Functional oxidase models based on dioximatomanganese(II)

Ph.D. Thesis

Imola Csilla Szigyártó

Supervisor: Prof. László Simándi

Loránd Eötvös University, Ph.D. School of Chemistry
Prof. György Inzelt (Ph.D. School leader)
Synthetic Chemistry, Material Science and Biomolecular Chemistry
Prof. István Tamás Horváth (Program leader)

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I. Introduction

The different metalloenzymes play important roles in nature. The oxidoreductase enzymes belong to one of its biggest subgroup. Some important examples are: catechol oxidase and dioxygenase, which catalyze the oxidation of catechols to quinones and are also involved in the formation of melanin pigments in human skin; or phenoxazinone synthase, which plays an important role in biosynthesis of the actinomycin; or manganese superoxide dismutase, which catalyzes the disproportionation of $O_2^{\bullet -}$ and protects the organism against oxidative stress.

Modeling the biological system is a widely used method in bioinorganic chemistry. If metalloenzymes are regarded as metal complexes embedded in proteins, the active sites can be modeled with low-molecular metal complexes. Enzyme models may be of two kinds, *viz*. structural and functional. Structural models mimic enzyme structure, whereas functional models mimic the mechanism of enzyme action.

The background of my doctoral work has been the research conducted in the *Biomimetic Catalysis* group on two functional models *viz*. [bis(dimethylglyoximato)-bis(triphenylphosphine)cobalt(II)], in short, cobaloxime(II), and [bis(dimethylglyoximato)-bis(*N*-methylimidazole)iron(II)], also known as ferroxime(II), which exhibit catechol oxidase and phenoxazinone synthase activity.
We use 3,5-di-*tert*-butylcatechol (H_2dbcat) and 2-aminophenol (H_3ap) as model substrate.

II. Objectives

The objective of my work was to model oxidase enzymes (catechol oxidase and phenoxazinone synthase) using a new manganese complex. The two dimethylglyoximato ligands of ferroxime(II) were replaced by dioximato ligands with less rigid coordination

spheres and the effects of changes on the kinetic parameters of catechol oxidase and phenoxazinone synthase activities were investigated. The main objectives are listed below.

1. Synthesis and characterization of the new manganese(II) complex using the H_2L ligand, where H_2L is $[HON=C(CH_3)C(CH_3)=NCH_2\text{-}CH_2]_2NH$. Role of the ligand: formation a new type of manganese complex, which has a practical applications.

2. The base-catalyzed oxidation of H_2dbcat to 3,5-di-*tert*-butyl-1,2-benzoquinone by O_2 take place in the presence of dilute NaOH. Detailed kinetic investigation of the remarkable accelerating effect that the manganese complex exerts on the triethylamine (TEA)-catalyzed oxidation of H_2dbcat in MEOH.

3. Temperature and pH effects on the kinetics of 2-aminophenol auto-oxidation in aqueous solution have been known. Elaboration of suitable reaction mechanism for the reaction in the presence of TEA and manganese complex consistent with the results of structural and kinetic studies.

4. The presence of anion radical and free radical in the case of cobaloxime(II) and ferroxime(II) complexes have been detected. The monitoring of the ESR spectra during the catalytic reaction provides information on the state of the catalyst complex and on the reaction intermediates.

5. The rate-limiting step can be supported by the kinetic isotope effect (KIE). Previous examples of ferroxime(II) and cobaloxime(II) complexes exhibited KIEs, indicating H-atom transfer from hydroxy group to coordinated O_2 in the rate-limiting step. Determination of the KIE value in the manganese catalyzed oxidation.

III. Experimental section

The ligand and the products were characterized by spectroscopic methods. The NMR spectra were recorded on a Varian Unity-Inova (400 MHz) spectrometer in a $CDCl_3$ solution. The IR spectra of ligand and metal complex were recorded on Avatar 320 (Thermo Nicolet) FT-IR spectrometer. The molecular structure of the dimeric manganese complex was determined on an Enraf-Nonius CAD4 diffractometer. Electrospray ionization mass spectrometry (ESI-MS) experiments were performed on a Perkin Elmer-Sciex API 2000 triple-quadrupole mass spectrometer. Samples were dissolved in MeOH. Electron spin resonance (ESR) spectra were recorded on a Bruker ELEXYS E500 CW-EPR spectrometer in MeOH and acetonitrile solutions.

The oxidation was followed by (i) recording the time evolution of UV/vis spectra on Hewlett-Packard 8453 diode-array spectrophotometer, at room temperature and (ii) by measuring the volume of O_2 absorbed. The rates of O_2 absorption uptake were measured in a constant-pressure gas-volumetric apparatus.

IV. New scientific results

1. We have demonstrated that the H_2dmdt ($[HON=C(CH_3)C(CH_3)=NCH_2\text{-}CH_2]_2NH$) ligand can react with manganese-chloride in methanol solution and after the addition of $NaB(C_6H_5)_4$ a dimeric Mn(II) was formed.

$$2\ MnCl_2 + 2\ H_2dmdt + 2\ NaBPh_4 \longrightarrow [Mn_2(HL)_2](BPh_4)_2 + 2\ NaCl$$

We have characterized the complex by elemental analysis, electrospray ionization mass spectrometry, electron spin resonance spectroscopy and X-ray diffraction.

2. The molecular structure shows that each monomeric unit contains one HL^- ligand, having one oximato(1-) and one oxime group. The coordination spheres of both Mn atoms are strongly distorted octahedra. The two monomeric units are bound together via *two oximato bridges*. The intramolecular H bonding conferring additional stability on the dimer.

   Upon dissolution in methanol, the dimer dissociates into monomeric units at room temperature. This observation is supported by mass spectrometry and ESR spectroscopy.

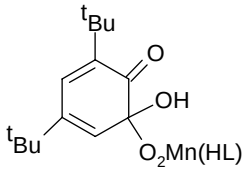
3. We have established that the complex $[Mn(Hdmdt)(MeOH)]^+$ does not react with O_2 nor does it catalyze the oxidation of 3,5-di-*tert*-butylcatechol (H_2dbcat) to the corresponding *o*-benzoquinone (dtbq) in methanol solution. However, when the complex added to a solution in which the triethylamine (TEA)-catalyzed oxidation of H_2dbcat is in progress, it brings about a remarkable increase in the oxidation rate. This is due to a new reaction path, which we termed "manganese-enhanced base catalysis" and it was first observed by us.

4. For establishing the kinetic parameters we have carried out a detailed kinetic study of the oxidation by measuring the rate of dioxygen absorption under pseudo-first order conditions by the gas-volumetric technique.

   We have established the partial kinetic orders with respect to the individual reactants. The rate is of first order with respect to the catalyst and O_2, and shows saturation behavior with respect to the substrate and TEA concentration.

5. Based on the empirical rate law, we have proposed a reaction mechanism. The oxidation of H_2dbcat in the presence of TEA takes place *via* deprotonation to the monoanion followed by the formation of the O_2 adduct $HdbcatO_2^-$. This compound is capable of coordinating to the manganese complex *via* its peroxo group.

The active ternary complex has the following structure:



In the rate-limiting step this active intermediate eliminates a semiquinone anion radical ($dbsq^{\bullet -}$), which has been detected by ESR spectroscopy. In a subsequent fast step $dbsq^{\bullet -}$ is oxidized to the final product dtbq.

6. The proposed reaction mechanism is consistent with the observed kinetic behavior and leads to the following kinetic equation:

$$V_k = \frac{(k_B K_B + k_7 K_6 K_B [Mn]_o)\,[O_2]_o[H_2dbcat]_o}{1 + \{(K_C/K_T)\,[H_2dbcat]_o / [TEA]_o\}}$$

We have established that both reaction paths (base-catalyzed and Mn-enhanced oxidation paths) contribute to the oxidation of 3,5-di-*tert*-butylcatechol to the corresponding *o*-benzoquinone.

7. The kinetic isotope effect (KIE) of the combined base-catalyzed and Mn-enhanced catecholase reaction was found to be 1.02. The lack of a significant KIE indicates no direct transfer of a H atom from the aromatic OH group to the superoxo ligand in the rate-limiting step.

8. We have established that the complex $[Mn(Hdmdt)(MeOH)]^+$ does not react with O_2, nor does it catalyze the oxidation of 2-aminophenols (H_3ap) to 2-amino-3*H*-phenoxazine-3-one (apx) in methanol solution However, when the complex added to a solution in which the triethylamine (TEA)-catalyzed oxidation of H_3ap is in progress, it brings about a

remarkable increase in the oxidation rate. This is an other example of a new reaction path, which we termed "manganese-enhanced base catalysis".

9. For establishing the reaction mechanism, we have carried out detailed kinetic studies of the catalytic oxidation by measuring the initial rate of amino-phenoxazinone formation as a function of the catalyst, dioxygen, substrate and triethylamine concentration. The initial rates of amino-phenoxazinone formation were measured by UV-spectroscopy. The rate is of first order with respect to catalyst and O_2, and shows a saturation behavior with respect to the substrate and TEA concentration.

10. We have established that the kinetic results are consistent with the reaction mechanism. The kinetic equation of "manganese-enhanced base catalysis" can be given as the contribution of both reactions paths in the following form:

$$V_k = \frac{(k_B + k_6 K_5 [Mn]_o) K_B [O_2]_o [H_3ap]_o}{1 + \{(K_A/K_T) [H_3ap]_o / [TEA]_o\}}$$

The free radical ($Hapx^{\bullet}$) has been detected by ESR spectroscopy.

Publications based on the thesis work

1. **Imola Cs. Szigyártó**, László I. Simándi, László Párkányi, László Korecz, Gitta Schlosser: Biomimetic Oxidation of 3,5-Di-*tert*-butylcatechol by Dioxygen *via* Mn-Enhanced Base Catalysis.
   Inorg. Chem., **2006**, *45*, 7480-7487. (IF:3.851)

2. **Imola Cs. Szigyártó**, Tatiana M. Simándi, László I. Simándi, László Korecz, Nóra Nagy: A functional phenoxazinone synthase model based on dioximatomanganese(II). Kinetics and mechanism of the catalytic oxidation of 2-aminophenols by dioxygen.
   J. Mol. Catal. A: Chem., **2006**, *251*, 270-276. (IF: 2.348)

3. Tatiana M. Simándi, Zoltán May, **Imola Cs. Szigyártó**, László I. Simándi: Hydrogen atom *vs* electron transfer in catecholase-mimetic oxidations by superoxometal complexes. Deuterium kinetic isotope effects.
   Dalton Trans., **2005**, 365-368. (IF:3.003)

Conference posters and lectures

1. Szigyártó I.Cs., Simándi L.: Dioximáto-mangán(II)komplex előállítása, szerkezetvizsgálata és katalitikus aktivitása
   ELTE-TTK Kémia Doktori Iskola, Budapest 2003. november 7 (lecture)

2. I.Cs. Szigyártó, G. Schlosser, L. Párkányi and L.I. Simándi: Synthesis, structure and reactivity of hydroxyimino Schiff's base complexes of manganese(II)
   28th International Conference on Solution Chemistry, August 24-29, 2003 Debrecen, Hungary (poster)

3. I.Cs. Szigyártó, G. Schlosser, L. Párkányi and L.I. Simándi: Synthesis, structure and reactivity of hydroxyimino Schiff's base complexes of manganese(II)
   4th International School of Organometallic Chemistry, September 6-10, 2003 Camerino, Italy (poster)

4. L.I. Simándi, T.M. Simándi, Z. May and I.Cs. Szigyártó: Biomimetic activation of dioxygen by iron and manganese complexes
   COST-Workshop, April 24-25, 2004 Girona, Spain (lecture)

5. Szigyártó I.Cs., Simándi L., Párkányi L., Győr M., Schlosser G.: Dioximáto-mangán(II) komplex előállítása, szerkezetvizsgálata és katalitikus aktivitása
   MTA-KKKI, VII. Doktori Kémiai Iskola, Tahitótfalu 2004. április 27-28 (lecture)

6. Szigyártó I.Cs., Simándi L., Párkányi L., Győr M., Schlosser G.: Dioximáto-mangán(II) komplex előállítása, szerkezetvizsgálata és katalitikus aktivitása
   XXXIX. Komplexkémiai Kollokvium, Gárdony 2004. május 26-28 (lecture)

7. Szigyártó I.Cs., Simándi L., Párkányi L., Győr M., Schlosser G.: Dioximáto-mangán(II) komplex előállítása, szerkezetvizsgálata és katalitikus aktivitása
   MTA Kutatóközponti Tudományos Napok, Budapest 2004. június 2-3 (lecture)

8. L.I. Simándi, T.M. Simándi, Z. May and I.Cs. Szigyártó: Catalytic activation of dioxygen by dioximatoiron(II) and -manganese complexes. Hydrogen atom *vs* electron transfer mechanisms
   14^{th} International Symposium on Homogeneous Catalysis ISHC-14, July 7-9, 2004 München, Germany (lecture)

9. I.Cs. Szigyártó, L.I. Simándi, L. Párkányi, Gy. Miklós, G. Schlosser: Synthesis, structure and reactivity of a dioximatomanganese(II) dimer
   MTA Nemzetközi Tudományos Tanácsadó Testület tudományos ülése, Budapest 2004. szeptember 1-3 (lecture)

10. I.Cs. Szigyártó, L.I. Simándi: A new dioximatomanganese(II) dimer: synthesis and catecholase-mimetic activity
    COST-D21 Workshop, September 25-30, 2004 Seggau, Austria (lecture)

11. Szigyártó I.Cs., Simándi L.: A dioxigén biomimetikus aktiválása dioximátomangán(II) komplexszel
    XL. Komplexkémiai Kollokvium, Dobogokő 2005. május 18-20 (előadás)

12. I.Cs. Szigyártó, L.I. Simándi: A dioximatomanganese(II) model of phenoxazinone synthase-related metalloenzyme action
    COST-D21 Workshop, May 26-29, 2005 Roma, Italy (lecture)

13. Szigyártó I.Cs., Simándi L.: A dioxigén biomimetikus aktiválása dioximátomangán(II) komplexszel
    MTA Kutatóközponti Tudományos Napok, Budapest 2005. június 1-2 (lecture)

14. I.Cs. Szigyártó, L.I. Simándi, L. Párkányi, L. Korecz and G. Schlosser: Biomimetic activation of dioxygen by a dioximatomanganese(II) complex
9th International Symposium Activation of Dioxygen and Homogeneous Catalytic Oxidation (ADHOC 2005), July 25-29, 2005 Cologne, Germany (poster)

15. I.Cs. Szigyártó, L.I. Simándi: Activation of dioxygen by a manganese(II)-based phenoxazinone synthase model
9th International Symposium Activation of Dioxygen and Homogeneous Catalytic Oxidation (ADHOC 2005), July 25-29, 2005 Cologne, Germany (lecture)

16. I.Cs. Szigyártó, T.M. Simándi, L. Korecz and L.I. Simándi: ESR studies of free radical intermediates in the catalytic oxidation of aminophenol and catechol derivatives
1st Joint Working Group Meeting and 2nd Management Committee Meeting of the COST P15 action, October 26-28, 2005 Budapest, Hungary (poster)

17. Szigyártó I.Cs., Simándi L.: Metalloenzim modellezés átmentifém komplexekkel, MTA-KKKI, IX. Doktori Kémiai Iskola, Tahitótfalu 2006. április 24-26, (lecture)

18. Szigyártó I.Cs., Simándi L.: Metalloenzim modellezés mangán(II) komplexszel, XLI. Komplexkémiai Kollokvium, Mátrafüred 2006. május 31-június 2, (lecture)