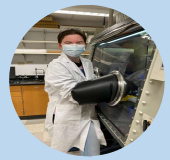
Synthetic Models of Ring Cleaving Dioxygenase Enzymes

Ellie L. Balensiefen, Laxmi Devkota, Adam T. Fiedler

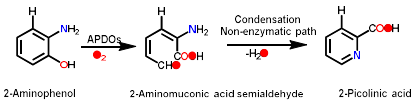
Department of Chemistry, Marquette University , Milwaukee, WI, 53233



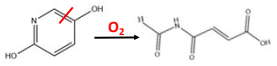
Background

- Large amounts of pollutants in freshwater and the soil create a need for bioremediation.
- Ring-cleaving dioxygenases are enzymes that catabolize (i.e., break down) pollutants by cleaving aromatic and heterocyclic rings. The enzymatic reactions incorporate both atoms of O_2 into the product.
- Two types of ring-cleaving are aminophenol dioxygenases (APDO) and 2,5-dihydroxypyridine dioxygenases (NicX).

Reaction catalyzed by APDO:

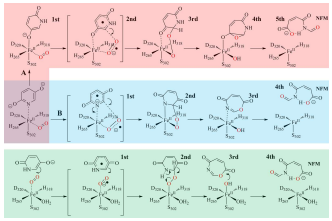


Reaction catalyzed by NicX:



Liu, G., Zhao, YL., He, F. *et al.* Structure-guided insights into heterocyclic ring-cleavage catalysisof the non-heme Fe (II) dioxygenase NicX. *Nat Commun* **12**, 1301 (2021)

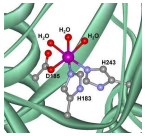
Potential NicX mechanisms



- The active sites of APDO and NicX contain a mononuclear nonheme iron(II) center.

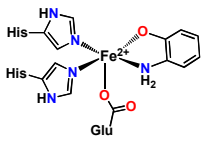
Active Site Structures

APDOs



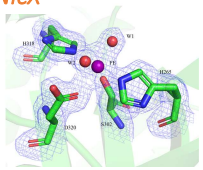
2-His-1-carboxylate (2H1C) facial triad

Koehntop, K. D. et al., *J. Biol. Inorg. Chem.*, **2005**, *10*, 87.



Enzyme-substrate complex

NicX



Liu, G., Zhao, YL., He, F. *et al.* *Nat Commun* **12**, 1301 (2021)

Our Approach

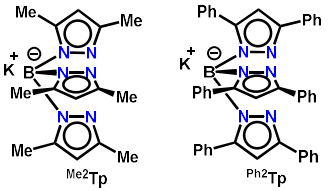
- **Motivation:** The development of synthetic APDO models has the potential to reveal key aspects of enzymatic structure and mechanism. Additionally, to our knowledge there are no functional or structural models of the NicX active site which could shed light on its catalytic function

Bio-Inspired Inorganic Chemistry

- Synthetic models are more easily prepared, characterized, and modified than the enzymes themselves.
- Trapping intermediates is more easily done with synthetic methods since there is greater control over reaction conditions (solvent, temperature, etc.).

Hydrotrispyrazolyl (Tp) Ligands

- Tp ligands mimic the 2-His-1-carboxylate facial triad of nonheme iron dioxygenases (see above for APDO).
  - Facially-coordinating and monoanionic.
- Easily modified by changing the substituents on the pyrazole ring. Ligands with diphenyl- and dimethyl- groups are commonly used in our experiments.
- Accessible ligands: easy to synthesize, modify, and characterize.



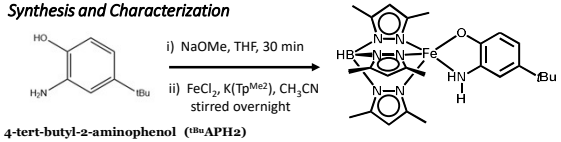
Structure of Tp ligands used in my research

APDO Active-Site Models

Experimental Techniques:

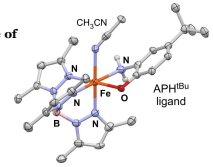
- X-Ray crystallography provides molecular structure.
- ^{1}H NMR spectroscopy is also used to identify structure and check for possible impurities
- Reactivity with dioxygen is explored with UV-vis absorption spectroscopy.
- Observed intermediates are further characterized with resonance Raman and electron paramagnetic resonance (EPR) spectroscopies.

Synthesis and Characterization



4-tert-butyl-2-aminophenol (^{tBu}APH2)

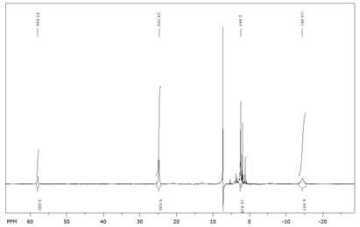
X-ray crystal structure of [Fe^{II}(Tp^{Me2})(^{tBu}APH)



- Acetonitrile solvent coordinates to Fe in the crystal.
- The Fe-N/O bond distances indicate the Fe(II) center is high-spin (S = 2).

Paramagnetic ^{1}H NMR spectrum of [Fe^{II}(Tp^{Me2})(^{tBu}APH)

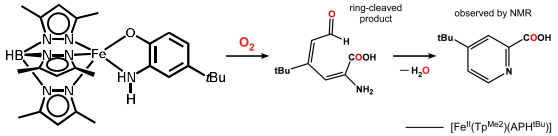
- Spectrum was measured at room temperature in $CDCl_3$.



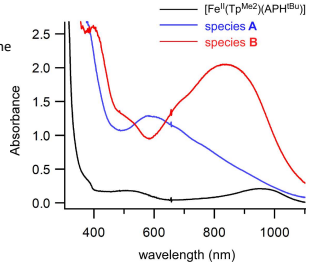
- Paramagnetic NMR: Interactions between the unpaired electrons of the Fe(II) center and the proton nuclei causes a widespread in chemical shifts (over 100 ppm). In contrast, peaks in spectra of diamagnetic molecules are typically between 0-10 ppm.
- Integration of the peaks indicates the number of equivalent hydrogens that contribute to the peak.

Dioxygen Reactivity

- Exposure of the Fe(II) complex to O_2 results in cleavage of the aminophenolate ring.



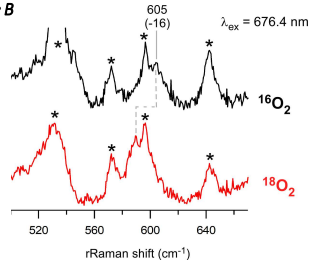
- Two intermediates are observed when the O_2 reaction is performed at low temperature (203 K) in Me-THF solvent.
  - Intermediate **A**: blue line.
  - Intermediate **B**: red line



Spectroscopic Studies of Intermediates

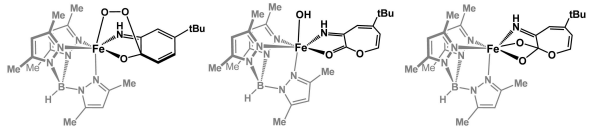
Resonance Raman Studies of Intermediate B

- Resonance Raman spectroscopy reveals the energies of molecular vibrations. Useful for identifying molecular structure.
- Samples for resonance Raman studies were generated with $^{16}O_2$ (black) or $^{18}O_2$ (red).
- Spectra were measured in frozen THF solutions with Kr+ laser excitation (λex = 676.4 nm).
- Peaks marked with an asterisk (*) arise from solvent.



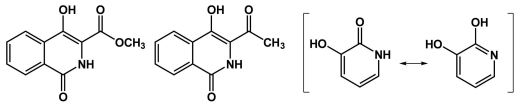
- Downshift in the peak at 605 cm^{-1} by 16 cm^{-1} upon $^{16}O_2$ / $^{18}O_2$ substitution shows that atoms from O_2 must be incorporated into product.

Possible Structures of Intermediate B based on resonance Raman data:



Synthesis of Ligands for NicX models

- Generated the dihydroxypyridine compounds shown here, which can serve as ligands for NicX models.



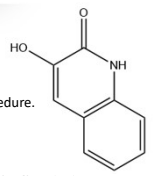
- Attempted to generate NicX models using these ligands. Procedure: (i) deprotonated ligand using sodium methoxide, (ii) reacted deprotonated ligand with $FeCl_2$ and Tp ligand.
- ^{1}H NMR data suggest that the target NicX models were generated. Current efforts are directed at obtaining X-ray crystal structures.

Conclusions

- We have learned more about the structure and reactivity of APDOs through the development of synthetic models.
- Along the way I learned about ligand synthesis, coordination chemistry, crystal growing, and spectroscopic techniques
- I am ready to work more independently in the coming semester.

Future Work

- Synthesize the 3-hydroxyquinolin-2(1H)-one ligand using a literature procedure.
- Prepare Co and Fe complexes using this ligand and the Tp scaffold.
- Study the structure and O_2 reactivity of the resulting NicX models.



3-Hydroxyquinolin-2(1H)-one

Acknowledgements

(CHE- 1900562)

Dr. Thomas Brunold at UW-Madison (access to resonance Raman instrument)