside hydrolase family GH-64 based on sequence analysis. This investigation is aimed to determine the crystal structure of LPHase for a detailed structure-function study. The native and seleno-protein crystals were grown by the hanging-drop vapor diffusion method. The native crystal belongs to space group $P2_12_12_1$, with the unit cell parameters a=46.16 Å, b=60.68 Å, c=149.40 Å, $\alpha=\beta=\gamma=90.00^\circ$. There is one LPHase molecule per asymmetric unit. A complete native data set was collected to 1.62 Å resolution at the National Synchrotron Radiation Research Center (NSRRC) beamline BL13C1. The phase was solved to 2.3 Å using multiwavelength anomalous dispersion (MAD) data from the SeMet-LPHase crystal collected at BL12B2 Taiwan beamline at SPring-8, Japan. The structure was refined to 1.62 Å and had an R-factor of 17.8% (R_{free}=21.6%). The LPHase structure adopts a novel fold consisting of a β-barrel domain and a mixed α/β domain. To our knowledge, this is the first structure of GH64 family. Three conserved carboxylates (E154, D170, and D377) are situated at the surface of a groove between the domains, in which E154 is close to D170 and D377. Site-directed mutagenesis supports the importance of these residues. These results together suggest that E154, D170, and D377 may serve as potential catalysts to hydrolyze the glycosidic bond via an inverting mechanism.

Keywords: crystallographic structure, glycosyl hydrolases, MAD phasing

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The crystal structure of N-terminal domain of plant NADPH oxidase

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Reactive Oxygen species (ROS) produced by NADPH oxidase play critical roles in various cellular activities including defense against pathogens by plant. To generate ROS in defense response against pathogen, plant cells need oxygen in large amounts called respiratory burst. Plant NADPH oxidase named Rboh (respiratory burst oxidase homolog) is a homolog of mammalian phagocyte NADPH oxidase catalytic subunit NOX2/gp^{91phox} (NADPH oxidase 2 / glycoprotein 91kDa phagocyte oxidase). Rboh exists in plasma membrane. The phagocyte NADPH oxidase consists of 6-transmembrane helices and C-terminal nucleotide binding domain, and forms multi-protein complex containing several membrane and cytosolic regulatory factors and small GTPase Rac. However, in plants, no homolog of these regulatory factors except Rac have been found. The regulation mechanism of NADPH oxidase is different between plants and mammals. Rboh possesses an extended N-terminal domain including two EF-hand motifs (EF1 and EF2) which does not exist in gp^{91phox}, and this N-terminal domain interacts with Rac directly. It is suggested that Rboh is regulated by Ca^{2+} and Rac. Moreover, recently it was suggested that Ca^{2+} dependent protein kinase (CDPK) participates in Rboh regulation. To elucidate the regulation and recognition mechanism of Rhoh we determined the crystal structure of the N-terminal domain of *Oryza sativa*RbohB (OsRbohB(138-313)). Electron density of Ca^{2+} in EF1 was clearly detected but not in EF2. OsRbohB(138-313) formed homo dimer by swapping of EF2. Ca^{2+} did not affect this dimerization or interaction between Rboh and Rac. But The CD spectrum of OsRbohB(138-313) is different between a Ca^{2+} loaded and free form. These results suggest that conformational

change of EF1 play a key role of regulation.

Keywords: plants, EF-hand proteins, GTP-binding proteins

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Crystal structure and functional study of wild type and mutated *Bacillus cereus* NCTU2 chitinase

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Chitinases which hydrolyze chitin as carbon and nitrogen nutrient, occur in a wide range of organisms include in viruses, bacteria, fungi, insects, higher plants, and animals. Agene of family 18 chitinase from *Bacillus cereus* NCTU2 encodes a signal peptide (27amino acids) and a mature protein (333 amino acids), The gene of family 18 chitinase from *Bacillus cereus* NCTU2 was overexpressed by *E. coli* BL21 (DE3) strain. ChiNCTU2 and mutant E145Q of MW 36 kDa have been crystallized using the hanging-drop vapor diffusion method with solution consisted of polyethene glycerol 8000, sodium cacodylate and zinc acetate dihydrate. According to diffraction of ChiNCTU2 crystals at resolution 1.20 Å, the unit cell belongs to space group $P2_1$ and has parameters a = 50.789 Å, b = 48.788 Å and c = 66.867 Å. And E145Q crystal at resolution 1.49, the unit cell belongs to space group $P1$ and has parameters a = 61.306 50.820 Å, b =72.888 Å and c = 76.343 Å. The protein structure of ChiNCTU2 is monomer by using multiwavelength anomalous dispersion method and the crystal packing of E145Q is tetramer by using molecular replacement method. Four residues Asp143, Glu145, Glu190 and Gln225 bind with zinc atoms in the catalytic domain of ChiNCTU2 protein structure. We proved that zinc atoms decline activity of ChiNCTU2 by detecting the amount of chitobioside using DNS (3,5-Dinitrosalicylic acid). According to structure and mutagenesis we found that E145, Q225 and Y227 are the most important redidues for its function.

Keywords: chitin, chitinase, structure

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The structure of human diamine oxidase

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The crystal structure of human diamine oxidase (hDAO), the first reported structure of a diamine oxidase (DAO), has been determined to 2.9 Å resolution. DAO, a copper-containing amine oxidase (CuAO), contains a 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor derived by post-translational modification of a tyrosine residue [1]. DAO is distinguishable among members of the CuAO enzyme family in its ability to oxidize diamines, such as putrescine and cadaverine, as well as monoamines. DAO is involved in many biological processes. In mammals DAO is found in several tissues, with the highest reported expression levels found in the placenta, small intestine and kidneys. In particular, hDAO may play an important role in histamine metabolism (1). We have grown orthorhombic crystals of hDAO belonging to the space group $C222_1$, with unit-cell dimensions a=95.0, b=97.2, c=179.2 Å. These crystals diffracted to 2.9 Å in-house at 100 K. Data were integrated and scaled with the HKL suite of programs, DENZO and SCALEPACK. The data is 98.3% complete in the range 50-2.9 Å with an overall R_{merge} of 8.4%. The most reasonable Matthews' coefficient suggests there is one molecule in the asymmetric unit with 40% solvent content using 100 kDa as the molecular mass. The structure was solved by molecular replacement, PHASER v1.3 giving a Z-score of 26.2 with a search model created using CHAINSAW, with human vascular adhesion protein-1 (hVAP-1, PDB code 1US1) as the target. Initial rigid-body and restrained refinement has been carried out using REFMAC v5.2. 2*Fo-Fc* and *Fo-Fc* electron-density maps were inspected with, and modeled using COOT.

[1] Elmore, B. O., Bollinger, J. A., and Dooley, D. M. (2002) *J Biol Inorg Chem* **7**(6), 565-579

Keywords: diamine oxidase, amine oxidase, topaquinone

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A study of protocatechuate 3,4-dioxygenase muntants and substrate interactions

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Protocatechuate 3,4-dioxygenase is a nonheme, iron containing enzyme that catalyzes the intradiol oxidative cleavage of 3,4-dihydroxybenzoic acid to ß-carboxy-*cis*,*cis*-muconic acid via incorporation of molecular oxygen into the aromatic ring of the substrate. In an attempt to further understand the factors involved in substrate turnover and mechanism, a series of second sphere residue mutants has been created and structurally and kinetically examined. These crystals diffract to high resolution and show clearly that alterations of these second sphere residues can dramatically affect the interactions with substrate and substrate analogs. A detailed structural and kinetic comparison of these mutants will be presented.

Keywords: structure and function, structural enzymology, metalloenzymes

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Structural study of H_2O_2 reductase, rubperoxin

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Rubperoxin (Rpr) was identified as an O_2-induced protein in