Poster Presentation

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Crystal structures of bacterial flavin reductase GraD and its complex with NADH

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Rhizobium sp. strain MTP-10005 uses the aromatic compound γ-resorcylate as a sole source of carbon and energy for growth. Resorcinol hydroxylase, which converts resorcinol to hydroxyquinol, plays an important role in the aerobic microbial catabolism of γ-resorcylate. Resorcinol hydroxylase from Rhizobium sp. strain MTP-10005 is a two-component enzyme consisting of the reductase and the monooxygenase components. The reductase component (GraD) is an oxidoreductase containing a flavin molecule as a cofactor. GraD catalyzes the NADH-dependent reduction of free FAD according to a ping-pong bisubstrate-biproduct mechanism. The reduced FAD is then used by the monooxygenase component GraA to hydroxylate resorcinol to hydroxyquinol. We have determined the three-dimensional structures of recombinant GraD with a bound FAD and in complex with NAD. GraD was crystallized at 293 K by the sitting-drop vapour-diffusion method using a precipitant solution containing 13 - 14% (w/v) PEG 2000, 6 - 9% (v/v) 2-propanol, 100 mM sodium citrate pH 5.6, 100 mM DTT and 200 µM FAD. The approximate dimensions of the obtained crystals were 0.1 × 0.1 × 0.15 mm³. The crystal diffracted to 1.8Å and belongs to space group P41212 with unit-cell parameters of a = b = 77.8 Å and c = 124.2 Å. The crystal structure has been determined by the molecular replacement and refined at 1.8 Å resolution. GraD exists as a homodimer, and each monomer contains an FAD. The probable binding site for NADH is covered with the N-terminal sub-domain in chain A, whereas the site is completely exposed to bulk solvent in chain B. The NAD-complex crystals were prepared by soaking the GraD crystals in the reservoir solution supplemented with NADH. The crystal diffracted to 1.8 Å, and the crystal structure was determined at 1.8 Å resolution. The Fo-Fc maps for the crystal soaked with NADH showed the electron densities corresponding to the nicotinamide ring and the adenyl moiety in chain B.

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