

# **TRACE ELEMENTS IN THE FOOD CHAIN**

## **Vol. 3. Deficiency or Excess of Trace Elements in the Environment as a Risk of Health**



**Eds.: Mihály Szilágyi, Klára Szentmihályi**

Working Committee on Trace Elements  
of the Complex Committee Hungarian Academy of Sciences (HAS) and  
Institute of Materials and Environmental Chemistry of the HAS,  
Budapest, Hungary, 2009

**COMPLEXATION PROPERTIES OF OLIGOPEPTIDE H-GHKHG-H WITH  
COPPER(II) STUDIED BY THE DECOMPOSITION OF ELECTRON  
PARAMAGNETIC RESONANCE SPECTRA**

**Nóra Veronika Nagy<sup>1</sup>, Andrea Lakatos<sup>2</sup>, Béla Gyurcsik<sup>2</sup> and Antal Rockenbauer<sup>1</sup>**

<sup>1</sup>Chemical Research Center of the Hungarian Academy of Sciences, 1025 Pusztaszeri út 59-67, Budapest, Hungary, Email: [nagyn@chemres.hu](mailto:nagyn@chemres.hu); <sup>2</sup>University of Szeged, Aradi vértanúk tere 1. 6720 Szeged, Hungary

**ABSTRACT**

The copper(II) complexes of oligopeptide H-GHKHG-H was investigated in aqueous solution at different pH's by the decomposition of the Electron Paramagnetic Resonance spectra recorded at 77K. The data revealed that the coordination of amino, amid, and histidine nitrogens form the species CuLH. Macrochelate coordination of the remote histidine were suggested for the predominant CuL and CuLH-1 complexes as well. At highly alcalic pH a slight change in the EPR spectra shows that mixed hidroxocomplex formed with axially bound OH<sup>-</sup> ion. For the EPR spectrum of Cu<sub>2</sub>LH<sub>2</sub> and the minor component of Cu<sub>2</sub>LH<sub>4</sub> monomer-like signal were detected. This leads to suggest that considerable dipolar interaction did not occur between the two copper(II)ions in these molecules. In contrary the major component of the Cu<sub>2</sub>LH<sub>4</sub> spectra had a broad singlet signal, for which bridge connection between the metal centres by the help of an OH<sup>-</sup> ion were suggested.

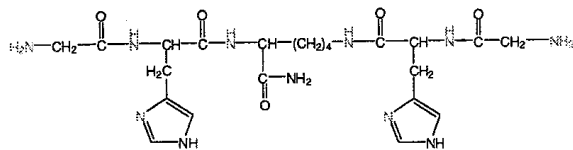
**INTRODUCTION**

There are several diseases in which accumulation of heavy metal ions were established, *eg.* Zn<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, accumulation in the Alzheimer disease. In these cases a prospective therapy could be the elimination of the accumulated metal ions. It is known that terminal amino nitrogens as well as histidine side-chains of peptides have high affinity to bind metal ions. Accordingly the strength of the complexation property can be increased by applying a ligand with a few histidine and two amino-terminal side. The small oligopeptide H-GHKHG-H was synthesised to produce a ligand with very high complexation feature, which can be a possible therapeutical agent.

Electron Paramagnetic Resonance (EPR) spectroscopy was used to determine the electronic and local geometric structure of paramagnetic species in the above system. In these overlapping equilibrium systems a number of complexes with different compositions and structures are formed by stepwise deprotonation of the donor groups, which necessitates the decomposition of the spectra. The developments of spectrum simulation programs have made these techniques even sensible for unravelling the structures not only for predominant but for minor complexes and isomers (complexes with different coordination modes but same constitution).

**MATERIALS AND METHODS**

The ligand was synthetized at the University of Szeged and clarified by HPLC technique. The purity was verified by NMR measurement. The structure of the ligand is shown in Figure 1.



**Figure 1.** The structure of the ligand H-GHKHG-H

21 anisotropic EPR spectra were recorded at 77 K at pH range 3-11 in the solutions of 1mM ligand and 1mM or 2mM of copper(II) concentrations. NaOH solution was added to the sample to adjust the chosen pH, which was measured with Methrom combined glass electrode. The titrations were carried out under argon atmosphere. 30 $\mu$ l of methanol was added to 100 $\mu$ l of the samples to avoid water crystallisation. EPR spectra were recorded in a dewar containing liquid nitrogen with a BRUKER EleXsys E500 spectrometer (microwave frequency 9.51 GHz, microwave power 12 mW, modulation amplitude 5 G, modulation frequency 100 kHz).

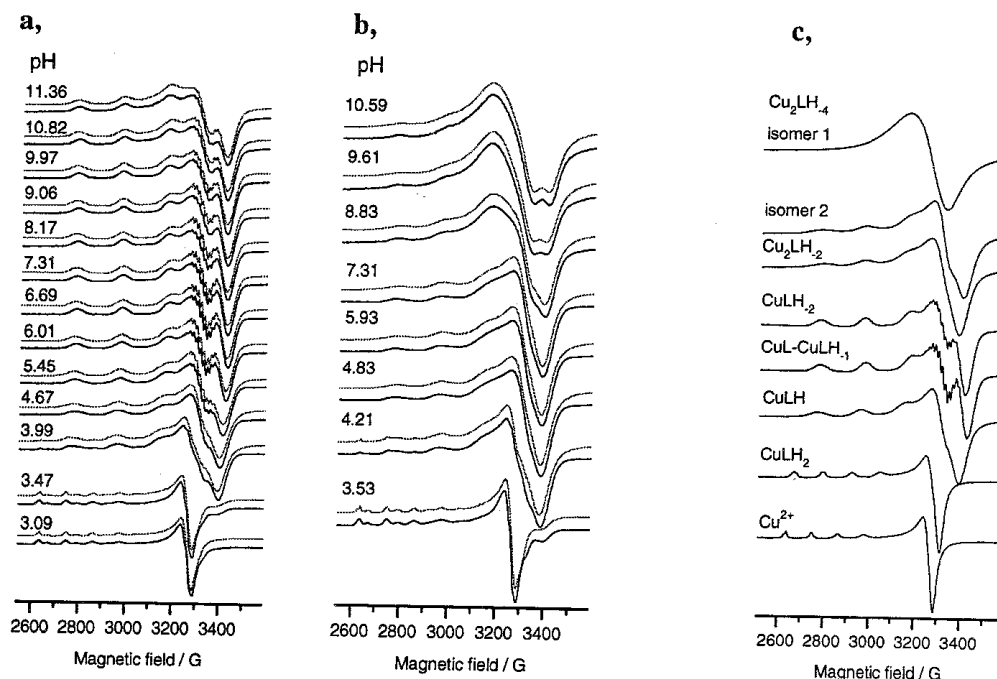
#### Decomposition of EPR spectra

All recorded EPR spectra were simulated with a spectrum decomposition method by the EPR [1] computer program. Anisotropic ( $g_{\parallel}$ ,  $g_{\perp}$ ,  $A_{\parallel}$ ,  $A_{\perp}$  copper couplings,  $a_N$  isotropic nitrogen couplings and magnetic field dependent linewidth) parameters were fitted in order to reduce the average square deviation between the experimental and the calculated spectrum. The computer program characterise the fit by the regression parameter (R) which was found to be between 0.990-0.997 for the simulated spectra. The program is able to take into account three component spectra for the maximum. Since the copper(II) used in the solutions was a natural mixture of the isotopes, the spectrum of each species was calculated as the sum of spectra containing  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$  in their natural abundances. The copper and ligand coupling constants are given in units of gauss (G; 1 G =  $10^{-4}$  T). The copper couplings refer to the  $^{63}\text{Cu}$  isotop. The details of the analysis were published previously[1].

## RESULTS AND DISCUSSIONS

EPR spectra could be described satisfactory by the help of 8 different component curves. Figure 2 shows the fit between experimental and simulated spectra recorded at different pH's at two different metal-to-ligand concentration ratios together with the resulted component spectra. Anisotropic EPR parameters of the components are collected in Table 1.

The complex  $\text{CuLH}_2$  were identified at very low pH with EPR parameters close to those of the aqua complex. This suggest a weak ligand field with one histidine coordination for this species. The formation of this complex seems to be more favourable at low temperature as pH potentiometric measurements could not detect it at room temperature. For the complex  $\text{CuLH}$  the coordination of amino, amid, and histidine nitrogens are supported by the significant decrease in  $g_{\parallel}$ ,  $g_{\perp}$  and increase in  $A_{\parallel}$ ,  $A_{\perp}$  values (see Table 1) which indicates much higher ligand field in the equatorial plane.



**Figure 2.** Comparison of the experimental (lower) and simulated (upper) EPR spectra for a,  $T_{Cu}=1mM$  and  $T_L=1mM$ ; b,  $T_{Cu}=2mM$  and  $T_L=1mM$  concentration ratios; and c, component spectra obtained from the simulation

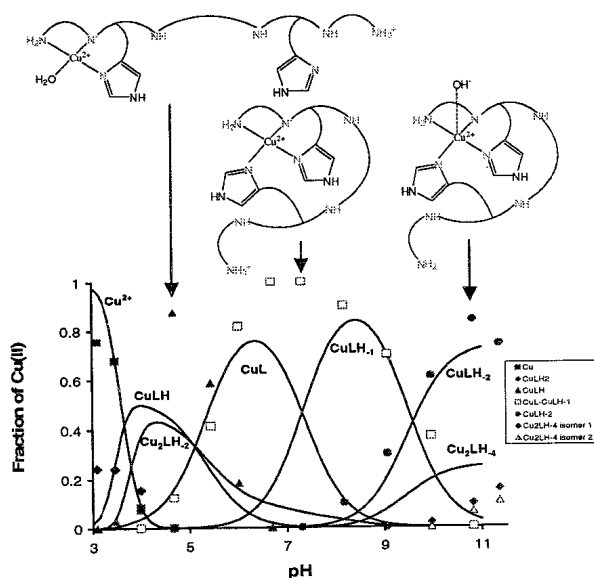
**Table 1.** Anisotropic EPR parameters of the components

Components	$g_{  }$	$g_{\perp}$	$A_{  }/G$	$A_{\perp}/G$	$a_N/G$
$Cu^{2+}$	2.424	2.081	111	7.1	
$CuLH_2$	2.379	2.071	122	11	
$CuLH$	2.228	2.056	184.5	16.6	13.7, 13.7, 14.6
$Cu_2LH_2$	2.227	2.059	179	18.9	14.2, 14.2, 14.2,
$CuL - CuLH_1$	2.212	2.046	195.0	20.0	12.9, 12.9, 12.6, 16.5
$CuLH_2$	2.218	2.048	190.4	19.3	12.9, 12.9, 12.6, 16.5
$Cu_2LH_4$ isomer 1 <sup>A</sup>	2.109		40		
isomer 2	2.221	2.056	189.0	17.2	15.1, 15.1, 14.4

<sup>A</sup> Singlet spectra simulated by isotropic  $g_0$  and  $A_0$  parameters

Further decrease in the  $g$  values and increase in  $A$  values support the coordination of four equatorial nitrogen donor groups in the  $CuL$  complex. This agrees with the fact that the well resolved nitrogen hyperfine lines could be simulated very well with taking into account four nitrogen donor atoms. It is likely that the  $CuLH$ -type of coordination is completed by the macrochelate coordination of the far histidin in this complex (however we cannot exclude the coordination of the amino nitrogen from the EPR parameters). The spectra of the  $CuLH_1$  complex does not show any changes in comparison with  $CuL$

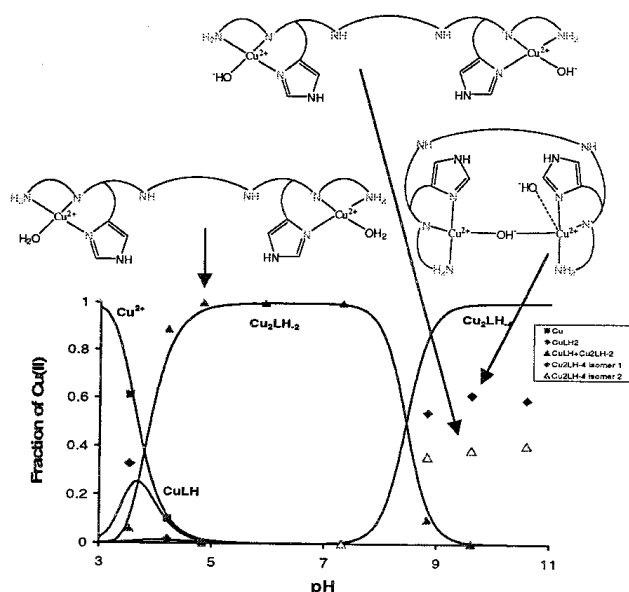
which indicates the deprotonation of the amino nitrogen without any coordination. The EPR parameters of the  $\text{CuLH}_2$  are slightly different from  $\text{CuLH}_1$  which makes it likely that this is a mixed hydroxo complex with axial  $\text{OH}^-$  ion coordination. Proposed coordination modes and the distribution of the copper(II) among the different species are depicted in Figure 3. Distribution curves shows good agreement between the pH-potentiometric and EPR results taking into account the difference between the experiment temperatures 295 and 77K, respectively.



**Figure 3. Distribution curves for  $T_{\text{Cu(II)}} = 1\text{mM}$  and  $T_L = 1\text{mM}$  obtained from pH-potentiometry (lines) and from the simulation of anisotropic EPR spectra (symbols) together with suggested coordination modes**

In metal excess the  $\text{Cu}_2\text{LH}_2$  complex predominates at 4-7 pH range. The EPR spectra parameters of this complex are surprisingly very close to those of  $\text{CuLH}$  only its spectrum is broader (compare spectrum  $\text{Cu}_2\text{LH}_2$  and  $\text{CuLH}$  in Figure 2.) From this we suggest the coordination of the amino, amid, histidine nitrogens for both copper(II) ions in this complex. The monomer-like signal indicates that the distance between the two copper(II) ions in the molecule is too far to cause a considerable dipolar interaction between them and only a line broadening effect could be observed for this dinuclear complex.

The spectra of the  $\text{Cu}_2\text{LH}_4$  complex could be decomposed into two component curves. The major component has a broad singlet signal (see Figure 2.  $\text{Cu}_2\text{LH}_4$  isomer 1) which reflect the dipolar interaction between the copper(II) ions in this complex suggesting a short distance between the metal ions. The minor component signal was again a monomer-like signal with parameters similar to the parameters of  $\text{CuLH}$  suggesting three nitrogen and one  $\text{OH}^-$  ion coordination in the equatorial plane without any connection between the metal ions in the molecule. Proposed coordination modes and the distribution curves obtained for metal excess from the pH-potentiometric and EPR measurements are shown in Figure 4.



**Figure 4.** Distribution curves for  $T_{Cu(II)} = 2mM$  and  $T_L = 1mM$  obtained from pH-potentiometry (lines) and from the simulation of anisotropic EPR spectra (symbols) together with suggested coordination modes

### CONCLUSIONS

The oligopeptide H-GHKHG-H containing two N-terminal site was synthesised to produce a ligand with very high complexation feature. Coordination properties with copper(II) were investigated in frozen aqueous solution at different pH's. By the decomposition of the EPR spectra the coordination of amino, amid, and histidine nitrogens and the macrochelate coordination of the remote histidine were suggested for the predominant CuL complex. Investigation of complexes formed at two fold metal excess surprisingly resulted in a monomer-like signal for the  $Cu_2LH_2$  and minor component of  $Cu_2LH_4$  dinuclear species. This suggested that copper(II) centres coordinates far enough from each other to have significant dipolar interaction between them.

### ACKNOWLEDGEMENT

This work was supported by the Hungarian Scientific Research Fund OTKA (Grant F-67581). The financial support is gratefully acknowledged.

### REFERENCES

1. Szabó-Plánka, T., Rockenbauer, A., Korecz, L. 1999. ESR study of the copper(II) - glycyglycine equilibrium system in fluid aqueous solution. Computer analysis of overlapping multispecies spectra. *Magn. Reson. Chem.* 37, 484-492.