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Structural and Kinetic Analysis of Mutants of an Active Site Base in a Non-Heme Extradiol Dioxygenase.

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The key step in aerobic microbial degradation of aromatic compounds is often performed by a dioxygenase enzyme, which catalyzes aromatic ring cleavage. The iron containing dioxygenase enzymes consist of two families, extradiol (Fe^{2+}) and intradiol (Fe^{3+}). These enzymes have shown very high substrate and cleavage specificity. Homoprotocatechuate 2,3-dioxygenase (HPCD) from the Gram-positive soil bacterium Brevibacterium fuscum is an extradiol dioxygenase that catalyzes the ring cleavage of 3,4-dihydroxyphenylacetate to α-OH-δ-carboxymethyl cis,cis-muconic semialdehyde. One residue thought to be important in ring cleavage specificity in this enzyme is the highly conserved H200. A series of mutations at this site have been created and, to date, three of these mutants H200N, H200Y and H200F have been crystallized. H200Y is red in color; H200N kinetic data reveals an oxygenated intermediate not seen in wild type enzyme; and H200F has been shown to switch from extradiol cleavage to intradiol cleavage of an alternate substrate. Data from these mutants and their complexes are currently being collected and analyzed. Insights into the molecular mechanism resulting from this analysis will be presented.