Transient Intermediates in the Extradiol Dioxygenase Reaction Cycle

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The mononuclear nonheme Fe(II)-containing enzyme homoprotocatechuate 2,3-dioxygenase (HPCD) catalyzes the insertion of both atoms of oxygen from O_2 into the substrate (HPCA) resulting in proximal extradiol ring cleavage. By using the chromophoric substrate 4-nitrocatechol (4NC), it is possible to observe a single turnover cycle using stopped flow techniques.[1] The anaerobic substrate binding process occurs in at least 3 steps, resulting in a chelated dianionic Fe(II)-4NC complex. In contrast, the natural substrate is known from spectroscopic studies to bind as a chelated monoanion. Nevertheless, both complexes react with O_2 to yield analogous ring cleavage products. This reaction occurs in at least 4 more transient steps, but the key oxy-intermediate for the WT-HPCD cannot be detected. Mutagenesis of the 2nd sphere active site residue His200 to Asn, Glu, Gln, Phe, and Ala cause little change in the rate constants for 4NC binding, but slows several steps in the O_2 reaction half cycle.[2] Substantial decrease in the rate constant for reaction of bound O_2 on substrate allows the oxy-intermediate to be detected for the first time in an extradiol dioxygenase. This species absorbs near 600 nm ($\varepsilon \approx 600\ M^{-1}\ cm^{-1}$) suggesting that it is an Fe(III)-superoxo or peroxo species with substrate also bound to the iron. The dominant product from 4NC turnover by the H200 mutants is the 4NC quinone rather than the ring-cleaved adduct. In contrast, HPCA oxidation still results in the usual ring-cleaved product. The results suggest that His200 plays an important role in the second half of the extradiol reaction cycle in which oxygen is inserted and the aromatic ring is cleaved. Non-acid/base residues are much less efficient in promoting the initial oxygen reaction with substrate, the Criegee rearrangement reaction that targets ring opening, and product release. Nevertheless, the nature of the substrate affects each of these steps, suggesting that both 2nd sphere residues and the substrate substituent inductive effects determine the reaction progress and outcome. Supported by NIH GM24689.

[1] Groce, S. L.; Miller-Rodeberg, M. A.; Lipscomb, J. D. *Biochemistry* **2004**, *43*, 15141-15153

[2] Groce, S. L.; Lipscomb, J. D. *Biochemistry* **2005**, *44*, in press.