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METAL ION INTERACTION WITH NATURAL OLIGOPEPTIDES

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Abstract - The spectroscopic and potentiometric studies of the coordination abilities of two hormons: thyrotropin releasing factor (Pyr-His-Pro-NH_2) and melanostatin (Pro-Leu-Gly) revealed among others the specific role of proline residue in the metallo-peptide systems. The characteristic feature of this residue is also discussed for the series of metal - peptide complexes with the tri- and tetra-peptide complexes containing proline residue in the different positions of a peptide molecule.

INTRODUCTION

The interaction of metal ions with naturally occuring peptides seems to be an exiting subject in the metallo-peptide chemistry for, at least, two reasons:

1. Nature choosing the specific peptide sequence creates simultaneously the interesting ligand molecule (chemical interest), and
2. metal ion when bound to peptide molecule may change distinctly its biological activity by changing e.g. the peptide structure (biological interest).

The recent studies on e.g. Gly-His-Lys (Ref.1), enkephaline (Ref.2) or glutathione (Ref.3) support both these points strongly.
Thyrotropin releasing factor (TRF, L-pyroglutamyl-L-histidyl-L-prolinamide) and melanostatin (MIF, L-prolyl-L-leucyl-glycinamide) are both oligopeptide hormons containing among others proline residue on C- and N-terminal, respectively. Its coordination ability in both molecules as well as the chelating properties of these peptides are the major subject of this work. The behaviour of proline residue is also discussed for the Cu(II) complexes with the series of tetrapeptides.

METAL - PEPTIDE COMPLEXES

TRF and its di- and tri-peptide analogues.

TRF and its dipeptide analogue, Pyr-His, behave much alike. At low pH the Cu(II) and Ni(II) ions coordinate to the imidazole nitrogen and then cause the ionization of the amide protons of both the peptide linkage and the pyroglutamic acid with equal ease. Hence the concentration of $MH_{-1}L$ species is always very low (Ref.4). The stability constants are given in Table 1 together with an indication of the pH regions at which the important species predominate. The potentiometric and spectroscopic data clearly show that the final complex is 3N species with three nitrogens bound around metal ion (Ref 4, 5).

Pyr-D-His-Pro-NH_2 is identical to TRF apart from the presence of D-histidine in place of the L-enantiomer. The complex species present in solution are similar to those with TRF itself, although a small concentration of the $MH_{-1}L$ intermediate (~10 %) was detected potentiometrically. The most surprising spectroscopic feature was observed for Ni(II)TRF square planar complex when pH varied from 9 to 11. The CD spectra have shown the presence of two planar complexes which may vary in their chelate ring conformation. The conformation change may derive most likely from the deprotonation of N1 imidazole nitrogen (Ref.5). The comparison between TRF and Pyr-His clearly shows that the prolinamide moiety in TRF takes little part in the

Table 1. Formation constant of complexes with , Cu(II) and Ni(II) at 22°C and I = 0.10 mol dm^{-3} (KNO_3). (standard deviations in parentheses)

Proton complexes:		log K_1 (HL)		log K_2 (H_2L)
Pyr-His-Pro-NH_2 (TRF)		6.38 (1)		
Pyr-D-His-Pro-NH_2		6.15 (4)		
Pyr-His		7.18 (1)		2.70 (2)
Pic-His		7.72 (3)		3.05 (4)
Pic-His-Pro-NH_2		6.40 (3)		
Pyr-Tyr		10.25 (2)		3.60 (3)
Pyr-Tyr-Pro-NH_2		9.82 (1)		
Cu(II) and Ni(II) complexes:				
		Cu(II)		Ni(II)
	species	log β	pH range	log β
Pyr-His-Pro-NH_2	ML	3.85 (3)	5-6	3.17 (9)
	$MH_{-2}L$	-7.67 (2)	> 6	-14.65 (8)
Pyr-D-His-Pro-NH_2	ML	3.77 (1)	5-6	3.9 (1)
	$MH_{-1}L$	-2.5	*	
	$MH_{-2}L$	-7.93 (1)	> 6	-13.7 (1)
Pyr-His	ML	4.23 (4)	4-7.5	3.33 (7)
	$MH_{-1}L$	-2.0	*	
	$MH_{-2}L$	-8.29 (3)	> 7.5	-15.22 (7)
Pic-His	ML	9.43 (4)		7.61 (9)
	$MH_{-1}L$	5.4	*	2.2 (2)
	$M_2H_{-1}L_2$	17.7	*	
	$M_2H_{-2}L_2$	14.01 (8)	4-10	
	$MH_{-2}L$	-4.3 (1)	> 10	-4.7 (3) ?
Pic-His-Pro-NH_2	$MH_{-1}L$	4.82 (3)	3-8	0.28 (2)**
	$MH_{-2}L$	-3.66 (3)	> 8	-6.7 (1) ?
Pyr-Tyr	ML	5.0	*	
	$MH_{-1}L$	-1.6 (1)	> 7	
Pyr-Tyr-Pro-NH_2	ML	5.0	*	
	$MH_{-1}L$	-1.62 (6)	> 7	

* species only a minor component of the equilibrium mixture
** $\log \beta_{ML} = 4.24$ (9)

coordination equilibria below pH 10 and may even have a small, destabilizing effect.

Replacement of pyroglutamic acid with picolinic acid in the hormon molecule causes a major change in the structure of its complexes (Ref.4).

The peptide analogue, Pic-His, forms two dimeric species with Cu(II) which are not found in Cu(II) Pyr-His or Cu(II) TRF solutions (Table 1). Their spectroscopic features (EPR and CD spectra) are very different to those of monomeric CuL and $CuH_{-2}L$ complexes, e.g. the positive Cotton effect at 385 nm is observed only for the solutions containing dimeric species and the $n \rightarrow \pi^*$ intraligand transition at 208 nm of dimeric complex exhibits a Cotton effect opposite in sign to that found for the free peptide or monomeric species. The latter result suggests the significant changes in the conformation of the peptide molecule. The introduction of the proline amide residue into the Pic-His peptide changes the complex equilibria in solution distinctly (Table 2). There are only two species in equimolar solutions, i.e. $CuH_{-1}L$ and $CuH_{-2}L$. Neither EPR nor potentiometric data show any dimer formation at any pH. The other specific feature of picolyl analogues of TRF is the apical coordination of one of the nitrogen donors which is rather unusual for Cu(II) peptide complexes and may result from the low basicity of the picolyl nitrogen atom.

Table 2. Spectroscopic data for Pyr-Tyr-Pro-NH_2 : Ni(II) 10 : 1 solution.

pH	vis		CD						μ_{eff}, BM
			d-d		CT		intraligand transitions		
	λ,nm	ε	λ,nm	Δε	λ,nm	Δε	λ,nm	Δε	
>9	420	130	508 424	-0.06 +0.21	270sh	+1.62	240 207	+6.36	0

The introduction of tyrosine residue in the TRF sequence in place of histidine can, in some cases, lead to the direct involvement of proline amide in the binding of metal ions, e.g. Ni(II)Pyr-Tyr-Pro-NH_2 (Table 2, Ref.4). The tyrosyl analogues form much weaker complexes than the other peptides studied. It seems interesting the formation of the square planar complex in Ni(II)Pyr-Tyr-Pro-NH_2 solution probably due to the involvement of amide nitrogen as well as the pyroglutamyl and peptide nitrogen atoms into the coordination of metal ion. The latter coordination mode was not found for the Cu(II) containing solutions.

Melanostatin

MIF forms a mono-hydrogen complex (log K = 8.80) through protonation of the prolyl nitrogen. This prolyl nitrogen may also be directly involved in the metal ion binding (Ref.6). With Cu(II), MIF forms a series of 1:1 complexes as the pH is raised by the successive ionization of amide protons. The 1N complex (with only prolyl nitrogen bound to metal ion) is a minor species existing between pH 4.5 and 7 (~15 %) in the Cu(II)MIF equilibrium. The stability constants and spectroscopic data are given in Tables 3 and 4.

Complex formation between Ni(II) and MIF was found to be exceedingly slow at 25 °C. Above pH 8 a $(NiL_{-3})^-$ complex could be identified clearly both from potentiometric and spectroscopic data. It is a planar 4N complex comparable to the Cu(II) analogue (Ref.6).

Table 3. The formation constants of complexes of MIF with H^+, Cu(II) and Ni(II) at 25 °C and I=0.10 M ($K[NO_3]$). $\log\beta_{xyz}$ values for the complexes $M_xL_yH_z$. (Standard deviations are given in parentheses).

H^+		$\log\beta_{011}$ = 8.80(3)
Cu(II)	1N:	$\log\beta_{110}$ = 5.69(2)
	2N:	$\log\beta_{11-1}$ = 0.02(1)
	3N:	$\log\beta_{11-2}$ =-7.23(2)
	4N:	$\log\beta_{11-3}$ =-16.30(2)
Ni(II)	4N:	$\log\beta_{11-3}$ =-18.1(1)

Cu(II) complexes with tetrapeptides containing proline residue in position one, three and four.

The spectroscopic studies of Cu(II) complexes with four tetrapeptides: Ala$_4$, Ala-Ala-Ala-Pro, Ala-Ala-Pro-Ala and Pro-Ala-Ala-Ala have shown clearly that proline residue is a specific structural factor in the formed complexes and, on the other hand, it may act as a break point in the metal ion coordination to the consecutive peptide bond nitrogens. The only position of proline residue in a peptide sequence that makes proline nitrogen available for the metal coordination is the N-terminal position as it was found for MIF complexes see above .

The CD spectra of the studied systems in the d-d region have shown among others that the presence of proline residue in a coordinated peptide ligand

Table 4. The spectroscopic characterization of copper(II) complexes with MIF peptide.

Species	vis		CD		EPR	
	λ,nm	ε	λ,nm	Δε	$A_{\parallel}$,G	$g_{\parallel}$
4O	800	10			131	2.367
					144	2.326
2N	660	40	695	-0.12	170	2.243
			565	-0.10		
			310	+0.44		
			260sh	-1.20		
			240	-1.50		
			207	+1.80		
3N	~570	80	580	-0.23	190	2.190
			310	+0.47		
			273	-0.93		
			231	-0.71		
			207	+2.85		
4N	503	130	503	-0.36	212	2.163
			293	+0.58		
			260	-1.28		
			233sh	+0.50		
			207	+7.03		
MIF,pH 6-10			230	-0.08		
			206	+1.59		

can lead to considerable decrease of the symmetry around the metal ion. This symmetry change causes splitting of the metal E transitions and enhancement of the A transition, which is forbidden in tetragonal systems (Table 5,Ref.7).

Table 5. CD spectra of Cu(II) complexes with tetrapeptides.

	d-d transitions			
Cu(II)A_4		B + E		
2N		685 (-0.24)		
3N		577 (-0.55)		
4N		525 (-1.11)		
Cu(II)A_3P		B + E		
2N		685 (-0.32)		
3N		588 (-0.87)		
3N,pH 11.15		573 (-0.74)		
	A	B	E	
3N,pH 11.95	657 (+0.04)	~560sh (-)	498 (-0.27)	
Cu(II)A_2PA		B	E	
2N,pH 5-6		695 (-0.15)	570 (-0.14)	
2N,pH 10.73		635 (+0.07)	528 (-0.23)	
2N,pH 12.00		640 (+0.08)	526 (-0.23)	354 (-0.01)
Cu(II)PA_3		B	E	
2N		695 (-0.15)	575 (-0.22)	
		B + E		
3N		563 (-0.47)		
	A	B	E(Γa)	E(Γb)
4N,pH 10.95		545 (-0.83)	460 (+0.05)	415 (-0.01)
4N,pH 11.95	646 (+0.07)	542 (-0.80)	460 (+0.02)	415 (-0.01)

Note: The band position is given in nanometers and the $\Delta\varepsilon$ ($\varepsilon_l - \varepsilon_r$) is presented in parentheses.

Cu(II) complexes with tetrapeptides containing proline and tyrosine.

The three tetrapeptides: Gly-Pro-Gly-Tyr (GPGT), Gly-Pro-Tyr-Gly (GPTG) and Tyr-Pro-Gly-Gly (TPGG) contain proline as the second amino acid subunit to act as a break-point in metal ion complex formation. The tyrosine residue was placed as the first, third and fourth amino acid in tetrapeptide and its position was critical for the coordination equilibria and the structure of complex formed (Ref.8).
The values calculated for the copper complex formation constants are given in Table 6. In all cases the $CuHL^+$ species is a major component below pH 7. Above pH 10.5 the $CuH_{-2}L^{-2}$ complex predominates in solutions of GPTG and TPGG; with GPGT the second peptide proton is less readily ionized so that the major species at high pH is $CuH_{-1}L^-$. Both the spectroscopic and the potentiometric results suggest the formation of only three major complexes in Cu(II)GPGT solutions over the pH range 5-10.5. These are $CuHL^+$, CuL and $CuH_{-1}L^-$. The absorption, CD and EPR spectra suggest that the metal ion is bound in $CuHL^+$ species via the terminal NH_2 group and the vicinal carbonyl oxygen. This coordination mode for $CuHL^+$ species is found for all three systems studied.

Table 6. Proton and copper(II) complex formation constants at 22°C and I = 0.10 mol dm^{-3} (KNO_3) (Standard deviations are given in parentheses).

Proton complexes:

ligand (H_2L)	log K_{OH}	log $K_{NH_3^+}$	log K_{COOH}
GPGT	9.98(1)	8.43(2)	?
GPTG	10.27(1)	8.51(2)	3.43(6)
TPGG	9.76(1)	7.59(2)	3.29(6)

Copper(II) complexes:

ligand	species (charges omitted)	log β (or log K)	pH range (main species)
GPGT	$CuHL$	15.68(3)	6.1 - 8
	CuL	7.73(5)	8.0-10.1
	$CuH_{-1}L$	-2.40(5)	above 10.1
	Cu_2HL	17.8 (2)	insignificant(<5%)
	$Cu + HL \rightarrow CuHL$	5.70	
	$CuH_{-1}L + H \rightarrow CuL$	10.15	
GPTG	CuL	rejected	
	$CuHL$	15.87(3)	6.2-7.2
	$Cu_2H_{-1}L$	5.26(6)	7.2-8.8
	Cu_2L_2	19.32(9)	8.8-9.0
	$Cu_2H_{-2}L$	-3.72(4)	9.0-9.9
	$CuH_{-1}L$	-1.32(8)	9.9-10.5
	$CuH_{-2}L$	-11.83(6)	above 10.5
	$CuH_{-2}L + H \rightarrow CuH_{-1}L$	10.51	
	$Cu + HL \rightarrow CuHL$	5.60	
TPGG	$CuHL$	14.71(2)	6.1-6.8
	CuL	7.91(3)	6.8-9.0
	$CuH_{-1}L$	-1.17(2)	9.0-10.1
	$Cu H_{-2}L$	-11.32(2)	above 10.1
	$Cu_2H_{-2}L$	-4.9 (4)	insignificant(<5%)
	Cu_2L_2	17.5 (2)	insignificant(<5%)
	$Cu + HL \rightarrow CuHL$	4.95	
	$CuH_{-1}L + H \rightarrow CuL$	9.08	
	$CuH_{-2}L + H \rightarrow CuH_{-1}L$	10.15	

The most surprizing coordination mode in the Cu(II)GPGT solutions and hence the complex structure is found for CuL species. In this complex besides N-terminal (NH_2,CO) coordination the formation of Cu – O (phenolate) bonding

is observed. From the spectroscopic and the potentiometric data it is concluded, that, in the CuL complex, the tetrapeptide molecule is bent to give tridentate coordination through the glycine-NH_2 terminal, the carbonyl oxygen and the phenolate oxygen. This is an unexpected structure but a study of models shows it can be formed without undue strains.

The Cu – O (phenolate) bond formation is also found in Cu(II)GPTG solutions. In this system, however, this bonding leads to the creation of the dimeric species. The ability to form a dimeric complex, bridging through the phenolate oxygen atom was also found in the Cu(II)Tyr-Gly solutions (Ref.9). The presence of the proline break-point in the peptide chain causes also the formation of binuclear complexes $Cu_2H_{-1}L$ and $Cu_2H_{-2}L$ in Cu(II)GPTG solutions in which one metal ion coordinates with glycine N-terminal donors while the other is bound through one or two peptide nitrogens and the terminal carboxylate. In the Cu(II)TPGG solutions the metal ion migrates from N-terminal tyrosine to glycine residues when the pH increases above 9.5 but only the monomeric complexes are the major species in the solution.

The spectroscopic and potentiometric results suggest that the pairing of proline (a break-point in metal coordination) and tyrosine in a peptide chain allows the phenolate oxygen atom to take part in a specific type of interaction with Cu(II). The presence of both amino acid residues appears to give complexes with relatively low symmetry around the metal ion, as evidenced by the multicomponent CD spectra in the d-d transition region (Table 7). The range and the kind of complex species formed is strongly dependent on the position of the proline and tyrosine in the peptide chain.

Table 7. Circular dichroism data for complexes of tetrapeptides with Cu(II). Values for $\varepsilon_1 - \varepsilon_r$ given in parentheses.

ligand	pH	major species	d-d transition λ,nm	CT transition λ,nm	assignment
GPGT	7	CuHL	760 (-0.02) 730 (-0.02)		
	9	CuL	760 (-0.14)		$B_1 \rightarrow A_1$, (A)
			730sh (-0.16)		$B_1 \rightarrow A_2$, (B)
			710 (-0.17)		$B_1 \rightarrow E(\Gamma_a)$, $E(\Gamma_a)$
			680sh (-0.13)		$B_1 \rightarrow E(\Gamma_b)$, $E(\Gamma_b)$
				410 (+0.4) 290 (+0.55)	O → Cu N → Cu
	>11	$CuH_{-1}L$		330 (-0.3) 290 (+0.5)	N → Cu N → Cu
GPTG	6.5	CuHL	760 (-0.1) 685 (-0.17) 660 (-0.16)		A[a] $E(\Gamma_a)$ $E(\Gamma_b)$
	>7	various		430 (+0.7)	O → Cu
	10.5	$CuH_{-2}L$	680 (+0.16) 580 (-0.18)		B E
				420 (+0.02) 330 (-1.5) 290 (+2.0)	O → Cu N → Cu N → Cu
TPGG	6.5	CuHL	760sh (-0.15) 680sh (-0.5) 655 (-0.6) 600sh (-0.35)		A B $E(\Gamma_a)$ $E(\Gamma_b)$
	7.5	CuL		330 (+1.0)	N → Cu
	10.4	$CuH_{-2}L$	665 (+0.05) 585 (-0.06)		B E

a) B transition likely to be overlap with A and/or E.

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