

# **REDOX-ACTIVE BIOINSPIRED CATALYSTS – SYNTHESES, CHARACTERISATION AND AN APPLICATION**

PhD Theses

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## 1. Introduction and the aims of the work

It is a general aim of modern catalysis to develop products that are able to act as highly active and, more importantly, highly selective catalysts throughout the field of chemical synthesis. In order to achieve this goal, various approaches are possible. One of the most promising ways is mimicking nature's most efficient catalysts, the enzymes. Even though living organisms contain several thousand enzymes for basically all conceivable chemical reactions, simply applying them in the synthesis of, *e.g.*, commodity or specialty chemicals has obstacles: they are very sensitive to the environment; they work in narrow temperature and pressure ranges and in physiological aqueous solution. The advantages can be retained, while the disadvantages can be eliminated by preparing a structural or a functional model of the active site of enzymes, among them metalloenzymes.

Previously, numerous homogeneous and, less frequently, heterogeneous bioinspired mimics have been synthesised and applied for the oxidation of organic molecules. In these biomimetic catalysts redox-active transition metal ions were used as the central ion, while amino acids or other molecules, with coordinating (donor) groups, as ligands.

On the basis of results published in the literature, and the previous experiences of the group, the following aims have been set out:

1. building immobilised Mn(II)–, Fe(III)–, Co(II)–, Ni(II)– and Cu(II)–C-protected amino acid complexes on chloropropylated silica gel through covalent grafting, using the active sites of the various types of the superoxide dismutase enzyme as functional models for the complexes,
2. structural characterisation of the surface complexes with as many chemical and instrumental methods as possible,
3. testing the superoxide dismutase activities of the surface-grafted complexes by a biochemical test reaction,
4. applying the heterogenised complexes in "real-life" catalysis, in the epoxidation/oxidation of cyclohexene.

## 2. Experimental

### Materials and synthesis methods

For the syntheses, C-protected (in the form of methyl ester) L-histidine, L-tyrosine, L-cysteine and L-cystine were used as ligands. The source of metal ions were  $\text{MnCl}_2 \cdot 5\text{H}_2\text{O}$ ,

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  and  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ . Chloropropylated silica gel (SG – particle size: 230–400 mesh, BET surface area:  $500 \text{ m}^2/\text{g}$ , functionalisation: 8%) was used as support. The solvent was 2-propanol.

The general features of the syntheses are as follows. The first step of immobilisation was the reaction of the appropriately protected amino acid and the support. The C-protected amino acids were covalently grafted onto the support through N-alkylation like reaction, under basic conditions. After 24 h, the solid substance was filtered, washed several times in order to remove the uncoupled amino acid excess and dried. Complexation followed the anchoring; the material was soaked in the 2-propanolic solution of the metal salt under continuous stirring at room temperature for 24 h. After filtering and washing, the obtained material was divided into two parts. Half of it was set aside (covalent grafting under ligand-poor conditions – only the immobilised protected amino acids were available for coordination). To the other half, 2-propanolic solution of the appropriately protected amino acid derivative was added in excess, and the suspension was continuously stirred at room temperature for 24 h. Then, the solid material was filtered, rinsed with 2-propanol several times and dried (covalent grafting under ligand-excess conditions – the surface-grafted complex might have rearranged in the presence of excess amino acid mixture).

Surface-grafted complexes were prepared having uniform or mixed amino acid derivatives (two protected amino acids were used) as ligands. Two methods were applied for the syntheses when mixed ligands were used. In method ‘A’, one of the protected amino acid ester was covalently anchored to the surface of the support; then, it was soaked in the metal salt solution, and after filtering and thorough washing the final substance was made by allowing complexation with excess amounts of the other amino acid ester. In method ‘B’ a 1:1 molar mixture of the protected amino acids was grafted onto the surface of the support; then, the metal complex was formed. Parts of the materials thus formed were further treated in excess 1:1 protected amino acid mixtures resulting in the formation of surface-anchored complexes under ligand-excess conditions.

## Methods of characterisation

The amount of metal ions on the surface-modified silica gel was measured by ICP–MS, while the nitrogen content of the samples was determined by the Kjeldahl method.

For the optical characterisation of the Ni(II)-containing samples, UV–Vis diffuse reflectance spectroscopy (UV-Vis DRS) was used.

EPR measurements were only used for the Mn(II)-containing samples. The spectra were recorded at room temperature. All recorded EPR spectra were simulated with a spectrum decomposition method.

X-ray absorption spectroscopy measurements were performed to determine the coordination numbers, geometries around the metal ions, and to find out whether the sulphur atom was coordinated to the central ions.

Structural information on each step of the synthesis procedure was obtained by far- and mid-range infrared spectroscopy. For evaluation, the difference spectra were used, *i.e.* the spectrum of the support was subtracted. The spectra in the far IR region provide direct information on metal ion–functional group coordination, although assignation of the vibrations in the far IR spectra is not a trivial exercise. For making it easier probe complexes having uniform, thus easily identifiable coordinating groups were prepared and their far IR spectra were registered. They were Mn(II), Fe(III), Co(II), Ni(II) and Cu(II) complexes of imidazole, isopropylamine and monosodium malonate.

### **Catalytic test reactions**

The Beauchamp-Fridovich biochemical test reaction was used for testing the superoxide dismutase activity of the complexes. In this reaction riboflavin, L-methionine and nitro blue tetrazolium were used. Under aerobic conditions reaction takes place on illumination between riboflavin and L-methionine. It is a reduction and the reduced form of riboflavin reacts with oxygen forming a peroxide derivative. This derivative decomposes giving the superoxide radical anion. This radical ion is captured by the nitro blue tetrazolium (NBT) and its original yellow colour turns blue. The transformation can be quantitatively followed by spectrophotometry, measuring the absorbance at 560 nm. If the enzyme mimicking material works well, it competes with NBT with success in capturing the superoxide radical ion. Thus, the photoreduction of NBT is inhibited.

The epoxidation/oxidation reaction of cyclohexene was the "real-life" catalytic test reaction. Cyclohexene is a suitable model substrate for oxidation reactions since it has C=C and C–H bonds as well. The formation of the allylic oxidation products 2-cyclohexene-1-ol and 2-cyclohexene-1-one indicates the favoured attack of the activated C–H bond, while if cyclohexene oxide is the main product epoxidation is the primary reaction on the C=C bond. The reaction products were monitored *via* gas chromatography.

### 3. Novel scientific results

**T 1. Most of the metal ion [Mn(II), Fe(III), Co(II), Ni(II), Cu(II)]-protected amino acid [mostly C-protected histidine, tyrosine, cysteine and cystine] complexes *via* covalent grafting onto silica support were synthesised for the first time.**

1.1 The overwhelming majority of the covalently anchored complexes with uniform protected amino acids are novel, while all of those containing mixed protected amino acids have not been prepared and published before.

**T 2. A novel experimental protocol has been elaborated for the construction of the covalently grafted complexes.**

2.1 Syntheses under ligand poor conditions: the protected amino acid or 1:1 two-component molar mixture of the protected amino acids was covalently anchored onto chloropropylated silica (N-alkylation, the excess amino acids were removed), then soaking in an 2-propanolic solution of the metal salt, followed by washing with 2-propanol and drying at room temperature.

2.2 Syntheses under ligand excess conditions: the complexes prepared under ligand poor conditions were stirred with the 2-propanolic solution of the protected amino acid or a two-component 1:1 molar mixture of protected amino acids for 24 hours at room temperature. Often, the original anchored complexes rearranged, however, occasionally, they remained unchanged.

**T 3. A many-component protocol has been developed for determining the coordination atoms or groups.**

3.1 The Kjeldahl method and ICP-MS measurements were applied to determine the anchored amino acid to metal ion ratio. UV-Vis DRS and/or EPR and/or X-ray absorption spectroscopies were used for determining the geometrical arrangement of the ligands around the metal ions. Mid IR spectroscopy was an efficient method in learning whether the carboxylate, carbonyl and phenolate oxygens were coordinated. The combination of X-ray absorption and mid IR spectroscopies revealed if the thiolate sulphur took part in the complexation or not. Far IR measurements allowed the direct observation of the metal ion-coordinating atom (group) interactions.

**T 4. The synthesis and far IR spectroscopy of purpose-built complexes (the same metal ions used in the synthesis of the covalently anchored complexes and ligands having only one type of coordinating groups) allowed the direct identification of metal ion-coordinating group vibration band; these results could be transferred to the far IR spectra of the silica-anchored metal ion-protected amino acid complexes (*i.e.*, leading to a far IR correlation table).**

**T 5. The Beauchamp- Fridovich superoxide dismutase biochemical test reaction, applied for these silica-anchored complexes for the first time, revealed that these immobilised complexes were able to dismutase the superoxide radical anion, some of them with very high efficiency.**

5.1 Nevertheless, none of the complexes had the activity of the relevant superoxide dismutase enzyme, however, some of the anchored Fe(III) complexes [SG–Tyr-OMe–Fe(III), SG–Cys-OMe–Fe(III) and SG–His-OMe;Cys-OMe–Fe(III)–H-His-OMe;H-Cys-OMe] came an order of magnitude close to it.

**T 6. The immobilised complexes (the Co(II) complexes were not investigated) were active in the selective epoxidation of cyclohexene. In the presence of the immobilised complexes, in most cases, the conversions were higher or in the range of the uncatalysed (stoichiometric) reaction, and, most importantly, in each case, the epoxide selectivity significantly surpassed that of the stoichiometric transformation.**

6.1 As far as the activities and selectivities are concerned the best performances were put out by the anchored Mn(II) and Fe(III) complexes. Interestingly, they were different from those having the highest activity in the superoxide dismutase biochemical test reaction.

6.2 The epoxide selectivities were not dependent significantly on either the metal ion or the ligands. They were quite probably controlled by steric effects, *i.e.*, the restricted room available in the coordination sphere of the metal ions.

## **4. Practical use of the results**

The results presented here are of fundamental nature. Nevertheless, the immobilised complexes constructed on the chloropropylated silica surface proved to be efficient catalysts in the dismutation reaction of the superoxide radical as well as in the epoxidation of cyclohexene. In this reaction the activity was often, and the epoxide selectivity was always better than in the generally used stoichiometric reaction. Quite probable that these materials can also be used in other oxidation reactions in the production of fine chemicals.

## 5. Publications

### 5.1. Papers related to the Theses published in refereed journals

1. Aranyi, A., **Csendes, Z.**, Kiss, J.T., Pálinkó, I.:  
Covalently grafted, silica gel supported C-protected cysteine or cystine copper complexes – syntheses, structure and possible surface reaction studied by FT-IR spectroscopy,  
*J. Mol. Struct.* **924-926**, 166–169 (2009).  
Impact factor: 1.555<sub>2009</sub> Independent citation: 4
2. **Csendes, Z.**, Bugris, V., Lackó, L., Labádi, I., Kiss, J.T., Pálinkó, I.:  
Superoxide dismutase mimicking Cu(II)–mixed amino acid complexes covalently grafted onto silica gel – an FT-IR study,  
*Anal. Bioanal. Chem.* **397**, 549–555 (2010).  
Impact factor: 3.841<sub>2010</sub> Independent citation: 2
3. **Csendes, Z.**, Lajter, I., Bugris, V., Kiss, J.T. Pálinkó, I.:  
Structural characterisation of silica gel anchored, biomimetic, mixed ligand Co(II)–amino acid complexes,  
*Vibr. Spectr.* **53**, 132–135 (2010).  
Impact factor: 2.083<sub>2010</sub> Independent citation: 4
4. **Csendes, Z.**, Földi, N., Kiss, J.T., Sipos, P., Pálinkó, I.:  
Covalently grafted, silica gel supported mixed amino acid iron complexes – syntheses, structural characterization and catalytic testing,  
*J. Mol. Struct.* **993**, 203–207 (2011).  
Impact factor: 1.634<sub>2011</sub> Independent citation: 2
5. **Csendes, Z.**, Dudás, Cs., Varga, G., Bajnóczi, É.G., Canton, S.E., Sipos, P., Pálinkó, I.:  
Superoxide dismutase inspired Fe–amino acid complexes covalently grafted onto chloropropylated silica gel – Syntheses, structural characterization and catalytic activity,  
*J. Mol. Struct.* **1044**, 39–45 (2013).  
Impact factor: 1.404<sub>2012</sub> Independent citation: –
6. Varga, G., **Csendes, Z.**, Peintler, G., Berkesi, O., Sipos, P., Pálinkó, I.:  
Using far IR spectra for the unambiguous identification of metal ion–ligand coordination sites in purpose-built complexes,  
*Spectrochim. Acta, A* **122**, 257–259 (2014).  
Impact factor: 1.977<sub>2012</sub> Independent citation: –
7. **Csendes, Z.**, Varga, G., Nagy, N.V., Bajnóczi, É.G., Sipiczki, M., Carlson, S., Canton, S.E., Metzinger, A., Galbács, G., Sipos, P., Pálinkó, I.:  
Synthesis, structural characterisation, and catalytic activity of Mn(II)–protected amino acid complexes covalently immobilised on chloropropylated silica gel,  
*Catal. Today* (in press).  
Impact factor: 2.980<sub>2012</sub> Independent citation: –
8. **Csendes, Z.**, Varga, G., Schmehl, Timár, Z., Carlson, S., Canton, S.E., Bajnóczi, É.G., Elek, G., Sebők, D., Dékány, I., Sipos, P., Pálinkó, I.:  
Superoxide dismutase inspired immobilised Ni(II)–protected amino acid catalysts – synthesis, characterisation, and catalytic activity with improved selectivity,  
*J. Mol. Catal. A* (to be submitted).

## 5.2. Papers related to the Theses published as full papers in conference proceedings or as progress report

1. **Csendes, Z.**, Bugris, V., Sebők, P., Major, Zs., Kiss, J.T., Pálinkó, I.:  
Covalently immobilized, silica gel or resin-supported C-protected cysteine or cystine Fe and Ni complexes – synthesis and structural characterization with FT-IR spectroscopy,  
*Insights into Coordination, Bioinorganic and Applied Inorganic Chemistry*, (Melník, M., Segl'a, P., Tatarko, M.), ISBN 978-80-227-3085-3, Press of Slovak University of Technology, Bratislava, 2009, pp. 54–61.
2. **Csendes, Z.**, Elek, G., Zahorán, K., Gyuris, K., Varga, G., Kiss, J.T., Pálinkó, I.:  
Co-protected amino acid complexes covalently grafted onto solid supports – syntheses, structural characterization and testing of catalytic activity,  
*Proc. 10th Pannonian Int. Symp. Catal.*, ISBN: 978-83-929430-4-4, Cracow (Poland), 2010, pp. 37–44.
3. **Csendes, Z.**, Pálinkó, I.:  
Biomimetikus elektrontranszfer katalizátorok készítése (The preparation of biomimetic electron transfer catalysts),  
*Kémiai Előadói Napok (Chemistry Lectures), Program és előadásösszefoglalók*, ISBN 978-96-3315-020-7, pp. 56–59 (2010).
4. **Csendes, Z.**, Csőr, Á., Szécsényi, Á., Sipos, P., Pálinkó, I.:  
Cu(II)–C-protected mixed amino acid complexes covalently immobilized onto silica gel – synthesis, structural characterization and catalytic activity,  
*New Trends in Coordination, Bioinorganic and Applied Inorganic Chemistry*, (Melník, M., Segl'a, P., Tatarko, M.), ISBN 978-80-227-3509-4, Press of Slovak University of Technology, Bratislava, 2011, pp. 41–52.
5. **Csendes, Z.**, Sipos, P., Pálinkó, I.:  
Biomimetic metal ion–amino acid complexes – functional modeling of superoxide dismutase enzymes,  
*New Trends in Coordination, Bioinorganic and Applied Inorganic Chemistry*, (Melník, M., Segl'a, P., Tatarko, M.), ISBN 978-80-227-3509-4, Press of Slovak University of Technology, Bratislava, 2011, pp. 431–441.
6. Varga, G., **Csendes, Z.**, Sipos, P., Pálinkó, I.:  
Egyfajta koordinálódó csoportot tartalmazó fémion-komplexek távoli IR spektrumai – kísérlet korrelációs táblázat összeállítására, (The far IR spectra of metal ion complexes containing ligands capable of coordination *via* one type of functional group),  
*Kémiai Előadói Napok (Chemistry Lectures), Program és előadásösszefoglalók*, ISBN 978-963-315-099-3, pp. 135–137 (2012).
7. H. Dudás, Cs., **Csendes, Z.**, Bajnóczi, É.G., Canton, S.E., Sipos, P., Pálinkó, I.:  
Módosított szilikagél felületén kovalens kötással rögzített vas–aminosavkeverék komplexek készítése, szerkezeti jellemzése és katalitikus aktivitásának vizsgálata, (Synthesis, structural characterisation and catalytic activities of iron–mixed amino acid complexes covalently anchored on modified silica gel),  
*Kémiai Előadói Napok (Chemistry Lectures), Program és előadásösszefoglalók*, ISBN 978-963-315-099-3, pp. 138–142 (2012).



8. Varga, G., **Csendes, Z.**, Peintler, G., Berkesi, O., Sipos, P., Pálinkó, I.:  
Using far IR spectra for the unambiguous identification of metal ion–ligand coordination sites in purpose-built complexes,  
*Recent Developments in Coordination, Bioinorganic and Applied Inorganic Chemistry*, (Melník, M., Segľa, P., Tatarko, M.), ISBN 978-80-227-3918-4, Press of Slovak University of Technology, Bratislava, 2013, pp. 429–433.
9. **Csendes, Z.**, Bajnóczi, É.G., Canton, S.E., Carlson, S., Sipos, P., Pálinkó, I.:  
Structural details of bioinspired catalysts: Detailed description of the coordination spheres of Cu(II)–, Co(II)–, Ni(II)–, Mn(II)– and Fe(III)–amino acid complexes covalently grafted onto modified silica gel,  
*MAX-LAB Activity Report 2012* (Johansson, U., Nyberg, A., Nyholm, R., eds.), 2013, I811\_1.
10. Timár, Z., **Csendes, Z.**, Sipos, P., Pálinkó, I.:  
Cu(II)–C-védett aminosav komplexek előállítása, szerkezetvizsgálata és felhasználása a ciklohexén oxidációjában (Synthesis and structural characterisation of immobilised Cu(II)-C-protected amino acid complexes and their use in the oxidation of cyclohexene),  
*Kémiai Előadói Napok (Chemistry Lectures), Program és előadásösszefoglalók*, ISBN 978-963-315-145-7, pp. 218–222 (2013).

### 5.3. Other papers published in refereed journals

1. Pallagi, A., Dudás, Cs., **Csendes, Z.**, Forgo, P., Pálinkó, I., Sipos, P.:  
Structure and equilibria of  $\text{Ca}^{2+}$  complexes of glucose and sorbitol from multinuclear ( $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{43}\text{Ca}$ ) NMR measurements supplemented with molecular modelling calculations,  
*J. Mol. Struct.* **993**, 336–340 (2011).  
Impact factor: 1.634<sub>2011</sub> Independent citation: 4
2. Lakatos, A., Gyurcsik, B., Nagy, N.V., **Csendes, Z.**, Wéber, E., Fülöp, L., Kiss, T.:  
Histidine-rich branched peptides as Cu(II) and Zn(II) chelators with potential therapeutic application in Alzheimer's disease,  
*Dalton Trans.* **41**, 1713–1726 (2012).  
Impact factor: 3.806<sub>2012</sub> Independent citation: –
3. **Csendes, Z.**, Kiss, J.T., Kutus, B., Sipos, P., Pálinkó, I.:  
Ni-S(uper)O(xide)D(ismutase) inspired Ni(II)–amino acid complexes covalently grafted onto Merrifield's resin – synthesis, structure and catalytic activity,  
*Mater. Sci. Forum* **730-732**, 1012–1017 (2013).  
Impact factor: – Independent citation: –
4. Pallagi, A., **Csendes, Z.**, Kutus, B., Czeglédi, E., Peintler, G., Forgo, P., Pálinkó, I., Sipos, P.:  
Multinuclear complex formation in aqueous solution of Ca(II) and heptagluconate ions,  
*Dalton Trans.* **42**, 8460–8467 (2013).  
Impact factor: 3.804<sub>2012</sub> Independent citation: –
5. Ruiz-Cabello, F.J.M., Trefalt, G., **Csendes, Z.**, Sinha, P., Oncsik, T., Szilagyi, I., Maroni, P., Borkovec, M.:  
Predicting aggregation rates of colloidal particles from direct force measurements,  
*J. Phys. Chem. B*, **117**, 11853–11862 (2013).  
Impact factor: 3.471<sub>2012</sub> Independent citation: –

6. Oncsik, T., Trefalt, G., **Csendes, Z.**, Szilagyi, I., Borkovec, M.:  
Aggregation of negatively charged colloidal particles in the presence of multivalent cations,  
*Langmuir* **30**, 733-741, (2014).  
Impact factor: 4.187<sub>2012</sub> Independent citation: –
7. Sipiczki, M., Ádám, A.A., Anitics, T., **Csendes, Z.**, Peintler, G., Kukovecz, Á., Kónya, Z., Sipos, P., Pálinkó, I.:  
The catalytic epoxidation of 2-cyclohexen-1-one over uncalcined layered double hydroxides using various solvents,  
*Catal. Today* (in press).  
Impact factor: 2.980<sub>2012</sub> Independent citation: –

#### 5.4. Other full paper published in conference proceedings

1. Pallagi, A., **Csendes, Z.**, Forgo, P., Sipos, P., Pálinkó, I.:  
Structural features of some Ca(II)–sugar complexes studied by NMR spectroscopy and computation,  
*New Trends in Coordination, Bioinorganic and Applied Inorganic Chemistry*, (Melník, M., Segľa, P., Tatarko, M.), ISBN 978-80-227-3509-4, Press of Slovak University of Technology, Bratislava, 2011, pp. 442–451.

full journal papers, total: 14

cumulative impact factor, total: 35.354

independent citations, total: 16

related to the topic of the Theses: 7

related to the topic of the Theses: 15.470

related to the topic of the Theses: 12