Structural Studies on 3-hydroxyanthranilate-3,4-dioxygenase

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3-Hydroxyanthranilate-3,4-dioxygenase (HAD) catalyzes the oxidative ring opening of 3-hydroxyanthranilate in the tryptophan-based quinolinate biosynthetic pathway. The enzyme requires Fe^{2+} as a cofactor and is inactivated by 4-chloro-3-hydroxyanthranilate (ClHAA). The structure of HAD from *Ralstonia metallidurans* was determined at 1.9 Å resolution. The structures of HAD complexed with the inhibitor ClHAA and either molecular oxygen or nitrous oxide were determined at 2.0 Å resolution, and the structure of HAD complexed with the substrate 3-hydroxyanthranilate was determined at 3.2 Å resolution. HAD is a homodimer with a subunit topology that is characteristic of the cupin barrel fold. Each monomer contains two iron binding sites. The catalytic iron is buried deep inside the cupin barrel. The other iron site forms an FeS_4 center close to the solvent surface. The two iron sites are separated by about 24 Å. Based on the crystal structures of HAD, mutagenesis studies were carried out and a new mechanism for the enzyme inactivation by 4-chloro-3-hydroxyanthranilate is proposed.

Keywords: dioxygenase, cupin, enzyme mechanism