Copper(II) Complexes with Canine Tuftsinyltuftsin Octapeptide. A Novel Mode of Metal–Peptide Coordination

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Our recent studies on Cu(II) interaction with proline containing peptides revealed that the proline residue may act as a 'break-point' in the peptide sequence unless it is on a N-terminal position [1–5]. This feature of the proline residue may be critical to the binding ability of a peptide ligand, resulting in the unique binding mode in the formed metal–peptide complexes [1].

The most important position of the proline residue in the tetrapeptide sequence was found to be the second position, in which the proline 'break-point' divides the ligand molecule into two fragments, able to bind the metal ions independently, *i.e.* via N-terminal amino acid and/or via C-terminal residues [1]. It is likely that a similar effect of the proline residues could also be observed in the longer peptides. The latter assumption is being checked for the Cu(II) canine tuftsinyltuftsin octapeptide system (Thr-Lys-Pro-Lys-Thr-Lys-Pro-Lys) containing two proline residues in the third and seventh positions of the ligand sequence; the results are presented in this communication.

The ligand used, as well as its human analogue, besides its interesting coordination abilities, also shows promising anti-tumor activity [6–8].

Experimental

Octapeptide (pentaacetate) was synthesized by fragment condensation, by the azide method [6]. $Cu(ClO_4)_2 \cdot 6H_2O$ (Merck) was used as a metal ion source. The absorption spectra were recorded on a Beckman UV 5240 spectrophotometer. The CD spectra were measured on a Mark III Jobin-Yvon Dichrographe in the 800–200 nm region. The EPR spectra (at 9.12 GHz) were recorded on a JES-ME-3X JEOL spectrometer at 130 °K. Solutions with the molar ratio of Cu(II): octapeptide = 1:1 were used for all measurements, with the Cu(II) concentration equal to 0.005 *M*.

Results and Discussion

The absorption spectra of Cu(II) octapeptide solution at pH 4.2 show two d–d bands at ~750 and ~600 nm, suggesting two metal–metal peptide species (Table I). The increase in pH causes the disappearance of the 750 nm band and the d–d

TABLE I. Absorption and EPR Spectral Data for the Cu(II): Thr-Lys-Pro-Lys-Thr-Lys-Pro-Lys 1:1 Solutions.

pH	d–d transitions		EPR		Dominant species[a]
	λ, [nm]	ε [M^{-1} cm^{-1}]	$A_{\parallel}$, [Gauss]	$g_{\parallel}$	
3.7	750	~26			1N
	600sh	~25			2N
3.9	750sh	~26	164	2.302	1N
	600	31			2N
4.4	750sh	<10	162	2.300	1N
	610	42	174	2.232	2N
5.8	620	103	176	2.230	2N
6.9	612	148	176	2.226	2N
8.8	573	161	170	2.212	3N
10.4	552	170	170	2.212	3N
11.0	550	165	170	2.212	3N

[a]1N, 2N and 3N correspond to Cu(II) peptide complexes with 1, 2 and 3 nitrogens bound to metal ion, respectively.

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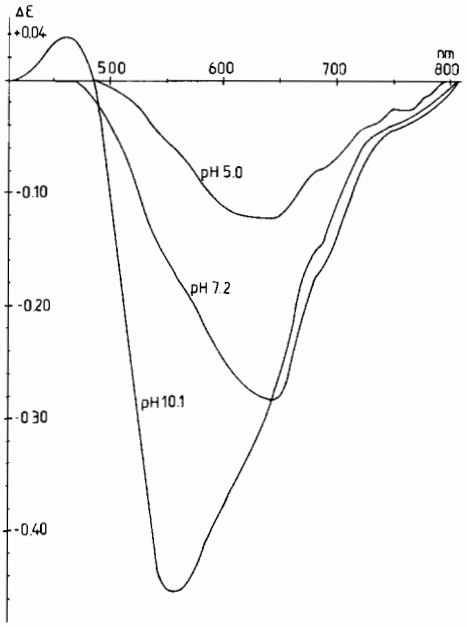

Fig. 1. The d–d region of CD spectra of Cu(II)–tuftsinyl-tuftsin equimolar solutions at different pH.

transition at ~600–620 nm is observed up to pH 7. These two transitions are most likely to correspond to two metal–octapeptide species with one and two nitrogen donors bound to cupric ions, respectively [1–5]. The EPR spectra support these conclusions and the $A_{\parallel}$ and $g_{\parallel}$ values (Table I) are typical for the 1N and 2N metal–peptide complexes [1, 3–5, 9].

The CD spectra give additional evidence for the presence of 1N and 2N species in the 4–7 pH range (Fig. 1). At pH = 5 the d–d multicomponent band is centered at 640 nm with shoulders at 760, 735 and 690 nm. The two former shoulders disappear when the pH increases and they correspond to the 1N complex [1]. The appearance of the 280 nm band ($\Delta\epsilon$ = –1.7, pH = 10) at low pH, which is usually assigned as $NH_2 \rightarrow Cu(II)$ charge transfer transition [1, 3–5, 10, 11] indicates that in the 1N complex a cupric ion is bound with octapeptide molecule via the NH_2 group of N-terminal threonine residue. The increase in pH activates the other charge transition at 325 nm ($\Delta\epsilon$ = +0.4, pH = 10) which may be assigned as $N^- \rightarrow Cu(II)$ transition [1, 3–5, 10, 11]. The latter band suggests that in the 2N complex the two bound nitrogen donors are NH_2 and N^- of the vicinal peptide linkage (Lys). Both charge transfer bands are observed for the species formed in the whole pH range studied. It could suggest that the two N-terminal nitrogen donors are bound to the cupric ion in all major species formed in Cu(II) octapeptide solutions.

The increase of pH above 7 causes a considerable variation in the d–d region of CD spectra. The $\Delta\epsilon$ of the broad band at 640 nm decreases and the new Cotton effect at 560 nm becomes predominant at pH > 8.5. The increase of the d–d transition energy observed in the CD and absorption spectra (Fig. 1) in basic solutions strongly suggests the involvement of the third nitrogen donor in metal ion binding. The energy of the d–d transitions centered at 560 nm in the CD spectrum and ~550 nm in the absorption spectrum corresponds well to the 3N complex [3–5, 9–12].

The involvement of the side lysine NH_2 group in the metal ion binding is usually reflected in the CD spectra. The $NH_2 \rightarrow Cu(II)$ charge transfer transition in the 250–270 nm region is observed for the system with cupric ion bound to side lysine amino groups [10, 12]. Such a band was not observed for the system described in this work. Thus, the third nitrogen donor in the 3N species may derive from one of the peptide linkages. Our earlier studies on Cu(II) tetrapeptide systems with proline at a second position [1] may suggest the involvement of the C-terminal lysine residue in the cupric ion binding via its COO^- and N^- donors. The latter two donors would create the stable, five-membered chelate ring. The involvement of the C-terminal lysine in the cupric ion binding could also be supported by the fact that the pH increase up to 11.5 does not cause any other nitrogen binding, which could happen in the case of the involvement of the N^- donor(s) derived from the central part of octapeptide, *i.e.* Lys-Thr-Lys unit. In the latter case the migration of cupric ion from N-terminal to the middle part of a ligand molecule is likely (see the Cu(II)Tyr-Pro-Gly-Gly system in Ref. 1).

Thus in the 3N species a metal ion binds the octapeptide molecule via two nitrogen donors of N-terminal Thr and Lys residues (NH_2, N^--five membered chelate ring) and nitrogen and carboxylate of C-terminal Lys (N^-, COO^--five membered ring). This unusual coordination of metal ion caused by two proline residue 'break-points' leads to the loop-like conformation of five unbound residues (-Pro-Lys-Thr-Lys-Pro-). It seems likely that the octapeptide itself has the bent structure which could be additionally stabilized by metal ion coordination.

The above results show that the proline residue acts as a 'break-points' and even if not bound directly to the metal ion it leads to very unusual binding properties of the peptide ligand.

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References

1 H. Kozłowski, M. Bezer, L. D. Pettit, M. Bataille and B. Hecquet, *J. Inorg. Biochem.* (in press).
2 B. Jeżowska-Trzebiatowska, E. Matczak-Jon and H. Kozłowski, *Bull. Acad. Pol. Sci., Ser. Sci. Chim., 26,* 145 (1978).
3 G. Formicka-Kozłowska, H. Kozłowski, I. Z. Siemion, K. Sobczyk and E. Nawrocka, *J. Inorg. Biochem., 15,* 201 (1981).
4 G. Formicka-Kozłowska, M. Bezer and L. D. Pettit, *J. Inorg. Biochem.,* (in press).
5 G. Formicka-Kozłowska, H. Kozłowski, M. Bezer, L. D. Pettit, G. Kupryszewski and J. Przybylski, *Inorg. Chim. Acta, 56,* 79 (1981) and references therein.
6 D. Konopińska, V. A. Najjar and M. Łuczak, *Proceed. 17th Eur. Pept. Symp. Prague 1982* (in press).
7 V. A. Najjar, M. K. Chaudhuri, D. Konopińska, B. D. Beck, P. P. Layne and L. Linehan, in 'Augmenting Agents Cancer Therapy', ed. E. M. Hersh *et al.,* Raven Press, New York, N.Y., 1981, p. 478.
8 V. A. Najjar, D. Konopińska, M. K. Chaudhuri, D. E. Schmidt and L. Linehan, *Mol. Cell. Biochem., 41,* 3 (1981).
9 G. Formicka-Kozłowska, H. Kozłowski and B. Jeżowska-Trzebiatowska, *Inorg. Chim. Acta, 25,* 1 (1977) and references therein.
10 A. Garnier, L. Tosi, L. Mosoni, C. Toniolo, G. M. Bonora and E. B. Paniago, *Biopolymers, 20,* 951 (1981) and references therein.
11 R. B. Martin, in 'Metal Ions in Biological Systems', H. Sigel Ed., N.Y. Basel (1979), vol. 9, p. 1.
12 S. Salardi, L. Tosi, A. Garnier-Suillerot, C. Toniolo, B. M. Bonora and F. Marchiori, *Biopolymers, 21,* 1229 (1982).