THESIS OF THE DISSERTATION

SELECTIVE METALLOENZYME-INSPIRED OXIDATIONS

Written by:

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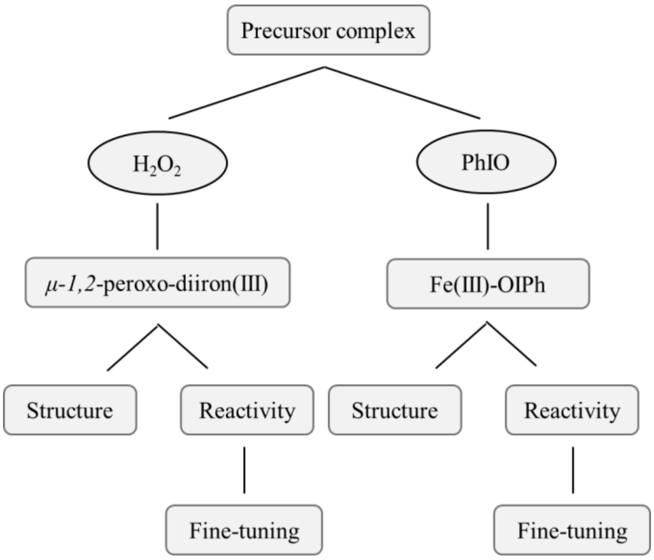
I. Introduction and objectives

Despite significant research efforts, oxidation processes catalyzed by reactive intermediates occurring in the active sites of enzymes remain poorly understood to this day. In oxidoreductase enzymes, the activation of dioxygen can give rise to a variety of intermediates. These may include high-valent iron(IV)-oxo species, which typically exhibit strong electrophilic character. The formation of such intermediates is a multistep process that can proceed through the transformation of several precursors, such as μ-peroxo-diiron(III) and Fe(III)-OR species. In many cases, these intermediates display ambiphilic reactivity, meaning they are capable of oxidizing both nucleophilic and/or electrophilic substrates.

My goal was to develop structural and functional models for these enzymatic systems. As structural models, I aimed to prepare and spectroscopically characterize *μ-1,2-peroxo*-diiron(III) and Fe(III)-OR intermediates by employing appropriately chosen ligands, oxidants, and coligands, taking into account the previous work of our research group. Furthermore, I investigated the influence of ligands and coligands on the redox properties of the complexes. For these structural models, I utilize iron(II) salts, bidentate ligands containing nitrogen donor atoms, monodentate nitrogen-donor heteroaromatic coligands (five- and six-membered rings), and various oxidants (e.g., H_2O_2 and PhIO). A central aim is the fine-tuning of the reactivity of the individual intermediates by modulating the electron density of the metal center through the coordinated ligands and coligands.

As functional models, I intend to investigate the oxidation of electrophilic and nucleophilic substrates via detailed kinetic studies, with a focus on correlating spectral and redox properties with reactivity and selectivity. An additional objective is the identification of the active intermediate that serves as the oxidant in the studied processes.



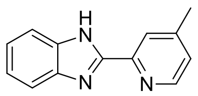
II. Experimental methods

The precursor complex was synthesized under inert conditions using standard Schlenk techniques. The enzyme model intermediates were generated *in situ* using either hydrogen peroxide (H_2O_2) or iodosobenzene (PhIO). The structures of the precursor complexes and the corresponding intermediates were characterized by UV-Vis spectrophotometry and cyclic voltammetry.

To determine catalase-like activity, gas volumetric measurements were performed using a thermostated gas burette. By monitoring the amount of O_2 evolved during the reaction, the kinetics of the process could be evaluated. Hydrogen atom transfer (HAT) and oxygen atom transfer (OAT) reactions were monitored by UV-Vis spectrophotometry, based on the characteristic absorption band changes of the intermediates. The effects of substrate concentration, substrate structure, and temperature on the reaction rate were also investigated. Experiments under catalytic conditions were carried out in glass reaction vessels, and the resulting products were identified using gas chromatography coupled with mass spectrometry (GC-MS).

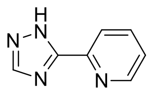
III. New scientific results

1. During my PhD work, I synthesized two Fe(II) complexes using ligands with two nitrogen donoratoms and different structures, two of which are new compounds. The structures of the complexes were thoroughly investigated by UV-Vis spectrophotometry and cyclic voltammetry, and in one case, the structure was also confirmed by single-crystal X-ray diffraction analysis.



L_2

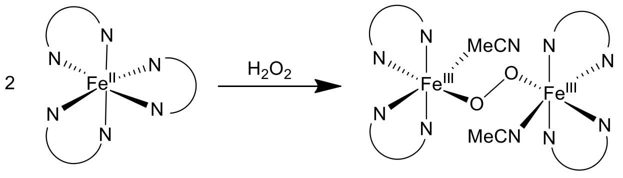
$[Fe^{II}(L_2)_3]^{2+}$ **(K_2)**

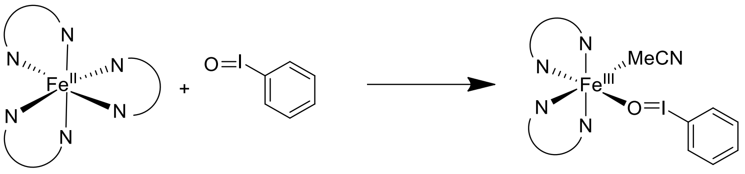


L_4

$[Fe^{II}(L_4)_3]^{2+}$ **(K_4)**

From the precursor complexes, I was able to generate μ-1,2-*peroxo*-diiron(III) intermediates using H_2O_2, which exhibit characteristic absorption bands in the 710–730 nm range. Furthermore, Fe(III) adducts were formed using iodosobenzene (PhIO), showing distinctive bands between 750–760 nm. The structures of these reactive intermediates were thoroughly investigated in all cases.



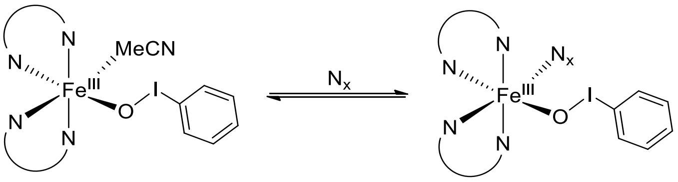


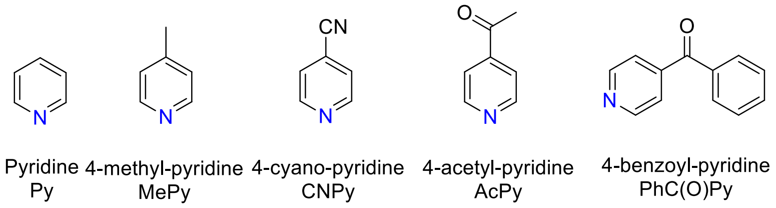
2. The *μ*-1,2-*peroxo*-diiron(III) intermediates were investigated as catalase enzyme models. The kinetics of the reaction were monitored using gas volumetric measurements by quantifying the amount of O_2 produced during the decomposition of H_2O_2. Two of the complexes had previously been partially studied as catalase models, which allowed for a direct comparison with the newly synthesized complexes. The observed second-order rate constants were 2.16×10^{-3} $M^{-1}s^{-1}$ (K_2), 9.67×10^{-2} $M^{-1}s^{-1}$ (K_4), and 1.13×10^{-2} $M^{-1}s^{-1}$ (K_5). According to the proposed mechanism, the *μ*-1,2-*peroxo*-diiron(III) intermediate is formed via reaction with the first H_2O_2 molecule. Subsequent cleavage of the peroxo bridge generates a high-valent Fe^{IV}-*oxo* species, which then reacts with another H_2O_2 molecule. Ultimately, the Fe^{II} complex is regenerated at the end of the catalytic cycle.

3. I investigated the reactivity of the adducts generated from the precursor complexes using PhIO in various oxidation reactions. Benzaldehyde and triphenylmethane were applied as substrates. The reactions mediated by the adducts were characterized by detailed kinetic measurements. Through the analysis of linear free energy relationships (*Hammett* correlations), I confirmed the electrophilic character of the intermediates (ρ_{K1} = -0,56, ρ_{K2} = -0,76). Furthermore, determination of the activation parameters provided evidence that the oxidation reactions in the studied systems proceed via a similar mechanism. The high kinetic isotope effect (*KIE* = 12-14) indicates a hydrogenatom abstraction process. No formation of high-valent Fe^{IV}-*oxo* intermediates was observed; instead, the adduct itself served as the active oxidant in the reactions.

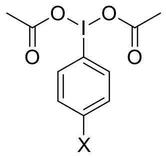
4. The reactivity of the Fe^{III}-iodosyl adduct was also investigated in the oxidation of thioanisole and styrene. The adduct proved to be active in both cases. Using *Hammett* correlation with thioanisole as the substrate, the electrophilic character of the adduct was confirmed ($\rho_{\text{thioanisole}}$ = -1,13), and based on the kinetic studies, a direct oxygenatom transfer (DOT) mechanism was proposed. In case of styrene, a characteristic 'V' shaped *Hammett* plot was obtained, indicating a radical-type mechanism, which was further supported by additional experiments. For both substrates, the Fe^{III}-iodosyl adduct was identified as the active oxidant.

5. Various *para*-substituted pyridine derivatives were applied as coligands to investigate their influence on the structure and reactivity of the Fe^{III}-iodosyl adduct. The use of different coligands allowed for fine-tuning of the reactivity to a significant extent, and the intermediate proved to be highly sensitive to changes in the electron density around the central metal ion.





The highest yields were obtained with pyridine derivatives bearing electron-withdrawing groups, such as CNPy. The electrophilic character of the adduct was further supported by the variation in its reactivity in the presence of different coligands. In addition, the effect of the oxidant's electron-withdrawing or electron-donating nature was also investigated. The Fe^{III}-iodosyl adduct was generated using various iodobenzene diacetate ($XPhI(OAc)_2$) derivatives.

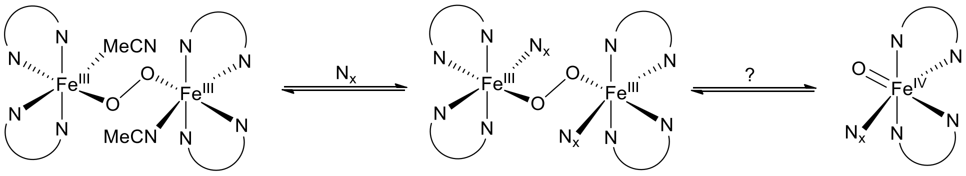


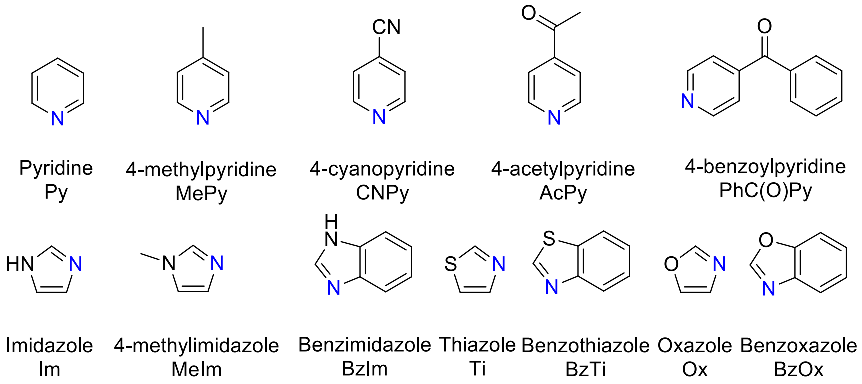
X = -OMe, -Me, -H, -Cl, -Ac

The results in this case also confirmed the electrophilic character of the adduct and provided clear evidence that the adduct itself acts as the active oxidant. The studied system represents one of the first well-characterized models in which the Fe^{III}-adduct functions as the active oxidizing species, rather than a subsequently formed Fe^{IV}-*oxo* intermediate.

6. I was able to confirm the nucleophilic character of the P_1 μ-1,2-*peroxo*-diiron(III) intermediate, generated from the K_1 precursor complex, in the oxidation of *p*-substituted benzaldehydes ($\rho = 0.67$). Furthermore, I also verified the ambiphilic nature of the P_5 intermediate, generated from the K_5 complex, toward both electrophilic and nucleophilic substrates. In the oxidation of benzaldehyde derivatives, the P_5 intermediate acted as a nucleophilic reagent ($\rho = 2.34$), whereas in the oxidation of *N,N*-dimethylamine derivatives, it exhibited electrophilic behavior ($\rho = -1.86$).

7. In the *μ*-1,2-*peroxo*-diiron(III) intermediate, one of the coordination sites of the Fe^{III}-ions remains essentially vacant. I investigated how the occupation of this free coordination site influences the structure and reactivity of the intermediate. Two groups of coligands were applied to study the effect of their electronic properties as well as the influence of additional heteroatoms present in the coligand structure.





The characteristic absorption band of the *μ*-1,2-*peroxo*-diiron(III) intermediate exhibited a significant, predominantly hypsochromic shift upon coligand coordination. The half-wave potential of the intermediate also shifted notably toward more negative values. These findings clearly demonstrate that the structure of the *μ*-1,2-*peroxo*-diiron(III) intermediate undergoes substantial changes depending on the nature of the coligands. The reactivity of the modified intermediates was investigated in the oxidation of benzaldehyde, 2-phenylacetaldehyde, and 2-phenylpropanal. My experiments confirmed that the inherently nucleophilic *μ*-1,2-*peroxo*-diiron(III) intermediate can be transformed into an electrophilic species through the influence of coligands. As such, the system provides a valuable model for enzymatic processes involving O_2 activation via peroxo intermediates at the active site.

IV. The significance of the research

My results contribute to a deeper understanding of the function of oxidoreductase enzymes, the formation of reactive intermediates, and the underlying chemical processes. I successfully demonstrated how a precursor complex can exhibit ambiphilic properties toward different oxidants. I also showed that the reactivity of the studied intermediates can be fine-tuned through the ligand framework of the precursor complex and by employing various monodentate coligands. To the best of my knowledge, this work provides the first description of a dinuclear synthetic enzyme model in which the μ-peroxo-diiron(III) intermediate displays nucleophilic character. Furthermore, I was among the first to describe catalytic systems in which the Fe(III)-OIPh intermediate acts not only as a precursor, but also as an active oxidant in the investigated oxidation reactions. These findings may open up new opportunities for the development of selective and finely tunable catalysts.

V. Publications forming the basis of the dissertation

Publications Underlying the Dissertation:

B. Kripli, F. V. Csendes, P. Török, G. Speier & J. Kaizer, Chem. Eur. J. **2019**, 25, 14290-14294. (**IF: 4,86**)

P. Török, D.Unjaroen, F. V. Csendes, M. Giorgi, W. R. Browne & J. Kaizer, Dalton Trans. **2021**, 50, 7181-7185. (**IF: 4,39**)

P. Török, D. Lakk-Bogáth, & J. Kaizer, Molecules, **2023**, 28(4), 1855. (**IF: 4,2**)

P. Török, D. Lakk-Bogáth, & J. Kaizer, Molecules, **2023**, 28(7), 2905. (**IF: 4,2**)

P. Török, D. Lakk-Bogáth, & J. Kaizer, Molecules, **2023**, 28(12), 4745. **(IF: 4,2)**

D. Lakk-Bogáth, D. Pintarics, P. Török, & J. Kaizer, Molecules, **2024**, 29(1), 58. **(IF: 4,2)**

P. Török, D. Lakk-Bogáth, D. Unjaroen, W. R. Browne, & J. Kaizer, J. Inorg. Biochem., **2024**. 112620. **(IF: 3,9)**

P. Török, & J. Kaizer, Molecules, **2024**, 29(16), 3842. **(IF: 4,2)**

Publications Not Directly Related to the Dissertation Topic:

D. Lakk-Bogáth, P. Török, F. V. Csendes, S. Keszei, B. Gantner & J. Kaizer, Molecules **2021**, 26, 4501. (**IF: 4,9**)

D. Lakk-Bogáth, P. Török, M. Giorgi, & J. Kaizer, J. Mol. Struct., **2022**, 1262, 133100. (**IF: 3,84**)

D. Lakk-Bogáth, M. I. Szávuly, P. Török, & J. Kaizer, Molecules, **2022**, 27, 2814. (**IF: 4,6**)

B. I. Meena, D. Lakk-Bogáth, P. Török, & J. Kaizer, Catalysts, **2023**, 13(1), 194. (**IF: 4,5**)

D. Lakk-Bogáth, P. Török, D. Pintarics, & J. Kaizer, Molecules, **2024**, 29(15), 3470. **(IF: 4,2)**

Presentations and Conference Attendance:

- ***May 26–28, 2025*** – 58th Colloquium on Coordination Chemistry, Balatonszárszó, Hungary (oral presentation)
- ***May 9–10, 2025*** – 18th Albert Szent-Györgyi Conference, Budapest, Hungary (oral presentation)
- ***November 11, 2024*** – *VEAB* Chemical Section: Innovative Chemical Research at the Faculty of Engineering, University of Pannonia, Veszprém, Hungary (oral presentation)
- ***October 23–26, 2024*** – 30th International Conference of Chemists, Cluj-Napoca, Romania (oral presentation)
- ***May 27–29, 2024*** – 57th Colloquium on Coordination Chemistry, Szeged, Hungary (oral presentation)
- ***October 25–28, 2023*** – 29th International Conference of Chemists, Târgu Mureș, Romania (oral presentation)
- ***June 27, 2023*** – Institutional ÚNKP Conference, University of Pannonia, Veszprém, Hungary (oral presentation)
- ***May 31, 2023*** – 56th Colloquium on Coordination Chemistry, Szeged, Hungary (oral presentation)
- ***December 9, 2022*** – Coordination Chemistry Working Group, Hungarian Academy of Sciences – 2nd Annual Meeting, Budapest, Hungary (oral presentation)
- ***June 8, 2022*** – Institutional ÚNKP Conference, University of Pannonia, Veszprém, Hungary (oral presentation)
- ***October 26–27, 2021*** – 44th Chemistry Presentation Days, Hungary (oral presentation)