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Can chicken and human PrPs possess SOD-like activity after β-cleavage?

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Abstract

The prion protein is a membrane attached glycoprotein that is involved in binding of divalent copper ions. *In vivo* human and chicken PrPs exhibit SOD-like activity associated with octarepeat and hexarepeat regions, respectively, when bind Cu(II) ions. However, the species of Cu(II)–PrP involved in the Cu(II) center which determines the highest SOD-like activity is still unknown. The data presented here clearly show that the single Cu(II) ion bound to PrP octapeptide repeat region of mammalian prion and hexapeptide repeat region of avian prion via 4 His side-chain imidazoles reveals the highest SOD activity.

Keywords: Prion protein; SOD activity; Copper(II) complexes

Prion protein (PrP) is a cell membrane anchored glycoprotein expressed in most cell types with major abundance in the neuronal and glia tissues [1–3]. *In vivo*, PrP occurs in two isoforms (i) PrP^{C}, cellular form possessing biological activity and (ii) PrP^{Sc}, scrapie form, which is responsible for accumulation and the extracellular deposits characteristic for the neurodegeneration syndrome [4,5]. It is believed that the conversion $PrP^{C} \rightarrow PrP^{Sc}$ causes neuro-disorders and the loss of physiological function resulting in the apoptotic cell death. However, in spite of huge biological research effort, not much is known about natural role of PrP^{C} in the cell biology. Evidence suggests that one of the most likely functions of the PrP^{C} in presynaptic space is SOD-like activity [6–10]. *In vivo* studies have shown that mice with Prnp-knockout gene ($Prnp^{0/0}$) possess reduced SOD activity [6]. This likely function may be abolished by the removal of the octarepeat region involved in the copper ion binding or by addition of copper chelator diethyldithiocarbamate [8]. Cu(II) ion may form several species with octapeptide tetra-repeat fragment (4Oct) of mammalian PrP. In the case of 4:1 Cu(II):4Oct molar ratio, the major species formed around pH 7 is the complex with Cu(II) bound to imidazole and two or even three amide nitrogens [11–14]. This Cu(II) coordination mode differs strongly from that observed in cellular Cu, Zn–SOD copper site [15]. However, in the equimolar solutions of Cu(II) ion and tetrameric repeat fragment of both human (4Oct) and chicken (4Hex) PrPs around physiological pH the multi-imidazole coordination pattern, closely resembling the active centre of Cu, Zn–SOD enzyme, occurs [11–16].

The SOD-like activity was reported for both the full-length mouse and chicken recombinant PrPs, which were refolded in the presence of copper [7]. However, recent studies have shown that certain population of PrP^{C} anchored at the cell membrane is proteolytically cleaved in the neurotoxic region near position 110 termed α-cleavage, while the other cleavage process depending on ROS (radical oxygen species) occurs near position 89 termed β-cleavage at the end of the copper-binding peptide repeat domain [17–19]. One of the potential functions of PrP might be acting as a receptor which after the binding of extracellular copper and the β-cleavage of the Cu(II)–peptide complex may play a relevant function in extracellular matrix as a SOD-like enzyme. In this work, the SOD-like activity of the copper complexes with the whole tetra-repeat region and its fragments both for human and avian PrPs was studied.

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Materials and methods

Peptide synthesis and purification. All peptides were synthesized according to published methods using standard solid-phase synthesis techniques with a manual methodology [20,21], and the analytical data were reported earlier [11,12,16].

Determination of the superoxide dismutase activity. The *in vitro* SOD activities of the copper complexes were evaluated at 298 K, in samples containing $Cu(NO_3)_2$ and solutions containing Cu(II)–peptide system in different molar ratios (Tables 2 and 3) in Tris–HCl buffer (25 mM, pH 7.4 and pH 6.6, respectively). The enzymatic activity was examined indirectly using nitroblue tetrazolium (5×10^{-5} M) assay [22]. The superoxide anion was generated *in situ* by the xanthine/xanthine oxidase reaction, and detected spectrophotometrically by monitoring the reduction of NBT band at 550 nm. The concentration of xanthine was 1×10^{-4} M, and the reaction was evoked by adding appropriate amount of xanthin oxidase to form absorption change at 550 nm (ΔA_{550}) by 0.025–0.026 min^{-1}. The reduction rate of NBT was measured in the presence as well as in the absence of the studied system ($[Cu^{2+}]_{tot} = 0–1.5 \times 10^{-6}$ M). The control experiment was carried out in the presence of horseradish Cu, Zn–SOD ($0–7 \times 10^{-8}$ M). The xantine oxidase activity was monitored by the following of urate production, spectrophotometrically at 298 nm, in order to exclude any inhibition induced by the copper(II)–peptide complexes. The SOD-like activity is expressed by IC_{50} values, indicating the concentration of SOD mimic causing 50% inhibition of NBT reduction.

Results and discussion

The studied fragments of human and chicken PrPs and the Cu(II)–peptide ratios are listed in Table 1.

The SOD activities of the above-mentioned complexes are listed in Table 2 as the IC_{50} values.

In pH 7.4, chicken hexapeptide forms two different species due to the Pro residue at third position (Fig. 1a) [23]. The most abundant isomer (*trans*-Pro) binds Cu(II) via imidazole nitrogen and one deprotonated amide nitrogen of His residue, while in the case of minor isomer (*cis*-Pro) the phenolate oxygen of Tyr residue together with His imidazole participates in the metal ion chelation [23]. Human octapeptide in the same conditions forms Cu(II) complex with the $\{N_{im}, 2N^-, CO\}$ donor set (Fig. 1b) [11,24]. The fifth apical site binds the water molecule which is interacting with the indol ring of Trp residue via hydrogen bond [11,24]. The IC_{50} values of 1Hex (0.261 μM) and 1Oct (0.492 μM) differ strongly. The difference could derive from the involvement of two amide nitrogens bound to Cu(II) in mammalian peptide, which stabilizes the Cu(II) complex making it difficult to be reduce to Cu(I). Therefore, the copper–chicken 1Hex with one amide coordinated possesses twofold higher SOD-like activity when compared to Cu(II)–1Oct.

The impact of a bound amide on the SOD activity is also observed in the peptide dimers. In the case of equimolar chicken 2Hex–Cu(II) system the main complex in physiological pH involves the $\{2N_{im}, N^-\}$ donor sets whereas in the case of human 2Oct–1Cu(II) the $\{2N_{im}, 2N^-\}$ donor set coordinates (Fig. 1c and d, respectively) [12,16]. The IC_{50} values of dimers differ by about 0.03 μM probably because of the extra amide nitrogen coordination in 2Oct species. Comparing SOD activity of monomeric and dimeric peptide systems it is worthy to note that the involvement of the higher number of imidazole nitrogens in the metal ion coordination causes the increase of the enzymatic activity (Table 2). This is seen even better in the case of tetrameric peptide complexes, 4Hex and 4Oct for which the SOD-like activity is the highest among all systems studied (Table 2). In the latter cases in the equimolar solutions both 4Hex and human 4Oct coordinate Cu(II) ion via the $\{4N_{im}\}$ donor set (Fig. 1e and f) [12,16].

In the case of twofold excess of Cu(II), the SOD-like activity increases in the 2Hex–2Cu(II) system but decreases in the 2Oct–2Cu(II) one. The coordination pattern of 2Hex–2Cu(II) system represents two dominating complexes in physiological pH: CuH_2L with $\{2N_{im}\}$ and $Cu_2H_{-1}L$ with $\{N_{im}, N^-\}$ and $\{N_{im}, N^-, O_{Tyr}^-\}$ donor sets involved (Fig. 1A, a—supplementary material). As it was previously shown (*vide supra*) the multi-imidazol complexes are enzymatically more active. In the case of 2Oct–2Cu(II) system, this activity decreases, because of the lack of the multi-imidazol species. The $Cu_2H_{-5}L$ complex dominating in this pH range involves the $\{N_{im}, 2N^-\}$ and $\{N_{im}, 3N^-\}$ donor sets for coordinated metal ions (Fig. 1A, b) [12,16]. Thus, the presence of amide nitrogens have the critical impact on the SOD-like activity of the system studied. In the case of 4Hex and 4Oct the metal excess results in the decrease of SOD activity. Additional Cu(II) ions compete for imidazole binding sites and amount of bound imidazoles per one Cu(II) ion decreases, while the number of the involved amide nitrogens increase (Fig. 1A, c–f) [12,16]. This is especially clear in the case of 4Oct. The chicken PrP tetra-hexapeptide forms Cu_2H_2L complex mainly in pH 7, 4 with $\{N_{im}, N^-, O_{Tyr}^-\}$ donor coordinating to each copper ion. Comparing with the chicken dimer peptide, the lack of the multi-imidazole complexes leads to the relative decrease of enzyme activity. But in the case of human octapeptide tetramer system, the main species dominating in physiological pH is the same as that observed for the dimeric peptide system. The significant difference between the activities

Table 1
The tested fragments of human and chicken PrPs and the Cu(II)–peptides ratios

Chicken PrP		Human PrP	
Peptide fragments	Cu(II)–peptide molar ratio	Peptide fragments	Cu(II)–peptide molar ratio
Ac-HNPGYP-NH_2(1-Hex)	1 ÷ 1	Ac-PHGGGWGQ-NH_2(1-Oct)	1 ÷ 1
Ac-$(HNPGYP)_2$-NH_2(2-Hex)	1 ÷ 1, 2 ÷ 1	Ac-$(PHGGGWGQ)_2$-NH_2(2-Oct)	1 ÷ 1, 2 ÷ 1
Ac-$(HNPGYP)_4$-NH_2(4-Hex)	1 ÷ 1, 2 ÷ 1, 3 ÷ 1, 4 ÷ 1	Ac-$(PHGGGWGQ)_4$-NH_2(4-Oct)	1 ÷ 1, 2 ÷ 1, 3 ÷ 1, 4 ÷ 1

Table 2
IC_{50} (μM) values of the Cu(II)–peptide complexes in pH 7.4, with respect to the native Cu, Zn–SOD enzyme,[a] and $Cu(NO_3)_2{\cdot}6\,H_2O$ [b]

Chicken PrP					Human PrP				
	1Cu(II)	2Cu(II)	3Cu(II)	4Cu(II)		1Cu(II)	2Cu(II)	3Cu(II)	4Cu(II)
1Hex	0.261				1Oct	0.492			
2Hex	0.188	0.158			2Oct	0.217	0.242		
4Hex	0.177	0.314	0.330	0.207	4Oct	0.175	0.310	0.225	0.316

[a] IC_{50} of the native Cu, Zn–SOD 0.0044.
[b] IC_{50} of $Cu(NO_3)_2{\cdot}6H_2O$ 0.980.

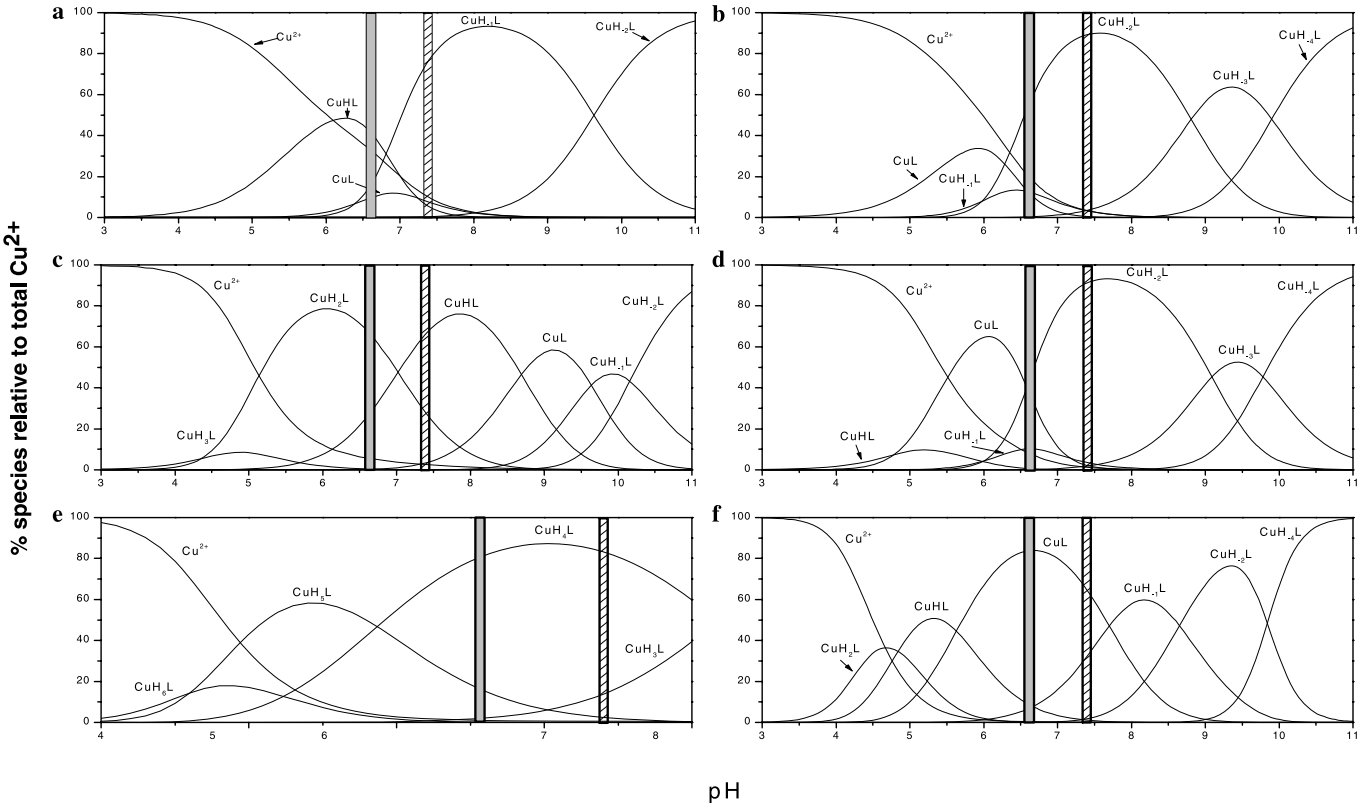

Fig. 1. Species distribution profiles for Cu^{2+} complexes of: (a) Ac-HNPGYP-NH_2, (b) Ac-PHGGGWGQ-NH_2, (c) Ac-$(HNPGYP)_2$-NH_2, (d) Ac-$(PHGGGWGQ)_2$-NH_2, (e) Ac-$(HNPGYP)_4$-NH_2 and (f) Ac-$(PHGGGWGQ)_4$-NH_2, at 25 °C and $I = 0.1$ M KNO_3·$[Cu^{2+}] = 1 \times 10^{-3}$ M, metal to ligand ratio 1:1. pH 7.4 was marked by dense column and pH 6.6 by light gray column [11,12,16].

may result from the impact of other minor species on the SOD-activity.

Threefold excess of copper ions in chicken PrP tetramer changes the SOD-like activity only slightly, because of the same coordination pattern as that observed in Cu_2H_2L (*vide supra*). The tandem repeat domains of both human and chicken PrP fragments fully saturated by metal ions exhibit the same chelating mode per copper ion as that observed in monomeric fragments (Fig. 1A, g and h). But the formation of the oligomeric structures with 4 or 3 Cu(II) ions may also have influence on the SOD activity and the precise correlation of the complex structures with the SOD activity is more difficult than in the case of monomeric Cu(II) species.

In order to confirm the negative effect of amide coordination on the SOD activity we determined the SOD-like activity also at lower pH 6.6. Lower pH destabilizes amide binding. The IC_{50} values for pH 6.6 are listed in Table 3. As expected the comparison of the IC_{50} values of Cu(II) with chicken and human PrP monomers (1Hex and 1Oct) at pH 6.6 reveals that the SOD-like activity is strongly

Table 3
IC_{50} (μM) values of the Cu(II)–peptide complexes in pH 6.6, with respect to the native Cu, Zn–SOD enzyme,[a] and $Cu(NO_3)_2{\cdot}6H_2O$ [b]

Chicken PrP		Human PrP	
	1Cu(II)		1Cu(II)
1Hex	0.355	1Oct	0.242
2Hex	0.191	2Oct	0.221
4Hex	0.223	4Oct	0.121

[a] IC_{50} of the native Cu, Zn–SOD 0.0044.
[b] IC_{50} of $Cu(NO_3)_2{\cdot}6H_2O$ 0.965.

Table 4
IC_{50} (μM) values of reports on SOD-like activity of copper(II) complexes

Complex	IC_{50} (μM)	Ref.
Cu(II)-HisValHis 1:1	0.20	[26]
Cu(II)-Ac-HisValHis-NH_2 1:1	0.16	[26]
Cu(II)-HisHisGlyHis 1:1 (pH 6.8)	0.13	[27]
Cu(II)-HisHisGlyHis 1:1 (pH 7.5)	0.15	[27]
Cu(II)-HisHisGlyHis 1:10 (pH 7.5)	0.25	[27]
Cu-(cyclo-HisHis)	0.11	[28]
Cu(II)–4Oct 1:1 (pH 6.6)	0.121	This work
Cu, Zn–SOD (pH 6.8)	0.0045	[27]
Cu, Zn–SOD (pH 7.4)	0.0084	[29]
Cu(HPO_4) (pH 7.4)	1.06	[27]

depended on the pH. Lower pH leads to protonation of some of amide nitrogens removing them from the coordination pattern.

There is a distinct decrease of SOD activity for Cu(II)–1Hex system from 0.261 to 0.355 μM which derives from the fact that at pH around 6.6 the amount of free Cu(II) increases considerably from several to about 30% of total copper (Fig. 1a) [16]. The amount of free copper in the case of Cu(II)–2Hex also increases but only to about 10% (Fig. 1c). In the case of Cu(II)–4Hex system the amount of CuH_4L species with four imidazoles bound to Cu(II) is slightly lower at pH 6.6 when compared to pH 7.4 (Fig. 1e). These slight differences may cause the relatively small differences between the SOD activities of two latter systems at pH 6.6 when compared to pH 7.4.

In the case of human octapeptides 1Oct and 2Oct at pH 7.4 two amide nitrogens are involved in the metal ion coordination and pH decrease removes partly amides from the coordination sphere (Fig. 1b and d) improving SOD activity which increases from 0.492 to 0.242 μM (Tables 2 and 3). The distinct increase of SOD activity of Cu(II)–4Oct system (from 0.175 to 0.121 μM) is due to distinct increase of the CuL species concentration when pH decreases from 7.4 to 6.6 (Fig. 1f). In the latter complex, the binding mode involves four imidazoles characteristic for the Cu(II) site in the real Cu, Zn–SOD enzyme.

The data presented above clearly show the relation between the Cu(II) donor set and the SOD activity of the formed species. The multi-imidazole binding favors the SOD activity, while the amide nitrogen coordination has a contrary effect.

The relation between the SOD activity and Cu(II) binding mode follows closely the copper reduction ability by the octapeptide repeat region of prion protein by Trp residue [25].

The comparison of the SOD activity of Cu(II)–4Oct system with the other Cu(II) His containing peptide systems (Table 4) clearly shows that PrP octarepeat region is a very effective SOD center when binds one Cu(II) ion.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.11.003.

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