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Substrate-Specific Coupling of O_2 Activation to Hydroxylations of Aromatic Compounds by Rieske Non-heme Iron Dioxygenases

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Abstract:

Rieske dioxygenases catalyze the initial steps in the hydroxylation of aromatic compounds and are critical for the metabolism of xenobiotic substances. Because substrates do not bind to the mononuclear non-heme Fe^{II} center, elementary steps leading to O_2 activation and substrate hydroxylation are difficult to delineate, thus making it challenging to rationalize divergent observations on enzyme mechanisms, reactivity, and substrate specificity. Here, we show for nitrobenzene dioxygenase, a Rieske dioxygenase capable of transforming nitroarenes to nitrite and substituted catechols, that unproductive O_2 activation with the release of the unreacted substrate and reactive oxygen species represents an important path in the catalytic cycle. Through correlation of O_2 uncoupling for a series of substituted nitroaromatic compounds with ^{18}O and ^{13}C kinetic isotope effects of dissolved O2 and aromatic substrates, respectively, we show that O_2 uncoupling occurs after the rate-limiting formation of Fe^{III}-(hydro)peroxo species from which substrates are hydroxylated. Substituent effects on the extent of O_2 uncoupling suggest that the positioning of the substrate in the active site rather than the susceptibility of the substrate for attack by electrophilic oxygen species is responsible for unproductive O_2 uncoupling. The proposed catalytic cycle provides a mechanistic basis for assessing the very different efficiencies of substrate hydroxylation vs unproductive O_2 activation and generation of reactive oxygen species in reactions catalyzed by Rieske dioxygenases.

Keywords: non-heme ferrous iron oxygenases, nitrobenzene dioxygenase, bio catalysis, O_2 uncoupling, isotope effects, xenobiotics