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TRANSITION METAL COMPLEXES OF PEPTIDE FRAGMENTS OF PRION PROTEINS

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ABSTRACT

Prion related diseases are associated with the conversion of a normal cellular isoform of prion protein (PrP^C) into an abnormal scrapie isoform (PrP^{Sc}). The highly conserved region encompassing the amino acids 106-126 of PrP^C (KTNMKHMAGAAAAGAVVGGLG) is believed to play a key role in such a conformational change. In this study we report the results of combined pH-potentiometric, UV-Vis, CD and EPR spectroscopic studies on the copper(II), zinc(II), cadmium(II) and palladium(II) complexes of 106-114 nonapeptide (KTNMKHMAG and KTNFKHVAG) and 109-112 tetrapeptide fragments of human (HuPrP) and chicken (ChPrP) prion proteins, respectively. It was found that these peptides are very efficient ligands for copper(II) and palladium(II) ions, while only weak interactions were detected with zinc(II) and cadmium(II). Histidine residues are the major metal binding sites in all cases and the presence of methionine makes some difference between the complexation reactions of HuPrP and ChPrP. Metal ion speciation and structure of the complexes formed with copper(II) and palladium(II) are discussed in detail.

INTRODUCTION

Prion diseases are neurodegenerative disorders associated with a conformational change in the normal cellular isoform of the prion protein, PrP^C, to an abnormal scrapie isoform, PrP^{Sc} [1,2]. Unlike the α-helical PrP^C, the protease-resistant core of PrP^{Sc} is predominantly β-sheet and possesses a tendency to polymerize into amyloid fibrils [3,4]. These rod-shapes fibrils accumulate in the nervous system giving rise to protein depositions or plaques, often associated with neuronal death [5] through the triggering of the activation of the apoptotic program [6] and astrogliosis [7]. Unfortunately structural studies of the pathogenic form have been hampered by the insolubility, heterogeneity and complexity of PrP^{Sc} isoforms. Thus, synthetic peptides derived from discrete PrP regions have been extensively used to identify

protein domains that could be involved in the conformational transitions of PrP^{C} to PrP^{Sc} and in disease pathogenesis [8].

Previous studies have shown that a synthetic peptide encompassing human PrP residues 106-126 (KTNMKHMAGAAAAGAVVGGLG) exhibits some of the pathogenic and physicochemical properties of PrP^{Sc} [9]. PrP(106-126) is highly fibrillogenic and is toxic to neurons in vitro, requiring PrP^{C} expression for neurotoxicity [10,11]. Moreover, this peptide was reported to induce apoptotic death in cortical [12] and cerebellar neurons [13], showing to be toxic in vivo [14]. PrP(106-126) is highly conserved among various species and it has been suggested to represent one of the key domains where conformational changes are started leading to the conversion of PrP^{C} to PrP^{Sc}. In particular, attempts to define the toxic region of the protein have identified the palindrom region AGAAAAGA, representing the residues 113-120 of PrP(106-126) fragment, as being necessary for both fibril formation and PrP^{Sc}-like toxicity [15]. Recently, using circular dichroism analysis of PrP(106-126) combined with in vitro neurotoxicity studies [16], it was suggested that the peptide activity is tuned by the hydrophobic 113-120 residues. Due to the decrease in the β-sheet content and in cellular toxicity, bearing mutations in the amino acid residues comprised in the hydrophobic core of the PrP(106-126), it has been hypothesized that the hydrophobic sequence may similarly affect aggregation and toxicity in prion diseases [16]. Further studies on the molecular determinants responsible of the conformation polymorphism and fibrillogenic properties of PrP(106-126) brought in evidence that His(111) plays a central role in the structural features of this PrP fragment with the amidation of the C-terminus driving the peptide to a predominant random coil conformation and decreasing the tendency of PrP fragment to generate amyloid fibrils [17]. The role of this His(111) has been proved again in a paper describing the copper (II) and zinc (II)-assisted fibrilization of PrP(106-126), inhibited in the His(111)S mutant [18]. These results gave further evidence on the role of copper(II) in the neurotoxicity of PrP(106-126).

The previous studies on the metal complexes of peptide fragments were focused on the complexation with copper(II) and other metal ions were only scarcely studied. The aim of this paper is to characterize the binding of copper(II), zinc(II), cadmium(II) and palladium(II) to PrP(106-126) with both termini protected, also by using its blocked polar fragment PrP (106-114). In addition, to identify key residues of metal coordination, the complexing features of blocked PrP(106-114) of the avian prion protein have been investigated. This fragment does not comprise Met residues. To complete the picture of the binding location sites the tetrapeptides covering the amino acid residues in positions 109-112 of both human and

chicken prion proteins have also been synthesized and their complexing properties investigated.

EXPERIMENTAL PART

Reagents and solutions

The peptides were synthesized at the University of Catania (Italy) according to standard procedures described elsewhere [19]. The purity of the ligands was checked by NMR measurements and their concentrations were determined by potentiometric titrations. Metal ion stock solutions were prepared from the corresponding chloride salts and their concentration checked gravimetrically via oxinates.

Apparatus and equipment

Potentiometric measurements

The pH-potentiometric titrations were performed in 3-5 cm^3 samples in the concentration range $2\times10^{-3} - 4\times10^{-3}$ mol dm^{-3} with the metal ion to ligand ratios between 1:2 and 2:1. The peptide HuPrP(106-126) with the scrambled sequence (Ac-NGAKALMGGHGATKVMVGAAA-NH_2) was well soluble under acidic conditions, but precipitation started around pH 7 following the deprotonation of the imidazolium group. Thus, pK values of this ligand were obtained in diluted samples (10^{-4} mol dm^{-3}) and standard deviation of the equlibrium data is significantly higher than those of the other peptides. Copper(II) complexes of the scrambled peptide were soluble at any pH values and the milimolar concentration range was used in all measurements. During the titration argon was bubbled through the samples to ensure the absence of oxygen and carbon dioxide and also to stir the solutions. All pH-potentiometric measurements were carried out at 298 K and at a constant ionic strength of 0.2 M KCl with a Radiometer pHM84 pH-meter equipped with a 6.0234.100 combination glass electrode (Metrohm) and a Dosimat 715 automatic burette (Metrohm) containing carbonate-free potassium hydroxide in known concentration. The pH readings were converted into hydrogen ion concentration and protonation constants of the ligands and the overall stability constants (log β_{pqr}) of the systems were calculated by means of a general computational program, PSEQUAD [20], using eqn. (1) and (2).

$$pM + qH + rL = M_pH_qL_r \qquad (1)$$

$$\beta_{pqr} = \frac{[M_pH_qL_r]}{[M]^p \cdot [H]^q \cdot [L]^r} \qquad (2)$$

Spectroscopic measurements

UV-Vis spectra of the copper(II) complexes were recorded on a Hewlett Packard HP 8453 diode array spectrophotometer in the same concentration range as used for pH-potentiometry. CD spectra of copper(II) complexes were recorded on a JASCO J-810 spectropolarimeter using 1 or 10 mm cells in the 200-800 nm range in the same concentration range as used for potentiometry. CD spectra of the individual species were calculated by the same general program (PSEQUAD) as used for the evaluation of potentiometric measurements.

Frozen solution EPR spectra were recorded on a Bruker ER 200D spectrometer equipped with the 3220 data system at 150 K. Copper(II) complex solutions (1 mM) were prepared in situ by mixing the necessary volume of a standard solution of $^{63}Cu(NO_3)_2$ with solutions of the peptide ligands in a 1:1.1 metal to ligand ratio, and adjusting the pH of the resulting solution to the desired value by adding 10 mM KOH or HNO_3. Methanol, circa 10%, was added to the aqueous copper(II) complex solutions to increase resolution. ^1H NMR spectra of the free ligands and palladium(II) complexes were taken in D_2O on a Bruker AM360 FT-NMR spectrometer using tetramethylammonium tetrafluoroborate (3.18 ppm) as internal reference.

RESULTS AND DISCUSSION

Protonation constants of the ligands and stability constants of the copper(II) complexes of the nonapeptide fragments of human and chicken prion proteins and HuPrP(106-126, scrambled) have been determined by potentiometric titrations and the equilibrium data are collected in Table 1. It can be seen from Table 1 that both the nonapeptides HuPrP(106-114), ChPrP(106-114), and the twenty one-mer peptide, HuPrP(106-126, scrambled) have three protonation sites; the imidazole nitrogen atom of His(111) and the ε-amino groups of Lys(106) and Lys(110). Protonation constants of the imidazole nitrogen atoms of the ligands are very similar to each other and the values are in good agreement with other histidine-containing fragments of prion proteins [21-23]. Deprotonation of the lysyl ammonium groups takes place in the pH range 9 to 11 and the values corresponds well to other peptides of lysine [24]. Potentiometric titration curves of the copper(II)-peptide systems have been recorded at three different metal ion to ligand ratios: 1:1, 2:1 and 1:2. The number of base equivalents of titration curves and the computer evaluation of the data, however, indicated that only 1:1 complexes are formed at any metal ion to ligand ratios. The metal ion speciation of the three systems are very similar to each other and it is represented by Figure 1 for the copper(II)-ChPrP(106-114) system.

It is clear from Figure 1 and Table 1 that complex formation starts around pH 4 in all systems with the formation of the doubly protonated species $[CuH_2L]^{4+}$. Taking into account the number of protonation sites of the ligands and the spectral parameters in Table 2 the monodentate coordination of the His(imidazole) residue can be expected in this species. Any CD activity can not be observed below pH 5.5 providing further support that imidazole-N donor atoms are the primary binding sites of the ligands. The stability constants of the species $[CuHL]^{3+}$ can be calculated only in the copper(II)-HuPrP(106-114) system, but the concentration of the complex is rather low even in this case. As a consequence, the formation of this species largely overlaps with $[CuL]^{2+}$ and $[CuH_2L]^{4+}$ and spectral parameters can not be obtained for the species $[CuHL]^{3+}$. The pK_{-1} values in Table 2 correspond well to those of a 2N complex suggesting the metal ion coordination of the imidazole and one amide nitrogen donor atoms in $[CuHL]^{3+}$(= $[Cu(H_{-1}L)H_2]^{3+}$).

Table 1: Equilibrium data of the copper(II) complexes of peptide fragments of prion proteins. I = 0.2 mol dm^{-3} KCl, T = 298 K

Species	ChPrp(106-114)	HuPrP(106-114)	HuPrP(106-126, scrambled)
pK(His)	6.22(1)	6.24(1)	6.43(5)
$pK(Lys_1)$	9.89(1)	9.91(1)	9.61(15)
$pK(Lys_2)$	10.62(2)	10.54(2)	10.24(20)
$[CuH_2L]$	24.19(2)	23.54(3)	23.52(5)
[CuHL]	–	17.61(9)	–
[CuL]	12.39(2)	12.39(2)	11.75(3)
$[CuH_{-1}L]$	5.34(1)	4.56(1)	4.40(3)
$[CuH_{-2}L]$	–4.65(2)	–5.45(2)	-5.13(4)
$[CuH_{-3}L]$	–15.25(3)	–16.11(3)	-15.51(5)
pK_{-1}	–	5.93	–
pK_{-2}	–	5.22	–
pK_{-12}	5.90	5.58	5.89
pK_{-3}	7.05	7.83	7.35
$pK(Cu\text{-}Lys_1)$	9.99	10.01	9.53
$pK(Cu\text{-}Lys_2)$	10.60	10.66	10.38

In the case of ChPrP(106-114) and the scrambled peptide the 2N complex can not be identified at all and the formation of the species $[CuL]^{2+}$ from $[CuH_2L]^{4+}$ takes place in one step, in a cooperative manner. Thus pK_n values of successive deprotonation of amide functions can not be calculated for these ligands, only an average of the first two deprotonation (pK_{-12}) can be given. The unusual trend of pK_{-1} and pK_{-2} values, however, reveals that the deprotonation of first two amide functions is cooperative for all ligands. This reaction is accompanied with a significant blue shift of the absorption spectra supporting the deprotonation and coordination of two amide functions; most probably those from His(111) and Lys(110). The CT bands, in the CD spectra, due to $N_{im} - Cu^{2+}$ (λ_{max}= 240-260 nm) and $N^- - Cu^{2+}$ (λ_{max}=330-350) contribute to conclude that $[CuL]^{2+}$ is a 3N complex with [N_{im}, 2xN(amide)$^-$] binding sites, while the lysyl ε-ammonium groups are still protonated. In fact, the stoichiometry of the species is $[CuL] = [Cu(H_{-2}L)H_2]^{2+}$. The g_{II} and A_{II} values (Table 2) are similar to those reported for similar species of copper(II) complex with prion octarepeat [21]. These observations support previous findings that the coordination of imidazole nitrogen atoms promotes the deprotonation and coordination of amide functions [25]. Of course, we can not distinguish between the deprotonation reactions on the N- or C-terminal sides of His residues. In the case of the octarepeat segments of prion proteins the deprotonation took place towards the C-termini in the form of a 7-membered chelate and it was explained by the presence of proline residue [21,22]. The nonapeptides PrP(106-114), however, do not contain proline, thus the formation of the 6-membered chelate towards the N-terminus is more probable. It is obvious from the Tables that both equilibrium and spectral parameters of Cu(II)-HuPrP and Cu(II)-ChPrP systems are very similar, but a slight difference between the CD spectra can be observed in the pH range 6 to 8. Circular dichroism spectra of the systems have been recorded as a function of pH and the individual spectra of each species (see Figure 2) were calculated by means of the general computational program, PSEQUAD.

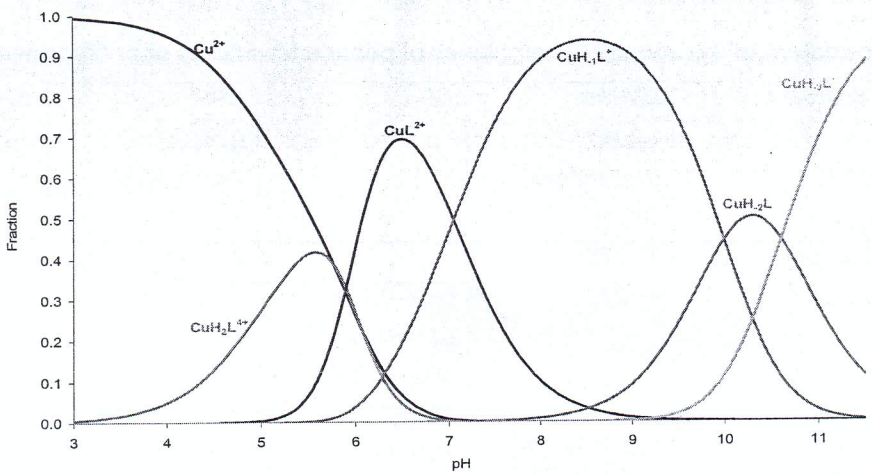


Fig. 1. Species distribution of the complexes formed in the copper(II) – ChPrP(106-114) system at 1:1 metal ion to ligand ratio (c = 2 mM)

In the case of Cu(II)-HuPrP(106-114) system a weak positive Cotton effect was observed at λ = 380 nm and the intensity of this band varied parallel with the concentration of the species $[CuL]^{2+}$. The charge transfer transitions from the copper(II)-thioether interactions are generally appear in the same range of UV-Vis spectra [26-28], thus a weak equatorial binding of the thioether donor atom of Met(109) residue can be suggested in the species $[CuL]^{2+}$ as shown by Scheme 1.

Previous studies on the copper(II) complexes of the neurotoxic peptide PrP(106-126) came to different conclusions on the binding ability of thioether residues. Met(109) and/or Met(112) residues were suggested as important bridging ligands in the corresponding copper(II) complexes [18], while neither methionyl thioether nor lysyl amino groups were suggested as metal binding sites in a more recent study on the terminally free peptides [29].

Table 2:

Spectroscopic parameters of the copper(II) complexes of peptide fragments of prion proteins

Ligand	Species	UV-Vis $\lambda_{max}(\varepsilon)$ [nm(dm^3mol^{-1} cm^{-1})]	CD	EPR $g_{\|\|}/A_{\|\|}$ (10^{-4} cm^{-1})
ChPrP(106-114)	$[CuH_2L]$	772(28)	–	2.366/135
	[CuL]	610(72)	615(–0.19) 525(+0.42) 350(–0.77) 248(+7.21) 224(-7.69)	2.231/169
	$[CuH_{-1}L]$	538(117)	645(+1.27) 500(–1.35) 325(+0.97) 293(-0.75) 260(+7.14) 224(+25.4)	2.201/194
	$[CuH_{-2}L]$			
	$[CuH_{-3}L]$			
HuPrP(106-114)	$[CuH_2L]$	760(28)	–	2.366/133
	[CuHL]	–	–	2.272/173
	[CuL]	616(77)	760(–0.22) 535(+0.39) 385(+0.11) 330(–0.61) 249(+8.29)	2.220/174
	$[CuH_{-1}L]$	532(108)	631(+1.05) 495(–1.22) 317(+1.27) 292(+0.16) 256(+7.82)	2.198/198
	$[CuH_{-2}L]$			
	$[CuH_{-3}L]$			
HuPrP(106-126, scrambled)	$[CuH_2L]$	760(28)	–	2.358/141
	[CuHL]	–	–	–
	[CuL]	610(79)	328(+0.68) 225(-9.20)	2.232/173
	$[CuH_{-1}L]$	550(125)	600(-0.32) 505(+1.05) 305(-0.75) 247(+1.00) 229(-1.40)	2.201/195
	$[CuH_{-2}L]$			
	$[CuH_{-3}L]$			

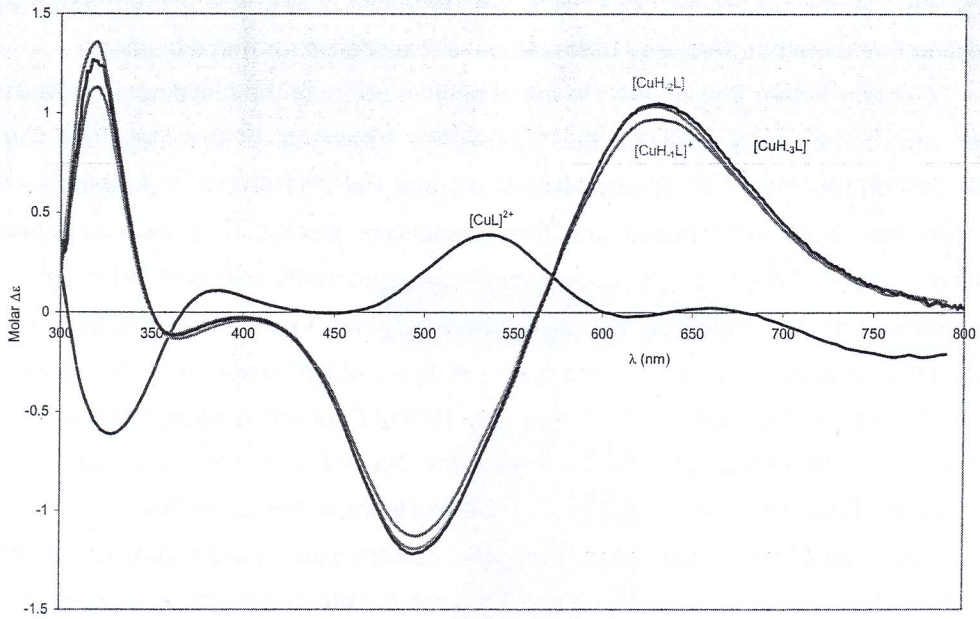
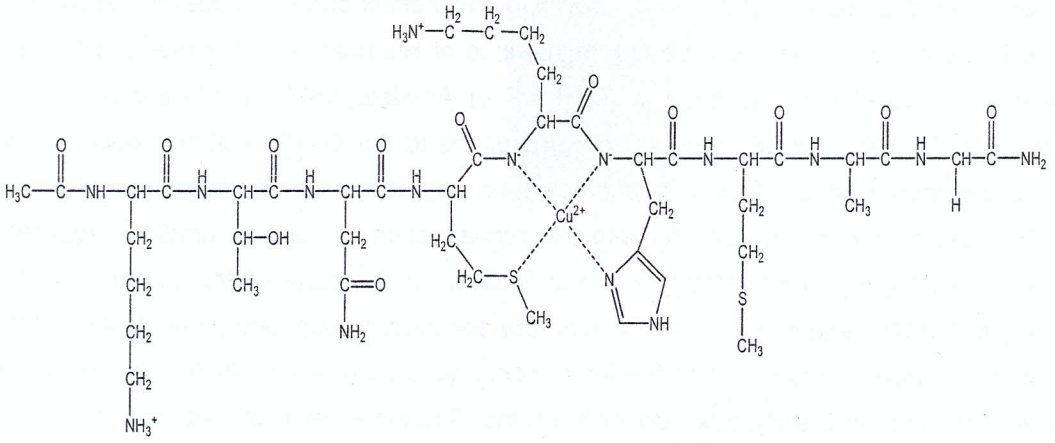


Fig. 2. Molar CD spectra of the species formed in the copper(II) – HuPrP(106-114) system. The spectra are calculated using the computational program PSEQUAD.



Scheme 1. $[CuL]^{2+} = [Cu(H_{-2}L)H_2]^{2+}$

Now, our studies reveal that thioether binding occurs in a pH-dependent manner. Namely, the thioether residues can occupy the fourth coordination site of the metal ion in the 3N-coordinated complex. It is, however, a weak interaction and not able to prevent the deprotonation and metal ion coordination of the subsequent amide residues. The spectroscopic data also reveal that the metal ion coordination of the thioether donor function

takes place only in the species $[CuL]^{2+}$ (the "3N complex") in a narrow pH range (6 < pH < 8) suggesting that thioether from Met(109) residue is responsible for metal binding.

To obtain further insight into the metal binding ability of the histidine(111) residues of chicken and human prion proteins the tetrapeptide fragments of the major binding core, namely ChPrP(109-112) = Ac-PheLysHisVal-NH_2 and HuPrP(109-112) = Ac-MetLysHisMet-NH_2 have also been synthesized and their complexes studied. The data obtained from potentiometric and UV-Vis and CD spectroscopic measurements are collected in Table 3. For the comparison of the stability constants included in Tables 1 and 3 it should be considered that the tetrapeptides contain only one lysyl residues, which results in a difference in the stoichiometry of the corresponding species (e.g. $[CuH_2L]^{4+}$ of the nonapeptides corresponds to $[CuHL]^{3+}$ with the tetrapeptides). Otherwise, the speciation of the nonapeptides is very similar to that of the tetrapeptides suggesting the existence of the same binding modes in the corresponding complexes. Both equilibrium and spectroscopic parameters of the species $[CuHL]^{3+}$ of tetrapeptides suggest that it is a 1N complex with monodentate binding of histidyl residue, while the ε-ammonium group of lysine remains protonated at low and medium pH values. The spectroscopic parameters of the species $[CuH_{-1}L]^{+}$ correspond to those of 3N complexes suggesting the cooperative deprotonation of two amide functions and the formation of 3N complexes. The pH range for the formation of the 3N complexes of nona- and tetra-peptides are almost the same providing and indirect proof that deprotonation of the amide functions takes place on the N-terminal side of His residues. It is also important to note that CD spectra of the species $[CuH_{-1}L]^{+}$ of Ac-MetLysHisMet-NH_2 exhibit a weak charge transfer band at 380 nm, which corresponds to the Cu–S(thioether) coordination. The development of this CT band is characteristic only of the peptide fragments of HuPrP and its intensity is changing parallel with the concentration of the 3N complex suggesting again the involvement of Met(109) in metal binding. In the case of the tetrapeptide the intensity of the CT band is higher than that for the nonapeptide supporting that binding of the thioether is a weak interaction and the fourth coordination site of the 3N complexes is only partially occupied by the thioether donor functions. The increase in the intensity of the CT band suggests that the ratio of the closed and open forms is higher for the tetrapeptide in agreement with the smaller size of the molecule.

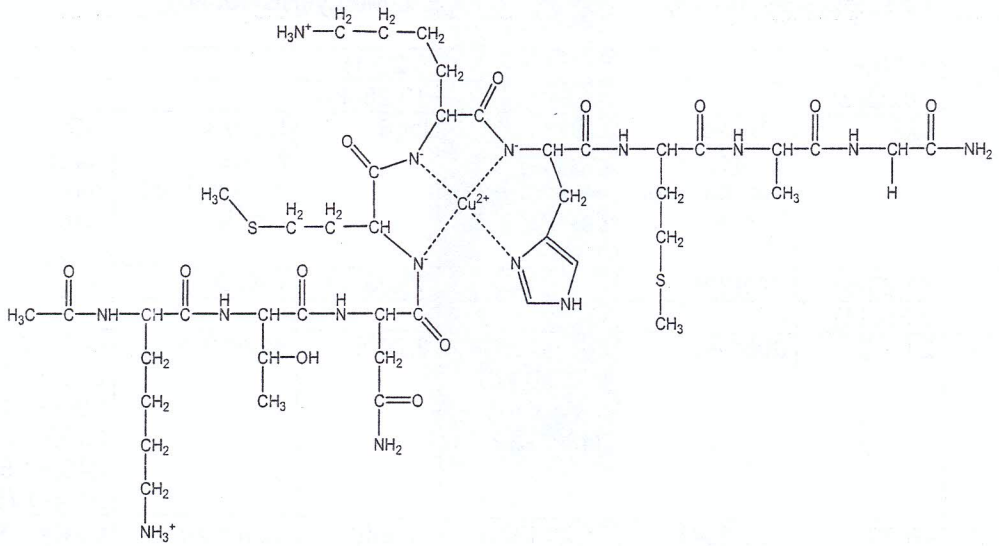
Further increase of pH results in another base consuming process with the concomitant changes of all spectral parameters in the case of all five ligands. The spectral changes can be best interpreted by the deprotonation and coordination of the third amide residues (Met(109) and Phe(109) for HuPrP and ChPrP, respectively) resulting in the formation of 4N complexes as shown by Scheme 2.

The amino groups are still protonated in these species indicating that the real stoichiometry of the species are $[Cu(H_{-3}L)H_2]^+$ and $[Cu(H_{-3}L)H]$ for the nonapeptides and tetrapeptides, respectively. Deprotonation of the lysyl side chains takes place in the same pH range as for the free ligands (pH > 9). These deprotonation reactions are, however, not accompanied with any spectral changes supporting that the Lys(106) and Lys(110) residues are not metal binding sites at any pH values. The equilibrium data provide further support for this conclusion because the protonation constants of the complexes and the free ligands (see pK(Cu-Lys) and pK(Lys) values in Tables 1 and 3) are almost the same in all cases.

Table 3. Stability constants and spectroscopic parameters of the copper(II) complexes of the tetrapeptide fragments of human and chicken prion proteins.

Species	ChPrP(109-112) Ac-PheLysHisVal-NH_2			HuPrP(109-112) Ac-MetLysHisMet-NH_2		
pK(His)	6.29(3)			6.22(1)		
pK(Lys)	10.28(2)			10.28(1)		
	logβ	UV-Vis $\lambda_{max}(\varepsilon)$ [nm($dm^3mol^{-1}cm^{-1}$)]	CD $\lambda_{max}(\Delta\varepsilon)$ [nm($dm^3mol^{-1}cm^{-1}$)]	logβ	UV-Vis $\lambda_{max}(\varepsilon)$ [nm($dm^3mol^{-1}cm^{-1}$)]	CD $\lambda_{max}(\Delta\varepsilon)$ [nm($dm^3mol^{-1}cm^{-1}$)]
[CuHL]	13.88(6)	770(30)	–	13.98(11)	760(30)	–
[CuL]	7.38(11)	–	–	–	–	–
$[CuH_{-1}L]$	2.02(3)	606(98)	605(-0.35) 510(+0.40) 345(-1.31) 243(+12.3)	2.70(5)	622(123)	660(+0.15) 530(+0.33) 380(+0.16) 325(-1.27) 248(+7.56) 218(-0.75)
$[CuH_{-2}L]$	–6.50(4)	528(134)	650(+1.00) 500(-1.33) 360(-0.17) 320(+1.10) 290(-0.10) 260(+4.49) 220(+23.9)	–6.26(9)	524(125)	654(+0.36) 488(-0.69) 354(-0.21) 318(+0.49) 250(+5.60) 219(-6.23)
$[CuH_{-3}L]$	–16.69(4)	525(142)	655(+1.27) 503(-1.84) 355(-0.16) 320(+0.65) 290(-0.86) 260(+4.82) 222(+29.9)	–16.32(9)	521(127)	652(+0.92) 494(-1.56) 354(-0.17) 316(+0.74) 289(-0.81) 256(+4.80) 229(-2.93)
$pK_{-1\,2}$	5.93			5.64		
pK_{-3}	8.52			8.96		
pK(Lys)	10.19			10.06		

The only difference in the complex formation processes of the nonapeptides and tetrapeptides is reflected in the deprotonation of the third amide functions (pK_{-3} values) of peptide molecules. In the case of tetrapeptides the metal ion coordination of the third amide functions takes place at least one log unit higher pH values than with the nonapeptides. It should be considered, however, that in the case of tetrapeptides the third amide nitrogen belongs to the acetamido group, which is much less acidic than the common peptide amide groups. So, the high pK_{-3} values of tetrapeptides provide an additional proof for the assumption that the deprotonation of the amide functions goes towards the N-termini from the internal histidyl residues. On the other hand, it is also clear from the data that the peptide fragments of HuPrP always have higher pK_{-3} values than those of chicken fragments. It is probably the effect of the weak thioether coordination, which is not able to prevent, but slightly suppresses the binding of the third amide residues.



Scheme 2. $[CuH_{-1}L]^+ = [Cu(H_{-3}L)H_2]^+$

The visible absorption bands of the 3N and 4N complexes are rather unusual. In the case of the 3N complexes the absorption maxima are at 610 and 616 nm which correspond to lower energies than those reported for the common 3N complexes. On the other hand, the 4N complexes have wide absorption bands with well-defined shoulders on the low energy side. All of these parameters support significant distortion of the coordination geometry of copper(II) in these peptide complexes.

Palladium(II), zinc(II) and cadmium(II) complexes of the tetrapeptides have also been studied by potentiometric measurements. In the case of palladium(II) both titration curves and NMR measurements clearly indicate the metal ligand interaction in very acidic solutions.

All free ligands are titrated by pH 3 with the consumption of two extra bases suggesting the formation of $[PdH_{-2}L]$ as the major species in acidic solution. A third equivalent of base is titrated around pH 7.5 which corresponds to the deprotonation and coordination of the third amide functions of the tetrapeptides.

NMR measurements reveal significant differences in complex formation processes of HuPrP(109-112) and ChPrP(109-112) with palladium(II), especially in acidic solutions. It can be best explained by the metal ion coordination of the thioether residues at low pH values. The increase of pH, however, results in the increase of the number of coordinated nitrogen donors and $[PdH_{-3}L]$ complexes with 4N-coordination are present in alkaline solutions of both tetrapeptides. On the contrary, the potentiometric titrations curves of zinc(II) and cadmium(II)-tetrapeptide systems indicate only a weak interaction between the metal ions and ligands. Log K = 2.75 and 2.44 can be calculated for the reactions M + HL, where HL stands for HuPrP(109-112) with protonated lysyl side chain and M is zinc(II) and cadmium(II), respectively. These stability constants correspond well to those of monodentate imidazole coordination, which results in about 20 % complexation in the milimolar concentration range and it is not able to prevent the hydrolysis of metal ions at higher pH values.

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