New Copper(II) Complexes with Methionine

HENRYK KOZŁOWSKI and TERESA KOWALIK

Institute of Chemistry, University of Wrocław, Joliot-Curie 14, 50 - 383 Wrocław, Poland

The methionine molecule contains three functional groups which can act as coordination sites for metal ions. Only two of these groups, *viz.* the carboxyl and amino groups, have been found to be involved in the coordination of Cu(II) in low molecular weight complexes [1–5]. From a biological point of view, however, thioether sulfur can be an important binding site for copper ions. Recent structural studies on two 'blue' copper proteins [6], have shown that 'blue' copper centers involve a methionine sulfur, as well as a cysteine sulfur and two imidazole nitrogens as ligands. The coordination of Cu(II) by methionine sulfur was not considered in earlier explanations of the unusual EPR and UV–visible spectra of the 'blue' copper proteins [7], and a reinterpretation of the spectroscopic data therefore appears necessary.

In this communication model studies are reported with the aim of exploring the spectroscopic characteristics of Cu(II)–methionine complexes with metal–sulfur bonds. In particular it is pertinent to see wether the contributions of a cysteine sulfur and a methionine sulfur to the spectra of the 'blue' copper proteins can be separated.

Previous studies on Cu(II) complexes with methionine containing dipeptides [8] have shown that it is possible to force a Cu(II)–sulfur bond formation. The description of the Cu(II) ion methionine sulfur interaction, however, has been limited only to the observation of a S → Cu(II) charge transfer transition at 450 nm. We present here more detailed results for the solutions containing Cu(II) and methionine methyl ester (MetOMe).

Experimental

$Cu(ClO_4)_2 \cdot 6H_2O$ and L-methionine-metylester hydrochloride were obtained from Fluka. The measurements were performed in aqueous methanol and ethanol solutions which were 10^{-2} to 10^{-3} M with respect to Cu(II). EPR spectra were recorded on a JEOL-ME-3X spectrometer at 9.12 GHz at 120 K. The absorption spectra were recorded on a Beckman UV 5240 spectrophotometer. ^1H NMR measurements were performed on a JEOL 100 MHz JNM-PS-100 spectrometer. A Varian CF 90 MHz instrument was used for the T_1 measurements. Simulated EPR spectra were generated on a JEC-6 computer.

Results and Discussion

Absorption spectra of yellow Cu(II)–MetOMe solutions exhibit two characteristic bands at 380 and 450 nm (Fig. 1). The intensities of both bands
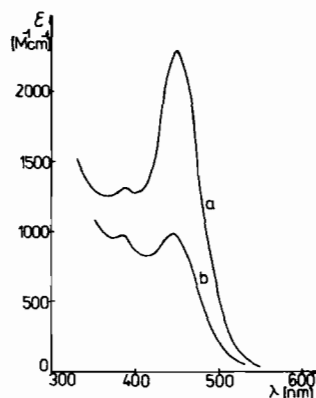

Fig. 1. Charge transfer transitions observed in ethanol solution containing 0.01 M Cu(II) and 1.1(a) and 0.5(b) M MetOMe.

vary considerably with the ligand concentration. The intensity variations depend also on the solvent used (Fig. 2). Ethanol solutions have the highest
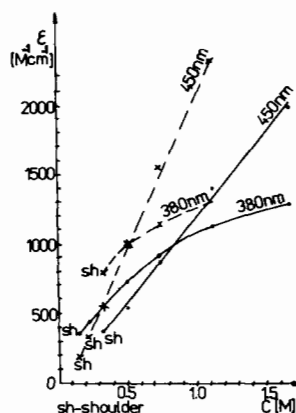

Fig. 2. Dependence of the charge transfer band absorbencies on the ligand concentration in methanol (solid lines) and ethanol (dotted lines) solutions.

absorbencies and aqueous solutions have the lowest absorbencies for any given ligand concentration. All absorption coefficients refer to total cupric ion concentration.

The 380 and 450 nm bands are not observed either in the metal-free MetOMe solution or in solutions containing the Cu(II) ions with the other amino acids (*e.g.* glutamic acid). Thus considering the NMR studies *vide infra* as well as the previous results for the Cu(II) complexes with thiotheric sulfur containing ligands [10, 11], it could be sug-

gested that both bands mentioned above derive from the CT S → Cu(II) transitions.

The relative intensity changes of both transitions show that at least two species with Cu(II)—sulfur bonds exist in the solutions. One species is characterized by the 380 nm bnad (species 1) and it is the only species which is observed at low ligand concentration (Fig. 2).

The Cu(II) d—d transition energies and absorbencies also depend on the ligand concentration in a way which indicates the presence of at least two major species under the conditions of the experiment (Fig. 3). $Cu(ClO_4)_2 \cdot 6H_2O$ dissolved in ethanol
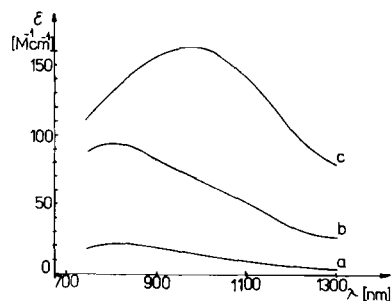

Fig. 3. The d—d transition bands for Cu(II) ions in ethanol (a), in ethanol solutions containing 0.15(b) and 1.65 M(c) MetOMe and 0.01 M Cu(II).

(Cu(II) = 0.01 M) shows a weak d—d transition at 808 nm with $\epsilon = 22.5 \, M^{-1} \, cm^{-1}$ (Fig. 3a). Addition of MetOMe causes an increase of the absorbence and the appearance of a new d—d transition below 800 nm, $\epsilon = 95 \, M^{-1} \, cm^{-1}$, for [MetOMe] = 0.15 M (Fig. 3b). The d—d band is very broad and a shoulder can be observed on a lower energy side. Further increase of the ligand concentration to 1.65 M transforms the shoulder into a clear broad band at 972 nm with an absorbence of 164 $M^{-1} \, cm^{-1}$ (Fig. 4a).
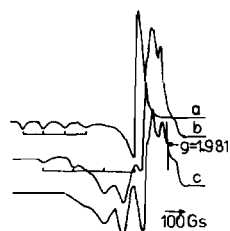

Fig. 4. EPR spectra of Cu(II) in ethanol (a) and of ethanol solutions containing 0.15(b) and 1.65 M(c) MetOMe and 0.01 M Cu(II).

The EPR spectra of Cu(II)—MetOMe solutions support the conclusions drawn from the absorp-

tion spectra. The comparison of EPR spectra for several solutions with different ligand concentrations, [Cu(II)] = 10^{-2} M, indicates that after ligand addition to Cu(II) containing solutions two major species are formed. The complex species which exist at low ligand concentration (species I) is characterized by axial g and A tensors with $g_\parallel = 2.270$ and $A_\parallel = 155$ G (Fig. 4b). These properties as well as the d—d transition at about 800 nm are consistent with an approximate tetragonal symmetry of that species. The second complex species (species II) which becomes dominant at high ligand concentration has orthorhombic spin Hamiltonian tensors with $g_1 = 2.007$, $g_2 = 2.112$, $g_3 = 2.190$ and $A_1 = 50$ G, $A_2 = 85$ G, $A_3 = 90$ G [15], (Fig. 4c). The small value of $A_\parallel$ and the much lower d—d transition energy of this second species suggests that the geometry of the complex is near tetrahedral or considerably distorted trigonal bipyramid [12—14]. Such a geometry also provides an explanation for the fact that the charge transfer transition of the second complex occurs at a longer wave-length (450 nm) than that of the first complex (380 nm).

Further evidence for interactions between MetOMe and the Cu(II) ions was provided by NMR measurements. Addition of small amounts of Cu(II) ions to solutions containing 1.50 M of MetOMe (Cu(II): MetOMe = 1:1000) causes a considerable broadening of the S-CH₃ and γ-CH₂ proton resonances but leaves the lines of α and β protons unaffected (Fig. 5).
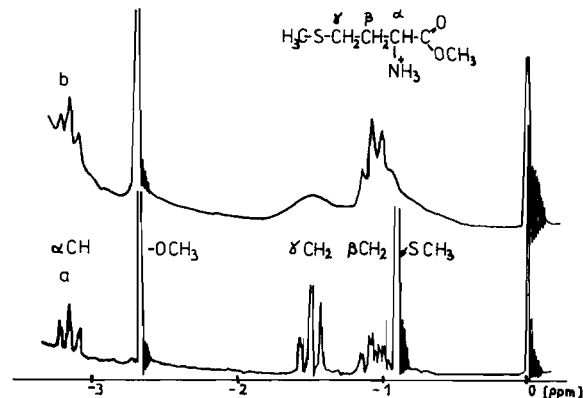

Fig. 5. The broadening effect of the MetOMe ^1H NMR spectrum when 0.001 M Cu(II) is present in the solution.

This effect is observed in aqueous solution at pH ~ 1 as well as in methanol and ethanol. While definite conclusions cannot be drawn from paramagnetic line-broadening [9] our observations strongly support the absorption spectral data that the thioetheric sulfur is a metal-binding locus. The T_1^{-1} values of the OH protons of methanol decrease from 5.8 to about 3 sec⁻¹ when the ligand concentration is varied from 0.0 to 2.3 M ([Cu(II)] = 2.6 × 10^{-2} M). T_1^{-1} is almost constant for ligand concentrations from 0.1 to about 2.3 M, suggesting that some

solvent molecules may remain in the coordination sphere of the cupric ion. Diamagnetic values of T_1^{-1} are below 1 sec^{-1}.

Acknowledgements

References

1 C. A. McAuliffe, J. W. Quagliano and L. M. Vallarino, *Inorg. Chem.*, 5, 1996 (1966).
2 C. A. Bear and H. C. Freeman, *Acta Cryst.*, B32, 2534 (1976).
3 H. Yokoi, M. Sai, T. Isobe and S. Ohsawa, *Bull. Chem. Soc. Japan*, 45, 2189 (1972).
4 Ch-Ch. Ou, D. A. Powers, J. A. Thich, R. Felthouse, D. N. Hendrickson, J. A. Potenza and H. J. Schugar, *Inorg. Chem.*, 17, 34 (1978).
5 M. R. Harrison and F. J. C. Rossotti, *Chem. Comm.*, 175 (1970).
6 P. M. Colman, H. C. Freeman, J. M. Guss, M. Murata, V. A. Norris, J. A. M. Ramshaw and M. P. Ventatappa, *Nature*, 272, 319 (1978).
E. T. Adman, R. E. Stenkamp, L. C. Sieker and L. H. Jensen, *J. Mol. Biol.*, 123, 35 (1978).
7 E. I. Solomon, J. W. Hare and H. D. Gray, *Proc. Natl. Acad. Sci. USA*, 73, 1389 (1976).
8 H. Kozłowski and T. Kowalik, *Inorg. Nucl. Chem. Letters*, 14, 201 (1978).
9 J. K. Beattie, D. J. Fenson and H. C. Freeman, *J. Am. Chem. Soc.*, 98, 500 (1976).
W. G. Esperson and R. B. Martin, *J. Am. Chem. Soc.*, 98, 40 (1976).
10 A. R. Amundsen, J. Whelan and B. Bosnich, *J. Am. Chem. Soc.*, 99, 6730 (1977).
11 V. M. Miskowski, J. A. Thich, R. Solomon and H. J. Schugar, *J. Am. Chem. Soc.*, 98, 8344 (1976).
12 V. K. Voronkowa, M. M. Zaripov, V. A. Kogan and Yu. V. Yabłokov, *Phys. Stat. Sol. b*, 55, 747 (1973).
13 A. Bencini, I. Bertini, D. Gatteschi and A. Scozzafava, *Inorg. Chem.* (in press).
14 H. Kozłowski, *Chem. Phys. Letters*, 46, 519 (1977).
15 A Q-band spectrum analysis supports only partially an X-band spectrum (Fig. 4c) simulation as the g_2 and g_3 regions in a Q-band are not resolved. Thus the A_2 and A_3 constant values could be obtained only from an X-band spectrum simulation.