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# EPR, NMR and Absorption Spectroscopy Studies of Ni(II) and Cu(II) Complexes with Thr–Lys–Ala–Ala Peptide in Aqueous Solution

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*The PMR, EPR and absorption spectroscopy methods have been used to propose the structure of Ni(II) and Cu(II)–Thr–Lys–Ala–Ala complexes formed in the aqueous solution over a broad pH range. The binding sites in tetrapeptide to both metal ions are shown to be the $NH_2$–Thr, and three deprotonated nitrogens of peptide linkages. In the case of Ni(II) ions the cooperative interaction was observed.*

## Introduction

As a part of our studies on the metal–peptide complexes [1–5] as possible models for metal–enzyme interaction we have examined the Cu(II) and Ni(II)–Thr–Lys–Ala–Ala systems. Both metal ions used in these studies have the ability to promote the proton ionization of the peptide linkage [1, 2, 6, 7, 10]. It was interesting for us to compare the copper and nickel ion interaction with peptide over a broad pH range.

## Experimental

Tetrapeptide was synthesized by the method given in ref. [8]. The $Cu(NO_3)_2 \cdot 2H_2O$ and $Ni(NO_3)_2 \cdot 6H_2O$ were used as metal ion sources. The absorption spectra were recorded on a Unicam SP-700 spectrophotometer at room temperature. Magnetic susceptibility was determined by the Evans method [9] at the temperature 25 ± 1 °C. The EPR spectra were recorded on a JES-ME-3X JEOL spectrometer at 130 and 298 °K (X band). The pH was measured on a MERA ELMAT N-512 pH-meter.

The solutions of a peptide–Cu(II) and Ni(II) molar ratio equal to 2.5:1 and 3.0:1 respectively, were prepared for EPR and absorption spectroscopy measurements, to avoid the precipitation at high pH. The concentration of Ni(II) ions was 0.01 *M* and 0.03 *M* for the absorption spectra and magnetic moment measurements, respectively. The solutions containing 0.15 *M* of tetrapeptide and 0.075 *M* of Ni(II) were used to get NMR spectra. The concentration of copper(II) in all investigated solutions was 0.015 *M*.

## Results and Discussion

For symmetry determination of the tetrapeptide complexes with Ni(II) ions formed in aqueous solution over a broad pH range the electron spectroscopy method was applied and the magnetic moment was determined. The absorption band positions and the molar extinction coefficients are listed in Table I. At pH below 7.7 the bands of the octahedral nickel complexes were observed. The magnetic moment of a solution at pH 3–7.9 is 3.1 BM, what corresponds to octahedral Ni(II) complexes coordinated only with the carboxyl groups and/or water molecules, as in other aminoacid and peptide systems [1, 2, 17, 18].

TABLE I. Absorption Spectra for Solutions Containing Tetrapeptide and Ni(II) Ions in 3:1 Molar Ratio.

| pH | $\bar{\nu}$ ($cm^{-1}$) | $\epsilon$ ($M^{-1}$ $cm^{-1}$) |
|---|---|---|
| | 9 200 | 5.3 |
| 6.5 | 15 200 | 5.8 |
| | 25 800 | 18.8 |
| | 9 300 | 6.8 |
| 7.2 | 15 600 | 7.3 |
| | 25 800 | 24.0 |
| | 9 500 | 6.3 |
| 7.7 | 15 800 | 7.2 |
| | 24 600 | 31.9 |
| 8.4 | 24 000 | 199.9 |
| 9.4 | 24 000 | 258.2 |
| 10.0 | 24 000 | 238.6 |
| 11.6 | 24 000 | 238.3 |

From pH 8.4 one intense band characteristic of planar nickel complexes [2, 6, 10] appears at 24.000 $cm^{-1}$. At pH 8.4 there are visible also the traces of the octahedral structure bands, but they disappear completely at higher pH. Magnetic susceptibility measurements indicated the solution at pH > 8.4 to be diamagnetic. In order to establish the coordination sites of the Ni(II) ion in the square planar complex the NMR spectra of the metal: tetrapeptide = 1:2 molar ratio and of the metal-free peptide solutions were analysed at pH = 9.5. The analysis of the NMR

spectrum of the metal-free tetrapeptide has been made by consideration of the signal intensities, of signal multiplicity and by comparison of the polypeptide spectrum with the spectra of the individual amino acids at the related pH (Fig. 1). It is noteworthy that the CH protons of both alanine residues, as well as the $CH_3$ ones, are chemically equivalent at pH = 9.5. The NMR spectrum of the solutions containing nickel(II) and tetrapeptide (1:2) consisted of uncoordinated and coordinated (shifted upfield) ligand signals (Fig. 1). Some peptide excess was necessary to get a clear solution. There was no difference between the chemical shifts of $\epsilon$-$CH_2$–Lys protons for coordinated and uncoordinated tetrapeptide, what excluded the coordination of $NH_2$–Lys group to Ni(II) ion. The α-CH–Thr doublet of coordinated peptide molecule is shifted upfield by 0.25 ppm due to the $NH_2$–Thr coordination. Another broad signal overlapped with this doublet corresponding to one CH group (stated by intensity measurements) belongs most likely to the α-CH–Lys of coordinated ligand molecule. The upfield shift of 0.85 ppm is rather large, but also the conformational changes of the tetrapeptide molecule (due to the coordination) should be greatest around the lysine residue. There are two other multiplets of coordinated ligand close to each other. The first one is the clear quartet of alanine residue shifted upfield by 0.52 ppm. The second one, unresolved, is likely to be also the alanine α-CH proton, shifted upfield by 0.42 ppm. This assumption is in agreement with the Ni(II)–tetraglycine case [22], in which there is a rather small difference in chemical shifts of two adjacent glycine residues at the carboxyl terminal. The chemical shift of β-CH–Thr of the coordinated peptide is almost the same as that of the uncoordinated one.

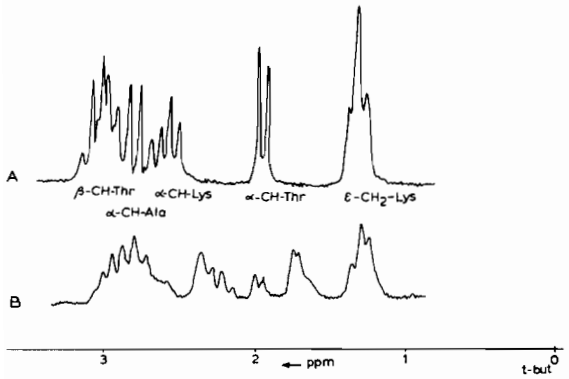


Figure 1. A) The PMR spectrum of metal-free tetrapeptide in $D_2O$ solution at pH 9.5, with respect to tert-butyl alcohol. B) The PMR spectrum of Ni(II)–tetrapeptide 1:2 molar ratio solution at pH 9.5. The $CH_3$–Thr, $CH_3$–Ala and β, γ, δ-$CH_2$–Lys lines do not change considerably during complexation and are omitted here.

The data presented above proved the coordination of $NH_2$–Thr and of three deprotonated peptide linkage nitrogens and the formation of the square planar Ni(II) complex in almost a single step (cooperative interaction). The proposed structure is given in Fig. 2. Planar Ni(II) complexes with peptide containing more than two amino acid residues after the peptide linkage deprotonation seem to be the most stable [10, 24].

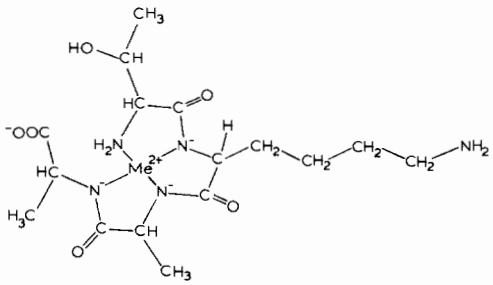

Figure 2. The structure proposed for the complex formed by Ni(II) and Cu(II) ions with Thr–Lys–Ala–Ala in the high pH region.

The electron spectroscopy and the EPR methods proved to be very useful for the metal–polypeptide systems [13, 20, 25, 27]. In the Cu(II)–Thr–Lys–Ala–Ala system one band corresponding to the d–d transition of the tetragonal Cu(II) complexes is observed over the whole pH range in the visible.

The band contains several bands due to transitions between the lowest lying $^2B_{1g}$ and the higher lying $^2A_{1g}$, $^2B_{2g}$, $^2E_g$ terms resulting from the splitting of the $^2E_g$ and $^2T_{2g}$ levels in a tetragonally distorted field [11–13]. The electron spectroscopy data obtained for the system are presented in Fig. 3 and Table II. The diagram of the pH dependence of the d–d band position exhibits four shoulders. The first one begins at pH 6, the second one – very faint –

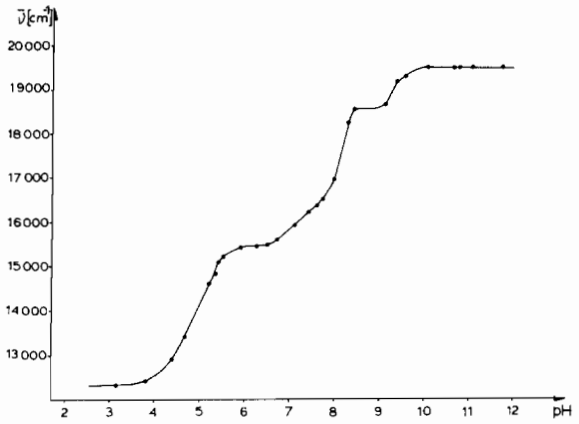


Figure 3. The pH dependence of the d–d transition band position for the Cu(II) complex with tetrapeptide.

TABLE II. Absorption Spectra and Molar Extinction Coefficients for Solutions Containing Tetrapeptide and Cu(II) Ions in 2.5:1 Molar Ratio.

| pH | $\bar{\nu}$ (cm$^{-1}$) | $\epsilon$ |
|---|---|---|
| 3.2 | 12300 | 14 |
| 3.8 | 12400 | 18 |
| 4.4 | 12900 | 18 |
| 4.7 | 13400 | 22 |
| 5.2 | 14300 | 32 |
| 5.5 | 15100 | 83 |
| 5.6 | 15200 | 85 |
| 6.0 | 15400 | 97 |
| 7.2 | 15900 | 107 |
| 7.5 | 16200 | 107 |
| 7.8 | 16500 | 117 |
| 8.1 | 16900 | 126 |
| 8.4 | 18200 | 153 |
| 9.3 | 18600 | 157 |
| 9.5 | 19100 | 185 |
| 10.2 | 19400 | 200 |
| 10.8 | 19400 | 200 |
| 11.2 | 19400 | 200 |

at pH about 7.5, the third one at pH 8.5, and the last one at pH about 10. Simultaneously there grows the intensity of the d–d absorption band (Table II). The stepwise change of a band position towards higher energies with increasing pH means the stepwise nitrogen uptaking by the Cu(II) ion [13, 21]. The $NH_2$ group of the threonine residue is bonded first. Next the nitrogens of the subsequent peptide bondings coordinate gradually forming the five-membered chelate rings. The peptide nitrogens are bound to the metal ion after the hydrogen ionization, what is in agreement with the characteristic $\epsilon_{mol}$ increase on complex formation [11, 13].

With the purpose to characterize the complexes formed by the coordination of the tetrapeptide the EPR spectra of frozen solutions were measured. The EPR spectra for a pH higher than 4.6 have axial symmetry. No signals are observed near g = 4 what indicates the complexes to be monomers. The $A_{\|}$ and $g_{\|}$ parameters determined from the EPR spectra are given in Table III. Their values vary in the pH ranges where the stepwise change of the d–d band position is observed.

Over the pH range 5.3–6.3 two overlapped and mutually shifted EPR spectra are observed (Fig. 4). They derive from two kinds of Cu(II) complexes being in an equilibrium. In accordance with the

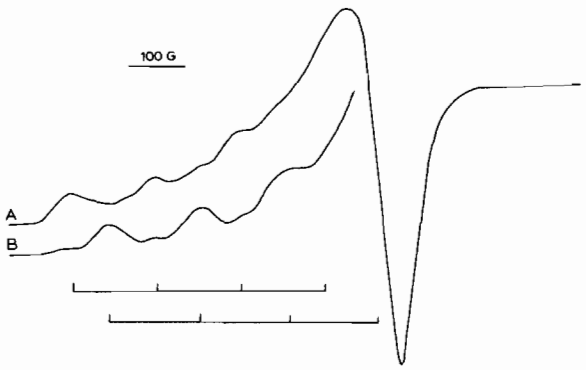


Figure 4. The EPR spectra of 2.5:1 Thr–Lys–Ala–Ala to Cu(II) molar ratio solutions at 130 °K. A) at pH = 5.3. B) at pH = 6.3.

TABLE III. EPR Parameters for Frozen Solutions of Tetrapeptide and Cu(II) Ions With 2.5:1 Molar Ratio. $A_{\|}$ is Given in Gauss.

| pH | 4N | | 3N | | 2N | | 1N | | 4O | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $A_{\|}$ | $g_{\|}$ | $A_{\|}$ | $g_{\|}$ | $A_{\|}$ | $g_{\|}$ | $A_{\|}$ | $g_{\|}$ | $A_{\|}$ | $g_{\|}$ |
| 11.2 | 203.2 | 2.170 | | | | | | | | |
| 10.9 | 205.1 | 2.164 | | | | | | | | |
| 9.7 | 210.4 | 2.171 | | | | | | | | |
| 9.4 | 207.1 | 2.163 | 188.3 | 2.218 | | | | | | |
| 9.1 | 205.1 | 2.164 | 185.3 | 2.218 | | | | | | |
| 8.9 | | | 182.1 | 2.216 | | | | | | |
| 8.4 | | | 185.9 | 2.218 | | | | | | |
| 8.1 | | | 185.0 | 2.217 | | | | | | |
| 8.0 | | | 185.3 | 2.213 | | | | | | |
| 7.8 | | | | | 175 | 2.223 | | | | |
| 7.6 | | | | | 175 | 2.224 | | | | |
| 6.9 | | | | | | | 168.6 | 2.235 | | |
| 6.3 | | | | | | | 162.0 | 2.242 | 157.8 | 2.303 |
| 5.8 | | | | | | | 162.0 | 2.242 | 157.8 | 2.303 |
| 5.5 | | | | | | | | | 159.2 | 2.294 |
| 5.3 | | | | | | | 162.0 | 2.242 | 157.8 | 2.303 |
| 5.1 | | | | | | | | | 154.0 | 2.299 |
| 4.7 | | | | | | | | | 154.0 | 2.299 |

diagram 3, in the discussed pH range the complexes containing one nitrogen in the copper coordination sphere (1N) and complexes in which the Cu(II) ions are surrounded by oxygen donors only "4O" [23] are in equilibrium.

At pH 6.9 the Cu(II) complex with one coordinated nitrogen atom (1N) is the predominant form. It remains in equilibrium with the "2N" complex, and the weak EPR spectrum is overlapped with the spectrum of the "1N" one.

At pH 7.6 and 7.8 the EPR spectrum indicates that the predominant species in solution is the copper (II) complex with two nitrogen atoms coordinated to metal ions (2N) (see diagram 3). Simultaneously, there are observed the weak signals from the "3N" type complex, which is the main species in solution over the pH range 8.0–8.9. At pH 9.1 and 9.4 the complexes of the "3N" and "4N" type are in equilibrium. The EPR spectra of both complexes vary considerably in their $A_{\parallel}$ and $g_{\parallel}$ parameters, so they are mutually shifted (Fig. 5). Thus, the EPR

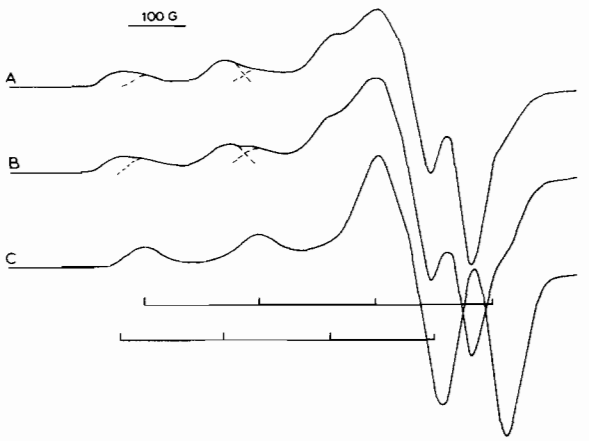


Figure 5. The EPR spectra of 2.5:1 Thr–Lys–Ala–Ala to Cu(II) molar ratio solutions at 130 °K. A) at pH = 9.1; B) at pH = 9.4; C) at pH = 10.9.

data are consistent with those obtained from the electron spectroscopy, and fully confirm the stepwise formation of complexes with an increasing number of nitrogen donors (1N, 2N, 3N and 4N).

Differences in spin Hamiltonian parameters for complexes "1N", "2N" and "3N" are too small to allow the clear observation of two mutually shifted EPR spectra. For their determination (Table III) the spectra of the solutions containing each complex as a predominant form were taken into consideration. The data obtained for all complexes are gathered in Table IV.

For the complex "2N" the EPR data were helpful in determining such a pH value at which that complex was predominant in solution. On that basis the $\bar{\nu}$ value used for $\beta_1^2$ calculation was estimated (see below).

The approximate contribution of the metal $d_{x^2-y^2}$ orbital to the molecular orbital was determined from the approximate expression [14]:

$$\alpha^2 = \frac{A_{\parallel}}{P}(g_{\parallel} - 2.0023) + \frac{3}{7}(g_{\perp} - 2.0023) + 0.04$$

$$P = 0.04\ cm^{-1}$$

For estimation of $\pi$-bonding character in the complex plane the MO coefficient $\beta_1^2$ ($d_{xy}$ orbital contribution) was calculated from the equation for $g_{\parallel}$ [14],

$$g_{\parallel} = 2.0023 - 8\rho[\alpha\beta_1 - \alpha'\beta_1 S - \alpha'(1-\beta_1^2)^{1/2} T(n)/2]$$

where $\rho = \lambda_o \alpha\beta_1 / \Delta E_{xy}$

$$\lambda_o = -828\ cm^{-1}$$

$\alpha'$ was determined from the normalization term:

$$\alpha^2 - 2\alpha\alpha' + \alpha'^2 = 1$$

The overlap integral S and the T(n) values were determined according to Kivelson and Neiman [14]:

$S_o = 0.076$, $T(O) = 0.220$ for four oxygen donors, and

$S_N = 0.093$, $T(N) = 0.333$ for four nitrogen donors.

The comparison of data in Table IV for complexes with an increasing amount of nitrogen donors coordinated with Cu(II) ions indicates the energy increase of the d–d band, the increase of $\epsilon_{mol}$ and of the $A_{\parallel}$, and the lowering of $g_{\parallel}$ and $g_{\perp}$ parameters. As the nitrogen donors number increases from 1 to 3, the

TABLE IV. Spectroscopic Data for Copper(II) Complexes With Tetrapeptide.

| | $\bar{\nu}$ ($cm^{-1}$) | $A_{\parallel}$ ($\times 10^4$ $cm^{-1}$) | $g_{\parallel}$ | $g_{\perp}$ | $\alpha^2$ | $\beta_1^2$ | $\lvert\lambda\rvert$ ($cm^{-1}$) |
|---|---|---|---|---|---|---|---|
| 4N | 19400 | 208.7 | 2.166 | 2.044 | 0.74 | 0.74 | 414 (50%) |
| 3N | 18500 | 192.0 | 2.217 | 2.055 | 0.76 | 0.88 | 505 (61%) |
| 2N | 16400 | 181.6 | 2.223 | 2.057 | 0.74 | 0.83 | 456 (55%) |
| 1N | 15400 | 171.1 | 2.240 | 2.061 | 0.73 | 0.83 | 466 (57%) |
| 4O | 12300 | 168.3 | 2.300 | 2.075 | 0.79 | 0.76 | 461 (56%) |
| | | | | | | | $\lvert\lambda_{Cu}\rvert$ = 828 $cm^{-1}$ |

$\alpha^2$ increases slightly. For the transition from the "3N" complex to the "4N" one a lowering of the $\alpha^2$ is observed. In the case of $\pi$-bonding the ionic character increases also as the nitrogen donor increases from 1 to 3. It decreases considerably at the transition to the "4N" complex.

The $\lambda$ parameter was determined from the approximate expressions for $g_{\|}$ and $g_{\perp}$ [15]. The comparison of the $\lambda$ parameter values obtained in that way with that for the free Cu(II) ion gives results similar to those obtained from the observation of changes in $\alpha^2$ and $\beta_1^2$ values. The $\lambda$ complex parameter is the lowest for the "4N" complex (50% of the value for the Cu(II) free ion), which reflects the greatest delocalization of metal–ligand bondings.

## Conclusions

In both systems examined, Ni(II) and Cu(II) with the peptide Thr–Lys–Ala–Ala the complexes are formed with contribution of the $NH_2$–Thr group and of the deprotonated peptide linkage nitrogens. The structure of the final complexes (at high pH) is similar for both metals, but they vary in the way of their formation. The Ni(II) ions are responsible for the deprotonation of the $NH_3^+$ group and of three nitrogens of the peptide bondings over the narrow pH range. The Cu(II) ions coordinate stepwise to the $NH_2$ and to the subsequent peptide bondings at pH 5.5–10. From among the metal ions which promote the peptide hydrogen ionization in tri- and tetraglycine only the Ni(II) ions exhibit the cooperative interaction [16, 22] accompanied by the spin state change and change of symmetry from the octahedral to planar.

Differences between the character of interaction of Cu(II) and Ni(II) ions with the peptide bondings are the reason, why within the physiological pH range in the case of Ni(II) the planar complex already formed exists in solution.

In the case of Cu(II) ions which easier promote the ionization of the first peptide linkage, mainly the "2N" and "3N" complexes are in equilibrium over the same pH range.

It is also necessary to note that in the Cu(II) complex the fifth and/or sixth position might be occupied by solvent molecules through the whole pH range.

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