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ESR and Optical Absorption Studies on the Copper(II) Interaction with Small Peptides Containing Aromatic Amino Acids*

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Abstract. The interaction of Cu(II) with di- and tripeptides each containing phenylalanine, tryptophan or histidine in the amino acid chain has been investigated by means of electron spin resonance (ESR) and optical absorption spectroscopy. Cu(II) complexes of dipeptides and tripeptides exhibit different magnetic and optical parameters. Dipeptide complexes have larger $g_{\|}$-values and smaller $|A_{\|}|$-values than tripeptide complexes. When compared to dipeptide complexes, the d-d band of the central metal ion is blue shifted for tripeptide complexes. There are no significant differences in the behavior of Cu(II) peptide complexes containing phenylalanine or tryptophan. Complexes of histidine containing peptides, however, show modified spectra caused by the participation of the imidazole nitrogen in the coordination to Cu(II). The imidazole nitrogen seems to coordinate in-plane with other coordinating atoms or in an axial position depending on the kind of peptide.

Key words: Cu(II)-complexes – Peptides – ESR – Optical absorption.

Introduction

The transition metal ion Cu^{2+} is very important for the structure and function of many proteins. In most cases the binding sites in the protein are not known yet though many proposals have been made during the last few years [8, 22, 24]. A special problem concerns the participation of aromatic amino acid residues of a protein chain in the complexation with the metal ion. Fluorescence quenching, magnetic resonance, and titration techniques have been applied mainly in order to elucidate this problem. The results obtained reveal that often tryptophan, thyrosine or histidine are located adjacent to Cu^{2+} depending on the protein investigated [5, 13, 16, 19]. The involvement of the aromatic residues in the complexation, however, is only known for histidine to some extent.

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Because of the very complicated situation in proteins, many investigations were carried out on small peptides only. In sulfur-free peptides containing no histidine residues, the binding sites are found to be the amino and peptide nitrogen and the carboxyl oxygen as well [1, 4, 6, 9, 14, 20]. If the peptide contains a histidine residue, the imidazole nitrogen acts as an additional binding site [2, 3, 11, 12, 25] occupying one of the four planar positions of the Cu(II) ion. It was not observed that the axial position was occupied although it should be possible. This is supported by results obtained recently with Cu(II)-histidine complexes in which the amino acid was used as a monomer [21]. The aim of this work has been to obtain some more additional knowledge on the influence of aromatic residues in the peptide complexes with Cu(II). Especially, the influence of tryptophan and phenylalanine residues on such a complexation as well as a possible binding of the imidazole nitrogen of histidine in an axial position of Cu(II) have been determined by means of electron spin resonance (ESR) and optical absorption studies.

Materials and Methods

L-phenylalanine (Phe), L-histidine (His), and DL-tryptophan (Try) as well as $Cu(NO_3)_2 \cdot 3\ H_2O$ and methanol were purchased from Merck, Darmstadt, Germany.

Glycyl-L-histidine (Gly-His), glycyl-L-phenylalanine (Gly-Phe), glycyl-L-tryptophan (Gly-Try), glycylglycyl-L-histidine (Gly-Gly-His), and glycylglycyl-L-phenylalanine (Gly-Gly-Phe) were purchased from Serva, Heidelberg, Germany. Glycyl-L-histidyl-glycine (Gly-His-Gly), and glycyl-L-phenylalanyl-L-alanine (Gly-Phe-Ala) were purchased from Sigma, St. Louis, USA. Glycylglycyl-L-tryptophan (Gly-Gly-Try), and glycyl-L-tryptophan-glycine (Gly-Try-Gly) were purchased from Cyclo Chemicals, Los Angeles, USA.

The substances have been used without further purification. The solutions were prepared using doubly destilled water as solvent. The pH value was adjusted to 7.2 by adding NaOH. The optical and ESR measurements as well were performed within a few hours after preparation of the solutions. During this time no change in the optical and ESR spectra could be observed. The ESR measurements were carried out with an X-band Varian E-9 spectrometer using 100 Kc field modulation. The microwave power was 10 mW and the modulation amplitude 2 G for all samples investigated. An aqueous solution sample cell was used for the measurements at room temperature (RT) whereas the low temperature spectra were obtained by measuring the samples in a Dewar flask containing liquid nitrogen. In order to reduce dipolar broadening due to aggregation of the copper complexes at low temperature 300 µl of methanol [18, 27] were added to 5 ml of solution. DPPH ($g = 2.0037$) was used as a reference for the g-value measurements. For this purpose, a small DPPH crystal was attached to the outside of the cell.

The optical measurements were done with a Zeiss DMR 10 spectrometer. The spectra in the red wavelength region were taken vs. water in the reference cuvette. In the UV region, relative difference spectra of Cu(II)-peptide solutions vs. peptide solution of the same molarity were recorded. In this way, most of the peptide ab-

sorption can be balanced out, that is the complex absorption becomes the major component.

Results

ESR *Measurements.* ESR spectra of some characteristic Cu(II) complexes with di- and tripeptides are shown in Figure 1 [room temperature (RT)] and in Figure 2 (liquid nitrogen temperature). Spectra of Cu(II)-tripeptide complexes (Fig. 1B, D) are shifted to a higher magnetic field and exhibit a well resolved ligand hyperfine structure if they are compared with those ones of Cu(II)-dipeptide complexes (Fig. 1A, C). The liquid nitrogen spectra (Fig. 2) show a similar behavior with the exception of Gly-Gly-His (Fig. 2D) which does not exhibit a well resolved superhyperfine (shf) structure. According to their line splitting parameters $|A_{\parallel}|$ and $|A_0|$ and their $g_{\parallel}$- and g_0-values the Cu(II)-ligand complexes can be divided into two main

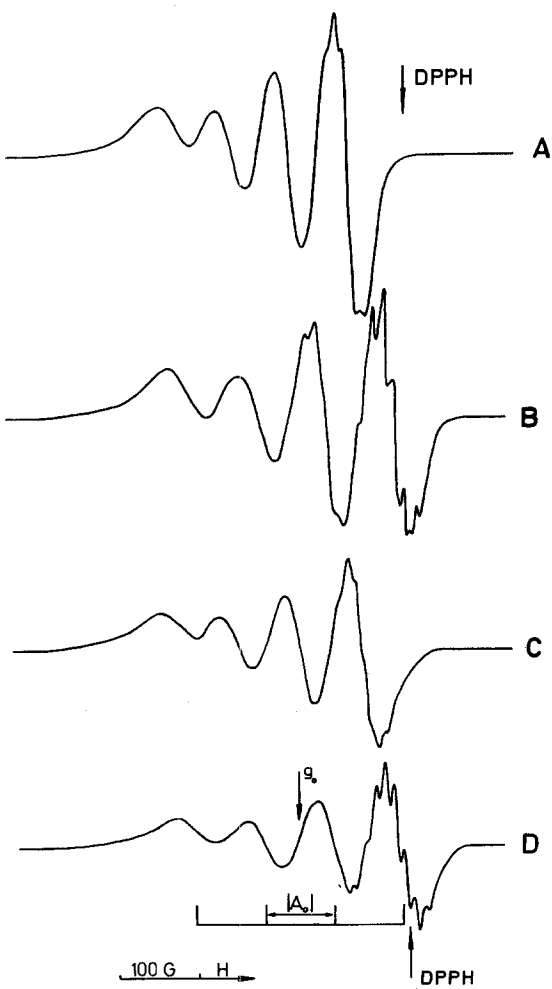

Fig. 1. ESR spectra of aqueous solutions of Cu^{2+}, 1 mM, measured at room temperature, in the presence of different peptides, 2 mM each, pH 7.2. **A:** Gly-Phe. **B:** Gly-Gly-Phe; a similar spectrum is obtained in the presence of Gly-Phe-Ala. **C:** Gly-His; a similar spectrum is obtained in the presence of Gly-His-Gly. **D:** Gly-Gly-His

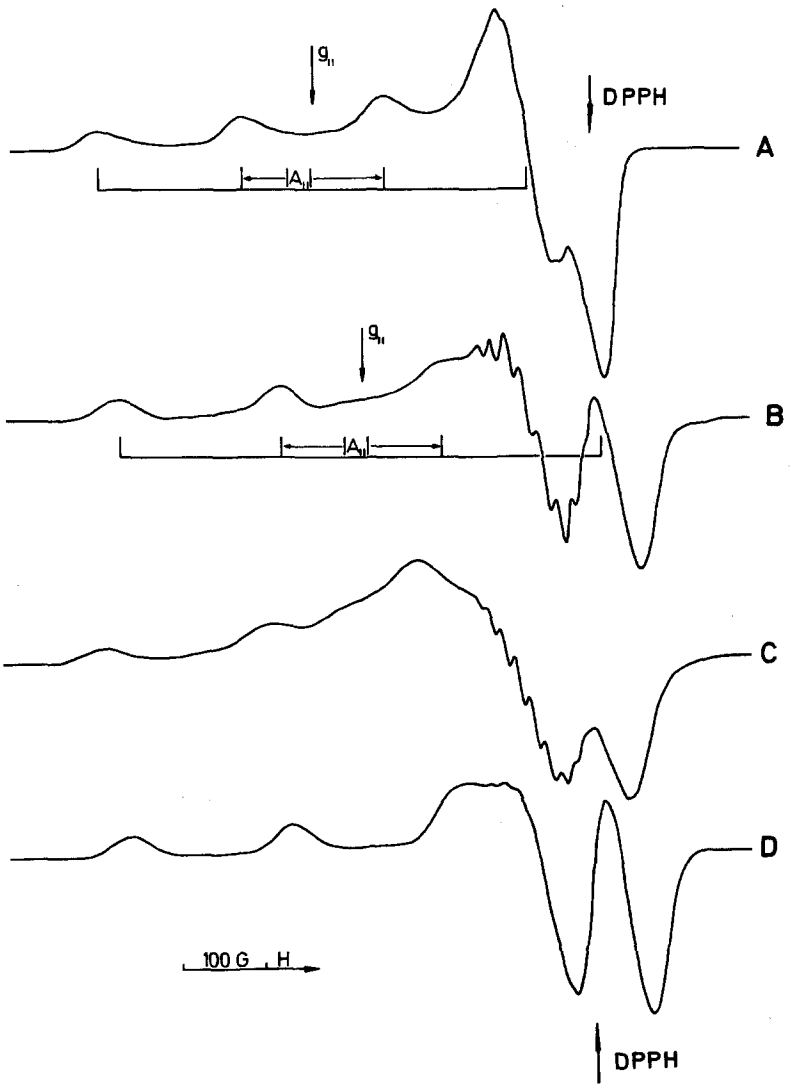

Fig. 2. ESR spectra of aqueous solutions of Cu^{2+}, 1 mM, 77 K, in the presence of different peptides, 2 mM each, pH 7.2. **A:** Gly-Phe. **B:** Gly-Gly-Phe; a similar spectrum is obtained in the presence of Gly-Phe-Ala. **C:** Gly-His; a similar spectrum is obtained in the presence of Gly-His-Gly. **D:** Gly-Gly-His

classes (Table 1): In the case of dipeptides and aromatic amino acids, the A-values are remarkably smaller and the g-values significantly larger than the corresponding values for tripeptides. The only exceptions are Gly-His and Gly-His-Gly which behave neither di- nor tripeptide like.

Optical Measurements. Absorption spectra and data of the visible spectral region, attributed to d-d transitions in the central Cu(II) ion, are shown in Figure 3 and

Table 1. Resonance parameters and optical absorption energies for different Cu(II)-ligand complexes

Ligand (2 mM)	77 K				RT		
	$\lvert A_{\parallel}\rvert$ ± 2 Gauss	$\lvert A_{\perp}\rvert$[a] ± 4 Gauss	$g_{\parallel}$ ± 0.002	$g_{\perp}$[b] ± 0.004	$\lvert A_0\rvert$ ± 2 Gauss	g_0 ± 0.002	ΔE ± 50 (cm^{-1})
Try	164	21.5	2.257	2.053	69	2.121	15,700
Gly-Try	173	18.5	2.234	2.058	70	2.117	16,000
Gly-Try-Gly	190	31	2.186	2.048	84	2.094	18,400
Gly-Try-Gly	195	31.5	2.184	2.049	86	2.094	18,400
Phe	169	20.5	2.254	2.051	70	2.119	15,700
Gly-Phe	172	20.5	2.236	2.057	71	2.117	16,000
Gly-Phe-Ala	191	32	2.195	2.043	85	2.094	18,400
Gly-Gly-Phe	195	31.5	2.192	2.040	86	2.091	18,500
His	175	14.5	2.239	2.056	68	2.117	15,800
Gly-His	195	16.5	2.209	2.063	76	2.112	17,000
Gly-His-Gly	191	15.5	2.212	2.063	74	2.113	16,900
Gly-Gly-His	195	30	2.187	2.035	85	2.086	19,200

[a] $\lvert A_{\perp}\rvert = 1/2\ (3\ \lvert A_0\rvert - \lvert A_{\parallel}\rvert)$; $\lvert A_0\rvert$ obtained from RT spectra
[b] $g_{\perp} = 1/2\ (3\ g_0 - g_{\parallel})$; g_0 obtained from RT spectra

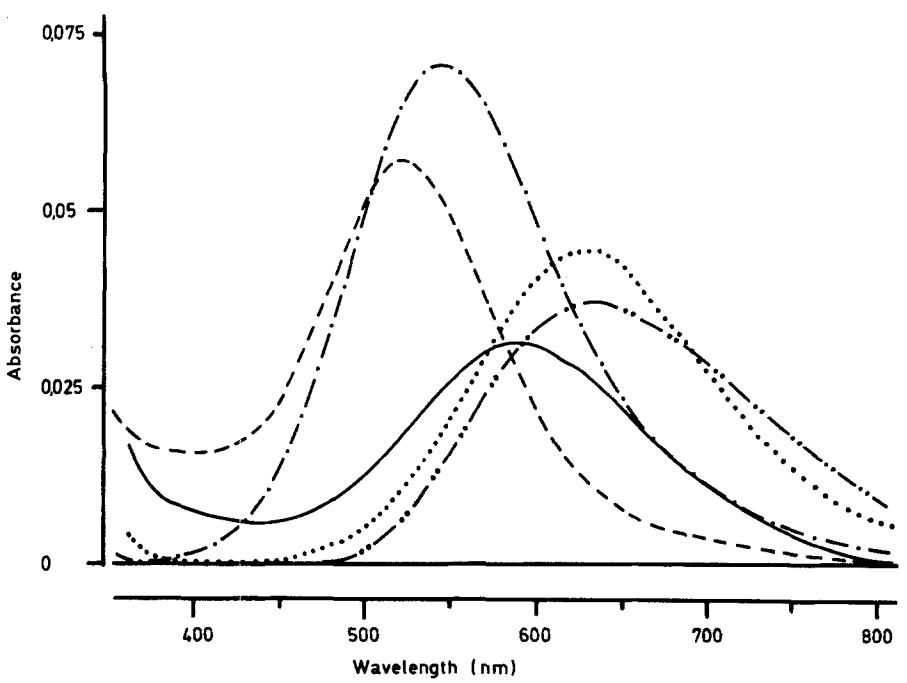

Fig. 3. Optical absorption spectra (d-d transitions) of aqueous solutions of Cu^{2+}, 1 mM, in the presence of different peptides, 2 mM each, pH 7.2. – · · · – His; · · · · Gly-Phe; —— Gly-His; – · – · – Gly-Gly-Phe; – – – Gly-Gly-His

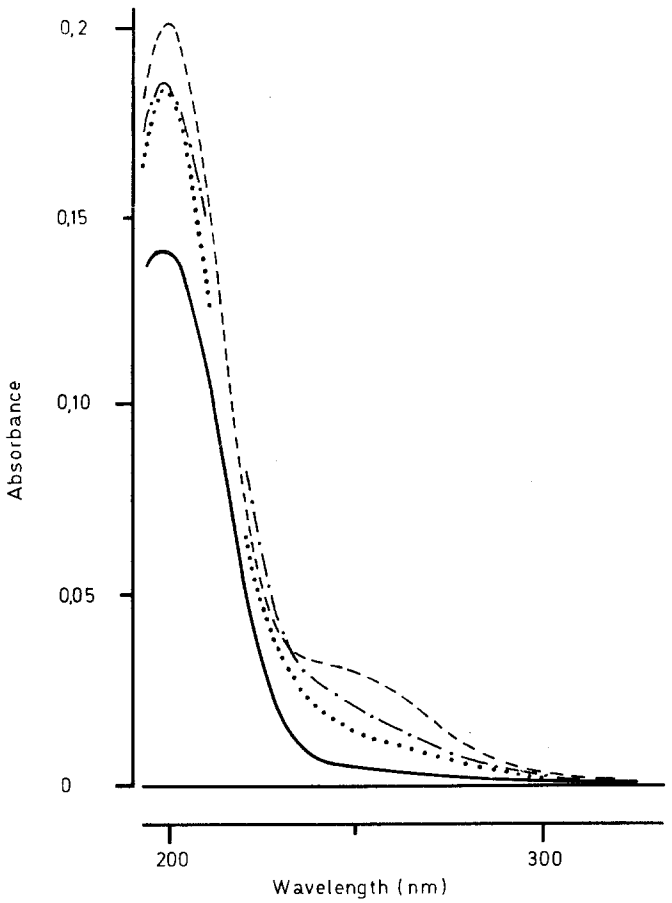

Fig. 4. Difference spectra of a Cu^{2+} (1 mM)-peptide (2 mM each) mixture vs. the corresponding peptide in an aqueous solution, pH 7.2. —— no peptide; – · – · – Gly-Gly-Phe; · · · · Gly-His, a similar spectrum is obtained in the presence of Gly-His-Gly; – – – Gly-Gly-His, a similar spectrum is obtained in the presence of His

Table 1. In general, a blue shift (ΔE) and an increase in intensity can be observed for tripeptide complexes if they are compared with dipeptide or amino acid complexes (Fig. 3). Another remarkable fact is the similarity in ΔE of copper complexes with peptides containing tryptophan or phenylalanine residue. Histidine containing peptides behave differently. In the case of these substances the classification mentioned above cannot be applied.

The UV spectra of the complexes shown in Figure 4 characterize the charge transfer (CT) interaction between the ligands and the central metal ion. All peptides and aromatic amino acids investigated show nearly the same CT spectra. The only exceptions are Gly-Gly-His and His [21]. In these cases an additional CT absorption band is present at 250 nm.

Discussion

Recently ESR investigations on the complexation of Cu(II) with aromatic amino acids have been reported [21]. According to these findings phenylalanine, tryptophan, and thyrosine coordinate to Cu(II) via the nitrogen of the amino group and one of the carboxyl oxygen to give approximately square planar 2 : 1 complexes. No significant influence of the three different aromatic rings was found on the g- and A-values. In the case of histidine, there is slightly different situation since the nitrogen in the imidazole ring can, in general, coordinate in-plane [17, 26] or out-of-plane [21, 23, 26]. Thus, in the case of di- and tripeptides it has to be distinguished between histidine and phenylalanine or tryptophan containing peptides.

From Table 1, the following conclusions can be drawn: the directly measurable g-values, g_0 and $g_\parallel$, decrease, in general, in the sequence amino acid $\geqslant$ dipeptide $>$ tripeptide while the $|A_0|$ and $|A_\parallel|$ values increase; the wavelength of the d-d transition of the central copper ion decreases (Fig. 3).

These results might give some indications in regard to the structure of the Cu^{2+} complexes. According to the ESR theory on copper complexes a correlation exists between the g values and the energy of the d-d transitions on one side and the covalency of the metal-ligand bond as well as the symmetry of the complexes on the other side [7, 10, 15, 24]. From the theory it is understandable that oxygen ligands will result in larger g-values than nitrogen ligands, and in longer wavelengths of the d-d transitions, if one compares in a complex with a nearly unchanged symmetry. The symmetry of the complexes investigated can be assumed to be a square planar arrangement since two g-values [with $g_\parallel > g_\perp > 2$] were observed only in the spectra registered. Finally, a blue shift of the absorption band of the d-d transition with increasing chelation of the complex can be observed [3].

Unfortunately, the dipeptides show a poorly resolved shf-structure which makes it impossible to determine the number of the coordinated nitrogen atoms. However, it seems to be reasonable to assume a two nitrogen coordination with the participation of one amino and one peptide nitrogen. The third binding position is probably occupied by one of the carboxyl oxygens, while on the fourth one a water molecule might be coordinated. This assumption is supported by the findings that the g- and A-values as well as the absorption maxima of the d-d transitions of the Cu(II) complexes with amino acids and dipeptides are very similar.

Cu(II) complexes with tripeptides containing phenylalanine or tryptophan residues have, as mentioned above, smaller g-values. ΔE is shifted to about 18,400 cm^{-1} and at least 7 lines of nitrogen hyperfine splitting with a coupling constant of about 15 Gauss are resolved. The data suggest an interaction of the unpaired Cu(II) electron with three approximately equivalent nitrogen atoms, that is one amino and two peptide nitrogens. In this case the fourth position is probably occupied by the carboxyl oxygen. The increased chelation may be responsible for the better resolved nitrogen hf splitting.

Though there are nearly identical parameters found for peptides containing tryptophan or phenylalanine residues and though the position of the aromatic amino acids in the tripeptide chain has no effect on the complexation parameters, the parameters found for aliphatic peptides are slightly different. For glycylglycine and diglycylglycine for example the hf-splittings are smaller, the $g_\parallel$ values are greater

and the d-d transitions exhibit smaller energies. This shows, that there is an influence of the aromatic residues; the kind of interaction, however, could not be found from this measurements. Experiments are in progress to solve this problem.

In the case of peptides containing histidine residues the situation is different as can be seen from the parameters measured $|A_{\parallel}|$, $g_{\parallel}$, and ΔE (Table 1). The ESR parameters of the Cu(II)-(Gly-Gly-His) complex resemble those ones of a tripeptide while its d-d transition exhibit a higher energy. At least 7 shf lines can be resolved at room temperature (Fig. 1D), at 77 K (Fig. 2D) they are poorly resolved.

Two different types of CT-spectra are obtained. His and Gly-Gly-His exhibit an additional band at about 250 nm (Fig. 4), which cannot be seen in the case of Gly-His and Gly-His-Gly. Based on rotatory dispersion and optical absorption measurements this CT-band was assigned to a charge transfer from imidazole to Cu(II) in the Cu(II)-His complex [21, 23]. It seems reasonable to correlate the appearence of this CT band with the different ESR parameters. The new CT band might be due to a change in the ligand sphere, resulting in a coordination of the imidazole nitrogen in an axial position of the central Cu(II) ion (Fig. 5A). A similar behavior has been observed in the case of Cu(II)-His complexes [21].

Both of the Cu(II)-(Gly-His) and Cu(II)-(Gly-His-Gly) complexes have the same $|A_{\parallel}|$, $g_{\parallel}$, and ΔE values and a poorly resolved shf structure at room temperature (Fig. 1C); at low temperature (Fig. 2C), this structure is resolved slightly better. This supports the idea that both of them have similar geometrical configurations. It has been suggested that both of the peptides coordinate via one amino nitrogen, one peptide nitrogen, and one imidazole nitrogen in a planar arrangement (Fig. 5B). The

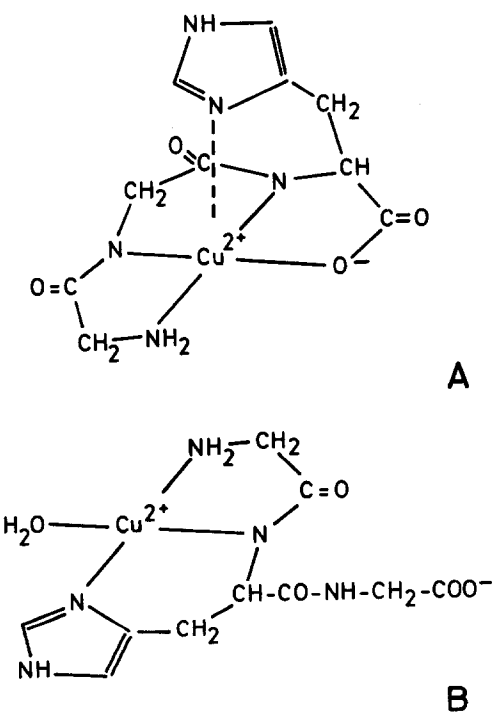

Fig. 5. Structures suggested for Cu(II) complexes with Gly-Gly-His (**A**) and Gly-His-Gly (**B**)

second peptide nitrogen in Gly-His-Gly doesn't seem to be involved in the coordination to the central Cu(II) ion. It might coordinate to an adjacent metal ion.

In summary, the aromatic amino acid residues in small peptides have an influence on their complexation behavior to Cu(II). Di- and tripeptides containing tryptophan or phenylalanine seem to react very similar. A suggestion on the mechanism of the interaction of the aromatic residues with Cu(II), however, cannot be done from the measurements presented. Copper complexes with peptides containing histidine show different complex parameters. From the CT-measurements it might be concluded that the histidine residue can coordinate at planar or axial positions depending on the position of histidine in the peptide chain.

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