# Sampling Catalysis Research in the Pannonian Region

*Proceedings of the 8th Pannonian International Catalysis Symposium*

*Supported by*


Jointly organised by

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***and***
***The Catalysis Club of the Hungarian Academy of Sciences***

**2006**
**Szeged, Hungary**

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ISBN 963 06 0138 9

Published by the Hungarian Zeolite Association

# The synthesis and the catalytic (*catalase* and *tyrosinase*) activities of amino acid copper complexes covalently grafted onto silica gel

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## Abstract

In this work the synthesis, structure and certain catalytic properties of amino acid copper complexes covalently grafted onto silica gel are described. The following enzyme mimicking complexes were synthesized and characterised by experimental (FT-IR) and computational (mainly MM+) methods: BOC-His-Cu/silica gel, BOC-Tyr-Cu/silica gel, His-OMe-Cu/silica gel, Tyr-OMe-Cu/silica gel, H-His-Cu/silica gel, H-Tyr-Cu/silica gel His-OH-Cu/silica gel and Tyr-OH-Cu/silica gel. The activities of these substances were also tested in the decomposition of hydrogen peroxide. The majority of the substances proved to be good enzyme mimics displaying either *catalase* or *tyrosinase* activity.

## 1. Introduction

Amino acid copper complexes often resemble the active sites of certain enzymes. It is especially so, if one or more ligands are L-histidine. Enzyme mimics are promising catalysts since high catalytic activities and, what is even more important, high selectivities can be expected under mild conditions. The catalytic and related (like work-up) properties may be further improved if the enzyme mimicking complexes are immobilised on a solid support. Immobilisation may occur by enforcing steric constraints (encapsulation) [1] or through electrostatic (ion exchange) [2], covalent bonding or secondary forces (hydrogen bonding or adsorption) [3, 4]. In this contribution the synthesis, structure and certain catalytic properties of amino acid copper complexes covalently grafted onto silica gel are described.

## 2. Experimental

The central ion for the complexes was $Cu^{2+}$ and the amino acids applied as ligands (products of Aldrich Co.) were N-protected (with BOC, tert-butoxycarbonyl that is) or C-protected (in the form of methyl ester) L-histidine or L-tyrosine: BOC-His-OH, BOC-Tyr-OH, H-His-OMe and H-Tyr-OMe (Fig. 1). The source of $Cu^{2+}$ ions was the

aqueous solution of $Cu(NO_3)_2$ – product of Reanal. The amino acids were used as received.

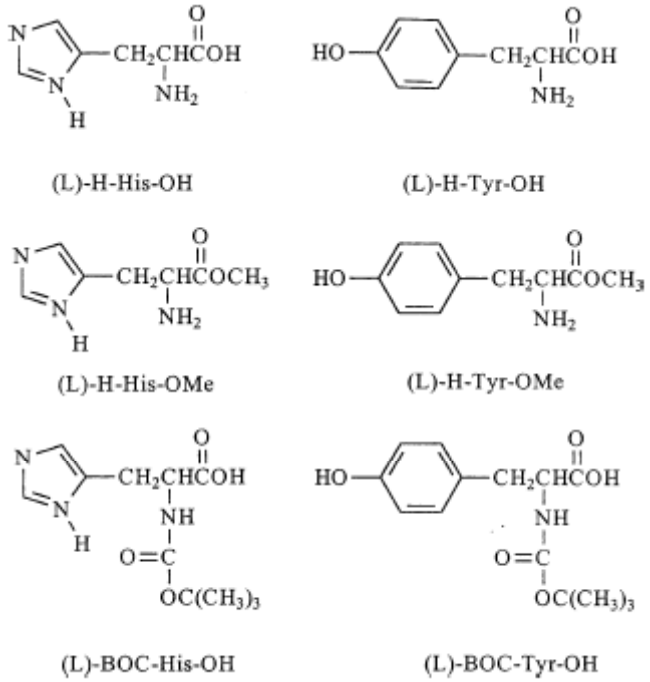



Fig. 1. The amino acids and their protected derivatives used in this study.

The host material was 3-chloropropyl silica gel – abbreviated as SG – (Aldrich, 230–400 mesh, BET surface area: ~500 $m^2/g$, ~8% functionalised).

The first step of immobilisation was the reaction of the appropriately protected amino acid with the chloropropylated silica gel. General recipe is as follows: certain amount of functionalised silica gel was suspended in water and excess protected amino acid solution was added. Coupling with the ester or the BOC-amino acid was achieved by refluxing the mixture under basic conditions during constant stirring. After 3 h the solid material were filtered washed several times and dried. The resulting material was divided into two parts. The first one was left unchanged, the other one was treated with sulfuric acid, in order to hydrolyse the ester bond or was refluxed under vigorous stirring at moderate temperature (338 K) for 2 h in an 1:1 mixture of $CH_2Cl_2$ and $CF_3COOH$ in order to remove the BOC protecting group. Then, the samples (four different substances) were soaked in $Cu(NO_3)_2$ solution under stirring overnight. After filtering solution of the appropriate amino acid derivatives were added in excess. The suspension was refluxed for an hour and stirred for 4–5 more hours at room temperature. Finally, the solid material was filtered rinsed with deionised water 5–6 times, dried and stored in a vacuum desiccator.

Substances obtained were studied by FT-IR spectroscopy by the KBr technique. The KBr pellets (1.2 mg of the substances in 200 mg KBr) were pressed from the materials and these were applied for monitoring changes in the IR spectra of the samples. The FT-IR spectra of the host (SG), the amino acid derivatives, the substances containing the anchored amino acid (BOC-His-O-SG, BOC-Tyr-O-SG, SG-His-OMe, SG-Tyr-OMe) and material with the covalently grafted Cu(amino acid) complexes (Cu-

BOC-His-O-SG, Cu-BOC-Tyr-O-SG, Cu-SG-N-His-OMe, N-protected or C-protected L-histidine or L-tyrosine as ligands were taken and compared. The 3800–480 $cm^{-1}$ range was investigated. Spectra were recorded by a Mattson Genesis I spectrophotometer with 2 $cm^{-1}$ resolution. For a spectrum 126 scans were collected. Spectra of the KBr pellets were taken at 298 K in air. Spectra were evaluated by the Win-IR package.

The covalently grafted complexes were modelled by them MM+ force field included in the HyperChem package [5]. The samples were tested in the decomposition of hydrogen peroxide. The reaction was performed in the liquid phase at 343 K and followed by UV-VIS spectroscopy (Perkin–Elmer). The well-stirred reaction mixture contained 100 mg of the catalysts and 20 $cm^3$ of 2% hydrogen peroxide aqueous solution. This way both the *catalase* ($H_2O_2$ decomposition) and, with the tyrosine-containing anchored complex, the *tyrosinase* (oxidation of the phenolic ring of tyrosine to quinoidal structure) could be examined.

## 3. Results and Discussion

Covalent grafting of appropriately protected amino acids allows greater control over immobilisation than does ion exchange. BOC-protected amino acids can be bonded to chloropropylated silica gel through transesterification, while N-alkylation is the reaction of the amino acid esters. The reactions are easy just as deprotection (if it is required) of the anchored amino acids. Then, the covalently grafted copper complex can be built up without difficulties. Eight immobilised complexes were synthesized. They were as follows: BOC-His-Cu-SG, BOC-Tyr-Cu-SG, His-OMe-Cu-SG, Tyr-OMe-Cu-SG, H-His-Cu-SG, H-Tyr-Cu-SG, His-OH-Cu-SG and Tyr-OH-Cu-SG.

FT-IR spectroscopic measurements revealed that both the nitrogen(s) of the ring or the amino side chain and the carboxylic oxygens took part in ligation. As an example Figure 1 contains the overlay spectra of the chloropropylated silica gel, L-tyrosine, L-tyrosine covalently grafted to silica gel through its N-terminal and the copper complex of this covalently anchored amino acid (Fig. 2). For the IR spectra of the other anchored complexes and the related discussion, see ref. [6].

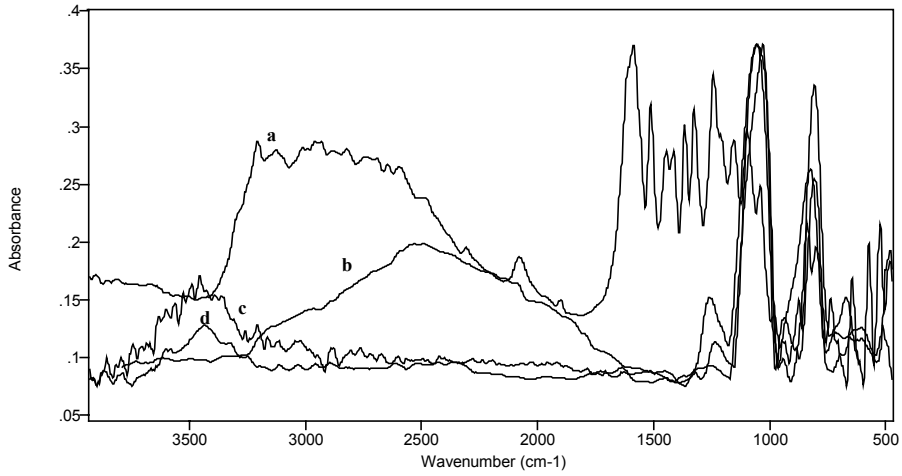
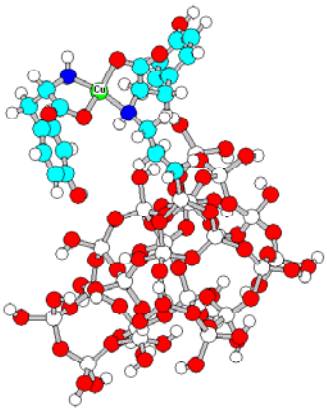
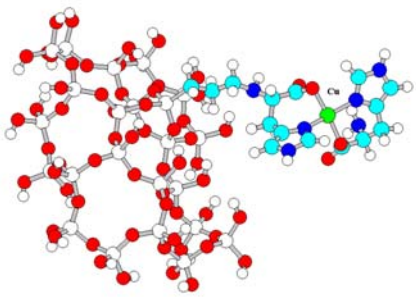


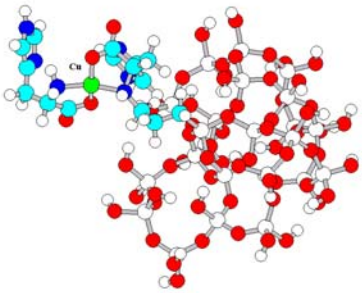

Fig. 2. The IR spectra of (a) L-H-Tyr-OH, (b) L-Tyr-OH-SG (L-tyrosine bonded to the silica gel *via* the N-terminal), (c) Tyr-OH-Cu-SG and (d) chloropropylated silica.

Computations indicated that the structure of the complexes were most probably distorted tetrahedron as shown for the grafted Cu-tyrosine and Cu-histidine complexes grafted on silica gel through the N-terminal of the amino acid ligand (Fig. 3). Related works published in the scientific literature [7-11] indicates that probably chelation occurred in most cases, i.e. the amino acid derivatives were coordinated to the copper(II) ion *via* their nitrogen and oxygen donor sites. During reaction this structure may be easily transformed to a pentacoordinated arrangement accepting temporarily the reactant, while changing the oxidation state of copper from two to one.



(a)





(b) (c)

Fig. 3. Calculated (MM+) structural models for the Tyr-OH-SG [(a)] and the His-OH-Cu-SG [(b) and (c)] substances. For structures (a) and (c) coordination is through the carboxylic oxygens and the amino nitrogens, while for structure (b) it is through the carboxylic oxygens and the imidazole nitrogens.

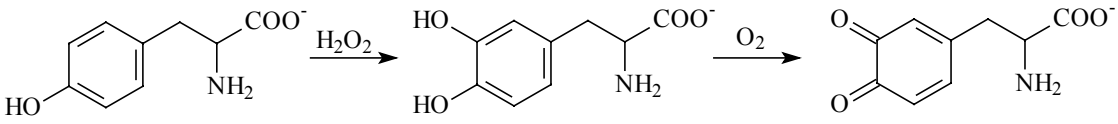
The activities of these substances were tested in the decomposition of hydrogen peroxide. The transformation was made visible with titanyl sulfate and the reaction thus could be followed by UV-VIS spectroscopy. Changes in the reaction mixture occurred with each immobilised material except H-His-Cu-SG and His-OH-Cu-SG. However, the phenomena observed were of two types. Either gas evolution took place and the colour of the reacting mixture did not change or gas evolution was accompanied with the browning of the reacting suspension. In the first case the gas evolved was oxygen

evolution accompanied by water formation – a disproportionation reaction of hydrogen peroxide (*catalase* activity).

$$2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$$

This was the reaction on BOC-His-Cu-SG, His-OMe-Cu-SG, Tyr-OMe-Cu-SG and H-Tyr-Cu-SG.

The other reaction could be observed on BOC-Tyr-Cu-SG and Tyr-OH-Cu-SG. It was found that the evolved oxygen oxidised the phenyl ring of tyrosine to an *ortho* quinoidal structure (dopaquinone derivative), which could be a precursor of a melanin-like material (*tyrosinase* activity) [12, 13]. The formation of the quinoidal structure is probably preceded by the hydroxylation of the phenolic ring in the *ortho* position [14].




The *catalase* activity decreased in the BOC-His-Cu-SG > His-OMe-Cu-SG > H-Tyr-Cu-SG > Tyr-OMe-Cu-SG sequence, while the *tyrosinase* activity decreased in the BOC-Tyr-Cu-SG > Tyr-OH-Cu-SG order.

## 4. Conclusions

Cu-amino acid complexes could be prepared under controlled conditions and were covalently anchored onto chloropropylated silica gel. The majority of the substances proved to be good enzyme mimics displaying either *catalase* or *tyrosinase* activity. Through modelling the protein skeleton as well by applying mobile functionalised resins instead of silica gel as the next step, even better enzyme mimics may be obtained.

## Acknowledgement

This work was financed by the National Research Fund of Hungary through grant K62288. The help is highly appreciated.

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