1 H. Kozłowski, M. Bezer, L. D. Pettit, M. Bataille and B. Hecquet, *J. Inorg. Biochem.*, (in press).
2 G. Formicka-Kozłowska, H. Kozłowski, I. Z. Siemion, K. Sobczyk, and E. Nawrocka, *J. Inorg. Biochem., 15*, 201 (1981).
3 H. Kozłowski, G. Formicka-Kozłowska and L. D. Pettit, *J. Pure Appl. Chem.*, (in press).

U16

Spectroscopic Studies of Cu(II) Complexes with Proline and Lysine Containing Octapeptide

G. FORMICKA-KOZŁOWSKA, D. KONOPIŃSKA, H. KOZŁOWSKI

Institute of Chemistry, University of Wrocław, Joliot-Curie 14, 50-383 Wrocław, Poland

and B. DECOCK-LE REVEREND

Lab. de Chimie Macromoléculaire, Université des Sciences et Techniques de Lille, 59-655 Villeneuve d'Ascq, Cedex, France

Recent studies on Cu(II) complexes with proline containing peptides have shown that the proline residue may act as a 'break-point' in the peptide sequence unless it is on a N-terminal position [1, 3].

The lysine residue, on the other hand, may in specific cases bind the metal ion *via* its lateral NH_2 group [2]. The dog double tuftsin octapeptide, Thr–Lys–Pro–Lys–Thr–Lys–Pro–Lys, contains both Pro and Lys residues and its coordination ability was checked in the system with cupric ions.

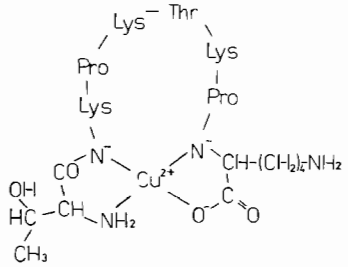

Fig. 1. Proposed structure of the 3N complex.

The EPR, absorption and CD spectra of Cu(II) octapeptide solutions suggest the presence of three different species formed at 3–10 pH range (Table I). The charge transfer region in the CD spectra in which two CT bands are observed at 280 nm ($\Delta\epsilon = -1.7$ for pH 10) and 325 nm ($\Delta\epsilon = +0.4$, pH 10) indicates the involvement of N-terminal NH_2 group (Thr) and one or two amide nitrogen donors [1–4]. The involvement of the lysine NH_2 lateral group seems to be less likely since no CT band for such coordination mode (at 250–270 nm, see ref. 2) could be observed.

The detailed considerations of the structure and the binding mode in 3N species (Table I, ref. 1, 4) lead to the unusual conclusion that the Cu(II) ion binds two octapeptide terminals (NH_2, N^- of N-terminal and N^-, COO^- of C-terminal) and that five central amino acid residues create a loop-like structure. It seems that octapeptide itself has a bent structure which could be additionally stabilized by the metal ion coordination.

TABLE I. Spectroscopic Characterization of Complex Species Formed in Cu(II) Thr–Lys–Pro–Lys–Thr–Lys–Pro–Lys System.

Species	d–d transition		CD		EPR	
	λ, nm	ε	λ (nm)	(Δε)	$A_\parallel$ (G)	$g_\parallel$
1N	750	25	780sh	(–)		
			760	(–0.02)		
2N	610	100	690sh	(–)	162	2.300
			640	(–0.28)		
3N	550	170	560	(–0.45)	170	2.212
			460	(+0.04)		

The results obtained may indicate the new way of synthesis of the model systems in which metal ion is bound *e.g.* to protein without formation of the subsequent 5- or 6-membered chelate rings characteristic for low-molecular weight models. It seems also to be evident that proline residue, even if not bound directly to metal ion, plays a critical role in the metal–peptide complexation mode.

1 H. Kozłowski, M. Bezer, L. D. Pettit, M. Bataille and B. Hecquet, *J. Inorg. Biochem.* (in press) and references therein.
2 S. Salardi, L. Tosi, A. Garnier-Suillerot, C. Toniolo, B. M. Bonora and F. Marchiori, *Biopolymers, 21*, 1229 (1982) and references therein.
3 G. Formicka-Kozłowska, H. Kozłowski, M. Bezer, L. D. Pettit, G. Kupryszewski and J. Przybylski, *Inorg. Chim. Acta, 56*, 79 (1981) and references therein.
4 G. Formicka-Kozłowska, D. Konopińska, H. Kozłowski and B. Decock-le Reverend, *Inorg. Chim. Acta, 78*, L47 (1983).

U17

Interaction of Metal Ions with Peptide Hormones

GRAZYNA FORMICKA-KOZŁOWSKA

Institute of Chemistry, University of Wrocław, Joliot-Curie 14, 50-383 Wrocław, Poland

LESLIE D. PETTIT

School of Chemistry, University of Leeds, LS2 9JT Leeds, U.K.

Among our studies concerning the coordination chemistry of naturally occurring peptides we have

been studying recently metal ion interaction with two hypothalamic hormones: thyroliberin (TRF) and melanostatin (MIF). These two hormones are the only tripeptides among all the pituitary hormones described until now [1–4]. TRF (Pyr–His–Pro–NH_2) and MIF (Pro–Leu–Gly–NH_2) have been extensively studied in various biological aspects [5], among others their possible use in the clinical treatment of mental diseases [6]. Many hormone analogues have been synthesized in order to solve the problem of the relation between biological activity and chemical structure [7]. In most papers the critical role of the histidine residue in TRF biological activity is suggested.

Our main interest concerns the coordination abilities of both hormones and some of their analogues and the relation between the binding modes and the ligand conformation (structure). Spectroscopic (NMR, EPR, absorption and CD spectra) and potentiometric techniques were used to solve these problems.

Though the MIF binding mode to Cu(II) and Ni(II) ions looks classical, the strongly basic Pro nitrogen donor on the N-terminal leads to the formation of very low concentration of 110 (1N) complex species [8].

The histidine residue of TRF ligand is a major binding site of metal ions. The other two residues however, have a critical importance for the complex equilibria and the structure of the formed species [9, 10].

The replacement of pyroglutamic acid with picolinic acid in the TRF molecule causes a major change in the structure of its Cu(II) and Ni(II) complexes. *E.g.*, in the Cu(II)Pic–His system a very specific equilibrium between dimeric and monomeric species was observed [11].

The introduction of tyrosine residue in the TRF sequence in place of histidine also changes the peptide coordination ability. In the Ni(II)Pyr–Tyr–Pro–NH_2 system a direct involvement of Pro–NH_2 residue in the metal binding was proposed [11].

TRF analogue with nicotinic acid in place of pyroglutamic acid forms very unstable Cu(II) complexes due to the unfavourable meta-position of the nitrogen donor with respect to carbonyl in the pyridine ring. Also the TRF analogue with Pro–NH–NH_2 residue in the C-terminal position forms much weaker Cu(II) and Ni(II) complexes than the TRF peptide.

The studies which were done for both hormones show that naturally occurring peptides may form relatively strong complexes with some metal ions and their chemical and physical properties may be very specific for each ligand. The latter conclusion becomes evident when the coordination abilities of the different chemical analogues (*e.g.* of TRF) are compared.

1 K. Folkers, F. Enzman, J. Boler, C. Y. Bowers and A. V. Schally, *Biochem. Biophys. Res. Commun.*, *37*, 123 (1969).
2 R. Burgus, T. F. Dunn, D. M. Desiderio and R. Guillemin, *C.R. Acad. Sci. (D) Paris, 269*, 1870 (1969).
3 R. M. G. Nair, A. J. Kastin and A. V. Schally, *Biochem. Biophys. Res. Commun.*, *43*, 1376 (1971).
4 M. E. Celis, S. Taleisnik and R. Walter, *Proc. Nat. Acad. Sci. U.S.A.*, *68*, 1428 (1971).
5 G. Grant and W. Vale, *Current Topics in Exp. Endocrinology*, *2*, 37 (1974).
6 M. Dimitrikoudi, E. Hanson-Narty and F. A. Jenner, *Lancet*, *1*, 456 (1974); R. H. Ehrensing and A. J. Kastin, *Arch. Gen. Psych.*, *30*, 63 (1974).
7 W. Voelter, Hypothalamus-Regulationshormone, W. Herz, H. Grisebach, G. W. Kirby (Eds.), Wien, N.Y. 1977, p. 439.
8 G. Formicka-Kozłowska, H. Kozłowski, M. Bezer, L. D. Pettit, G. Kupryszewski and J. Przybylski, *Inorg. Chim. Acta*, *56*, 79 (1981).
9 G. Formicka-Kozłowska, H. Kozłowski, G. Kupryszewski, J. Przybylski and B. Jeżowska-Trzebiatowska, *Inorg. Nucl. Chem. Letters*, *15*, 387 (1979).
10 G. Formicka-Kozłowska, H. Kozłowski, G. Kupryszewski, *Inorg. Chim. Acta*, *46* (B1), 29 (1980).
11 G. Formicka-Kozłowska, M. Bezer and L. D. Pettit, *J. Inorg. Biochem.*, submitted for publication.

U18

Iron Coodination Compounds with Glycine, Glycylglycine and Diglycylglycine

KH. M. YAKUBOV, G. M. VINNICHENKO, E. YA. OFFENGENDEN and A. N. ASTANINA

Tadjik State University, Dushanbe, U.S.S.R.

The investigation of biometals complex-forming processes with aminoacids and peptides is of interest as the model of metal–protein interactions.

In this work, the composition of coordination compounds at T = 308 K and pH interval from 0 to 11 units was established on the basis of the obtained partial dependences of oxidizing potential value (ϕ) on iron(III), iron(II), glycine (Gly), glycylglycine $(Gly)_2$, and diglycylglycine $(Gly)_3$ concentrations.

The fall of the oxidizing potential value with pH of the solution (Fig. 1) in pH sphere $< pK_1$ is accounted for by the stepped iron complex-forming processes in aminoacid and peptide aqueous solutions.

From the oxidizing potential dependence on (C_L)-glycine and glycylglycine and diglycylglycine ligand concentrations obtained at different pH values, it has been found that in pH sphere = 2.0 + 5.0 iron(III) complex compounds are formed where only one acidoligand is formed for the complex-forming ion and two acidoligands are formed as the aminoacid and peptide concentrations increase. These results confirm the competition for complex-formation between Fe(III) and Fe(II) in this pH sphere. The idea that protolytic processes proceed in aminoacid