STRUCTURAL AND FUNCTIONAL MIMIC OF METALLOENZYMES

Ph.D. Theses

István Szilágyi

University of Szeged

Department of Inorganic and Analytical Chemistry

SZEGED

2006

STRUCTURAL AND FUNCTIONAL MIMIC OF METALLOENZYMES

Ph.D. Theses

István Szilágyi

Supervisors: Dr. Tamás Kiss
Dr. Imre Labádi
Dr. István Pálinkó

University of Szeged
Department of Inorganic and Analytical Chemistry

SZEGED

2006

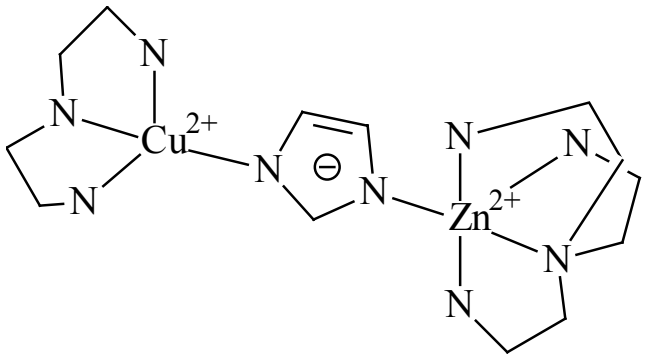
INTRODUCTION AND THE AIMS OF THE WORK

Generally, the active centre of native enzymes contains metal ions coordinated by donor groups of protein side chains. Further role of the protein is to organise a relatively mobile structure around the active centre. Several native enzymes contain two or more metal ions in their active centre (for example: superoxide dismutase, catalase, catechol oxidase). The metal ions cooperate when the enzyme works and they together with their immediate surroundings take part in the reactions of the substrates.

Since the active centre of metalloenzymes resembles a simple metal complex, the preparation of complexes with similar structure is a promising way of mimicking the enzymes. Structural and/or functional modelling the active sites are usually done by preparing complexes having the appropriate metal ion(s) and ligands of the same or similar structures found in the enzyme.

The aim of this work was to prepare and immobilise some, hopefully, enzyme mimicking metal complexes and test them in various enzymatic reactions. Inorganic materials (silica gel and montmorillonite) were used as the solid matrices in the immobilisation procedures. The guest materials were a heterobinuclear copper(II)-zinc(II) complex (Cu(II)-diethylenetriamino-μ-imidazolato-Zn(II)-tris(2-aminoethyl)amine perchlorate, denoted as Cu,Zn-complex in the followings) and its substructural complexes. The support-free as well as the immobilised materials were tested in the dismutation of the superoxide radical ion (SOD activity), the decomposition of H_2O_2 (catalase activity) and the oxidation of catechol (cathecolase activity). Subsystems (Cu(II)-diethylenetriamine, Cu(II)-diethylenetriamine-imidazole, Cu(II)-tris(2-aminoethyl)amine, Cu(II)-tris(2-aminoethyl)amine) of the Cu,Zn-complex were also studied. The Cu,Zn-complex bears some structural resemblance to the active centre of copper(II)-zinc(II) superoxide dismutase: both the complex and

the native enzyme contain an imidazolate bridge between the copper(II) and zinc(II) ions.



The structure of the Cu(II)-diethylenetriamino-μ-imidazolato-Zn(II)-tris(2-aminoethyl)amine complex

EXPERIMENTAL

The stability constant and composition of the evolving species in aqueous solution were calculated on the basis of experimental data of pH-potentiometric titration using the Psequad and Superquad computer packages.

The structures of these complexes were investigated by UV-Visible and EPR spectroscopies. The molecular weights of the complex molecules were determined by two mass spectrometric (ESI-MS and MALDI-MS) methods. The electrochemical properties of these materials were studied by cyclic voltammetry.

The amounts of the metal ions on/in the solid hosts were measured by atomic absorption spectrometry. The immobilisation of the complexes was followed by FT-IR and EPR spectroscopies, and the thermal behaviour of these materials was also investigated by thermal (TG and DTA) methods. The BET surface areas of the immobilised complexes were also measured.

The prepared and characterised substances were tested in various enzymatic assays; their superoxide dismutase, catalase and catechol oxidase activities were studied.

NEW SCIENTIFIC RESULTS

Investigations in aqueous solution

1. The speciation of the five-component system (and its subsystems) containing three ligands (diethylenetriamine, imidazole, tris(2-aminoethyl)amine), copper(II)- and zinc(II)ion in 1:1 ratio was investigated by pH potentiometric titration in the 2–11 pH region. The composition and the stability constants of the evolved species were calculated using a computer program. The pH-metric results indicated that the imidazolate-bridged Cu,Zn-complex is stable in the 7–11 pH range (deprotonation only occurred above pH 8 on the zinc(II) side, but the imidazolate bridge remained intact even under these conditions).

2. The EPR and UV-Vis spectra were also recorded in the same pH region. Complete structural characterisation was done for each species evolved. Two isomers were identified for the imidazolate bridged complex and a third one was also detected in the alkaline region in small amount. In our view, this complex also contains an imidazolate bridge between the metal ions and the only difference is the second deprotonation taking place on the copper(II) side.

3. The molecular weight of the Cu,Zn-complex was determined by two mass spectrometric methods (ESI-MS and MALDI-MS) at pH = 8. Some subsystems (Cu**A**, Cu**AB**, Zn**C**, Zn**CB**) were also scrutinised by mass spectrometry. Molecular weights and fragmentation pathways were determined.

4. The electrochemical behaviour of the Cu,Zn-complex was determined by cyclic voltammetry. The formation of the imidazolate-bridged compound was confirmed by the electrochemical results too. In all cases negative redox potentials were measured.

Investigations in the solid phase

5. The Cu,Zn-complex and some of its subsystems were immobilised on silica gel and in montmorillonite. Immobilisation was effected by electrostatic forces when montmorillonite was the host material (Mont–i–Cu,Zn). For silica gel two types of immobilisation method were applied: immobilisation *via* (i) hydrogen bonds (SG–h–Cu,Zn) or (ii) covalent linkage (SG–c–Cu,Zn). The prepared catalysts were characterised by FT-IR and EPR spectroscopy, atomic absorption spectrophotometry, BET surface are measurement and thermal (DTA, TG) methods.

6. The powder EPR spectra of the montmorillonite-immobilised material and the EPR parameters revealed that the complex did not fall apart upon immobilisation. The spectra of the host-free and the montmorillonite-immobilised complex were identical indicating that there was no change in the coordination geometry upon immobilisation. As concerns the SG–h–Cu,Zn and the SG–c–Cu,Zn materials the EPR spectra of the host-free and silica-immobilised complexes differ, in addition, two types of copper(II)-containing species were detected in these substances. The EPR data indicated change in the geometry around the copper(II) ion upon immobilisation. In one of the components the EPR parameters were very similar to those of the native superoxide dismutase enzyme.

7. The thermal behaviour of the materials was investigated by thermogravimetry (TG) and differential thermal analysis (DTA). The silica-immobilised complexes (SG–h–Cu,Zn and SG–c–Cu,Zn) were more stable thermally than the host-free and the montmorillonite-immobilised (Mont–i–Cu,Zn) complexes.

Enzymatic assays

8. It was found that the Cu,Zn-complex was more active than its copper(II) containing subsystems; however, it was significantly less active than the native enzyme. Nevertheless, the Cu,Zn-complex is a potent SOD mimic considering its very low molecular weight compared to that of the native enzyme. The complex-free supports were found to be inactive in the SOD test reaction. After immobilising the Cu,Zn-complex on silica gel via hydrogen bonding the material also displayed SOD activity. The SOD activity did not merely appear but was an order of magnitude higher than that of the host-free complex. Although the immobilised substructures of the Cu,Zn-complex also displayed SOD activity, it was significantly smaller than that of the silica-immobilised Cu,Zn-complex. They were even less active than the host-free Cu-Zn complex. After immobilisation in montmorillonite the complex displayed low SOD activity, however, it was comparable to that of the host-free complex.

9. The catecholase activity of the host–free and immobilised complexes was tested in the catalytic oxidation of 3,5-di-*tert*-butyl catechol (DTBC) to 3,5-di-*tert*-butyl quinone (DTBQ) in the presence of dioxygen. The cathecholase activity of the Cu,Zn-complex changed significantly upon immobilisation on either silica gel (SG–h–Cu,Zn and SG–c–Cu,Zn) or in montmorillonite (Mont–i–Cu,Zn). The most active material was the SG–h–Cu,Zn. Its activity was nearly 50 folds higher than that of the host-free complex.

10. The catalase activities of the host-free and immobilised complexes were tested in the decomposition reaction of hydrogen peroxide yielding water and molecular oxygen. It was found that the Cu,Zn-complex and its immobilised forms had no catalase activity under these conditions.

PUBLICATIONS

Papers directly related to the theses

1. **I. Szilágyi**, I. Labádi, K. Hernadi, I. Pálinkó, T. Kiss
   Synthesis and IR spectroscopic characterisation of immobilised superoxide dismutase (SOD) mimicking complexes
   Journal of Molecular Structure **744-747,** 495 (2005) IF: 1.200

2. **I. Szilágyi**, I. Labádi, K. Hernadi, I. Pálinkó, I. Fekete, L. Korecz, A. Rockenbauer, T. Kiss
   Superoxide dismutase activity of a Cu-Zn complex – bare and immobilised
   New Journal of Chemistry **29,** 740 (2005) IF: 2.735

3. **I. Szilágyi**, I. Labádi, K. Hernadi, T. Kiss, I. Pálinkó
   Montmorillonite intercalated Cu(II)-histidine complex - synthesis, characterisation and superoxide dismutase activity
   Studies in Surface Science and Catalysis **158,** 1011 (2005) IF: 0.489

4. **I. Szilágyi**, I. Labádi, K. Hernadi, I. Pálinkó, N.V. Nagy, L. Korecz, A. Rockenbauer, Z. Kele, T. Kiss
   Speciation study of an imidazolate-bridged copper(II)–zinc(II) complex in aqueous solution
   Journal of Inorganic Biochemistry **99,** 1619 (2005) IF: 2.225

5. **I. Szilágyi**, Z. Kele, I. Labádi, K. Hernadi, I. Pálinkó, T. Kiss
   ESI–MS and MALDI–MS investigation of a superoxide dismutase mimicking imidazolato–bridged Cu–Zn complex
   Rapid Communications in Mass Spectrometry **19,** 2878 (2005) IF: 2.789

6. **I. Szilágyi**, L. Horváth, I. Labádi, K. Hernadi, I. Pálinkó, T. Kiss
   Mimicking catalase and catecholase enzymes by copper(II)-containing complexes
   Central European Journal of Chemistry **4**, 118 (2006) IF: 0.171

Other papers

1. E. Princz, **I. Szilágyi**, K. Mogyorósi, I. Labádi
   Lanthanide complexes of ethylenediaminotetramethylene-phosphonic acid
   Journal of Thermal Analysis and Calorimetry **69,** 427 (2002) IF: 0.598

2. I. Labádi, **I. Szilágyi**, N. I. Jakab, K. Hernádi, I. Pálinkó
   Metal complexes immobilised in/on porous matrices – possible enzyme mimics
   Material Science **21,** 235 (2003) IF: 0.154

3. **I. Szilágyi**, G. Nagy, K. Hernádi, I. Labádi, I. Pálinkó
   Modeling copper-containing enzyme mimics
   Journal of Molecular Structure THEOCHEM **666,** 451 (2003) IF: 1.027

4. **I. Szilágyi**, E. Pál, L. Horváth, I. Labádi
   Az N-hidroxietil-glicin kölcsönhatása fémionokkal
   Magyar Kémiai Folyóirat **111,** 83 (2005) IF: –

Conference presentations

1. Imre Labádi and **István Szilágyi**
   N-hydroxyethyl-glycine - a new environmental benign complexing agent
   IVth International Symposium 'Young People and Multidisciplinary Research'
   Timisoara (14-15 November 2002) Romania Poster

2. Labádi I., **Szilágyi I.**, Sitkei E., Pálinkó I., Hernádi K.
   Enzimutánzó fémkomplexek immobilizálása pórusos mátrixokban,
   Erdélyi Magyar Műszaki Tudományos Társaság VIII. Nemzetközi Vegyészkonferencia
   Cluj (15-17 November 2002) Romania Lecture

3. **Szilágyi I.**, Száva J., Labádi I.
   Gyors biológiai lebomlású komplexképzők vizsgálata
   The IXth Symposium on Analytical and Environmental Problems
   Szeged (30 September 2002.) Hungary Poster

4. **István Szilágyi**, Imre Labádi, Klára Hernádi, István Pálinkó and Tamás Kiss
   SOD activity of immobilized enzyme mimicking complexes
   Vth International Symposium 'Young People and Multidisciplinary Research'
   Timisoara (6 November 2003) Romania Poster

5. **Szilágyi I.**, Labádi I., Hernádi K., Pálinkó I., Kiss T.
   Szuperoxid dizmutáz utánzó fémkomplex vizsgálata riboflavin/NBT tesztreakcióval
   Kémiai Előadói Napok (KEN)
   Szeged (27-29 October 2003) Hungary Lecture

6. I. Labádi, E. Pál, L. Horváth and **I. Szilágyi**
   N-hydroxyethylglycine – a new environmentally benign complexing agent
   20th International Conference on Coordination and Bioinorganic Chemistry
   Smolenice (5-10 June 2005) Slovakia Lecture

7. Labádi I., **Szilágyi I.**
   Gyors biológiai lebomlású komplexképzők vizsgálata
   XXV. Kémiai Előadói Napok (KEN)
   Szeged (28-30 October 2002) Hungary Lecture

8. I. Labádi, **I. Szilágyi**, K. Hernadi, I. Pálinkó and I. Kiricsi
   Modelling of enzymatic processes by metallocomplexes immobilized on microporous matrices
   XIIIth Winter School on Coordination Chemistry
   Karpacz (9-13 December 2002) Poland Lecture

9. **I. Szilágyi**, J. Szava, I. Labádi
   New environmental benign complexons
   XIIIth Winter School on Coordination Chemistry
   Karpacz (9-13 December 2002) Poland Poster

10. **I. Szilágyi**, G. Nagy, K. Hernádi, I. Labádi, I. Pálinkó
    Modeling copper-containing enzyme mimics
    The Role of Chemistry in the Evolution of Molecular Medicine (A Tribute of Professor Albert Szent-Györgyi)
    Szeged (27-29 June 2003) Hungary Poster

11. **I. Szilágyi**, I. Labádi, K. Hernádi, I. Pálinkó, T. Kiss
    Immobilizing enzyme mimicking complexes
    IXth International Symposium on Inorganic Biochemistry
    Szklarska Poreba (4-7 September 2003) Poland Poster

12. **Szilágyi I.**, Labádi I., Kiss T., Pálinkó I., Hernádi K.
    Enzimutánzó fémkomplexek immobilizálása pórusos anyagokon – szuperoxid dizmutáz aktivitás
    XXXVIII. Komplexkémiai Kollokvium
    Gyula (21-23 May 2003) Hungary Lecture

13. **István Szilágyi**, Imre Labádi, Klara Hernadi, István Pálinkó, Tamás Kiss
    Potentiometric and spectroscopic study of a superoxide dismutase (SOD) mimicking imidazolate-bridged Cu(II)-Zn(II) complex
    International Symposium Metals, Environment, Health
    Szklarska Poreba (24-27 June 2004) Poland Poster

14. **István Szilágyi**, Imre Labádi, Klara Hernadi, István Pálinkó
Synthesis and IR spectroscopic characterization of immobilised superoxide dismutase (SOD) mimicking complexes
XXVII. European Congress on Molecular Spectroscopy
Kraków (5-10 September 2004) Poland Poster

15. **Szilágyi István**, Labádi Imre, Hernádi Klára, Pálinkó István, Győr Miklós, Rockenbauer Antal, Kiss Tamás
Réz(II)-dietiléntriamin-imidazol-cink(II)-trisz(aminoetil)amin rendszer potenciometriai, spektrofotometriai és ESR spektroszkópiai vizsgálata
XXXIX. Komplexkémiai Kollokvium
Agárd-Gárdony (26-28 May 2004) Hungary Lecture

16. Czibulya Zsuzsanna, Sitkei Eszter, Pál Edit, **Szilágyi István** és Labádi Imre
Az imidazol átmenetifém-komplexeinek előállítása és vizsgálata termikus és IR spektroszkópiai módszerekkel
XXXIX. Komplexkémiai Kollokvium
Agárd-Gárdony (26-28 May 2004) Hungary Lecture

17. Edit Pál, László Horváth, **István Szilágyi**, Imre Labádi
N-hydroxiethyl-glycine – a new environmentally sound ligand
8th International Symposium on Interdisciplinary Regional Research
Szeged (19-21 April 2005) Hungary Poster

18. **I. Szilágyi**, I. Labádi, K. Hernadi, T. Kiss, I. Pálinkó
Montmorillonite intercalated Cu(II)-histidine complex – synthesis, characterisation and superoxide dismutase activity
Federation of the European Zeolite Association 3 Conference
Prague (22-26 August 2005) Czech Republic Poster