

CU(II)–C-PROTECTED MIXED AMINO ACID COMPLEXES COVALENTLY IMMOBILIZED ON SILICA GEL – SYNTHESSES, STRUCTURAL CHARACTERIZATION AND CATALYTIC ACTIVITY

Z. Csendes^a, Á. Csőr^a, Á. Szécsényi^a, P. Sipos^b, and I. Pálinkó^a

^a *Department of Organic Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary*

^b *Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7, H-6720 Szeged, Hungary*

e-mail palinko@chem.u-szeged.hu

ABSTRACT

In this study the synthesis, characterization and catalytic testing of bioinspired electron transfer catalysts are described. Cu(II)–mixed ligand complexes with three different amino acid esters (methylesters of L-histidine, L-cysteine and L-cystine) were covalently grafted onto chloropropylated silica gel. Conditions of the syntheses were altered: two methods were applied for the syntheses.

The obtained substances were studied by infrared spectroscopy and energy dispersive X-ray fluorescence coupled to scanning electron microscope (SEM–EDX). The superoxide dismutase activities of the materials were determined in a biochemical test reaction.

It was possible to prepare Cu(II)–mixed C-protected amino acid complexes anchored with covalent bonds onto modified silica gel. It was found that the structures of the complexes and the coordinating groups varied upon changing the experimental conditions. All the covalently immobilized Cu(II) complexes displayed superoxide dismutase activities, and some of them showed the promise of becoming efficient catalysts in electron transfer reactions in the laboratory or in fine chemical industry. Best of all was found to be the material when the 1:1 mixture of C-protected histidine and cysteine was attached to the chloropropylated silica and was soaked in a Cu(II) containing solution [SG–(His-OMe;Cys-OMe)–Cu(II)].

INTRODUCTION

Homogeneous catalysts are most often complex compounds containing a metal (ion) and various organic compounds. They can be highly active and, occasionally, extremely selective. However, during work-up, their separation from the reaction mixture and therefore their efficient recovery and reuse are usually difficult.

Metal ions are cofactors in many enzymes, most frequently in oxidoreductases. The ions there are capable of altering their redox state as well as their coordination number. The

ligands are bound to the metals through the donor atoms of various amino acids. Among them histidine residues are the most frequent. The cofactor together with the proteomic skeleton forms the most selective of all catalysts, the enzymes. They are semi-solid materials capable of working under relatively mild conditions: at near atmospheric pressure, in a limited temperature range and physiological aqueous solution. However, if we prepare metal ion–amino acid complexes inspired by the metal ion containing enzymes, and immobilize them on various supports, then we may be able to produce catalysts with activities and selectivities resembling those of the enzymes. This widens the range of conditions under which the heterogenized complexes are capable of working and, at the same time, facilitates easy recovery and recycling [1].

In order to approach these goals Cu(II)–C-protected amino acid (L-histidine, L-cysteine and L-cystine) complexes were covalently grafted onto modified silica gel. Our previous experience with successful synthesis of silica gel anchored Cu(II)–mixed ligand N- or C-protected histidine and tyrosine were encouraging [2]. The protective groups were not removed even after the synthesis by any of the methods, since our previous experience suggested that surface complexes with protective groups were more active than those having deprotected amino acids [3].

Results of this experimental work are described in the followings.

EXPERIMENTAL PART

Materials and methods of the synthesis

The components of the anchored complexes Cu(NO₃)₂, L-histidine methylester (H-His-OMe), L-cysteine methylester (H-Cys-OMe), L-cystine dimethylester (H-Cys-OMe)₂ and chloropropylated silica gel (SG – particle size: 230-400 mesh, BET surface area: 500 m²/g, functionalization: 8%) were commercial products just as isopropanol, which was used as solvent. They were purchased from Aldrich Chemical Co. All the chemicals were analytical grade and were used without further purification.

The amino acid esters (Fig. 1) were covalently grafted onto the modified silica gel with N-alkylation (Fig. 2) by refluxing the mixture in basic solution. After 24 h the solid substance was filtered, washed several times in order to remove the uncoupled amino acids. Then, the complexes were built with soaking the amino acid grafted supports in Cu(II) salt dissolved in isopropanol under continuous stirring at room temperature for 24 h. These were the complexes made under ligand-poor conditions (in this case, only the immobilized protected amino acids were available for coordination). The complexes made were allowed to rearrange in the presence of excess added amino acids under continuous stirring the

solution at room temperature for 24 hours. These were the complexes constructed under ligand-excess conditions.

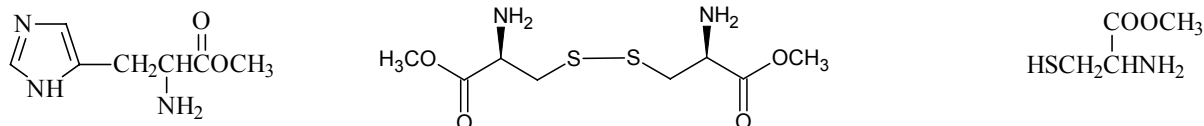


Fig. 1. The amino acid esters used as ligands: H-His-OMe, $[\text{H-Cys-OMe}]_2$, H-Cys-OMe.

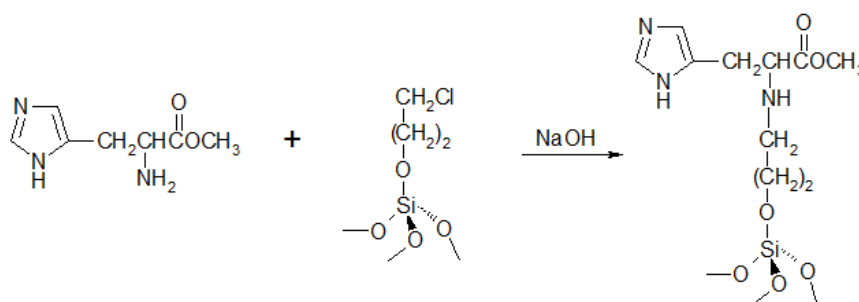


Fig. 2. The reaction sequence of grafting histidine methylester onto the modified silica gel.

For the preparation of covalently anchored mixed-ligand complexes, two methods were applied.

Method A: Grafting one of the protected amino acids was done first, then the obtained material was soaked in Cu(II) salt dissolved in isopropanol, and then the other protected amino acid was added in excess.

Method B: The first step of immobilization was the anchoring of a 1:1 molar mixture of the two protected amino acids, then the Cu(II) complex was formed under ligand-poor and ligand excess (1:1 molar ratio) conditions.

Infrared spectroscopy

Structural information on each step of the synthesis procedure was obtained by mid-range infrared spectroscopy, measuring diffuse reflectance. The $3800\text{--}600\text{ cm}^{-1}$ wavenumber range was investigated. Spectra were recorded with a BIO-RAD Digilab Division FTS-65 A/896 FT-IR spectrophotometer with 4 cm^{-1} resolution. For a spectrum 256 scans were collected. Spectra were evaluated by the Win-IR package. The spectra were treated thoroughly, they were baseline-corrected, smoothed (if it was necessary), the spectra of supports were subtracted and the resulting spectra were deconvolved. In the figures the more informative $1850\text{--}600\text{ cm}^{-1}$ region is depicted.

SEM-EDX

The elemental maps of the substances were determined with a Röntec QX2 energy dispersive X-ray fluorescence spectrometer coupled to the scanning electron microscope.

Testing the catalytic activity

Catalytic (SOD) activity was tested by the Beauchamp-Fridovich reaction [4]. For this biochemical test 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 followed by spectrophotometry, measuring the absorbance at 560 nm. If our enzyme mimicking material works well, it competes with NBT with success capturing the superoxide radical ion. Thus, the photoreduction of NBT is inhibited. The SOD probe reaction was carried out at room temperature in a suspension of the immobilized complex at pH=7 ensured with a phosphate buffer. The reaction mixture contained 0.1 cm³ of 0.2 mmol/dm³ riboflavin, 0.1 cm³ of 5 mmol/dm³ NBT, 2.8 cm³ of 50 mmol/dm³ phosphate buffer (Na₂HPO₄ and KH₂PO₄) containing EDTA (0.1 mmol/dm³), L-methionine (13 mmol/dm³) and the catalyst. Riboflavin was added last and the reaction was initiated by illuminating the tubes with two 15 W fluorescent lamps. Equilibrium could be reached in 10 minutes. EDTA removes the disturbing trace metal ions, since the metal ion-EDTA complexes have no SOD activity. From the resulting graph the volume of enzyme mimicking complex corresponding to 50% inhibition (IC₅₀) was registered to allow a comparison with the efficiency of the real enzymes and other SOD mimics. The enzyme mimics works the better when the IC₅₀ is the smaller. There was no reaction without illumination and the support did not display SOD activity either.

RESULTS AND DISCUSSION

Infrared spectroscopy

Surface-anchored Cu(II)-mixed histidine methylester and cystine dimethylester, histidine methylester and cysteine methylester, cystine dimethylester and cysteine methylester complexes were built on the surface of the chloropropylated silica gel support. Both the A and B methods were used for the syntheses.

Covalent grafting and the preparation of the silica gel anchored complexes were successful in all cases, since well-structured spectra were obtained after the subtraction of the spectrum of the support, and the obtained materials were colored.

The coordinating groups were determined through comparing the spectra of the pristine (uncomplexed) protected amino acids and the difference spectra (the spectrum of the support was subtracted) of the immobilized complexes, inspecting the color of the substances and chemical evidences.

C-protected histidine and cystine

As far as C-protected histidine is concerned, one of the imidazole nitrogens is always a Cu(II) coordinating site [5, 6]. Other candidates are the carbonyl oxygen or the methoxy oxygen of the ester group. Because of steric reason, the former is more probable than the latter. The amino nitrogen can also take part in complexation, but in anchored format this option can be discarded, because it is too close to the surface of the support.

For C-protected cystine, the most probable coordination sites are the sulfur atoms of the disulfide bridge, the carbonyl oxygens and the amino nitrogens.

In Fig. 3. the difference spectra of the materials prepared with *Method A* are displayed.

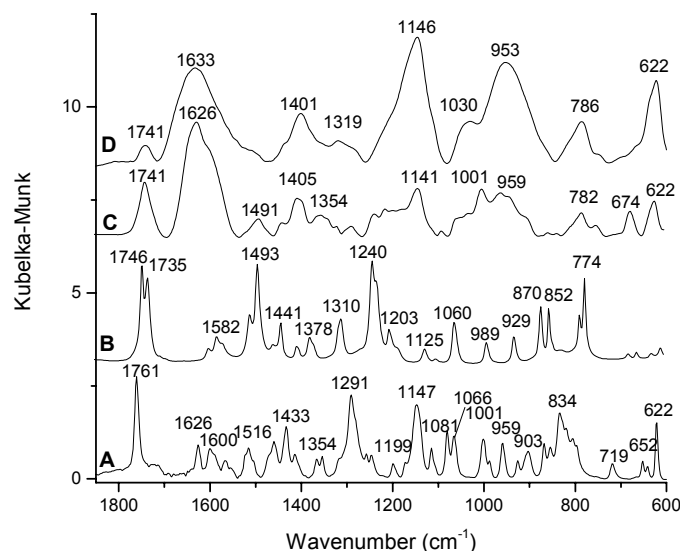
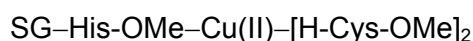
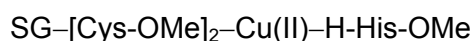


Fig. 3. *Method A*: the FT-IR spectra of A – H-His-OMe, B – [H-Cys-OMe]₂, C – SG-[Cys-OMe]₂-Cu(II)-H-His-OMe, D – SG-His-OMe-Cu(II)-[H-Cys-OMe]₂. The spectrum of SG is subtracted.

The similarity of the spectra of C and D is obvious. It means that the coordination sphere of the metal ion is similar. The carbonyl stretching vibration of H-His-OMe (1761 cm⁻¹) shifted towards lower wavenumbers (1625-1635 cm⁻¹ region) indicating that the carbonyl oxygen is

a sure coordinating site. The carbonyl bands attributed to [H-Cys-OMe]₂ (1746 and 1735 cm⁻¹) were not displaced, thus, they do not take part in the complexation. The N-H stretching vibration of cystine dimethylester shifted to higher frequencies (not shown), indicating that the amino group is coordinated to the central ion. The obtained substances were coloured, they were yellowish-brownish, meaning that the coordination number of Cu(II) is 4 [7]. On the basis of these results and the previously mentioned chemical considerations, the following structures for the covalently immobilized complexes may be proposed:



where surf stands for surface.

The FT-IR spectra of the materials obtained with *Method B* are displayed in Fig. 4.

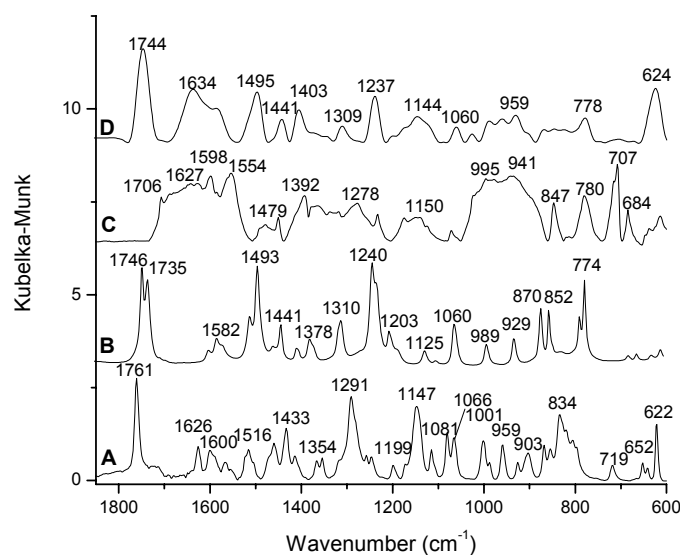
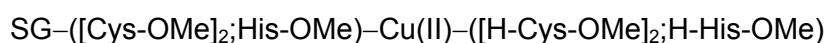
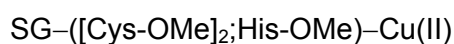


Fig. 4. *Method B*: the FT-IR spectra of A – H-His-OMe, B – [H-Cys-OMe]₂, C – SG-(His-OMe; [Cys-OMe]₂)-Cu(II), D – SG-(His-OMe; [Cys-OMe]₂)-Cu(II)-(H-His-OMe; [H-Cys-OMe]₂). The spectrum of SG is subtracted.

It is clear that the complexes made under ligand-poor (trace C) and ligand-excess (trace D) conditions significantly differ from each other. In these spectra one can identify bands of both components of the ligand mixture (e.g., compare bands at 1744 cm⁻¹ and 1746 cm⁻¹ in traces D and B, respectively; at 624 cm⁻¹ and 622 cm⁻¹ in traces D and A, respectively; at 1238 cm⁻¹ and 1240 cm⁻¹ in traces C and B, respectively; at 1150 cm⁻¹ and 1147 cm⁻¹, respectively). Under ligand-poor conditions the carbonyl vibrations of both protected amino acids are shifted to the 1720-1550 cm⁻¹ region, meaning that the carbonyl oxygens are coordinated to the central ion. The N-H stretching vibration of cystine dimethylester shifted to higher

frequencies (not shown), indicating that the amino group participates in the complexation. The obtained material is green, from literature data in these cases the coordination number of Cu(II) is more than four. Under ligand-excess conditions the surface anchored complex rearranged in the presence of added amino acids. The carbonyl oxygens of the C-protected cysteine were not coordinated, since the position of the carbonyl bands hardly changed relative to the free cysteine dimethylester. However, the carbonyl oxygen of histidine methylester takes part in the complexation. The N-H stretching vibration of the protected amino acids did not shift. The colour of the immobilized complex is brown. Putting together these pieces of information the following coordination environment are offered:



where surf stands for surface.

C-protected histidine and cysteine

The possible coordinating groups for histidine methylester were described in a previous paragraph. As far as cysteine methylester is concerned, the most probable coordination sites are the sulphur atom of the thiol/thiolate group, the carbonyl oxygen and the amino nitrogen of the added amino acids. The difference spectra of the anchored complexes prepared with *Method A* are shown in Fig. 5.

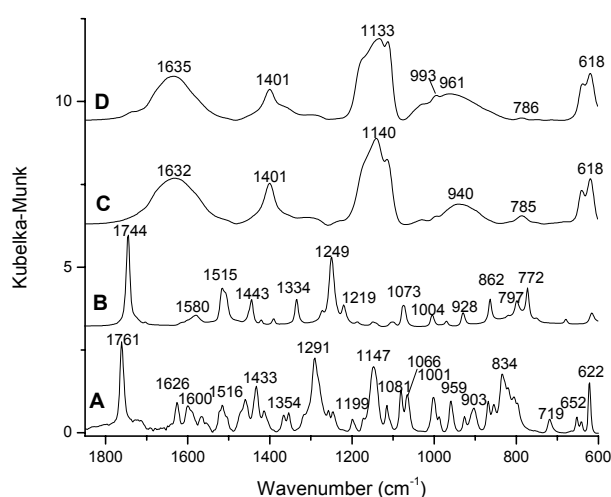
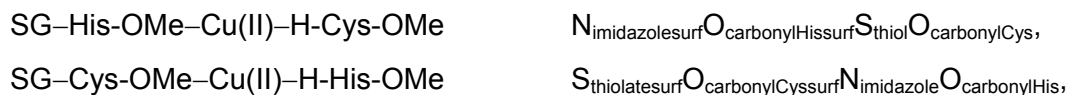


Fig. 5. *Method A*: the FT-IR spectra of A – H-His-OMe, B – H-Cys-OMe, C – SG-His-OMe-Cu(II)-H-Cys-OMe, D – SG-Cys-OMe-Cu(II)-H-His-OMe. The spectrum of SG is subtracted.

Spectrum C is practically the same as spectrum D, indicating that the coordination environment of the central ion in these two compounds is similar. It can be seen that the carbonyl oxygens takes part in the complexation. The corresponding bands shifted towards lower wavenumbers. The coordination number of Cu(II) is four again, since the obtained substances were brown. On the basis of these observations and accumulated knowledge the following structures for the covalently immobilized complexes can be proposed:



where surf stands for surface.

As far as *Method B* is concerned (Fig. 6) the two difference spectra are very different. The surface grafted complex rearranged under ligand-excess conditions.

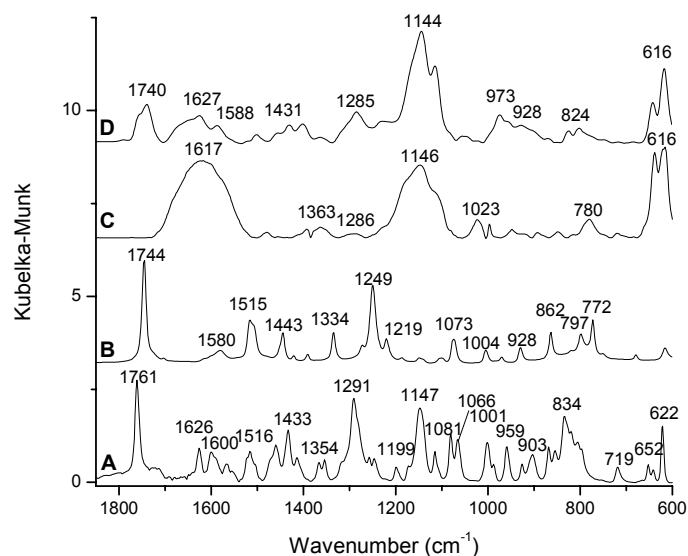


Fig. 6. *Method B*: the FT-IR spectra of A – H-His-OMe, B – H-Cys-OMe, C – SG-(His-OMe; Cys-OMe)-Cu(II), D – SG-(His-OMe; Cys-OMe)-Cu(II)-(H-His-OMe; H-Cys-OMe). The spectrum of SG is subtracted.

Under ligand-poor conditions (trace C) bidentate ligation is quite conceivable, there are not enough surface-anchored amino acids in close vicinity to each other for monodentate ligation. The carbonyl stretching vibrations shifted to lower wavenumbers (1650-1550 cm^{-1} range) indicating that the carbonyl oxygens are coordinating sites in both amino acids. A broad band can be observed at 3450 cm^{-1} (not shown) referring to the coordination of water molecules. Under ligand-excess conditions the carbonyl oxygen of cysteine methylester is no longer a coordinating site only that of histidine methylester.

The coordination number of Cu(II) is five or six in both cases, because the materials were green. On the basis of these results structural proposals for the surface-bound complexes are offered as follows:

SG–(His-OMe;Cys-OMe)–Cu(II)



SG–(His-OMe;Cys-OMe)–Cu(II)–(H-His-OMe;H-Cys-OMe)



where surf stands for surface.

C-protected cystine and cysteine

Visual inspection of Fig.7 reveals that the syntheses with *Method A* were successful.

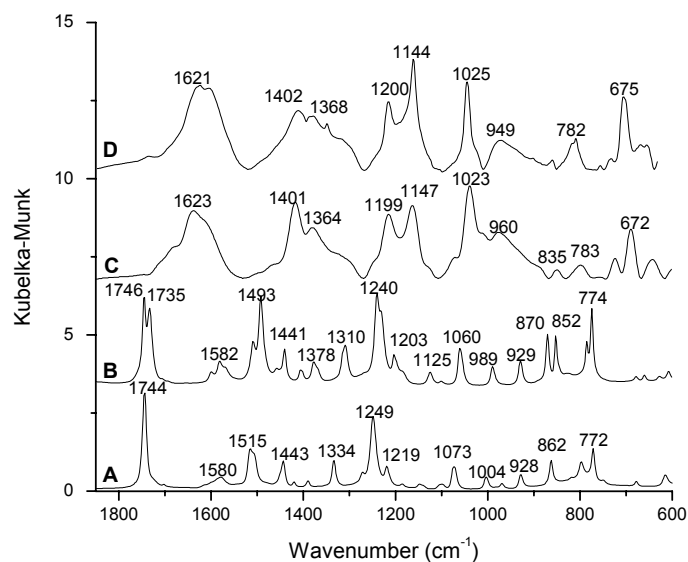
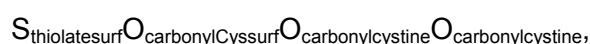


Fig. 7. *Method A*: the FT-IR spectra of A – H-Cys-OMe, B – [H-Cys-OMe]₂, C – SG-Cys-OMe-Cu(II)-[H-Cys-OMe]₂, D – SG-[Cys-OMe]₂-Cu(II)-H-Cys-OMe. The spectrum of SG is subtracted.

Spectra C and D look similar, *i.e.* the coordination sphere of Cu(II) is alike in both cases. The carbonyl oxygens of the protected amino acids are sure coordination sites, since their stretching vibrations shifted to the 1650-1550 cm⁻¹ region. All materials were yellowish-brownish indicating that the coordination number of Cu(II) was four. We propose a coordination environment as follows:

SG–Cys-OMe–Cu(II)–[H-Cys-OMe]₂



SG–[Cys-OMe]₂–Cu(II)–H-Cys-OMe



where surf stands for surface.

The difference spectra of the immobilized complexes when *Method B* was used for the syntheses are depicted in Fig. 8.

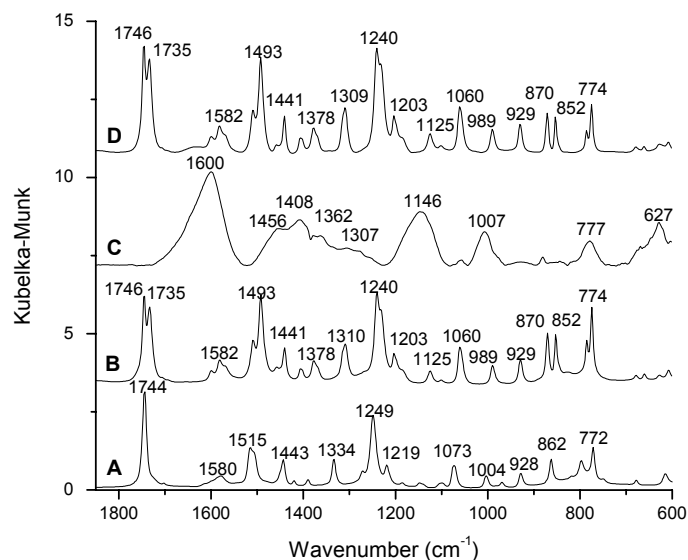
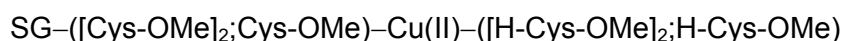
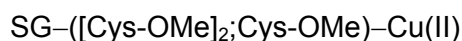


Fig. 8. *Method B*: the FT-IR spectra of A – H-Cys-OMe, B – [H-Cys-OMe]₂, C – SG-(Cys-OMe; [Cys-OMe]₂)-Cu(II), D – SG-(Cys-OMe; [Cys-OMe]₂)-Cu(II)-(H-Cys-OMe; [H-Cys-OMe]₂). The spectrum of SG is subtracted.

The two spectra are very different, added amino acids caused the rearrangement of the surface bound complex. It can also be learnt that the carbonyl oxygens of both amino acids are coordinated to the central ion under ligand-poor conditions because their positions shifted towards lower wavenumbers (to the 1650-1550 cm⁻¹ region). Under ligand-excess conditions spectrum D indicates that the cystine dimethylester is only coordinated from the 1:1 mixture of the added amino acids. The carbonyl oxygens of the added C-protected cystine are not coordination sites. The coordination number of Cu(II) was four again, the grafted complexes were yellowish-brownish. The offered coordination spheres are the followings:



where surf stands for surface.

SEM-EDX

The elemental map of the anchored complexes were prepared with the SEM-EDX measurements. In Fig. 9 the image of SG-Cys-OMe-Cu(II)-H-His-OMe can be seen (magnification: 10000). In some areas sulphur atoms and Cu(II) ions are evenly distributed in the sample, while in other places Cu(II) was accumulated. This clearly means that although mostly complexation with the amino acid derivatives takes place, but part of the Cu(II) ions can be firmly attached to the silica surface, probably to the surface OH groups.

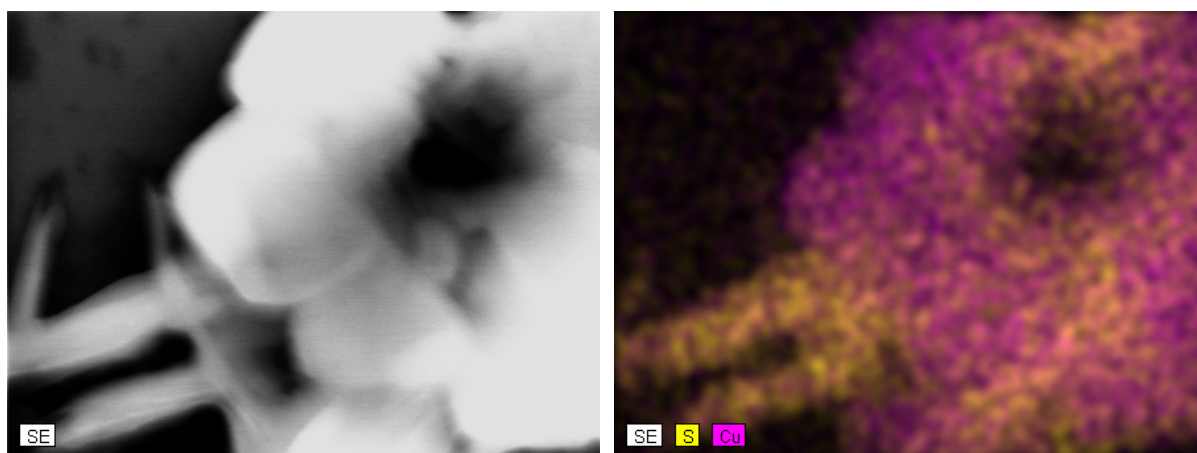


Fig. 9. SEM image at magnification of 10000 and S, Cu elemental map on the SEM image.

Testing the catalytic activity

All materials displayed catalytic activity, *i.e.*, could catalyze the dismutation of the superoxide radical anion. Catalytic activities differed widely though (Table 1).

Data reveal that there were catalysts coming close to the activity of the Cu-Zn SOD enzyme. The immobilized complexes made under ligand-poor conditions with *Method B* are more active than those prepared under ligand-excess conditions or with *Method A*. The most active anchored complex was SG-(His-OMe;Cys-OMe)-Cu(II). It seems that a more strained environment arising under ligand-poor conditions makes the complex more reactive.

Surface grafted complexes having mixed C-protected histidine and cysteine as ligands are more active than the others. They seem to have the optimum structures for promoting this reaction.

Table 1 The SOD activities of the surface complexes covalently grafted onto SG.

	IC ₅₀ (μM)
Cu-Zn SOD enzyme	0.4
SG-[Cys-OMe] ₂ -Cu(II)-H-His-OMe	177
SG-His-OMe-Cu(II)-[H-Cys-OMe] ₂	66
SG-([Cys-OMe] ₂ ;His-OMe)-Cu(II)	41
SG-([Cys-OMe] ₂ ;His-OMe)-Cu(II)-([H-Cys-OMe] ₂ ;H-His-OMe)	132
SG-His-OMe-Cu(II)-H-Cys-OMe	38
SG-Cys-OMe-Cu(II)-H-His-OMe	27
SG-(His-OMe;Cys-OMe)-Cu(II)	15
SG-(His-OMe;Cys-OMe)-Cu(II)-(H-His-OMe;H-Cys-OMe)	18
SG-Cys-OMe-Cu(II)-[H-Cys-OMe] ₂	30
SG-[Cys-OMe] ₂ -Cu(II)-H-Cys-OMe	102
SG-([Cys-OMe] ₂ ;Cys-OMe)-Cu(II)	63
SG-([Cys-OMe] ₂ ;Cys-OMe)-Cu(II)-([H-Cys-OMe] ₂ ;H-Cys-OMe)	149

Acknowledgements

Research leading to this contribution was financed by the National Research Fund of Hungary through grant OTKA 83889. The support is highly appreciated.

REFERENCES

1. A.J. Kirby, *Angew. Chem. Int. Ed. Engl.* 35 (1996) 706
2. Z. Csendes, V. Bugris, L. Lackó, I. Labádi, J.T. Kiss and I. Pálinkó, *Anal. Bioanal. Chem.* 397 (2010) 549
3. I. Szilágyi, O. Berkesi, M. Sipiczki, L. Korecz, A. Rockenbauer and I. Pálinkó, *Catal. Lett.* 127 (2009) 239
4. C. Beauchamp and I. Fridovich, *Anal. Biochem.* 44 (1971) 276
5. P.C.A. Bruijninx, M. Lutz, A.L. Spek, W.R. Hagen, B.M. Weckhuysen, G. van Koten and R.J.M.K. Gebbink, *J. Am. Chem. Soc.* 129 (2007) 2275
6. X. Bi and K.-L. Yang, *Langmuir* 23 (2007) 11067
7. J. Gazo, *Pure Appl. Chem.* 38 (1974) 279