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Voltammetric and Spectroscopic Behaviour of the Copper Adducts of Thiol and Thioether Ligands under Aerobic and Anaerobic Conditions

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Joint spectroscopic (ESR, electronic absorption and FT IR) and voltammetric (square-wave and cyclic voltammetry) techniques were employed to investigate the properties of copper adducts with cysteine, *N*-acetylcysteine and methionine under aerobic and anaerobic conditions. The metal oxidation state in the solid and solvated adducts adheres to the presence of oxygen, whereas ESR results ($g_{||} = 2.245$, $g_{\perp} = 2.059$, $A_{||} = 170$ Gauss) reveal tetragonally distorted octahedral copper(II) environment under aerobic circumstances. In presence of the thiol and thioether ligands under nitrogen voltammetric measurements evidence formation of copper(I) species.

Key words*:* aminoacids, copper complexes, redox reactions, spectroscopy, thioether, thiol, voltammetry

Reactions of cysteine and other biologically relevant thiol ligands with transition metal ions, particularly with copper(II), nickel(II) and cadmium(II), have been the subject of extensive research in the past [1–13]. Besides that the largest portions of the transition metals in biological systems are bound to proteins or other well-defined organic structures, redox reactions of "free" (uncoordinated, but yet solvated) metal ions in presence of thiols stimulate interest as a representative model of several biological processes. From the plethora of works, which documented complexation of copper ions with thiol ligands (*e.g.*, [3–13]), only a few account on comparison of the redox state and coordination environment of ligated copper ions in the solid state and aqueous solution in absence/presence of oxygen.

The present comparative study of both the electrochemistry and spectrophotometry of copper ion coordinated by biologically relevant thiol and thioether ligands cysteine, *N*-acetyl cysteine and methionine in the solid and aqueous phase is aimed to widen further the knowledge on its coordination properties. Structural preferences of adduct species, formed by even simple and flexible ligands comprising thiol or thioether functionalities as the ones we used here, are influenced strongly by the actual reaction conditions.

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EXPERIMENTAL

Synthesis: Cysteine, *N*-acetyl cysteine and methionine from commercial sources were applied without purification. All reactant solutions were prepared in deionized and triply-distilled water. The thiol ligands in stoichiometric amounts were added to the copper(II) solution in presence of oxygen. The adducts formed during subsequent stirring of the reaction mixtures at low temperature (about 298 K). The resulting solid complexes were removed by filtration, washed with small amounts of EtOH, dried *in vacuo*, and then their spectra were recorded.

Voltammetry: Square-wave voltammetric (SWV) experiments were performed with an EG&G PAR Model 384B polarographic analyser connected to an EG&G PARC Model 303A polarographic stand (Princeton, NJ, USA). A static Hg drop electrode (SMDE) and an Ag|AgCl|saturated KCl reference electrodes were used; the auxiliary electrode was a Pt wire. The voltammograms were recorded with a Houston Instrument DMP-40 plotter (Austin, TX, USA). The voltammetric measurements of each solution were carried out in 0.05 M Britton-Robinson buffer (pH 7.4) as supporting electrolyte. Before each investigation, the electrolyte was purged with N_2 for 8 minutes. A known volume of standard solution was added to the deaerated polarographic cell and voltammograms recorded. The experiments were carried out under a blanket of nitrogen at room temperature.

Spectroscopy: Electronic spectra of the mixtures with ambient mole ratio of both copper(II) and thiol/thioether ligand aqueous solutions in the 900–190 nm range were recorded with Unicam V2-100 UV/Vis spectrophotometer (cell length 1 cm) in presence of air. Absorbance variations at the maximum absorption wavelength of these mixtures were monitored. The FT IR spectra of the solid adducts in the 4000–400 cm^{-1} region were obtained with Jasco FT IR 350 interferometer from pressed KBr pellets at resolution of 4 cm^{-1} at ambient temperature, based on averaging 32 sample and 16 background scans. ESR spectra were collected using a Varian EC 109 spectrophotometer, the field being calibrated with diphenylpicrylhydrazyl (DPPH): microwave frequency 9.52 GHz; other analytical conditions: power 8 dB, field modulation amplitude 1.25 Gauss, scan field 2000 Gauss, field set 3000 Gauss, gain 2.5×10^2.

RESULTS AND DISCUSSION

Voltammetry: ***Cathodic SWV of the ligands in presence of copper (II):*** In absence of copper(II), the square-wave voltammogram of cysteine in the Britton-Robinson buffer of pH 7.4 showed two peaks at –0.112 V (mercuric cysteine thiolate, $Hg(SR)_2$, where R = $–CH_2CH(NH_2)COOH$) and –0.560 V (mercurous cysteine thiolate, $Hg_2(SR)_2$), respectively. Upon polarization of the mercury electrode at 0.00 V (*vs.* $Ag|AgCI|KCl_{sat}$), cysteine reacts with Hg in an ionic reaction producing $Hg(RS)_2$, which adsorbs at the Hg surface. In a subsequent scan to negative potentials, $Hg(RS)_2$ is initially reduced to $Hg_2(RS)_2$ and also adsorbed on Hg in a different way, and then $Hg_2(RS)_2$ is reduced to metallic mercury and free thiolate ion. The peak at –0.112 V is observed at both high cysteine concentrations and high scan rates [9,14,15]. The similar electrode reactions are obtained for *N*-acetyl cysteine [1,2,16] and methionine [17]. It is generally accepted that the voltammetric behaviour of methionine points on the following reaction [17]:

$$2R'SCH_3 + Hg + 2H_2O \rightleftarrows Hg(R'S)_2 + 2CH_3OH + 2H^+ + 2e^- \quad (1)$$

where $R' = –CH_2CH_2CH(NH_2)COOH$.

The voltammetric characteristic peaks of cysteine, *N*-acetyl cysteine and methionine in presence of copper(II) are given in Table 1. In the absence of the ligands, copper(II) gives one peak positioned at –0.190 V at pH 7.4 due to the electroreduction according to the scheme: $Cu(II) + 2e^- \rightleftarrows Cu(Hg)$. Addition of 2×10^{-6} M cysteine into a cell containing 1×10^{-5} M copper(II) induced two new cathodic peaks, at –0.086 and –0.698 V (Table 1). Similar peaks were also observed in the case of *N*-acetyl cysteine and methionine.

Table 1. The peak potentials of thiolate/thioether and their cuprous complexes in 0.05 M Britton-Robinson buffer (pH 7.4).

Compound	E_{p1}/V	E_{p2}/V
Cysteine	–0.112 [9]	–0.560 [9]
N-Acetyl cysteine	–0.120	–0.598
Methionine	–0.050	–0.438
Cu(I)–Cysteine complex	–0.086 [9]	–0.698 [9]
Cu(I)–*N*-Acetylcysteine complex	–0.090	–0.686
Cu(I)–Methionine complex	–0.098	–0.580

The peak at –0.698 V (peak 1) is explained [9] as the reduction of cuprous cysteinate complex (CuSR) to copper amalgam and free thiol (RSH) according to the reaction:

$$CuSR_{(ads)} + H^+ + e^- \rightleftarrows Cu_{(Hg)} + RSH_{(sol)} \quad (2)$$

No information is found in the literature on existence of a cupric complex under unaerobic conditions [18], although it has been indicated as an intermediate in the formation of the cuprous complex [19]. The cathodic peak at –0.086 V (peak 2) corresponds to a process, in which the adsorbed mercury complex is transformed in the presence of copper(II) ion into an adsorbed copper complex. The process proceeds [9] according to the reaction:

$$Hg(RS)_{2(ads)} + Cu^{2+} + 2e^- \rightleftarrows Cu(RS)_{2(ads)} + Hg \quad (3)$$

The reversibility of the electrochemical reaction of Cu(I)SR complex has been studied by cyclic voltammetry. The peak separation between the anodic and cathodic signals is about 57 mV. This value approaches closely the theoretical value (56 mV) for a reversible single-electron process [20]. The voltammetric information on stoichiometry and overall stability constant of the complex Cu(I)SR can be obtained from the peak potential shifts upon addition of cysteine. The logarithm of formation constant for the Cu(I)–thiolate complex was calculated as 21.64 [9]. Complexation of cysteine with Cu(II) was discounted as it is considered to be unstable with low formation constant ($\log\{K\} = 7.0$ [21]). The above mentioned reaction mechanisms are also proposed for *N*-acetyl cysteine and methionine in the presence of copper ions. Consequently, voltammetric measurements of the copper complexes show that the interaction of Cu(II) with the thiol and thioether ligands under anaerobic conditions yields copper(I) complexes.

Spectroscopy: ***Electronic absorption spectra:*** The electronic absorption spectra of the copper complexes in aqueous solution recorded at aerobic condition are presented in Fig. 1. All adducts exhibited absorptions in the 200–350 nm range that can be attributed to ligand absorption bands and amine-/amide- or carboxylate-to-copper ligand-to-metal charge transfer (LMCT) bands [22].

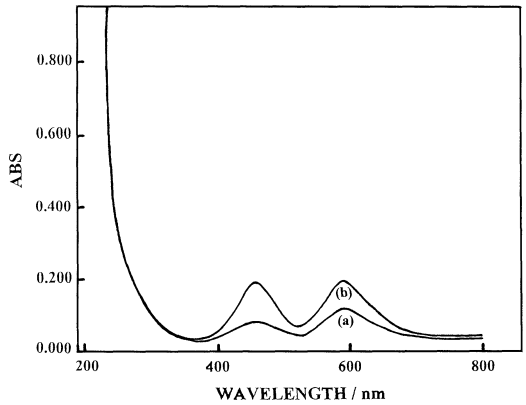

Figure 1. Electronic absorption spectra of the Cu(II) solution containing cysteine or *N*-acetyl cysteine: **(a)** methionine, **(b)** at room temperature.

Copper-thiol interaction is responsible for two main sulfur-copper charge transfer transitions, a weak one from the σ orbital around 460 nm and a strong one from the π bonding orbital around 600 nm, a pattern that is characteristic of a distorted tetrahedral geometry [5]. By modifying the ligand, a spectrum has been obtained, in which the intensity of two bands is reversed. The intensity ratio (A_{450}/A_{590}) for copper-cysteine or N-Ac is about 0.67, whereas the intensity ratio (A_{460}/A_{586}) for copper-methionine is about 1. This spectroscopic change corresponds with a change in the copper geometry from distorted tetrahedral to square planar. The distorted tetrahedral geometry are characterized by a number of unusual properties, *e.g.* an intense blue colour, usually high reduction potentials (Table 1), and distinctive electron spin resonance spectra. These extraordinary properties have been explained by the ligand forcing the Cu(II) ion into a geometry similar to that which is preferred by Cu(I) [23,24]. As the copper(II) form of the complex is by far the most accessible for spectroscopic characterization (as opposed to the Cu(I) form), all effects in the spectroscopic studies have focused on this form of the complexes.

Nevertheless, as firm conclusions about the actual ligation sites of the ligands could not be solely relied upon the absorption spectra, other techniques were employed to establish the possible adduct structures.

Vibrational spectroscopy is also useful in elucidating the characterization of copper compounds [25].

FT IR spectra: As expected from the ligand structures, the FT IR spectra of the cysteine complexes are more complex than that of the methionine adduct. The NH stretching resembling those of the parent zwitterions (located between 3040 and 3164

cm^{-1}) appear in the spectra of the cysteine compounds as absorption continua down to about 2400 cm^{-1}, indicating presence of NH_3^+ ends in the respective structures; correspondingly, broad prominent peaks are positioned at (in cm^{-1}) 3479 (b), 3092 (b), 2928 (w) for Cu-cysteine and 3576 (s), 3511 (s), 3424 (m), 3098 (b) for Cu-*N*-acetylcysteine. Nevertheless, it should be noted here that these absorption bands might also be resulted from carboxylic groups of the ligands. Contrary, overlapped sharp doublet appears in the methionine compound at 3299 and 3241 cm^{-1}, with a characteristic appearance of an unprotonated primary amine group. Furthermore, while the characteristic SH stretchings, expected in the 2450–2650 cm^{-1} interval, evolve in the spectrum of the *N*-acetylcysteine (2670, 2572 cm^{-1}) compound, no such bands could be found in the case of the cysteine compound. It may be assumed, therefore, that the resonant structure of the amino acid in the two compounds is different. The carbonyl stretching region in the methionine compound is particularly simple, featuring a single strong symmetric band at 1620 cm^{-1}. Such spectral picture implies a single crystallographic type of deprotonated carboxylate groups, positioned symmetrically around the metal. The cysteine complexes appear more complex, exhibiting at least three bands for cysteine (1620, 1593, 1391 cm^{-1}) and a strong triplet (1732, 1629, 1547 cm^{-1}) for *N*-acetylcysteine; the latter also employs contribution from the acetyl CO stretching, which might be identified by the highest-frequency ν(CO) band. The metal–ligands modes expected in the 300–500 cm^{-1} region as well as the methionine C–S–C stretching vibration are too weak to be assigned with confidence.

Search of the Cambridge Crystallographic Database (CSD)* [26] revealed 119 structures of metal complexes containing the cysteine fragment. In most of the cases the compound binds in a polydentate manner, featuring the NH_2/SH, NH_2/COO(H) or NH_2/SH/COO(H) modes. Octahedral coordination around the metal (as suggested by the ESR data, see below) in absence of other counter-ions, would imply either three cysteine ligands coordinated in a bidentate mode, or eventually two tridentate ligands. In the case of the methionine complex, according to the spectra, the fomer mode seems more probable. The CSD search yielded 52 metal complexes of methionine fragment. In the structurally characterized *cis* [27] and *trans* [28] isomers of bis-(methioninato)copper(II), the metal atom is tetracoordinated with nearly coplanar NH_2-C-C(=)OO^- residues of the two *N*:*O* bidentate ions. In catena-(bis(L-methionato)zinc(II) [29], two *N*:*O* bidentate and one O-donating ligands consist a square pyramid around the zinc atom. Nevertheless, the FT IR data in the present case do not afford firm evidence about the coordination type in the compounds studied.

ESR spectra: The ESR spectra of copper complexes with small ligands or macromolecules are well-known to provide valuable information about the hyperfine and superhyperfine structure, of particular importance when studying the coordination geometry, amino acid ligation sites to the metal and covalency of copper–ligand bonds [12,30].

*April 2001 version.

Room temperature ESR spectra of copper(II) complexes present broad high field signal [31,32] with a shoulder at *ca.* 3355 Gauss and poor resolution of the lower field signals; the fine structure in the $g_{||}$ region, however, is regularly clear. The parameters of the copper(II) thiolate complex with cysteine are as follows: $g_{||} = 2.245 \pm 0.005$, $g_{\perp} = 2.059 \pm 0.005$, $A_{||} = 170 \pm 5$ Gauss, $A_{\perp} = 44 \pm 5$ Gauss (Fig. 2). The spectrum is consistent with a tetragonally distorted *octahedral coordination* of the metal ion, with the unpaired electron occupying predominantly the $d_{x^2-y^2}$ orbital [12]. Similar results were obtained for the other investigated copper(II) complexes. These results are consistent with the literature data [33], *e.g.* with the copper(II) coordination in the fur receptor protein [34].

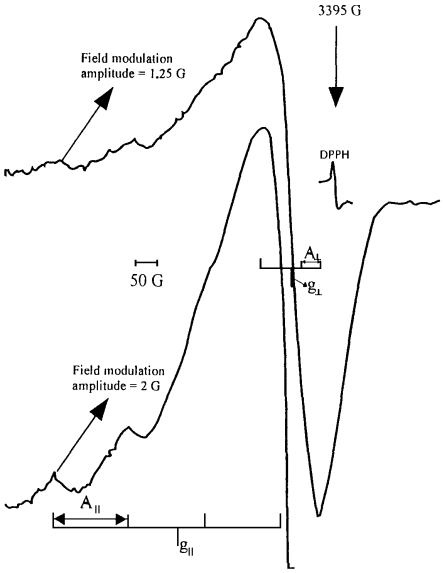

Figure 2. Room temperature ESR spectrum of microcrystalline powder of the Cu(II)–cysteine thiolate complex. The spectrum was recorded with microwave frequency of 9.52 GHz, power of 8 dB, scan field of 2000 gauss, field set of 3000 gauss and gain of 2.5×10^2 [12].

CONCLUSIONS

Cysteine, its *N*-acetylated analogue and methionine bind to copper ions in the solid state and in solution. The anisotropic g value with $g_{||} > g_{\perp}$ suggests distorted octahedral coordination of the copper(II) centers of the solid adducts. The cysteine ends probably exist as ammonium ions, while the methionine complex exhibits notably symmetrical coordination with deprotonated carboxylic group. There is no apparent evidence that the thiol group is retained in the cysteine adduct.

In sense of the coordination chemistry of amino acids, the present study shows that although Cu(I) complexes are known to be readily oxidized by atmospheric oxygen to Cu(II) species [35], reaction with thiol-containing amino acid can revert the redox process to formation of stable Cu(I) complexes.

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