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COVALENTLY IMMOBILIZED, SILICA GEL OR RESIN-SUPPORTED C-PROTECTED CYSTEINE OR CYSTINE Fe AND Ni COMPLEXES – SYNTHESIS AND STRUCTURAL CHARACTERIZATION WITH FT-IR SPECTROSCOPY

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ABSTRACT

In this work the syntheses of covalently grafted C-protected Fe(III)– or Ni(II)–L-cysteine and Fe(III)– or Ni(II)–L-cystine complexes onto a surface-modified silica gel or Merrifield's resin are described. Conditions of the syntheses were varied and the obtained structures were studied by classical analytical (titration) as well as spectroscopic (infrared, atomic absorption) methods. It was found that the sulphur atoms in the molecules acted as primary coordination sites, while the other coordinating groups varied depending on whether the complexes were formed under ligand-poor or ligand-excess conditions.

INTRODUCTION

In order to satisfy the need for novel highly active, and, even more importantly, highly selective catalysts various strategies may be followed. One promising way is the preparation of bioinspired catalysts, i.e., trying to copy the active sites of enzymes [1]. To make the work-up procedure easier and at the same time allowing the recovery of the catalyst, immobilizing these active site mimics seems to be advantageous. For immobilization rigid as well as flexible supports may be used. The latter may increase the resemblance to the enzymes, since the flexible support bears some similarities to the proteomic skeleton. In this contribution we describe such a biomimetic approach: our work concerns the preparation-covalent anchoring of Fe(III)– or Ni(II)–C-protected L-cysteine or C-protected L-cystine complexes onto a rigid modified silica gel or a flexible resin support.

Previously, we applied for anchoring of various copper–amino acid complexes onto silica gel [2] and montmorillonite [2-4] hydrogen bonding and ionic interactions, respectively. Although immobilisation was successful, we hoped for a better control of synthesis using covalent grafting. Our initial results with copper and N-protected tyrosine [5] or C-protected cysteine and cystine [6] and chloropropylated silica gel were encouraging.

Bioinspiration came from a paper [7] describing the discovery of a wild-type superoxide dismutase enzyme having nickel ion as cofactor coordinated by the cysteine and histidine amino acids of the surrounding peptide chains.

EXPERIMENTAL PART

Materials and the methods of the syntheses

The components of the anchored complexes [methylesters of L-cysteine and L-cystine (Figure 1), $FeCl_3.6H_2O$, $NiCl_2.6H_2O$ or $Cu(SO_4)_2.5H_2O$, chloropropylated silica gel (SG) or Merrifield's resin (RS)] were commercial products (Aldrich Chemical Co.) just as isopropanol, which was used as the solvent.

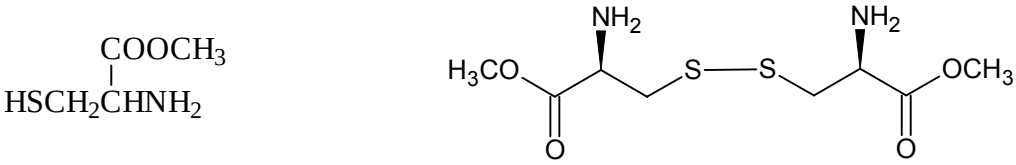

Fig. 1. The C-protected amino acids used as ligands.

Covalent grafting was performed at the N-terminal of the amino acid with the reaction of the amino group (N-alkylation-like transformation) with the chlorine of the chloropropylated silica gel or the Merrifield's resin. Example for the anchoring of C-protected cysteine onto the resin is displayed in Figure 2.

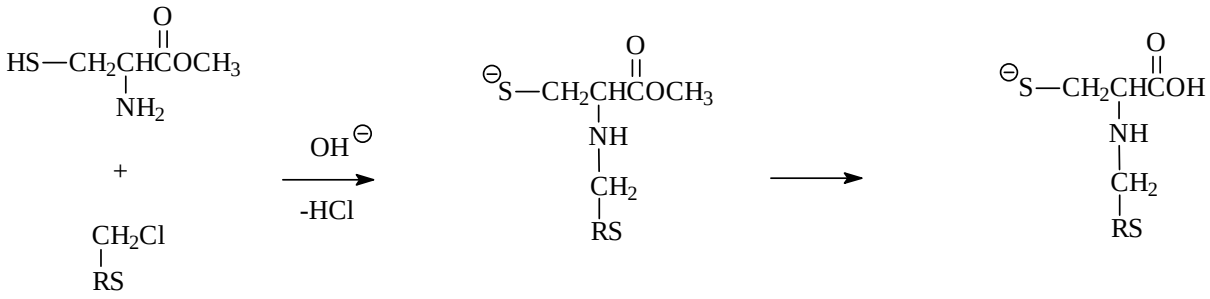

Fig. 2. The reaction sequence of grafting cysteine methylester onto Merrifield's resin.

Complexation followed the anchoring, applying either ligand-poor conditions (only the immobilized protected amino acids were available for coordination) or circumstances abundant in non-anchored C-protected amino acid molecules (ligand-excess conditions).

The general method was as follows: certain amount of functionalized silica gel was suspended in isopropanol and calculated amount of protected amino acid solution was added. Coupling with the ester was achieved by refluxing the mixture under basic conditions during constant stirring. After 24 hours the solid substance was filtered, washed several

times with isopropanol to remove unbound amino acid and dried. Then, it was soaked in the metal salt solution under stirring for another 24 hours at room temperature. After filtering and washing the filtrate thoroughly with the solvent and setting aside half the solid material thus obtained, excess amino acid and extra solvent was added to the other half. The suspension was stirred at room temperature for 24 hours again. Finally, the solid material was filtered, rinsed with isopropanol 5–6 times. Both portions of the solid substances (prepared under ligand-poor and ligand-excess conditions) were dried and stored in a vacuum desiccator.

Methods of structural characterisation

The amount of metal ions on/in the solid host was measured by atomic absorption spectrometry (AAS–Perkin Elmer 3110 instrument). Before measurements the solid materials were dissolved in aqua regia.

The nitrogen content was determined by the Kjeldhal method. The nitrogen content of the samples was turned to NH_3 by the sequential addition of cc. H_2SO_4 and NaOH solution. The ammonia formed was reacted with boric acid, then, it was titrated with aqueous HCl of known concentration.

Structural information on each step of the synthesis procedure was obtained by mid-range infrared spectroscopy, measuring diffuse reflectance. The 3800–550 cm^{-1} wavenumber range was investigated. Spectra were recorded with a BIO-RAD Digilab Division FTS-65 A/896 FT-IR spectrophotometer with 2 cm^{-1} resolution. For a spectrum 256 scans were collected. Spectra were evaluated by the Win-IR package after baseline correction smoothing (if it was necessary) and subtracting the spectra of the support.

RESULTS AND DISCUSSION

Metal ion to ligand ratios from AAS measurements and titrations

All the measurements indicated that covalent grafting and the preparation of surface-anchored complexes were successful either under ligand-poor or ligand-excess conditions. AAS measurements and the Kjeldahl method gave for metal ion to amino acid ratio 1:2 for the surface-bound Ni(II)– or Fe(III)–cysteine methylester complex either under ligand-poor or ligand-excess conditions. This ratio was the same for the surface-bound Ni(II)– or Fe(III)–cystine methylester complex under ligand-excess but 1:1 under ligand-poor conditions. Assuming four as the coordination number for the metal ions, we may state that under ligand-poor conditions two surface-bound cysteine methylester molecules were coordinated to the central ions and one surface-bound and added molecule were the ligands under ligand-excess conditions. As far as cystine methylester is concerned, one molecule

provided all the four coordinating groups under ligand-poor conditions, while the number of amino acid molecules was two in the presence of amino acid excess.

Coordination environment for the central metal ions in the surface-anchored complexes

FT-IR spectra, chemical reasoning and the pieces of information from AAS and titration measurements are used to envisage the coordination environments of the anchored complexes.

Exemplary FT-IR spectra for the resin-bound or the silica gel grafted Ni(II)– or Fe(III)–cysteine methylester complexes under ligand-poor and ligand-excess conditions and Ni(II)– or Fe(III)–cystine methylester complexes are depicted in Figure 3.

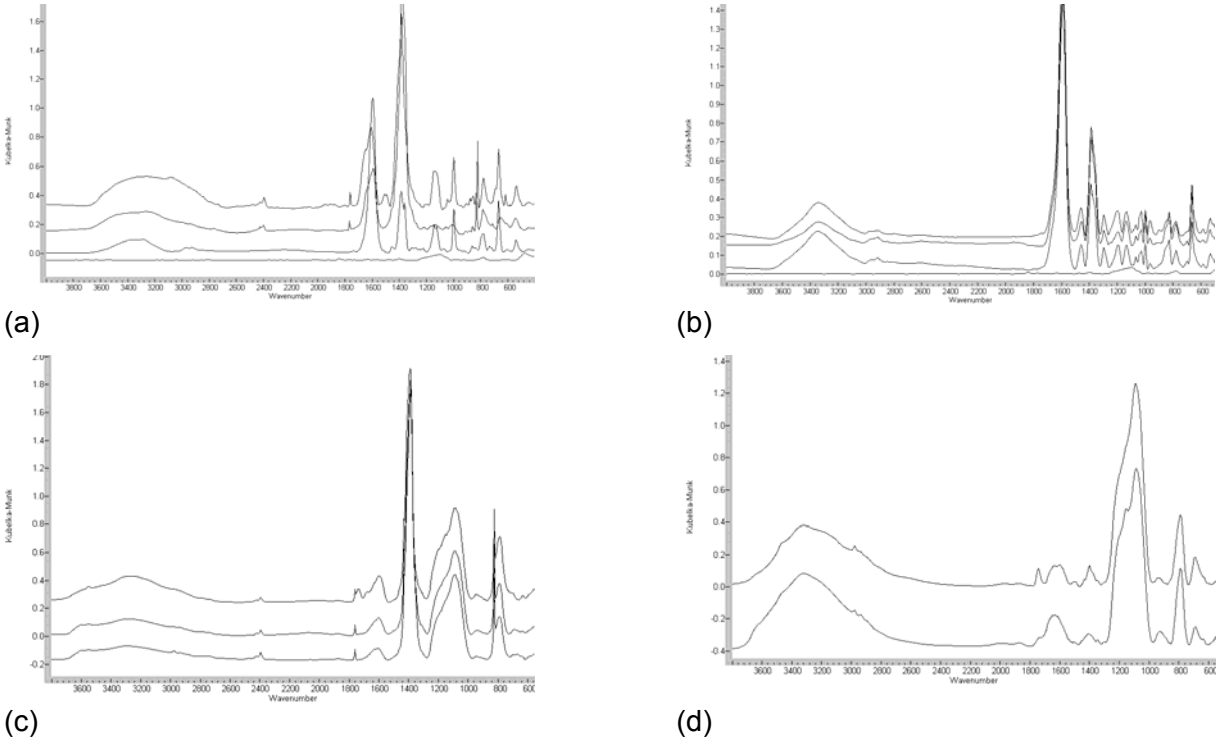

Fig. 3. The FT-IR spectra from the bottom to the top: (a) RS, cysteine methylester covalently grafted onto RS, the immobilized Ni-complex under ligand-poor and ligand-excess conditions, (b) RS, cystine methylester covalently grafted onto RS, immobilized Ni-complex under ligand-poor and ligand-excess conditions, (c) cystine methylester covalently grafted onto SG, the immobilized Ni-complex under ligand-poor and ligand-excess conditions (d) Fe(III)–cysteine methylester complex covalently grafted onto SG under ligand-poor and ligand-excess conditions.

Since it is difficult to evaluate the above spectra as shown (almost as registered after only minor transformations like baseline correction and smoothing), therefore, on the

example of the silica gel-anchored iron–amino acid complexes we show the way of analysis performed for each member of the four families of complexes (Ni-complexes on RS or SG, Fe-complexes on RS or SG). Let us put in advance that the conclusions proved to be the same for each family, therefore, the following statements have general validity for the substances of this study.

Analysis of the FT-IR spectra for the Fe(III) complexes covalently anchored onto silica gel

The major spectral transformation for the detailed assessment is the subtraction of the spectrum of the support and then the comparison of the results to the FT-IR spectra of the anchored (but not complexed) or the support-free C-protected amino acids.

In Figure 4 the spectrum of the SG-anchored cysteine methylester (spectrum at the bottom) and difference spectrum of the immobilized Fe(III)–C-protected cysteine prepared under ligand-poor conditions (spectrum at the top) are shown in the more informative 1850 cm^{-1} – 550 cm^{-1} wavenumber region. The carbonyl band and the bands corresponding to the C–S bonds shifted towards lower wavenumbers (1754 cm^{-1} → 1735 cm^{-1}, 795 cm^{-1} → 789 cm^{-1}, 698 cm^{-1} → 691 cm^{-1}), which means that surface complex was formed, indeed, and the two donor groups were the carbonyl and the thiolate groups. Taking four as the coordination number means that two surface-bound cysteine methylester as bidentate ligands coordinated to the Fe(III) central ion. The most probable structure of the immobilized complex was a distorted tetrahedron.

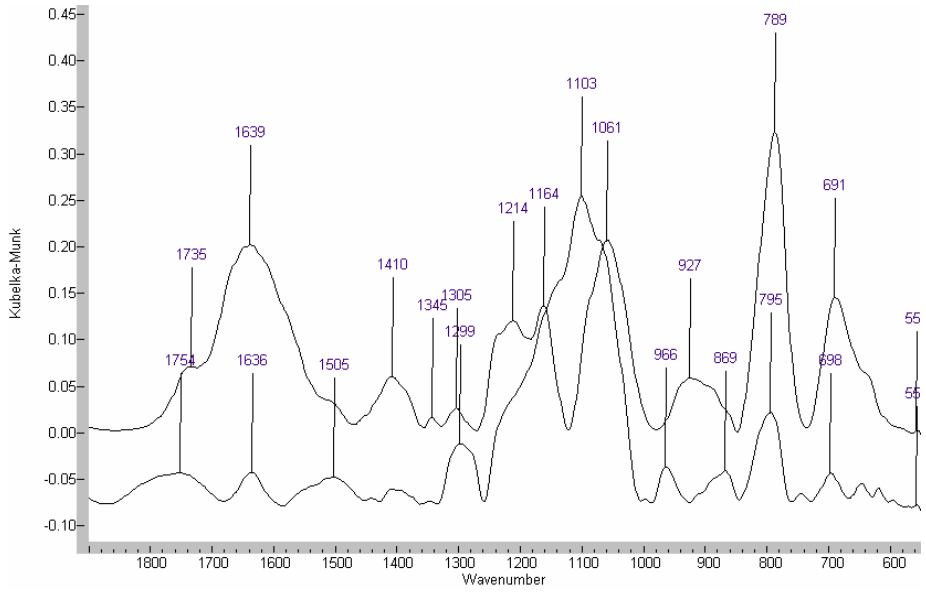

Fig. 4. The spectrum of the SG-anchored C-protected cysteine (at the bottom) and difference spectrum of the SG-anchored Fe(III)–C-protected cysteine complex prepared under ligand-poor conditions in the 1850-550 cm^{-1} region.

The structures of the SG-anchored complexes made under ligand-poor and ligand excess conditions were very similar to each other as Figure 5 attests. It contains the difference spectra for both materials in the 1850 cm^{-1} – 550 cm^{-1} wavenumber region.

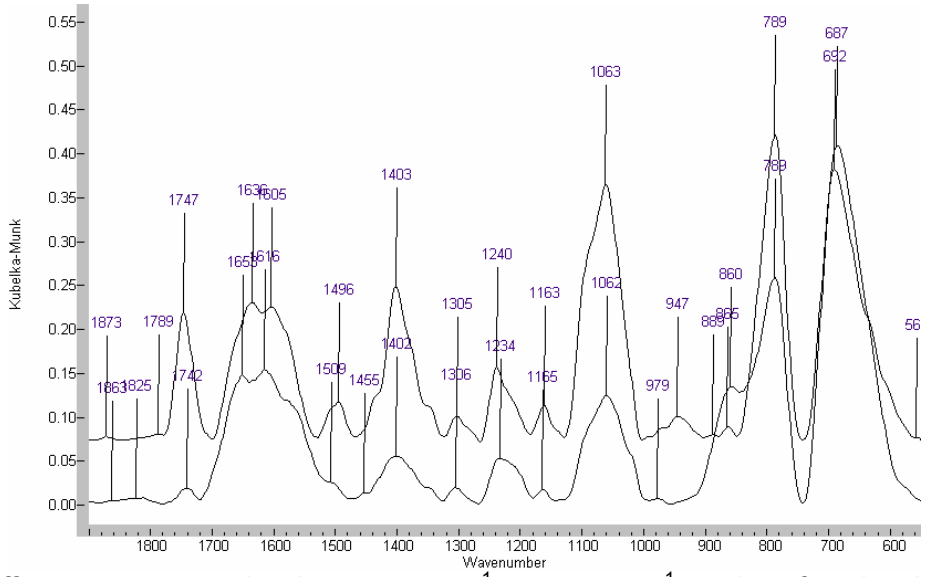

Fig. 5. Difference spectra in the 1850 cm^{-1} – 550 cm^{-1} region for the immobilized Fe(III)–C-protected cysteine complexes synthesized under ligand-poor (at the bottom) and ligand-excess (at the top) conditions.

This means that the structures were basically the same, i.e., two cysteine methylester molecules were coordinated as bidentate ligands irrespective to the conditions of the synthesis.

Figure 6 depicts (the slightly transformed) spectra of the SG-anchored Fe(III)–C-protected cystine complexes prepared under ligand-poor (at bottom) and ligand-excess (at the top) conditions. It is easy to observe that the two spectra differ from each other. However, the spectrum at the bottom is very similar to spectrum of Fig. 3(d) (also at the bottom), which means that under ligand-poor conditions the two anchored complexes (SG-anchored Fe(III)–C-protected cysteine and C-protected cystine) had very similar structure. In the latter case one surface-bound cystine methylester was able to provide the sulphur and the two carbonyl oxygens necessary for coordination, i.e. it behaved as a tetradentate ligand.

The comparison of the difference spectrum for the SG-anchored Fe(III)–C-protected cystine to the spectrum of the support-free cystine methylester (Figure 7) revealed that in this case the coordination mode changed, the added amino acid took over two coordination sites from the surface-grafted amino acid, thus, under ligand-excess conditions two cystine methylester molecules became coordinated as bidentate ligands. Most probably, coordination occurred through the sulphur atoms of the disulfide bridges.

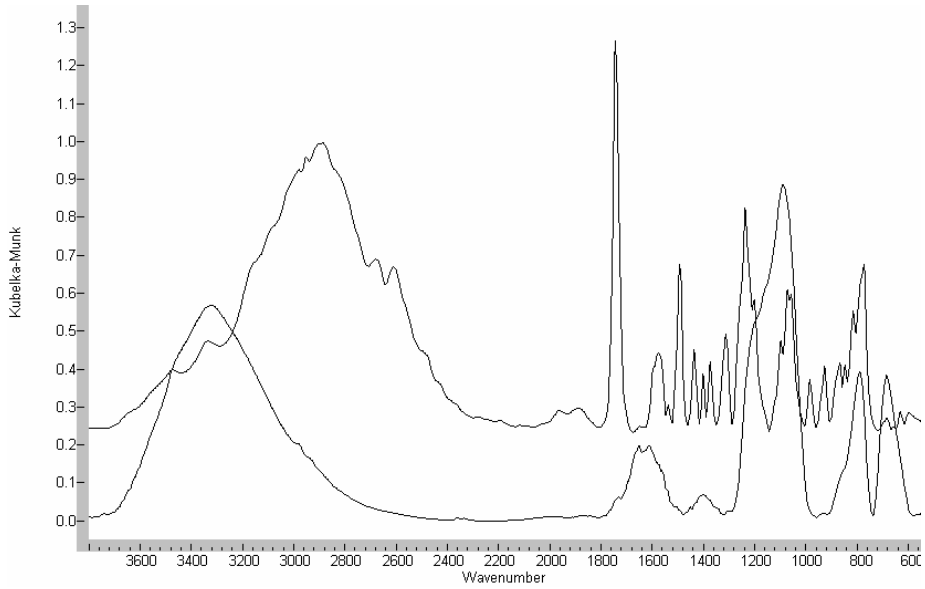

Fig. 6. The spectra of the SG-bound Fe(III)–C-protected cystine prepared under ligand-poor (at the bottom) and ligand-excess (at the top) conditions.

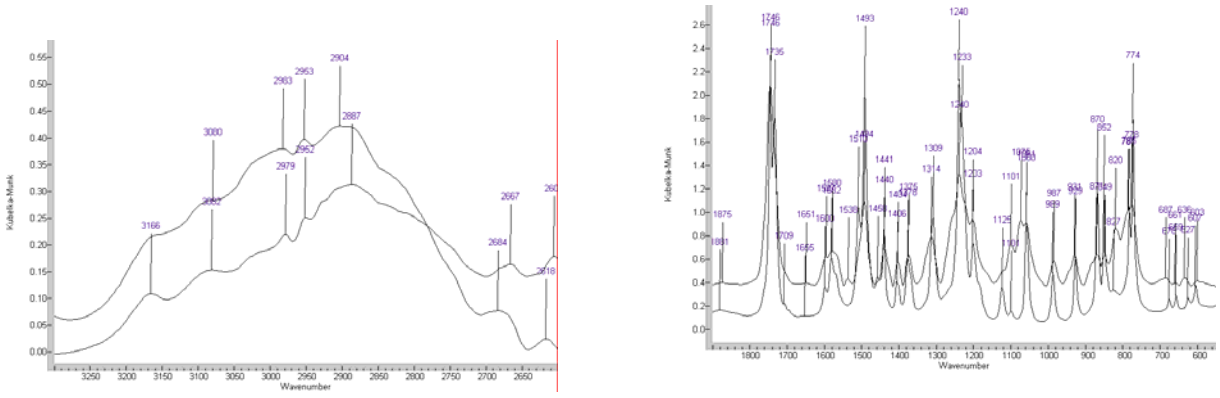

Fig. 7. The difference spectrum of the SG-anchored Fe(III)–C-protected cystine prepared under ligand-excess conditions (at the bottom) and the spectrum of the support-free C-protected cystine (at the top).

CONCLUSIONS

Surface-bound Ni(II)– or Fe(III)–cysteine methylester and Ni(II)– or Fe(III)–cystine methylester could be prepared under ligand-poor as well as ligand-excess conditions. A combination of chemical intuition, classical and instrumental analytical methods helped in finding possible coordinating groups. The major coordinating sites were proposed to be the sulphur atoms of the thiolate groups or the disulfide bridges. The other coordination sites depended on the conditions of the synthesis and the structures of the molecules.

Acknowledgements

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