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A voltammetric study of the binding of copper(II) to peptide fragments of prion

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ABSTRACT

Binding of copper to three peptide fragments of prion (Cu^{2+} binding sites: 60–91, 92–96 and 180–193 amino acid residues) was investigated by anodic stripping voltammetry to determine the stoichiometries of Cu^{2+}-prion peptide interactions. The method relies on the synthesis of N-terminally acetylated/C-terminally amidated peptide fragments of prion by solid-phase synthesis and direct monitoring of the oxidation current of copper in the absence and presence of each prion fragment. Titration curves of Cu^{2+} with Ac-PHGGGWGQ-NH_2, Ac-GGGTH-NH_2 and Ac-VNITKQHTVTTTT-NH_2 were obtained in concentrations ranging from 8.52×10^{-7} to 5.08×10^{-6}, 3.95×10^{-7} to 1.94×10^{-6} and 7.82×10^{-8} to 4.51×10^{-7} M, respectively. The acquired data were used to calculate the stoichiometries (one peptide per Cu^{2+} ion for all the three studied systems) and apparent dissociation constants ($K_d = 4.37 \times 10^{-8}$–$3.50 \times 10^{-10}$ M) for the three complexes.

1. Introduction

Metal ions are essential nutrients that play critical roles in a variety of biochemical processes but when over accumulated they can have toxic effects and result in an array of cellular disturbances characterized by oxidative stress, which in turn can trigger complex phenomena such as a neurodegenerative cascade [1–3]. The mechanism by which metal ions are pathogenic may be related to their pro-oxidant, free-radical generating action and to their association with metalloenzymes such as superoxide dismutases (SODs) [4]. Transition metal ions such as copper, iron or manganese are known to be involved in the oxidative and inflammatory events occurring in aging and in neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis and transmissible spongiform encephalopathies (TSEs) [4–5]. TSEs [6] result from the build-up of PrP^{sc}, which is a conformational variant of the normal cellular prion (PrP^{c}), in the brain and nervous system. It still remains unclear how PrP^{c} is converted into PrP^{sc} and what is the physiological function of the normal cellular prion. It has been suggested that PrP^{c} binds to Cu^{2+} ions and might act as a regulator of the concentration of intracellular copper and reactive oxygen species formed by reactions of this metal ion [4,7–11]. Moreover, copper coordination affects the structure of prion [12] and induces the transition from the native to the pathological isoform [6,13]. PrP^{c} is a 33–35 kDa glycosylphosphatidylinositol-anchored protein that is expressed in cells of the central nervous system. The amino terminus of PrP^{c} is highly flexible and contains a series of octapeptide repeats having the following consensus sequence: PHGGGWGQ (residues 60–91). This region is well conserved across species and has been shown to bind selectively to copper [5,7,14–17] with affinities ranging from 10^{-6} to 10^{-15} M for different peptides and proteins [7,17–23]. With regard to stoichiometry, the number of Cu(II) ion binding sites is reported to be between one and five, being also pH-dependent [7,15–16,23]. At pH 7.4, Cu^{2+} interacts with the octarepeat domain through three different modes (components 1, 2 and 3), depending upon the precise molar ratio of Cu^{2+} to protein [22–24]. Component 1 arises at full copper occupancy (4 M of Cu^{2+}) and involves one copper metal ion per octarepeat domain with dissociation constants ranging from 7.0 to 12×10^{-6} M [24]. The binding site of this component is composed by one nitrogen atom of the histidine imidazole, two deprotonated backbone nitrogens, an amide carbonyl, a tryptophan residue and a water molecule. Component 3 arises at the lowest copper occupancy (1 M of Cu^{2+}) and involves four octarepeats binding to a single Cu^{2+} metal ion through the four histidine imidazoles with dissociation constants ranging from 10^{-9} to 10^{-10} M. Component 2 arises at an intermediate copper occupancy (2 M of Cu^{2+}) with dissociation constants around 10^{-9} M. In this case, the coordination of copper is similar to that of component 1, but with an additional axially coordinated histidine. Quantification studies at physiological pH reveal a second copper-binding site (GGGTH) in the flexible region located after the octarepeats and before the PrP^{c} globular C-terminal domain (residues 92–96)

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[25–26]. Wells et al. [22] recently demonstrated that at pH 7.4, Cu^{2+} also interacts with PrP^{91-115} through two different modes (so-called components A and B). Component A arises at maximum copper occupancy (2 M of Cu^{2+}) and comprises two Cu^{2+}-binding sites with dissociation constants ranging from 1.0 to 2.0×10^{-7} M, each involving coordination by a single histidine imidazole, from either His96 or His111, and nearby backbone amide groups. Component B arises at low copper concentration (1 M equivalent of Cu^{2+}) and involves two peptide molecules binding to one Cu^{2+} metal ion. One imidazole from segment 1 ($K_d \sim 100$ nM) and two imidazoles from segment 2 coordinate the cooper ion. Recently, a third Cu^{2+}-binding site (VNITKQHTVTTTT) was identified in PrP^c second helical region (residues 180–193) [27] and the histidine residue 187 was identified as part of the binding site of Cu^{2+} metal ion.

Although many analytical techniques have been used to study the interaction between copper and prion (EPR, NMR, mass spectrometry, circular dichroism spectroscopy, Raman spectroscopy), questions concerning the stoichiometry, the specificity and the affinity of Cu^{2+} by prion peptide derivatives and also the role of copper in the aggregation process are still in debate. The electrochemical methods have been reported to have several advantages in determining the quantitative parameters for metal ion–macromolecule (DNA, proteins) complexes by studying the voltammetry of the metal ion in absence and presence of the macromolecule and recording shifts in standard potential caused by the interaction [28–34]. These advantages include determination of qualitative and quantitative data (stoichiometry, binding constants and metal ion binding sites) of metal ion–macromolecule complexes near physiological conditions.

In this paper, we report the formation of complexes between copper and three synthetic peptide fragments of prion (Ac-VNITKQHTVTTTT-NH_2; Ac-GGGTH-NH_2; Ac-PHGGGWGQ-NH_2) using anodic stripping voltammetry. Copper amperometric titrations were performed to determine the stoichiometry and apparent dissociation constant of Cu^{2+}-peptide fragments of prion complexes. This study was motivated by the idea that useful information concerning the aggregation process induced by copper ions in neurodegenerative disorders can be obtained by the investigation of their complexes with prion peptides.

2. Experimental

N-terminally acetylated and C-terminally amidated peptide fragments of prion (Ac-VNITKQHTVTTTT-NH_2; Ac-GGGTH-NH_2; Ac-PHGGGWGQ-NH_2) were manually synthesized by Fmoc (9-Fluorenylmethoxycarbonyl) solid-phase synthesis [35]. Couplings were performed for 1–2 h in *N,N*-dimethylformamide by using *N,N′*-diisopropylcarbodiimide/1-hydroxybenzotriazole (DIC/HOBt) as coupling additives. Final deprotection and cleavage from the resin were conducted by trifluoroacetic acid (TFA) in the presence of thioanisole, triisopropylsilane and 1,2-ethanodithiol.

After cleavage from the resin, the crude peptides were purified by reversed-phase high-performance liquid chromatography using a Shimadzu liquid chromatography system with a C_{18} preparative column (22 × 250 mm, 10 μm, Vydac) followed by a C_{18} analytical column (4.6 × 250 mm, 5 μm, Vydac). Gradients of 0.1% TFA in acetonitrile over 0.1% aqueous TFA, flow rates of 5.0 and 1.0 mL/min (for the preparative and analytical columns, respectively) and detection at 280 and 216 nm were employed. The resulting chromatographic fractions of Ac-VNITKQHTVTTTT-NH_2 were analyzed by electrospray ionization tandem mass spectrometry (ESI-MS/MS) using the Q-TOF ultima mass spectrometer (Micromass) at the following settings: 2.8 kV capillary voltage, 40 V cone voltage, 30 eV collision energy and 25 °C desolvation temperature. The resulting chromatographic fractions of Ac-GGGTH-NH_2 and Ac-PHGGGWGQ-NH_2 were analyzed by matrix-assisted laser desorption ionization tandem mass spectrometry (MALDI-MS/MS) using the Ultraflex II mass spectrometer (Bruker) at the following settings: operation on reflector mode, 13.8 kV accelerating voltage, N_2 laser, 1.2×10^{-7} mBar pressure at the ion source, 5.0×10^{-7} mBar pressure at the detector, α-cyano-4-hydroxycinnamic acid as matrix. The fractions containing pure peptides were pooled and lyophilized.

Prior to voltammetric measurements, the concentration of the peptide fragments was determined by UV measurements at 280 nm through the Beer–Lambert Law by using Edelhoch method [36–37] (for Ac-PHGGGWGQ-NH_2) or at 205, 215 and 225 nm through Murphy and Kies method [38] (for Ac-VNITKQHTVTTTT-NH_2; Ac-GGGTH-NH_2) by using the UV–Vis 911-A spectrophotometer (GBC).

The amperometric titration of Cu^{2+} with peptide fragments by anodic stripping voltammetry (ASV) were carried out on a 646 Metrohm Voltammetric Analyzer Processor connected to a 647 Metrohm electrochemical cell composed of a hanging mercury electrode (working electrode), Ag/AgCl (3.0 M KCl) electrode (reference electrode) and a platinum electrode (auxiliary electrode). All ASV measurements were performed in the potential range of −0.30 V (initial potential, E_i) to 0.20 V (final potential, E_f) at the following settings: $E_d = -0.3$ V accumulation potential, $t_d = 90$ s accumulation time, $t_e = 20$ s equilibration time, 0.4 mm^2 surface area of the mercury drop, 50 mV pulse amplitude and $v = 10$ mV s^{-1} scan rate. Additions of 165 μL of 9.56×10^{-6} M Ac-PHGGGWGQ-NH_2, 90 μL of 8.83×10^{-5} M Ac-GGGTH-NH_2 or 20 μL of 8.53×10^{-4} M Ac-VNITKQHTVTTTT-NH_2 to the electrochemical cell containing 2.00×10^{-7} M Cu^{2+}, 1.00×10^{-6} M Cu^{2+} or 2.0×10^{-6} M Cu^{2+} in 20 mL of 0.1 M KNO_3 were done. To avoid measurement interference due to peptide fragments and copper adsorption on the working surfaces of the electrode system, electrodes were submitted to periodic cleaning with 20% HNO_3 (by volume) followed by a generous wash with triple-distilled water. Experiments were performed at 23 °C and pH 7.4 and preceded by a gentle N_2 bubbling to prevent oxygen diffusion into the electrochemical cell (10 min for the 0.1 M KNO_3 supporting electrolyte and 100 s after each peptide fragment addition).

All chemicals were of reagent grade and all solutions were prepared with triple-distilled water from quartz still (quartex).

Two methods were used to determine the dissociation constant of Cu^{2+}-peptide complexes: the mass action law [39] and Saroff and Mark [40] approaches.

3. Results

Three main compounds were observed in the RP-HPLC chromatogram of the crude Ac-VNITKQHTVTTTT-NH_2 peptide. ESI-MS and ESI-MS/MS experiments confirmed that the major component ($t_r = 29.23$ min) corresponds to Ac-VNITKQHTVTTTT-NH_2. The peptide was obtained with purity higher than 98% by two-steps (preparative followed by analytical) reversed-phase purification. Its sequence was confirmed by ESI-MS–MS. Similar chromatographic and mass spectrometric results were observed for the two other prion synthetic peptides (Ac-PHGGGWGQ-NH_2 and Ac-GGGTH-NH_2). The two peptides were obtained with purity higher than 98% and their identities were verified by MALDI-MS–MS. The sequences and $[M+H]^+$ ions are presented in Table 1.

Ac-VNITKQHTVTTTT-NH_2, Ac-PHGGGWGQ-NH_2 and Ac-GGGTH-NH_2 peptides were then submitted to voltammetric studies to evaluate their ability of copper-binding. The method relies on the direct monitoring of the oxidation current of copper in the absence and presence of each prion fragment. The interaction of

Table 1
$[M+H]^+$ ions and sequences of peptide fragments of prion.

$[M+H]^+$ ions	Sequences
1484.80	Ac-VNITKQHTVTTTT-NH_2
469.10	Ac-GGGTH-NH_2
836.35	Ac-PHGGGWGQ-NH_2

Cu^{2+} with Ac-VNITKQHTVTTTT-NH_2, Ac-PHGGGWGQ-NH_2 and Ac-GGGTH-NH_2 peptides was attested by the decrease in the copper oxidation current after incremental additions of each prion fragment (Figs. 1–3). A complete decrease in the oxidation current of copper was found after the addition of 39.5 μL of 8.53×10^{-4} M Ac-VNITKQHTVTTTT-NH_2, 172.5 μL of 8.83×10^{-5} M Ac-GGGTH-NH_2 and 481.2 μL of 9.56×10^{-6} M Ac-PHGGGWGQ-NH_2 to the electrochemical cell. These amounts of Ac-VNITKQHTVTTTT-NH_2, Ac-PHGGGWGQ-NH_2 and Ac-GGGTH-NH_2 used in the copper complexation were determined through the inflection point (I.P.) of the titration curves and they were employed to calculate the stoichiometries of the reactions. By this method, the stoichiometric ratios were found to be one Ac-VNITKQHTVTTTT-NH_2 molecule per Cu^{2+} metal ion, one Ac-GGGTH-NH_2 peptide per Cu^{2+} metal ion and one Ac-PHGGGWGQ-NH_2 molecule per Cu^{2+} metal ion, thus showing strong agreement with electron paramagnetic resonance (EPR), X-ray crystallography, electron spin-echo envelope modulation (ESEEM), UV–Vis spectroscopy, circular dichroism (CD), nuclear magnetic resonance (NMR), dynamic light-scattering, fluorescence spectroscopy, equilibrium dialysis and electrospray ionization mass spectrometry (ESI-MS) data obtained for those and other prion peptides [5,15–17,21–27].

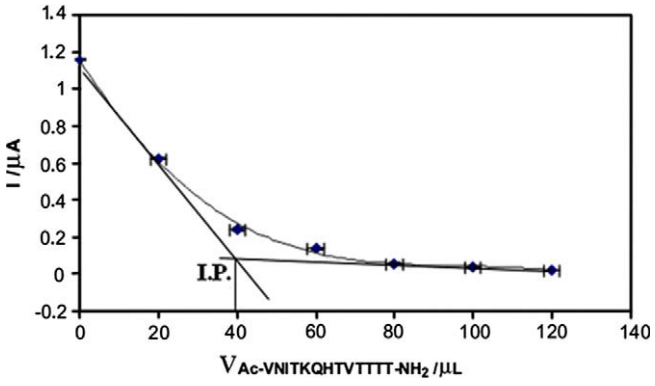

Fig. 1. Amperometric titration graph of 2.0×10^{-6} M Cu^{2+} in 20 mL of 0.1 M KNO_3 with Ac-VNITKQHTVTTTT-NH_2. Successive additions of 20 μL of 8.53×10^{-4} M Ac-VNITKQHTVTTTT-NH_2 was made. I.P. = Inflection point.

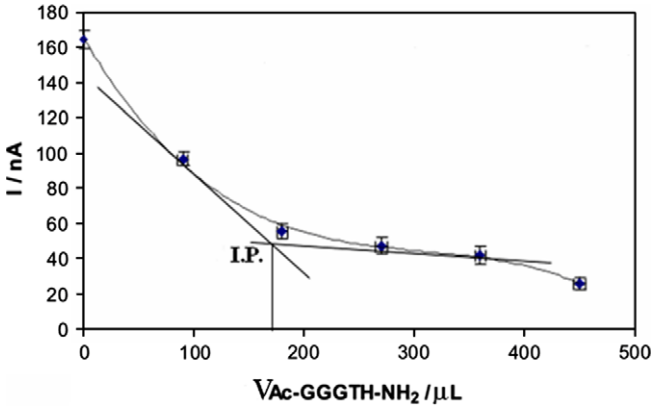

Fig. 2. Amperometric titration graph of 1.0×10^{-6} M Cu^{2+} in 20 mL of 0.1 M KNO_3 with Ac-GGGTH-NH_2. Successive additions of 90 μL of 8.83×10^{-5} M Ac-GGGTH-NH_2 was made. I.P. = Inflection point.

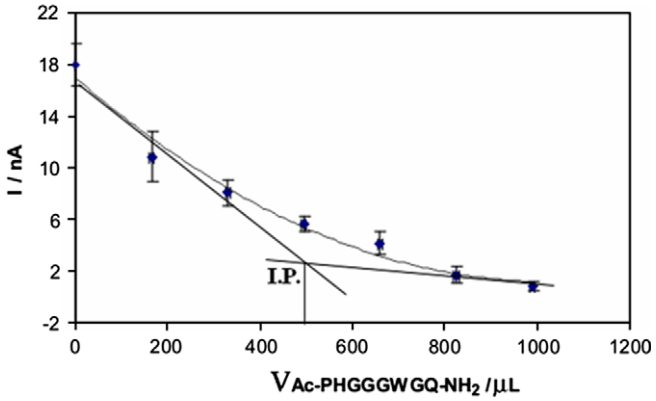

Fig. 3. Amperometric titration graph of 2.0×10^{-7} M Cu^{2+} in 20 mL of 0.1 M KNO_3 with Ac-PHGGGWGQ-NH_2. Successive additions of 165 μL of 9.56×10^{-6} M Ac-PHGGGWGQ-NH_2 was made. I.P. = Inflection point.

The interaction between Cu^{2+} and the peptide fragments of prion (L_t) at pH 7.4 can be represented by:

$$Cu^{2+} - L_t \overset{K_d}{\rightleftharpoons} Cu^{2+} + L_t \quad (1)$$

where, K_d = apparent dissociation constant of $Cu^{2+}L_t$ complex

$$K_d = \frac{\left[Cu^{2+}\right][L_t]}{\left[Cu^{2+}L_t\right]} \quad (2)$$

where, $[L_t]$ = total concentration of free peptide with protonated (L^+H) and unprotonated (L) histidine residues, $[Cu^{2+}L_t]$ = total concentration of $Cu^{2+}L_t$ complex involving one imidazole nitrogen donor (CuL) or one imidazole and one amide nitrogen donors ($CuH_{-1}L$).

K_d was considered to be an apparent dissociation constant because it involves two different forms of free peptide in the complex (L^+H and L) and different Cu^{2+}-peptide complexes (e.g. CuL, $CuH_{-1}L$ and $CuH_{-2}L$, where the index in H indicates the number of deprotonated amide ligands, probably with a predominant form at pH 7.4 [24]). The total concentration of the free peptide prion fragment ($[L_t]$) can be considered almost equal to the concentration of the free ligand with unprotonated histidine residue ([L]) because $[L^+H]$ represents 10% in the maximum of $[L_t]$ considering that our experiments were performed at pH 7.4 where the lysine residue is always protonated and that the pK_a values for histidine residue in peptides range from 5.5 to 6 [41]. The major component of the copper–ligand complex ($[Cu^{2+}L_t]$) is unknown but it can be considered to be a species involving one imidazole and at least one amide nitrogen donor ($[CuH_{-1}L]$) because we obtained complexes with stoichiometric ratios of one peptide molecule per Cu^{2+} metal ion. Thus, it is not possible the existence of a complex with two or more His ligands and also a complex of the type CuL must be very weak due to the absence of anionic groups in the ligand and it may contribute much less than $[CuH_{-1}L]$ or $[CuH_{-2}L]$ to the total concentration of the metal ion–peptide (bound species). This hypothesis is in agreement with Stańczak et al. [42] and is supported by the low K_d values obtained for the complexes.

The apparent dissociation constants for the three peptide–Cu^{2+} complexes were calculated by the mass action law and by the Sarroff and Mark method by using amperometric titration data (Table 2). The obtained apparent K_d values are unique in the literature for Ac-VNITKQHTVTTTT-NH_2–Cu^{2+} complex, are in agreement with those recently published in the literature for

Table 2
K_d values calculated for the interaction between copper ion and the three prion peptides (Ac-VNITKQHTVTTTT-NH_2, Ac-GGGTH-NH_2, Ac-PHGGGWGQ-NH_2) by using amperometric titration data.

Cu^{2+}-prion peptide complexes	$\bar{K}_d/M$[a]	$\bar{K}_d/M$[b]
Cu^{2+}-Ac-VNITKQHTVTTTT-NH_2	4.37×10^{-8}	2.68×10^{-9}
Cu^{2+}-Ac-GGGTH-NH_2	4.33×10^{-8}	1.83×10^{-9}
Cu^{2+}-Ac-PHGGGWGQ-NH_2	5.24×10^{-9}	3.50×10^{-10}

[a] $K_d = \frac{[Cu^{2+}]^2}{[Cu^{2+}L_t]}$, where: K_d = apparent dissociation constant of $Cu^{2+}L_t$ complex, $[Cu^{2+}]$ = concentration of free copper species in the equivalence point, L_t = total concentration of free peptide with protonated (L^+H) and unprotonated (L) histidine residues, $[Cu^{2+}L_t]$ = concentration of $Cu^{2+}L_t$ complex involving one imidazole nitrogen donor (CuL) and one imidazole and one amide nitrogen donors ($CuH_{-1}L$) in the equivalence point.

[b] $\bar{\nu} = \frac{(C_0 - C_m)}{P} = \frac{K_a C_m n}{1 + K_a C_m}$, where: K_a = apparent association constant of the complex $Cu^{2+}L_t$, C_0 = concentration of initial copper, C_m = concentration of free copper in the equivalence point, P = concentration of prion peptide forms in the equivalence point, n = number of copper-binding sites, $\bar{\nu}$ = stoichiometry determined by voltammetric techniques. N_d = 3 determinations.

Ac-GGGTH-NH_2–Cu^{2+} and Ac-PHGGGWGQ-NH_2–Cu^{2+}complexes [20–22] and demonstrate the high affinity of all the three prion fragments to copper ion in the absence of other potential ligands from the media.

The ASV measurements confirm that there are three different copper-binding sites in prion: PHGGGWGQ (site 1), GGGTH (site 2) and VNITKQHTVTTTT (site 3). The results presented here are in agreement with those already published [14–28] and attest, for the first time and in a unique study, the interaction between Cu^{2+} and all copper-binding sites of prion, near physiological conditions. The apparent K_d values (Table 2) demonstrate a high affinity of the three prion peptides towards copper metal ion and also reveal that Ac-PHGGGWGQ-NH_2–Cu^{2+} complex is more stable ($K_d = 5.24 \times 10^{-9}$ M: Mass Action Law; $K_d = 3.50 \times 10^{-10}$ M: Saroff and Mark) than the copper complexes formed with the two other prion peptides. The apparent K_d value for Ac-GGGTH-NH_2–Cu^{2+} complex is 4.33×10^{-8} M as calculated by the mass action law and 1.83×10^{-9} M if calculated accordingly to the Saroff and Mark method. For Ac-VNITKQHTVTTTT-NH_2–Cu^{2+}, the apparent K_d value found by the mass action law is 4.37×10^{-8} M and the value obtained by the Saroff and Mark method is 2.68×10^{-9} M. These data suggest that Cu^{2+} metal ions that bind to PHGGGWGQ repeats are those involved in the initial conformational change event that is responsible for the formation of PrP^{sc} from PrP^{c} and consequently for prion disease, which is in accordance to PrPs mechanistic function as copper sensor or transporting protein already proposed by others [13,43–46].

4. Discussion

Although many studies have contributed to the comprehension of the functions of PrP^{c} and the mechanisms associated with conversion of PrP^{c} to PrP^{sc}, there are a number of questions that still need to be answered: is copper needed for the normal function of PrP^{c}? What are the cellular mechanisms underlying copper-induced conversion of PrP^{c} to PrP^{sc}? What are the exact copper-binding sites involved in the normal function of PrP^{c} and in the conversion of PrP^{c} to PrP^{sc}? What are the stoichiometries and affinities of Cu–PrP^{c} interactions? To offer new insights for the elucidation of these questions, anodic stripping voltammetry was applied to study the interaction of Cu^{2+} metal ions with three synthetic peptides derived from copper-binding sites of prion (VNITKQHTVTTTT, GGGTH and PHGGGWGQ). This methodology was used in such a way that allowed the determination of quantitative data (stoichiometry and apparent dissociation constant) of copper–prion peptide systems near the physiologic conditions and it represents a new, sensitive and reliable alternative to study peptide–metal ion complexes.

The stoichiometric values obtained for the three complexes (1:1) are in close agreement with those determined by other techniques and give support to the idea that three copper-binding sites are involved in the normal function of PrP^{c} and in the conversion of PrP^{c} to PrP^{sc}: four tandem repeats of the fundamental eight residues sequence PHGGGWGQ in the amino terminus of PrP^{c} (residues 60–91) with affinities ranging from 10^{-6} to 10^{-15} M, depending upon copper concentration and pH since different modes of binding occur [5,23,42,47]; GGGTH in the flexible region following the octarepeats and preceding the PrP^{c} globular C-terminal domain (residues 92–96) with affinities around 10^{-9}–10^{-10} M and VNITKQHTVTTTT in PrP^{c} second helical region (residues 180–193), whose metal ion affinity has not been determined yet. In the calculation of the apparent dissociation constants by using the two proposed methods, it is reasonable to assume that: (a) the free prion peptide fragments of this study have a large predominance of a single species (L) at pH 7.4 where the lysine residue is always protonated and His residues of the peptides are mostly unprotonated. (b) the free prion peptide fragments of this study bind Cu^{2+} ions that can form different complexes (e.g. CuL and $CuH_{-1}L$ and $CuH_{-2}L$), probably with a species involving a deprotonated amide ligand being the major component at pH 7.4 (i.e. $CuH_{-1}L$), which is supported by our experimental results (stoichiometric ratios of one copper per peptide and low K_d apparent values) and is in agreement with published binding models [23–24,42]. The apparent dissociation constants determined in this work seems to be too low for short peptides with high conformational entropy. Nevertheless, it was found in the literature that a peptide containing the four copies of the octarepeat domain binds to Cu^{2+} metal ion with dissociation constant on the order of 10^{-14} M if unspecific binding due to the contribution of weak copper-binding sites is discounted [48]. An optimal condition for binding was achieved in our voltammetric measurements by using potassium nitrate as the supporting electrolyte, whose anion is unable to bind to copper. On the other hand, the buffers generally used in the measurements by spectroscopic techniques are weak copper-binding molecules and this competition for Cu^{2+} provides higher apparent dissociation constants for prion peptides–copper interactions. The low apparent K_d values found for the three copper–prion peptide complexes give support to the idea that prion and some peptides derived from it bind to Cu^{2+} and thus the metal ion has a role in copper transport or homeostasis and in the defense mechanism against oxidative damage [5,49]. The apparent K_d values also reveal that PHGGGWGQ–Cu^{2+} complex is more stable than those copper complexes formed with the two other prion peptides, suggesting that Cu^{2+} metal ions binding to PHGGGWGQ repeats is the initial event that trigger the conformational change events responsible for the formation of $PrP^{sc}$and consequently for prion disease. This result is in agreement with Kenward et al. [48] that showed by fluorescence spectroscopy that a synthetic peptide composed of four copies of the octarepeat domain linked to the GGGTH region aggregates in the presence of Cu^{2+} and especially in the presence of Zn^{2+}.

An important debate in literature refers to the pro-oxidant versus anti-oxidant effects of copper-binding to peptides. Concerning the prion peptides, a pro-oxidant effect due to Cu^{2+}-binding has been reported [49,50]. It has also been described that histatin, a histidine-rich peptide isolated from primate saliva is pro-oxidant upon Cu^{2+} complex formation. Other authors attribute an anti-oxidant effect to the Cu(II)-chelating capability of the prion protein and thus that is an undefined issue [5,12,45]. Nevertheless, thinking more broadly about copper-binding, there is a search in the literature for copper-chelating peptides to be used in diverse fields such as health sciences and food and agricultural sciences [51] and thus

anodic striping voltammetry can be a valuable technique to search for and evaluate the affinity of novel copper-binding peptides.

This contribution showed that small synthetic peptides can be useful to furnish relevant information about metal ion binding properties of misfolded, aggregated proteins such as the prion. In spite of being impossible to extrapolate the results for the binding of metal ions to the full-length protein and thus to its function in prion disease, ASV can be a valuable technique to select for metal-ion binding peptides in experimental conditions proximal to the physiological ones. Since many fibrillization phenomena of proteins depend upon short peptide sequences [47], voltammetry studies with peptides can contribute to the understanding of the role of Cu^{2+} and other metal ions on peptide aggregation.

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