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Structural Studies on Two Dioxygenases in the Prokaryotic Tryptophan-Based Quinolate Biosynthetic Pathway. Y. Zhang, K.L. Colabroy, S.A. Kang, S. Bale, T. Mukherjee, B.R. Crane, T.P. Begley, S.E. Ealick, Dept. of Chemistry and Chemical Biology, Cornell Univ., Ithaca, NY 14853.

It has been generally believed that the biosynthesis of quinolate from tryptophan is unique to eukaryotes, while in prokaryotes quinolate is derived from aspartate and dihydroxyacetone phosphate. However, the tryptophan-based quinolate biosynthetic pathway has been recently identified in bacteria including *Ralstonia metallidurans*. In the tryptophan-based quinolate biosynthetic pathway, tryptophan dioxygenase (TDO) catalyzes the first step to form N-formyl kynurenine; 3-hydroxyanthranilate-3,4-dioxygenase (HAD) catalyzes the last enzymatic step, which is the oxidative ring opening of 3-hydroxyanthranilate. The crystal structures of TDO and HAD from *R. metallidurans* were determined at 2.5 Å and 1.9 Å resolution, respectively. TDO is a tetramer with cofactor heme bound at the active site. Biochemical studies are underway. HAD is a dimer of cupin fold with a catalytic iron buried inside the cupin barrel in a distorted octahedral geometry. In addition, a FeS₄ center is found close to the solvent surface but its biochemical function is unknown. Based on the HAD crystal structures, mutagenesis studies were carried out and an enzymatic mechanism is proposed.