IMIDAZOLE AS AN ANCHOR DONOR GROUP IN OLIGOPEPTIDE – CU(II) EQUILIBRIUM SYSTEMS

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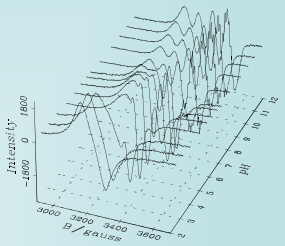
1. Introduction

In an aqueous solution of small peptides and metal ions a number of complexes of different compositions and structures are formed by stepwise deprotonation of the amid-NH groups. This deprotonation can be promoted by the coordination of a stronger anchor (terminal) donor group. In order to clarify the function of the imidazole-N and amino-NH_2 as anchor donor we aimed to compare the equilibrium systems of copper(II) – triglycyl-histamine (GGGHa) and – t-butyloxycarbonyl-triglycylhistamine (BGGGHa).

2. Experimental

A series of CW X-band EPR spectra were recorded in a circulating system at 298 K using a BRUKER EleXsys E500 spectrometer. NaOH solution was added to the sample to adjust the pH, and a peristaltic pump fulfilled the circulation of the solution from the pot to the EPR cavity in order to guarantee the same instrumental conditions during the measurement. 23 EPR spectra were recorded for the ligand BGGGHa and 27 for GGGHa at two different copper(II) to ligand concentration ratios in the pH range of 2-12.



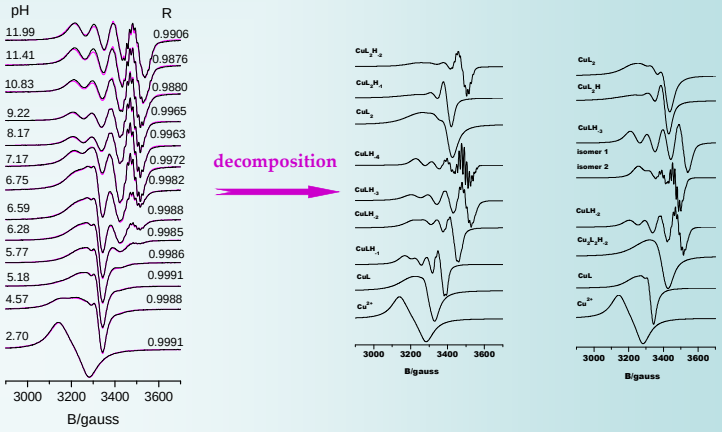
3. Simulation of EPR spectra

The complex formation can be characterized by the following equilibrium process, where M denotes the metal ion, L the non-protonated ligandum and **β** the formation constant:

$$pM + qH + rL \underset{}{\overset{\beta_{M_pH_qL_r}}{\rightleftharpoons}} M_pH_qL_r \qquad \beta_{M_pH_qL_r} = \frac{[M_pH_qL_r]}{[M]^p[H]^q[L]^r}$$

The series of EPR spectra recorded at different pH's were evaluated simultaneously by using the 2D_EPR program[1]. The program fits the EPR parameters g_o, copper (A_o) and nitrogen (a_N) coupling constants, and relaxation parameters **α** , **β** and **γ** relating to the linewidths of the copper hyperfine multiplet as $W_{MI} = \alpha + \beta M_I + \gamma M_I^2$. The spectra were calculated as the sum of the curves coming from isotope ^{63}Cu and ^{65}Cu weighted by their natural abundances. The ratio of the decomposed spectra were varied by fitting the formation constants of the components (**β**). The quality of the fit was characterized by the noise-corrected regression parameter R (= 1 for the perfect fit).

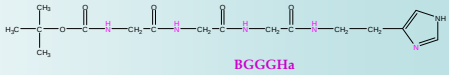
BGGGHa GGGHa



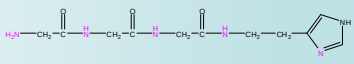
Recorded spectra together with simulation

Component spectra obtained by decomposition.

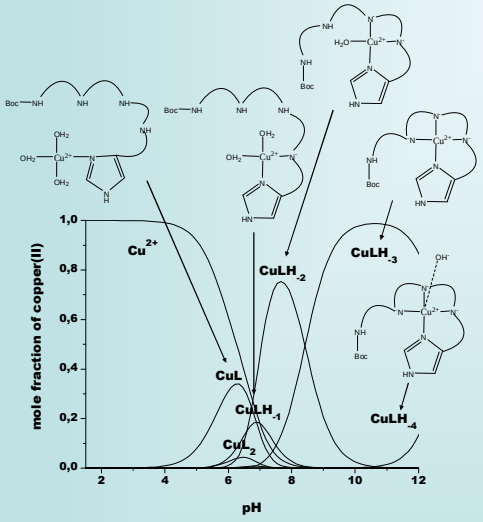
4. Equilibrium model and coordination modes



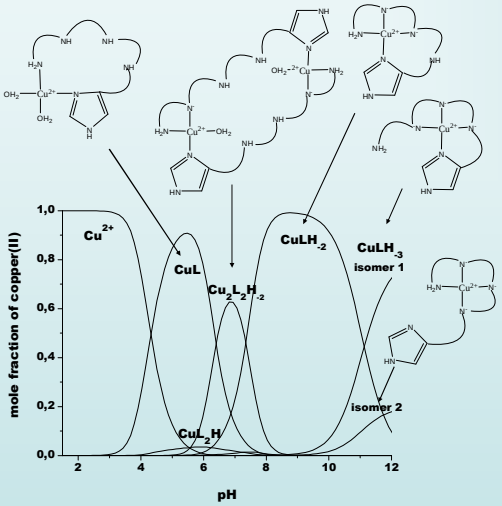
BGGGHa



GGGHa



BGGGHa



GGGHa

By the help of the formation constants distribution of the copper(II) among the different complexes can be depicted. From EPR data obtained for the different components equatorial arrangement of the ligand donor groups can be suggested. For BGGGHa, the stepwise deprotonation of the amid-NH groups starting from the imidazol-site of the ligand could be followed as the amino-site was protected.

For GGGHa both terminal nitrogens found to be coordinated to the same copper(II)ion or linked two different copper(II) centres forming dimers. There are two isomers (different coordination modes) for $CuLH_{-3}$ since the five donor groups of the peptide chain can coordinate to he copper(II)ion in two different way. One of them was found to have similar parameters to the same complex of BGGGHa indicating the same coordination mode.

5. Summary

A series of X-band CW EPR spectra were recorded at room temperature in different pH's and ligand to metal concentration ratios for copper(II) - triglycylhistamine (GGGHa) and – t-butyloxycarbonyl-triglycylhistamine (BGGGHa) systems. The two-dimensional simulation method allowed to identify the EPR parameters and formation constants of all the complexes formed in the solutions. Imidazol-N can only be a real anchor donor in BGGGHa – copper(II) system as for GGGHa amino-NH_2 was always found to be bound at the same time.

[1]Rockenbauer, A.; Szabó-Plánka, T.; Árkosi Zs.; Korecz, L.; *J. Am. Chem. Soc.* **2001**. 123, 7646-7654