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

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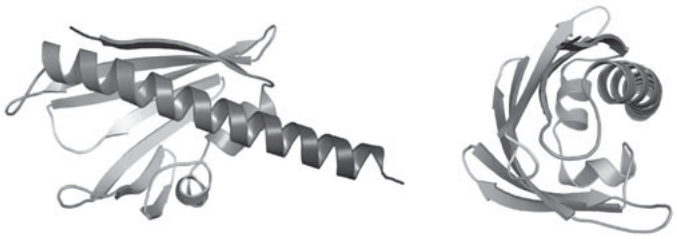
Many aromatic polyketide natural products have important pharmacological activity, including the antibiotic activity of the tetracyclines, and the anticancer activity of the anthracyclines. Aromatic polyketides are produced by the type II polyketide synthases (PKSs). Critical to the formation of aromatic 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