Analysis of Mutants of an Active Site Base in a Non-heme Extradiol Dioxygenase

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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 by insertion of both atoms of molecular oxygen into the ring. HPCD is an Fe^{2+} containing, colorless enzyme that has shown very high substrate cleavage fidelity. One of the residues thought to provide some of this specificity is the highly conserved H200 [1]. In the current mechanistic model, H200 acts as an active site base to activate substrate for oxygen addition [2]. 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 [1]. 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.

[1] Groce S.L., Lipscomb J.D., *JACS*, 2003, 11780. [2] Vetting M.W., Wackett L.P., Que L., Lipscomb J.D., Ohlendorf D.H., *Journal of Bacteriology*, 2004, 1945.

Keywords: enzymatic structure-activity relationships, enzyme mechanism kinetics, metalloenzymes