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Xiaoshi Wang

A Novel Heme-Thiolate Peroxygenase *Aae*APO and Its Implications for C–H Activation Chemistry

Doctoral Thesis accepted by
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X. Wang, S. Peter, R. Ullrich, M. Hofrichter and J. T. Groves, Driving Force for Oxygen Atom Transfer by Heme-Thiolate Enzymes, *Angew. Chem. Int. Ed.*, **2013**, 52 (35), 9238–9241

X. Wang, S. Peter, M. Kinne, M. Hofrichter and J. T. Groves, Detection and Kinetic Characterization of a Highly Reactive Heme-Thiolate Peroxygenase Compound I, *J. Am. Chem. Soc.* **2012**, 134 (31), 12897–12900

S. Peter, M. Kinne, X. Wang, R. Ullrich, G. Kayser, J. T. Groves and M. Hofrichter, Selective Hydroxylation of Alkanes by an Extracellular Fungal Peroxygenase, *FEBS J.*, **2011**, 278, 3667–75.

Dedicated to my loving parents

Supervisor's Foreword

In Dr. Wang's thesis, she examined the mechanism of action of a new extracellular heme-thiolate P450 peroxygenase. P450 enzymes are versatile catalysts that carry out a number of important and difficult modifications. For decades, the application of this enzyme class in the industry has been a major goal; however, their instability and lack of solubility has precluded wide-spread industrial use. Dr. Wang's thesis focused on an extracellular P450 enzyme that does not have many of these limitations. In her thesis she demonstrates that the peroxygenase catalyzes a wide scope of reactions, in some cases very difficult transformations in molecules that are highly inert. Her detailed investigations provide a mechanistic framework for how the peroxygenase catalyzes this wide array of reactions. A major highlight of her thesis is the identification of key short-lived intermediates in the catalytic cycle of the peroxygenase, using rapid kinetic and spectroscopic methods, as well as elucidation of the thermodynamic properties of these high-energy intermediates. Heme-thiolate P450 enzymes have been studied for over 40 years, yet the thermodynamic information gathered on the peroxygenase by Dr. Wang is a major 'first'. Her work adds new insight into an important class of enzymes.

Princeton
August 2015

Prof. Mohammad R. Seyedsayamdost

Abstract

*Aae*APO, a novel extracellular heme-thiolate peroxygenase, from the agaric fungus *Agrocybe aegerita* was recently discovered to catalyze the cytochrome P450-like monooxygenation of diverse organic compounds, using hydrogen peroxide as a cosubstrate. In this dissertation, the function and mechanism of alkane hydroxylation reactions catalyzed by *Aae*APO are addressed.

In Chap. 1, current studies on the functions and mechanisms of heme-thiolate enzymes are reviewed. In Chap. 2, *Aae*APO is found to catalyze various alkane hydroxylation reactions with high efficiency and selectivity. In Chap. 3, the hydroxylation event is probed with intramolecular kinetic hydrogen isotope effect substrates and radical clocks. Reasonable KIEs and the presence of radical rearranged alcohol products indicate the hydrogen atom abstraction step and the rebound mechanism. In Chap. 4, *Aae*APO compound I (oxo-Fe^{IV} porphyrin radical cation) is detected and kinetically characterized by using the UV-vis, rapid-mixing stopped-flow spectroscopy. The kinetics of *Aae*APO-I toward a panel of alkanes is directly measured and results in extraordinarily fast second-order rate constants. Both the shape and slope of Brønsted-Evans-Polanyi plot suggest that the reaction is entropically controlled with an early transition state for weaker C–H bonds. Additionally, in Chap. 5, the redox potentials of the couple *Aae*APO-I/ferric *Aae*APO are determined over a wide range of pHs, based on the reversible oxygen atom transfer between *Aae*APO-I and halide ions. This analysis has allowed the highly reactive *Aae*APO-I intermediate to be placed on an absolute energy scale for the first time. In Chap. 6, the rebound intermediate, *Aae*APO compound II (Fe^{IV}-OH), is generated with a high yield by a one-electron direct reduction of *Aae*APO-I, using nitroxides as the reducing reagents. *Aae*APO-II is characterized to have a basic pK_a of 10. The protonated nature of *Aae*APO-II at physiological conditions proves its role as the rebound intermediate. The kinetics of *Aae*APO-II is also investigated and compared with those of *Aae*APO-I. Finally, in Chap. 7, the *apo* gene is cloned into *E. coli* and over-expressed. The resulting recombinant *Aae*APO has opened doors for many high potential applications, including industrial usage of *Aae*APO as a biocatalyst, site-directed mutagenesis, protein engineering for better biocatalysts, and further mechanistic studies.

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