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Covalent Grafting of Cu(II)-histidine Complexes on Polystyrene Resin Studied by Photoacoustic Infrared Spectroscopy

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In order to mimic copper-containing enzymes, Cu(II)-histidine complexes were grafted onto chloropropylated polystyrene resin. The Cu(II)-histidine complexes and the mobile polymer were thought to resemble the active centre and the proteomic skeleton of the enzymes, respectively. The resulting heterogenised complexes were expected to be nearly so active, selective and more durable catalysts that are easier to recycle than their homogeneous counterparts. The intended area of use is oxidation and dismutation reactions. Control for the syntheses was exerted by protecting either the N-terminal or the C-terminal of the covalently grafted L-histidine molecules. However, since the resin was reported to be amino group selective, covalent grafting was performed with the unprotected amino acid as well. During the preparative work generally applied methods of synthetic organic chemistry (alkylation or esterification) were used. The whole procedure was performed in isopropanol to allow appropriate swelling of the host polymer. After deprotecting the immobilised amino acids the complexes were prepared and the emerging structures (just as the full synthetic procedure) were studied by Photoacoustic Infrared Spectroscopy. The photoacoustic spectra revealed that the grafting was successful in each case and the complexes were formed too. However, real control over the preparation could only be proven when the protected amino acids were applied. On the basis of the spectra and chemical reasoning structural features of the immobilised complexes could be described.

1 Introduction

Enzymes are perhaps the most active but definitely the most selective catalysts known today. Since the vast majority of chemical industry is based on catalytic reactions, learning about the activity and selectivity influencing factors in catalytic systems are of utmost importance. The accumulated knowledge may lead researchers to invent novel, more efficient catalysts.

For this invention a promising way is trying to mimic the active sites of enzymes if they are known. Quite often, especially in enzymes facilitating oxygen transfer, the active sites contain copper ion surrounded by amino acids of the proteomic skeleton [1]. The most frequently occurring amino acid is L-histidine. These two observations make copper-histidine complexes good approximation of the active site of oxygen-transfer enzymes.

However, the complex itself maybe a good catalyst but recovery is difficult if it is possible at all. Upon anchoring the complex by various methods (adsorption-hydrogen bonding [2], ion exchange [3], covalent grafting [4]) onto rigid supports (montmorillonite [2, 3], silica gel [2, 4], mesoporous materials like e.g., MCM-41 [3]) is a solution to this problem – a solid catalyst is always easier to handle than a homogeneous one.

Resemblance to an enzyme, however, may be further enhanced by replacing the rigid support with a more flexible one, since the proteomic skeleton is mobile and so are the amino acids serving as ligands to the metal ion cofactor. Swellable resins may such mobile supports. Choosing a chlorofunctionalised polystyrene resin allows covalent grafting of amino acids. Then, complex formation can be performed. If control on the synthesis of amino acid-copper complexes can be exerted one obtains a catalyst closely mimicking the structure of the enzymes, hopefully, with similar activity and selectivity in oxygen transfer reactions.

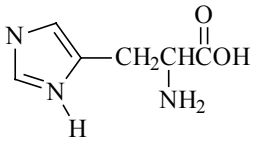
Results of the synthesis of such an enzyme mimic and its structural characterisation is reported in this contribution.

2 Experimental

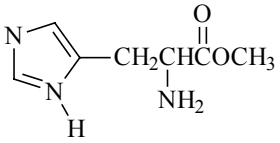
2.1 Materials

The central ion for the complexes was Cu^{2+} and the ligands (products of Aldrich Co.) were either L-histidine

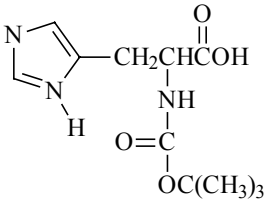
(H-His-OH), *tert*-butoxycarbonyl-L-histidine (BOC-His-OH) or L-histidine methyl ester (H-His-OMe) (Figure 1). The source of Cu^{2+} ions was the aqueous solution of $Cu(NO_3)_2$ B product of Reanal. The amino acids were used as received.



(L)-H-His-OH



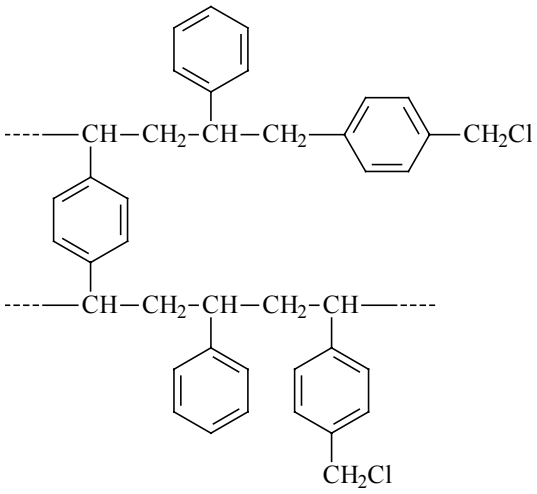
(L)-H-His-OMe



(L)-BOC-His-OH

Figure 1: The molecules to be immobilised

The host material was a chlorinated polystyrene resin [poly(styrene-co-vinylbenzyl chloride-co-divinyl-benzene with 3.8-4.2 mmol/g chlorine content] from Aldrich, abbreviated as PS-PheCH_2Cl in the followings (Figure 2).



PS-PheCH_2Cl

Figure 2: The chlorinated polystyrene resin host

Further materials used: cc. (96%) sulfuric acid (RPE; Farmitalia Carlo Erba S.p.A.), methylene chloride, trifluoroacetic acid (Aldrich Co.), solid NaOH, isopropanol, toluene (all from MOLAR CHEMICALS Ltd.).

2.2 Preparation of the covalently grafted complexes

The first step of immobilisation was the reaction of the appropriately protected amino acid with the chlorinated polystyrene. General recipe is as follows: certain amount of functionalised resin was suspended in isopropanol 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 three hours the solid material was filtered washed several times and dried. The resulting material was divided into two parts. The first one was left unchanged, the other one was either treated with sulfuric acid, in order to hydrolyse the ester bond or was refluxed under vigorous stirring at moderate temperature (338 K) for two hours in a 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 isopropanol 5-6 times, dried and stored in a vacuum desiccator.

Since the resin was reported to be amine-selective, anchoring of histidine and then complexation were performed with the unprotected amino acid as well.

2.3 Characterisation by Photoacoustic Spectroscopy

Substances obtained were studied by FT-IR spectroscopy. Spectra were recorded on a BIO-RAD Digilab Division FTS-65A/896 FT-IR spectrometer equipped with an MTEC 200 photoacoustic detector. The 4000 cm^{-1} – 400 cm^{-1} range was investigated. The resolution was 4 cm^{-1}. For a spectrum 256 interferograms were collected. Samples were loaded into a sample holder with 3 mm diameter. Measurements were performed under He atmosphere. The spectra were evaluated by the Win-IR package.

One advantage of using photoacoustic detector for recording the spectra is that the solid material has not to be pressed into a tablet or pellet, thus, heat evolving during pressurisation does not destroy our modified resin substances. The other one is the information from the interior of the sample unavailable to other IR methods.

3 Results and Discussion

3.1 Anchoring the amino acids to the polymer host

First as basis of comparison let us show the PAS IR spectra of L-histidine (Figure 3) and the chlorinated resin (Figure 4).

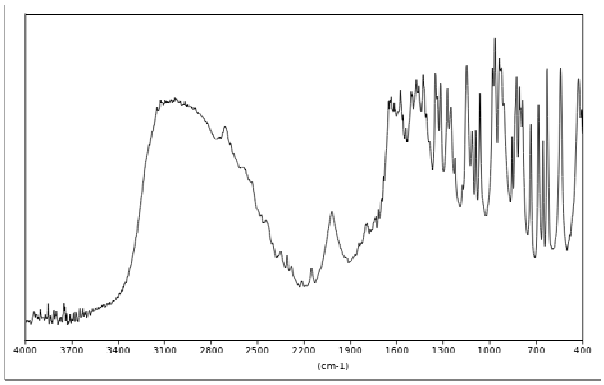


Figure 3: The PAS IR spectrum of H-His-OH

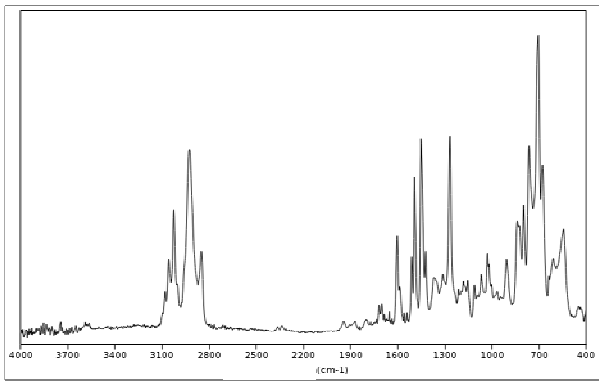
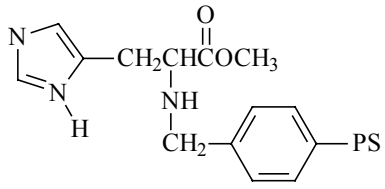


Figure 4: The PAS IR spectrum of PS-PheCH$_2$Cl

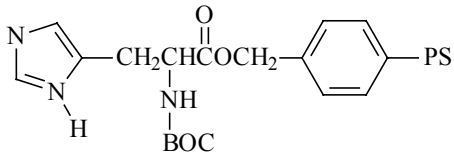
Since the chlorine functionality is attached to a benzylic group in our host polymer, coupling reactions between the resins and the amino acids should be relatively easy. To exert control on the syntheses protected amino acids were coupled first.



PS-PheCH$_2$-His-OMe

Figure 5: The covalently anchored C-protected histidine

Coupling of H-His-OMe resembles N-alkylation, while that of BOC-His-OH is an esterification. The anchored amino acids are depicted schematically in Figures 5 and 6.



BOC-His-OCH$_2$Phe-PS

Figure 6: The covalently anchored N-protected histidine

Since the resin was specified to be amino selective, the spectra of the resin (a), the anchored unprotected amino acid (b) and, most importantly, the difference spectrum (c) are shown in Figure 7.

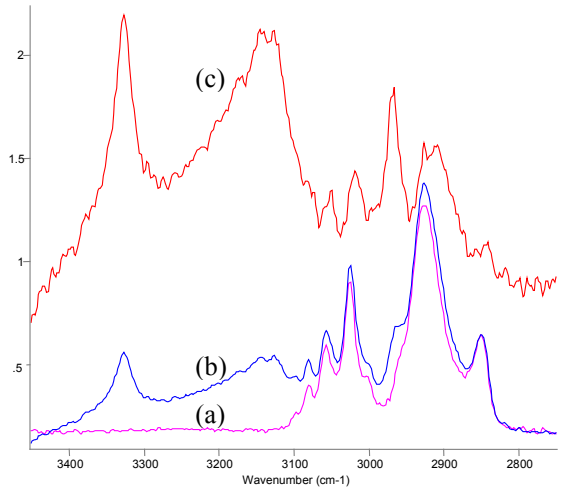


Figure 7: The PAS IR spectra of the resin (a), the anchored unprotected L-histidine (b) and the difference spectrum (c [b–a])

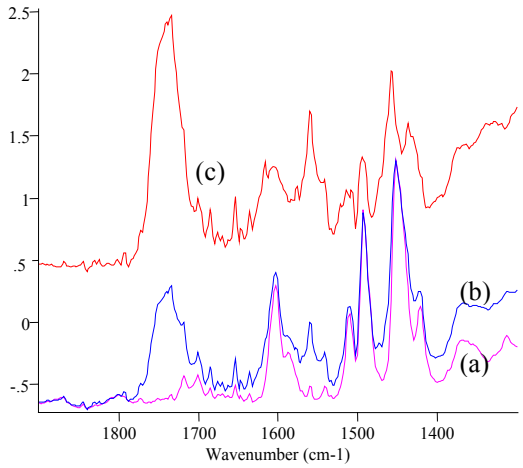


Figure 8: The PAS IR spectra of the resin (a), the anchored C-protected histidine after deprotection (b) and the difference spectrum (c [b-a])

The sharp band at 3330 cm^{-1} in the difference spectrum in Figure 7 clearly reveals that the unprotected histidine is anchored through its amino group, thus, the resin is amino selective, indeed.

The BOC-protected and the ester-protected amino acids were forced to be anchored *via* the carboxylic group and the amino group, respectively. The PAS IR spectrum of the latter, after deprotection, is depicted in Figure 8. Again, the spectra of the resin (a), the covalently grafted amino acid after deprotection (b) and the difference spectrum are displayed. The difference spectrum reveals that the amino acid is attached to the resin, indeed.

3.2 The covalently grafted complexes

Since the PAS IR spectra of immobilised complexes prepared from three different starting compounds resemble each other closely, first, as a representative example, the full spectrum of the covalently grafted complex derived from H-His-OMe is shown in Figure 9. Then, the PAS IR spectra of the anchored complexes derived from either the unprotected amino acid (H-His-OH) or the BOC-protected one (BOC-His-OH) are analysed in more detail.

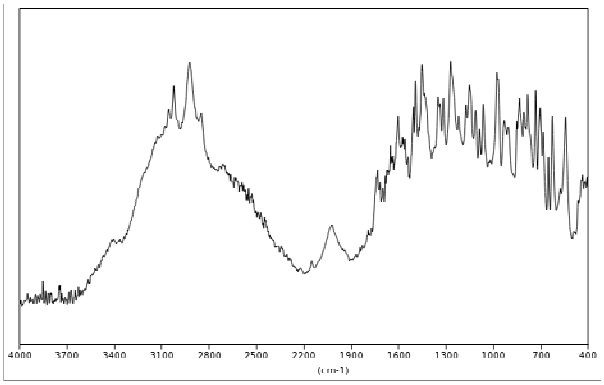


Figure 9: The PAS IR spectrum of the covalently anchored complex derived from H-His-OMe

The resemblance of the PAS IR spectra indicates that irrespective to the mode of anchoring coordination of the three immobilised complexes should be the same. Since the way of preparation disqualifies both the carboxylic group and the amino group from coordination the imidazole nitrogens should be responsible for making the complex. In order to prove this deductive statement let us compare the difference spectra obtained from subtracting the spectrum of the immobilised amino acid from that of the anchored complex for the BOC-His-OH related material (a) as well as the H-His-OH related substance (b) (Figure 10).

It is to be seen that in spite of the huge difference in the way of anchoring (grafting through the amino group for the H-His-OH related material vs. anchoring via the carboxylic group for the BOC-His-OH related substance) the difference spectra only slightly alter. The difference spectrum of the H-His-OH related substance shows the symmetric and asymmetric vibrations of the carboxylate group, while these are not seen in the spectrum of the H-His-OMe related anchored complex. It is, however, only due to the method of synthesis. The first material was prepared in a base-catalysed reaction, while the hydrolysis of the ester protecting group required acidic conditions.

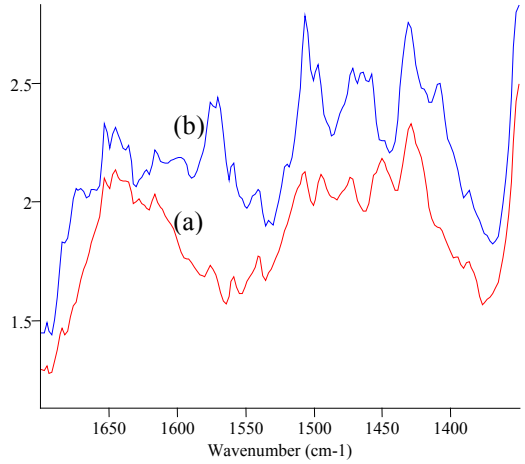


Figure 10: The difference PAS IR spectra of the BOC-His-OH related (a) and the H-His-OH related anchored complexes (the spectrum of the anchored amino acid was subtracted from that of relevant immobilised complex)

3.3 Modes of coordination in the covalently grafted complexes

Results displayed and discussed above indicate that no matter of whether anchoring occurred at the C- or N-terminal of histidine the covalently grafted amino acids are coordinated to the copper(II) ion through imidazole nitrogens. Thus, steric reasons require the coordination of four histidine molecules. Once again, steric reasons do not allow planar arrangement around the central ion, therefore, tetrahedral geometry is suggested (Figure 11).

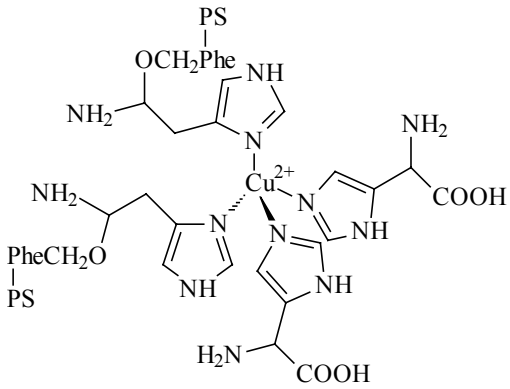


Figure 11: Covalently grafted Cu(II)-histidine complex anchored to the resin, for instance, *via* the C-termini

3 Summary

Immobilised Cu(II)-L-histidine complexes were prepared by covalently grafting the amino acid ligands onto chlorine-functionalised polystyrene. Control on the syntheses was exerted by applying protected amino acids, although, the resin displayed amine selectivity. Irrespective of the anchoring group the complexes assumed tetrahedral geometry. One ring nitrogen of all four histidine molecules was coordinated to the central ion. These immobilised complexes are promising catalysts in oxygen transfer reactions. Their activities, hopefully, are going to approach that of the Cu-containing oxygen transfer enzymes, since both the active site as well as the flexible proteomic skeleton were mimicked.

References

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