NOTIZEN

On the Influence of Aromatic Residues on the Interaction of Copper (II) with Small Peptides Containing Aromatic Amino Acids: ESR and Optical Studies *

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The complexation behaviour of Cu(II) with di- and tripeptides containing the aromatic amino acids phenylalanine or tryptophan has been investigated at different pH-values and compared with results obtained with di- and triglycine. The results obtained by means of ESR and optical absorption spectroscopy show an influence of the two different aromatic entities on the magnetic and optical parameters. A significant decrease of the $g_{||}$-value and, concomittantly, an increase of the energy of the d-d transition was measured when an aromatic entity is present in the peptide. A possible explanation for this observation is given.

The coordination of Cu(II) in copper proteins is a very complex problem. During the last few years several comparative investigations have been done on the coordination of Cu(II) in copper proteins and appropriate model systems [1, 2]. Di- and tripeptides have been used preferentially as model substances [3–7]. From the results obtained in a few investigations on some copper proteins it has been suggested that one tryptophyl residue is located adjacent to Cu(II) [8–10]; however, in model systems no extensive studies have been done yet on the involvement of such an aromatic residue in the coordination.

Recently we have reported on the interaction properties of Cu(II) with di- and tripeptides containing phenylalanine, tryptophan, or histidine in the peptide chain [11]. It could be shown that histidine imidazole was involved directly in the coordination to Cu(II) whereas it was difficult to decide if there is a participation of tryptophan or phenylalanine rings (s. Fig. 1).

In order to elucidate this problem the complexes of Cu(II) with either di- or triglycine compounds or di- and tripeptides containing tryptophan or phenylalanine have been investigated by means of electron spin resonance (ESR) and optical absorption techniques. Since the complex formation is influenced by the pH value the studies have been done at different pH values ranging from 4.3 to 9.8.

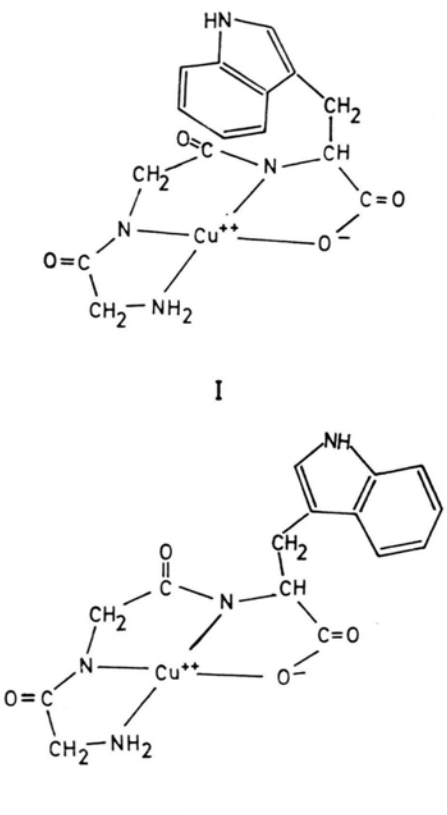

Fig. 1. Possible structures of the Cu(II)-(Gly-Gly-Try) complex. (I) Aromatic entity in a quasi-axial position to the copper ion. (II) Aromatic entity in a position outside of the coordination sphere of the copper ion.

In general, it may be stated that the parameters of the ESR and optical absorption spectra vary, depending upon the pH value, in a very similar manner for all compounds investigated (s. Tables Ia, Ib). As an example, the spectra of aqueous solutions of 1 mM Cu(II) containing 1 mM of Gly-Gly-Try [12] at three different pH values are shown in Figs 2 and 3.

In aqueous solution at pH 4.3 nearly no complexation exists between Cu(II) and the peptides investigated. The magnetic and optical parameters measured are those ones of the copper aquo complex [13].

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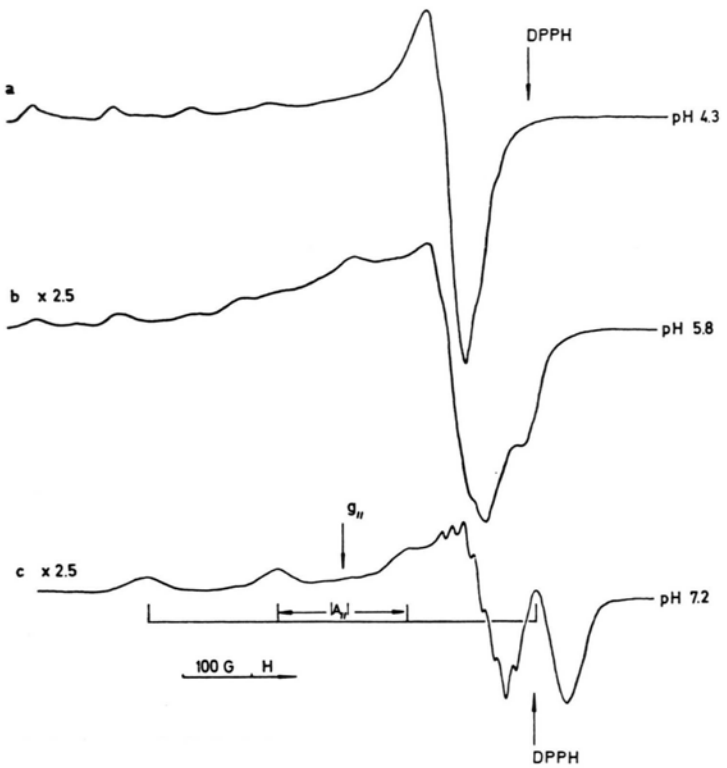

Fig. 2. ESR spectra of aqueous solutions of Cu(II), 1 mM, in the presence of Gly-Gly-Try, 1 mM, at different pH values and 77 K. pH-values were adjusted by adding NaOH or HNO_3.

Table Ia. ESR parameters and optical absorption energies of some Cu(II)-dipeptide complexes at different pH-values.

Ligand * [1 mM]	pH	$\|A_{II}\|$ ±1.5 [gauss]	g_{II} ±0.001	ΔE ±30 [cm^{-1}]
Gly-Gly		171	2.240	15.800
Gly-Phe	5.8	176	2.231	15.920
Gly-Try		177	2.229	15.920
Gly-Gly		165	2.242	15.820
Gly-Phe	7.2	170	2.233	15.920
Gly-Try		171	2.233	15.970
Gly-Gly		165	2.243	15.850
Gly-Phe	8.6	171	2.235	15.970
Gly-Try		170	2.234	16.000

* Ligands used contain Gly (glycine), Phe (phenylalanine), and Try (tryptophan).

Table Ib. ESR parameters and optical absorption energies of some Cu(II)-tripeptide complexes at different pH-values.

Ligand * [1 mM]	pH	$\|A_{II}\|$ ±1.5 [gauss]	g_{II} ±0.001	ΔE ±30 [cm^{-1}]
Gly-Gly-Gly		190	2.196	18.100
Gly-Gly-Phe		192	2.188	18.480
Gly-Phe-Ala	7.2	190	2.189	18.480
Gly-Gly-Try		197	2.182	18.520
Gly-Try-Gly		195	2.184	18.520
Gly-Gly-Gly		189	2.197	18.180
Gly-Gly-Phe		192	2.191	18.520
Gly-Phe-Ala	8.6	189	2.191	18.520
Gly-Gly-Try		195	2.184	18.590
Gly-Try-Gly		190	2.190	18.450
Gly-Gly-Gly		185	2.198	18.250
Gly-Gly-Phe		191	2.192	18.550
Gly-Phe-Ala	9.8	190	2.191	18.550
Gly-Gly-Try		192	2.185	18.650
Gly-Try-Gly		189	2.189	18.450

* Ligands used contain Gly (glycine), Phe (phenylalanine), Try (tryptophan), and Ala (alanine).

At pH 5.8, all solutions exhibit a blue color. However, only the dipeptide complexes show well resolved ESR spectra allowing the determination of the line splitting factor $|A_{II}|$ and the g_{II}-value of complexes with axial symmetry (Table Ia). Five poorly resolved superhyperfine (shf) lines of the nitrogen ligands can also be observed. In the case of Cu(II)-tripeptide solutions the ESR (Fig. 2b) and optical absorption (Fig. 3b) spectra seem to be caused by superposition of spectra of different copper complexes.

At pH 7.2 the two peptide nitrogens in the tripeptides have been deprotonated[14]. The ESR spectra of the Cu(II)-tripeptide complexes show in addition to the four Cu(II) hyperfine lines, at least 7 well resolved shf-lines of the nitrogen atoms in the high field region (Fig. 2c). The d-d band is blue shifted

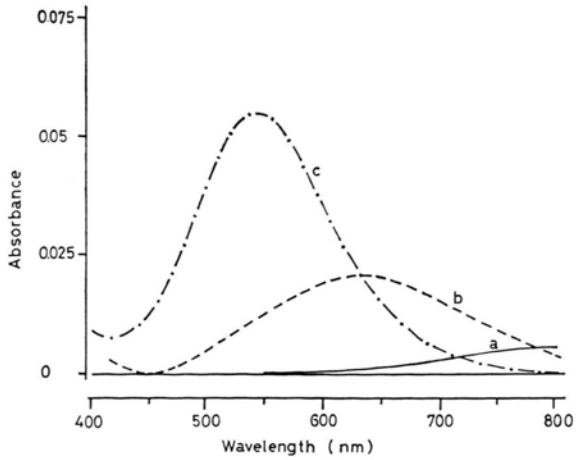

Fig. 3. Optical absorption spectra (d-d transitions) of aqueous solutions of Cu(II), 1 mM, in the presence of Gly-Gly-Try, 1 mM, at different pH values and room temperature, 5 mm cuvet; (a) pH 4.3, (b) pH 5.8, (c) pH 7.2.

(Fig. 3c). A coordination of Cu(II) to one amino and two peptide nitrogens is suggested in the case of tripeptide complexes while for dipeptide complexes one amino and one peptide nitrogen are involved only. In both cases, one oxygen atom of the carboxyl group might be coordinated, too.

As the pH is enlarged only small variations are observed in the magnetic parameters and in the energies of the d-d band of the Cu(II)-di- (Table Ia) and tripeptide complexes (Table Ib) as well. At pH 9.8, OH^- ions participate considerably at the coordination with dipeptides. This effect shall not be regarded here, therefore the spectra parameters are not listed in Table I a.

From the parameters obtained (Tables Ia and Ib) the following conclusions can be drawn:

1. Copper(II) complexes of tripeptides exhibit larger hyperfine splitting, smaller $g_{||}$-values, and d-d transitions with higher energies than copper complexes of dipeptides. This is a general effect of increasing chelation [13, 15].

2. Each type of complex seems to show a decreasing hyperfine splitting $|A_{||}|$, an increasing $g_{||}$-value, and an increasing energy of the d-d transition as the pH is enlarged. Gly-Try-Gly might show an exceptional behaviour.

3. At a certain pH, the presence of an aromatic entity in a copper dipeptide complex causes an enlarged hyperfine splitting, a smaller $g_{||}$-value, and a higher energy of the d-d transition. The effect obtained with either tryptophan or phenylalanine is about the same.

4. In the case of copper(II) complexes with tripeptides, this general behaviour shows some exceptions. The effect of tryptophan and phenylalanine on the parameters is no longer the same. With the exception of Gly-Try-Gly at pH 8.6 and 9.8, the presence of the tryptophan entity changes the parameters measured more than phenylalanine if compared with Gly-Gly-Gly.

The change of the parameters of the Cu(II)-di- and triglycine complexes with pH cannot be explained by an additional distortion of the symmetry of the ligand atoms being arranged almost planar. The results suggest an axial symmetry since $g_{||}$ and $g_{\perp}$ are greater than 2 and $g_{||} > g_{\perp}$ [16]. Therefore the results obtained might be explained by an increased covalency of the σ-bonds of complexes formed by the overlap of the $d_{x^2-y^2}$ copper ground state with the σ orbitals of the ligand atoms [17]. This, however, doesn't encounter the change of the parameters by the presence of an aromatic side chain at a given pH. It is known that in the Cu(II) complexes of phenylalanine and tryptophan the aromatic residues are not involved in the coordination to the planar bonds of the copper ion [18]. Therefore it is reasonable to suggest that also in di- or tripeptides these inities do not participate in the planar bonds. This means, that such peptides form Cu(II)-chelates with the same ligand atoms as do di- and triglycine [4, 19], if only the planar bonds are regarded.

The aromatic rings, however, might be able to occupy a quasi axial position (I) so that the π-electrons of the ring systems overlap with the d_{xz} and d_{yz} copper orbitals. This might cause an energy increase of the $d_{x^2-y^2} \rightarrow d_{xz}, d_{yz}$ transitions and, therefore, a decrease in the $g_{||}$ value. It is assumed that a quasi-axial position of the aromatic entities is favoured. If a configuration like (II) in Fig. 1 would be present the observed differences in the parameters should not be expected. Sterical reasons might be responsible that the influence of the aromatic entities in the Cu(II)-dipeptide complexes is less than in the copper tripeptide complexes.

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