INVESTIGATION OF OXIDOREDUCTASE MIMICS

THESES OF THE Ph.D. DISSERTATION

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I. INTRODUCTION AND AIM OF THE WORK

Chemical reactions in living organisms take place in the form of reactions consisting of several steps, generally each step is catalyzed by different enzymes. For many enzymes metal ions are essential cofactors and they are directly involved in the enzymatic reaction. Significant development of the so-called biomimetic chemistry has started in the last two decades, which aimed at determining the mechanism of metalloenzymes and the essential structural characteristics of the active center. This branch of science operates on the borderline of biology and chemistry tries to explore the structural, spectroscopic characteristics (structural models) of enzymes and also the processes at the active center, in the coordination sphere of the metal ion (functional models) with the help of compounds with small molecular mass.

Spectroscopic, structural and mechanistic investigation of metal complexes would thus be of great interest as they may give ideas to understanding the catalytic mechanism of the enzymatic systems.

II. EXPERIMENTAL METHODS

Handling of the air sensitive materials was done under a normal atmosphere of dry and deoxygenated argon utilizing standard Schlenk-techniques. Solvents were dried and distilled under an atmosphere of argon according to standard procedures. Numerous separational and structural methods (UV-VIS, IR, NMR, GC, MS, EPR, Resonance Raman, Mössbauer, X-ray, elemental analysis) were used to isolate and characterize the intermediates and products. Electron spectroscopy and GC methods were used for monitoring the conversion of substrates for kinetic measurements.

III. NEW SCIENTIFIC RESULTS

1. Flavonol 2,4-dioxygenase model systems

1.a. We synthesised and characterised (UV-VIS, IR, Mössbauer, X-ray, elemental analysis) iron- and manganese-containing complexes – namely $[Fe^{III}(4'MeOfla)_3]$, $[Fe^{III}(4'Rfla)(salen)]$, $[Fe^{III}(O\text{-bs})(salen)]$ and $[Mn^{II}(fla)_2(py)_2]$ – in order to obtain information on the possible binding of the substrate to the metal ion and to use them in oxygenation

reactions to study the oxidative ring splitting of the heterocyclic ring in the substrate. Com parison with the copper-containing models was also done.

1.b. After the characterization of the prepared complexes, their flavonol 2,4-dioxygenase activity was examined. The manganese and iron flavonolate complexes in DMF solutions are stable under anaerobic conditions and oxidisied upon addition of dioxygen at high temperatures. The CO content was determined in all cases by GC-MS (80-90%). The GLC-MS analysis of the residue of the hydrolyzed complexes, after treatment with etheral diazomethane, showed the presence of the *O*-benzoylsalicylic acid methylester. Provided that a mixture of $^{18,18}O_2$ and $^{16,16}O_2$ is used in the oxygenation, the composition of the product permits identification of the place for the dioxygen incorporation. As a conclusion it can be said that in the enzyme-like oxygenation of the coordinated flavonolate ligand (of manganese(II) and iron(III)) the formation of endoperoxide in bimolecular reactions can be assumed. The unique decomposition of this species accompanied by the loss of carbon monoxide results in the corresponding *O*-benzoylsalicylate complexes as yielding a good mimic of the enzymatic action. On the basis of the *k* values (compared to our earlier copper-containing systems), it can be said that the reactivity order is Fe > Mn > Cu.

1.c. We have found that the rate of dioxygenolysis is dramatically enhanced by various coligands, such as acetate ($CH_3CO_2^-$), phenyl- ($PhCH_2CO_2^-$), diphenyl- ($Ph_2CHCO_2^-$) or triphenyl acetate ($Ph_3CCO_2^-$). For example, addition of 10 equivalents of the bulky $Ph_3CCO_2^-$ to [Fe^{III}(fla)(salen)] accelerated its decay by two orders of magnitude (V_r = 171), and the reaction above can take place even at ambient temperature (20 ºC). The main mechanistic difference between the direct and carboxylate-enhanced dioxygenation of [Fe^{III}(fla)(salen)] is that in the latter case there is an electron transfer from [Fe^{III}(fla)(salen)] to dioxygen resulting in the formation of free $O_2^{\cdot-}$, which was proved by the test for free $O_2^{\cdot-}$ with nitroblue tetrazolium (NBT). On the basis of chemical, spectroscopic and kinetic data it can be said that bulky carboxylates as coligands dramatically enhance the reaction rate, which can be explained by two different pathways, caused by the formation of more reactive monodentate flavonolatoiron complexes.

2. Modeling of ACC oxidase enzyme

We reported for the first time an iron complex ([Fe^{III}(salen)Cl]) that efficiently catalyses the oxidation of amino acids (1-aminocyclopropane-1-carboxylic acid (accH), 2-aminoisobutyric acid (aibH) and alanine (alaH)) to ethylene and the corresponding carbonyl compounds in the presence of base (NH_4OH) with H_2O_2 as oxidant. The optimum conditions used for the amino acid oxidation by this catalytic system was catalyst, oxidant, base and substrate in a molar ratio of 1:5000:5000:5000, respectively. In the amino acid oxidation, the relative reactivities of the substrates used are in the following order: aibH > alaH > accH. To get direct evidence for the fact that high-valent oxoiron species are capable for the amino acid oxidation we have chosen the [Fe^{II}(N4Py)$(ClO_4)_2$] complex as a possible candidate, because its oxoiron(IV) intermediate was isolated and fully characterised by various methods. Comparision the acetone production rates employing [Fe^{II}(N4Py)$(ClO_4)_2$] and [Fe^{III}(salen)Cl] as catalysts, the conversion of aibH to acetone was found to be 2-3 times faster in the former case. The measured SIEs values for both catlysts above are identical within experimental error, with values ca. 1.4, suggesting a similar rate determining step. On the basis of chemical, spectroscopic and kinetic data a plausible mechanism can be proposed for both systems assuming ternary, high-valent oxoiron complex formation between catalyst, substrate and H_2O_2 in a fast pre-equilibrium step, followed by the PCET (proton-coupled electron transfer) between the coordinated substrate and the oxoiron moieties. Thus a substrate radical is formed in the rate-determining step.

3. Investigation of catechol oxidase mimics

3.a. The mononuclear [Mn(6'Me_2indH)$(H_2O)_2(CH_3CN)](ClO_4)_2$ (6'Me_2indH: 1,3-bis(6'-methyl-2'-pyridylimino)isoindoline) complex has been prepared and characterized by various techniques such as elemental analysis, IR, UV-vis and ESR spectroscopy. The Mn(II) ion is bound to a neutral tridentate isoindoline ligand, two waters and one acetonitrile molecule, forming a pseudooctahedral environment around the metal ion. Analytical data, the infrared and the electronic spectrum of the complex are all consistent with the presence of a neutral, nondeprotonated isoindoline ligand. In order to gain insight into the mechanism of these reactions we initiated studies on manganese-mediated oxidation of phenol derivatives.

3.b. The catalytic oxidation of 3,5-di-*tert*-butylcatechol has been studied as a model reaction for the catecholase activity of tyrosinase and catechol oxidase. The catecholase-like activity of the complex $[Mn(6'Me_2indH)(H_2O)_2(CH_3CN)](ClO_4)_2$ was examined in DMF at 40°C. The kinetic studies on the oxidation of 3,5-$DTBCH_2$ were carried out by the method of initial rates by monitoring the increase in the characteristic quinone (3,5-DTBQ) absorption band at 400.5 nm as a function of time. In the series of 3,5-$DTBCH_2$, 4-*tert*-butylcatechol (4-$TBCatH_2$), 4-methylcatechol (4-$MeCatH_2$) and catechol ($CatH_2$) the redox potentials increase due to the decreasing electron donating potential of the catechol substituents and the resulting decrease in Lewis basicity of the catechols. With a lower electron donating character (higher redox potential) of the substrate, the semiquinone character of the catechol is decreased. Under these circumstances the attack of dioxygen is disfavoured. This study has demonstrated that the $[Mn(6'Me_2indH)(H_2O)_2(CH_3CN)](ClO_4)_2$ complex is an efficient catalyst for the catechol oxidation, which means that the investigated systems serve as functional models for catecholase enzymes. On the basis of the kinetic data plausible mechanisms was proposed.

4. Examination of catalase model systems

As model compounds we prepared two homoleptic manganese(II) complexes with 4'-substituted 1,3-bis(2'-pyridylimino)isoindolines, $[Mn(4'R\text{-}ind)_2]$ [R = H; Me]. These complexes are stable under ambient conditions and are not oxidized by atmospheric dioxygen. Addition of H_2O_2 to DMF solutions of complexes $[Mn(ind)_2]$ and $[Mn(4'Me\text{-}ind)_2]$ causes the immediate vigorous evolution of dioxigen coupled to color changes. The initial rate of disproportionation of H_2O_2 was measured as a function of the complex and substrate concentration in DMF. This study has demonstrated that the $[Mn(4'R\text{-}ind)_2]$ complexes are efficient catalysts for the dismutation of H_2O_2, which means that the investigated systems above serve as functional models for catalase enzymes. On the basis of kinetic data plausible mechanisms was proposed.

5. SOD-like activity of hexacoordinate manganese and iron complexes

Hexacoordinate manganese- ($[Mn(4'R\text{-}ind)_2]$ [R = H; Me]) and iron-containing ($[Fe^{II}(1'Me_2bim_2ind)_2]$) complexes with isoindoline derivative ligands have been prepared and characterised by various spectroscopic methods (UV-VIS, IR, X-ray). The SOD-like activity of the prepared complexes was studied at 25°C by using a standard indirect method based on nitroblue tetrazolium (NBT), and cytochrome c reduction. The superoxide radical anion was

generated *in situ* by the xanthine/xanthine oxidase reaction, and detected spectrophotometrically by monitoring the formation of diformazan from NBT, or alternatively, the reduced form of cytochrome c. The SOD-like activity was then characterized by determining the IC_{50} values.

6. Catalytic activity of a non-heme iron complex in alcohol oxidation reactions

The oxidation of benzyl alcohol with hydrogen peroxide catalyzed by $[Fe^{II}(indH)(CH_3CN)(H_2O)_2](ClO_4)_2$ at room temperature occurs readily to form benzaldehyde in yield 34,4 %. Competitive reactions were also done with *para*-subsituted benzyl alcohol derivatives in order to evaluate the influence of electronic factors on the reaction. The relative reactivities show linear correlation with the σ constant of the Hammett equation. The reaction constant ρ was found to be large and negative (ρ = -1.3) demostrating that electron-releasing substituents enhance the reaction rate. This result suggests that oxidation of alcohols with H_2O_2 catalysed by $[Fe^{II}(indH)(CH_3CN)(H_2O)_2](ClO_4)_2$ proceed through polar transition states with charge transfer from the substrate to the iron. The addition of H_2O_2 to an acetonitrile (or acetone) solution of complex generates a transient green species, which decomposes at room temperature with in ~ 50 sec, but persists for over 0.5 h at -20°C. Based on spectroscopic studies (UV-VIS, EPR, Resonance Raman) we suggest that this green intermediate is a (μ-oxo)(μ-1,2-peroxo)-diiron(III) complex. Our experience in reaction kinetics will be utilized to reveal the mechanistic details, following the decay of the peroxo-diiron(III,III) species with UV-VIS spectroscopy at variable temperatures and different concentrations, and to provide plausible proposals for the mechanisms of the oxidative processes with respect to the active species involved.

7. SIGNIFICANCE OF THE SCIENTIFIC RESULTS

The presented work was carried out in order to get insight into the mechanism of the enzymes flavonol 2,4-dioxygenase, 1-aminocyclopropane-1-carboxylic acid oxidase, catechol oxidase, catalase and superoxide dismutase which play key role in the biosynthesis of various molecules in metabolic pathways and protect cells against such hazards. New manganese and iron complexes have been synthesized which were characterized with various spectroscopic and diffractometric methods. They served as good functional and structural models for the enzymes mentioned above. Detailed kinetic investigation of the catalytic reactions has been performed and reaction mechanisms were proposed.

V. SCIENTIFIC PUBLICATIONS AND PRESENTATIONS RELATED TO THE THESES

PUBLICATIONS

1. J. Kaizer, É. Balogh-Hergovich**, G. Baráth**, G. Speier, L. Párkányi, L. Que Jr.
Synthesis, structure and spectral properties of a novel iron(II) complex of 1,3-bis(2'-pyridylimino)isoindoline, and its catalytic activity on the alcohol oxidation with hydrogen peroxide
Inorg. Chem., (ready to be submitted)

2. **G. Baráth**, J. Kaizer, J. Pap, G. Speier, N. El Bakkari-Taheri, A. J. Simaan
Bio-inspired amino acid oxidation by a non-heme iron catalyst modeling the action of 1-aminocyclopropane-1-carboxylic acid oxidase
Chem. Commun., (accepted manuscript) DOI: 10.1039/c0cc01892a. (2010)

3. **G. Baráth**, J. Kaizer, L. Párkányi, G. Speier, E. Kuzmann, A. Vértes
One metal – two pathways on the carboxylate-enhanced, iron-containing quercetinase mimics
Chem. Commun., 3630-3632 (2009)

4. J. Kaizer, **G. Baráth**, R. Csonka, G. Speier, L. Korecz, A. Rockenbauer, L. Párkányi
Catechol oxidase and phenoxazinone synthase mimics of manganese(II) complex of 1,3-bis(6'-methyl-2'-pyridylimino) isoindoline, $[Mn(6'Me_2indH)(H_2O)_2(CH_3CN)](ClO_4)_2$
J. Inorg. Biochem., **102**, 773-780 (2008)

5. J. Kaizer, **G. Baráth**, J. Pap, G. Speier, M. Giorgi, M. Réglier
Manganese and iron flavonolates as flavonol 2,4-dioxygenase mimics
Chem. Commun., 5235-5237 (2007)

6. **G. Baráth**, R. Csonka, J. Kaizer, Á. Kupán, G. Speier
Catechol oxidase activity of copper and manganese isoindoline complexes
Achievements in Coordination, Bioinorganic and Applied Inorganic Chemistry;
Eds.; M. Melnik, J. Sima, and M. Tatarko, Slovak Technical University Press: Bratislava, Volume 8, 299-310 (2007)

7. J. Kaizer, **G. Baráth**, T. Hujber, J. Pap, G. Speier, R. Buják
Synthesis and oxidation of manganese and iron flavonolates with relevance to flavonol 2,4-dioxygenases
Achievements in Coordination, Bioinorganic and Applied Inorganic Chemistry;
Eds.; M. Melnik, J. Sima, and M. Tatarko, Slovak Technical University Press: Bratislava, Volume 8, 134-144 (2007)

8. J. Kaizer, **G. Baráth**, G. Speier, M. Réglier, M. Giorgi
Synthesis, structure and catalase mimics of novel homoleptic manganese(II) complexes of 1,3-bis(2'-pyridylimino) isoindoline, $Mn(4R\text{-}ind)_2$ (R = H, Me)
Inorg. Chem. Commun., **10**, 292-294 (2007)

FURTHER SCIENTIFIC PUBLICATIONS

1. B. Kripli, **G. Baráth**, É. Balogh-Hergovich, M. Giorgi, A. Jalila Simaan, M. Réglier, L. Párkányi, J. Pap, J. Kaizer
   Correlation between the SOD-like activity of hexacoordinate iron(II) complexes and their Fe^{3+}/Fe^{2+} redox potentials
   Inorg. Chem. Commun., (közlésre beküldve)

2. T. Csay, **G. Baráth**, B. Kripli, J. Kaizer, G. Speier
   Synthesis and catalase like activity of manganese(II) complexes with isoindoline-based ligands
   Insights into Coordination, Bioinorganic and Apllied Inorganic Chemistry; Eds.; M. Melnik, J. Sima, and M. Tatarko, Slovak Technical University Press: Bratislava, Volume 9, 120-132 (2009)

3. J. Kaizer, R. Csonka, **G. Baráth**, G. Speier
   Synthesis, properties, and catecholase-like activity of dimanganese(II) complex of (1,4-di-6'-methyl-2'-pyrydil aminophtalazine), $Mn_2(6'Me_2PAP)_2Cl_4$
   Transit. Met. Chem., **32**, 1047-1050 (2007)

PRESENTATIONS

1. **G. Baráth**, J. S. Pap, J. Kaizer, A. J. Simaan, G. Speier
   Studies on functional ACC oxidase mimics
   10th European Conference on Biological Inorganic Chemistry,
   Thessaloniki, Greece, 2010. June 22-26, 110 (Poster)

2. **G. Baráth**, G. Rácz, J. Kaizer, G. Speier
   Structural models for flavonol 2,4-dioxygenase
   10th International Symposium on Applied Bioinorganic Chemistry,
   Debrecen, Hungary, 2009. September 25-28, 96 (Poster)

3. **G. Baráth**, J. Kaizer, G. Speier
   One metal – two tales on iron-containing flavonol 2,4-dioxigenase mimics
   14th International Conference on Biological Inorganic Chemistry,
   Nagoya, Japan, 2009. July 20-25. (Poster, *J. Biol. Inorg. Chem.*, **14**, S139)

4. T. Csay, **G. Baráth**, B. Kripli, J. Kaizer, G. Speier
   Synthesis and catalase like activity of manganese(II) complexes with isoindoline-based ligands
   XXII. International Conference on Coordination and Bioinorganic Chemistry,
   Smolenice, Slovakia, 2009. June 7-12, 54 (Oral)

5. **G. Baráth**, J. Kaizer, G. Speier
   First manganese-containing structural models for flavonol 2,4-dioxygenase
   38th International Conference on Coordination Chemistry,
   Jerusalem, Israel, 2008. July 19-24, 357 (Poster)

6. **G. Baráth**, J. Kaizer, G. Speier
Carboxylate-enhanced reactivity in the oxygenation of flavonolate complexes
38th International Conference on Coordination Chemistry,
Jerusalem, Israel, 2008. July 19-24, 297 (Poster)

7. J. Kaizer, **G. Baráth**, G. Speier
Synthesis and oxidation of manganese and iron flavonolates with relevance to flavonol 2,4-dioxygenases
XXI. International Conference on Coordination and Bioinorganic Chemistry,
Smolenice, Slovakia, 2007. June 3-8, 49 (Oral)

8. G. Speier**, G. Baráth**, J. Kaizer
Catechol oxidase activity of copper and manganese isoindoline complexes
XXI. International Conference on Coordination and Bioinorganic Chemistry,
Smolenice, Slovakia, 2007. June 3-8, 90 (Oral)

9. **G. Baráth**, J. Kaizer, T. Hujber, J. Pap, G. Speier
Kinetic studies on the manganese(II)-mediated oxygenolysis of the flavonolate ligand with relevance to flavonol 2,4-dioxygenase
13th International Conference on Biological Inorganic Chemistry,
Vienna, Austria, 2007. July 15-20. (Poster, *J. Biol. Inorg. Chem.*, **12**, S165)

10. **G. Baráth**, G Speier, J. Kaizer, M. Giorgi, M. Réglier
Studies on iron-based flavonol 2,4-dioxygenase models
13th International Conference on Biological Inorganic Chemistry,
Vienna, Austria, 2007. July 15-20. (Poster, *J. Biol. Inorg. Chem.*, **12**, S176)