

Poster Presentation

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Crystal structure analyses of oxygenase component of resorcinol hydroxylase

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The resorcinol hydroxylase is involved in the first step of the resorcinol catabolic pathway and catalyzes hydroxylation of resorcinol to hydroxyquinol. The enzyme belongs to the two-component flavin-diffusible monooxygenase family and acts in the coexistence of two components: an oxygenase and a flavin reductase. The oxygenase component hydroxylates the substrate using molecular oxygen and reduced flavin produced by the reductase. To understand the structural basis for the catalytic mechanism, we analyzed the crystal structure of the oxygenase component (GraA) from *Rhizobium* sp. strain MTP-10005. The GraA subunit has 409 amino acid residues. Apo-form crystals were obtained in the tetragonal space group I4122 by a sitting-drop vapor-diffusion method with a reservoir solution of PEG3350 and K₂HPO₄. Holo-form crystals were obtained in the trigonal space group P3221 by a sitting-drop vapor-diffusion method with a reservoir solution of PEG3350 and KNO₃. Both structures were determined by molecular replacement and refined at 2.3 Å and 3.2 Å resolutions, respectively. GraA is a homotetramer with three molecular two-fold axes identical to crystallographic two-fold axes in the apo-form crystal. In the holo-form crystal, four tetramers exist in the asymmetric unit and each subunit binds one FAD. The subunit consists of three domains. The N-terminal domain has an α -structure mainly of antiparallel α -helices; the central domain has a β -structure of two β -sheets stacked together; the C-terminal domain has a four-helix-bundle structure of long antiparallel α -helices involved in tetramer formation. In the holo-form, the FAD is located in the space that is encompassed by these three domains. The loop region of 13 residues, which is disordered in the apo-form, is ordered and covers FAD of another subunit. The turn portion of the loop occludes the entrance of the putative active site.

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