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Prion Proteins Leading to Neurodegeneration

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Abstract

Prion diseases are fatal neurodegenerative disorders related to the conformational alteration of the prion protein (PrP^{C}) into a pathogenic and protease-resistant isoform PrP^{Sc}. PrP^{C} is a cell surface glycoprotein expressed mainly in the central nervous system and despite numerous efforts to elucidate its physiological role, the exact biological function remains unknown. Many lines of evidences indicate that prion is a copper binding protein and thus involved in the copper metabolism. Prion protein is not expressed only in mammals but also in other species such as birds, reptiles and fishes. However, it is noteworthy to point out that prion diseases are only observed in mammals while they seem to be spared to other species. The chicken prion protein ($chPrP^{C}$) shares about 30% of identity in its primary sequence with mammal PrP^{C}. Both types of proteins have an N-terminal domain endowed with tandem amino acid repeats (PHNPGY in the avian protein, PHGGGWQ in mammals), followed by a highly conserved hydrophobic core. Furthermore, NMR studies have highlighted a similar globular domain containing three α-helices, one short 3_{10}-helix and a short antiparallel β-sheet. Despite this structural similarity, it should be noted that the normal isoform of mammalian PrP^{C} is totally degraded by proteinase K, while avian PrP^{C} is not, thereby producing N-terminal domain peptide fragments stable to further proteolysis. Notably, the hexarepeat domain is considered essential for protein endocytosis, and it is supposed to be the analogous copper-binding octarepeat region of mammalian prion proteins. The number of copper binding sites, the affinity and the coordination environment of metal ions are still matter of discussion for both mammal and avian proteins.

In this review, we summarize the similarities and the differences between mammalian and avian prion proteins, as revealed by studies carried out on the entire protein and related peptide fragments, using a range of experimental and computational approaches. In addition, we report the metal-driven conformational alteration, copper binding modes and the superoxide dismutase-like (SOD-like) activity of the related copper(II) complexes.

Key words: Prion, chicken, avian, copper affinity, copper coordination environment, Molecular Dynamics, SOD-like activity

Prion Proteins Leading to Neurodegeneration

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1. Neurodegeneration, environment and metal complex species

A broad range of human diseases are associated with the conversion of a particular protein or peptide, from a normally soluble form to insoluble and well-defined fibrillar aggregates, known as amyloids [1-5]. Although the molecular mechanisms by which the pathologies develop may be different, for this common hallmark these pathologies are generally named protein misfolding diseases [6-10]. Among these there are different neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral schlerosis (ALS), Huntington's disease, and the transmissible spongiform encephalopathies (prion diseases) [11-18].

Many of these diseases exist as sporadic and inherited illnesses. For example, less than 1% of cases affected by AD are younger than 60 years old, but patients with early onset or presenile AD often have a positive family history, probably linked to mutations in genes for amyloid precursor protein (APP), presenelin 1 and presenelin 2, which affect the stability and metabolism of β-amyloid [19]. Actually the late-onset or senile Alzheimer's disease accounts for 95% of all Alzheimer's disease cases [20].

Within human prion diseases, sporadic Creutzfeldt-Jakob disease (sCJD) includes 85% of all cases of these pathologies [21]. Only 15% are inherited such as Gerstmann-Sträussler-Scheinker (GSS) or inherited forms of Creutzfeldt-Jakob disease (CJD), in which a point mutation in PrP^{C} results in an increase of the protein propensity to assume an abnormal conformation [22, 23]. However, in recent years the variant CJD (v-CJD), a prion disease supposed to be caused by transmission of bovine spongiform encephalopathy (BSE), appeared [24]. The majority of prion diseases affecting animals such as the BSE of cattle, scrapie of sheep and chronic wasting disease of elk and deer (CWD) are believed to be acquired through an alimentary route (BSE) or by horizontal transmission (CWD) between affected animals [25].

Taking into account the higher percentage of sporadic causes of these disorders, great efforts have been made in understanding what environment modifications and risk factors may be the cause of

protein misfolding [26]. In this regard, it has been proposed that the conversion likely occurs in the partially denaturing environment of endocytic compartment, where the lower pH may induce conformational changes that facilitate amyloid and prion self-assembly [27-29]. In general, pH, temperature, ionic strength, presence of chaotropic agents, oxidative stress and metal ions strongly influence the conformation adopted by the polypeptide [29-34].

Nowadays it is widely accepted that toxic aggregates are the soluble oligomers, intermediate species between monomers and amyloid fibrils [6, 35-37]. These oligomers can display different morphologies resulting in their different toxicity. The morphological variability is associated with various environmental conditions such as different interactions with metal ions [37-39].

Metal ions can be broadly classified as either “biometals” or “toxicological” metals based on whether they have a functional role or are detrimental to the organism. Transition metals such as iron, zinc and copper are present in the brain at concentrations ranging from 100-1000 μM, and dysfunction in homeostasis of these endogenous transition metals can result in significant neurological abnormalities [40-42]. In normal mice, age-related increases in copper (46% from 2.8 to 18 months), iron (51% from 2.8 to 18 months) and cobalt (66% from 2.8 to 18 months) levels in whole brain have demonstrated [43,44]. Other metals such as zinc and manganese did not change [45, 46].

Since age is one of the major risk factor for neurodegenerative diseases, alterations in the distribution or levels of metal ions with age might be important in the underlying disease pathogenesis. Actually, elevations (3-5 fold increase in the cortical and accessory basal nuclei of the amigdala) in zinc, copper and iron in the neuropil of AD patients, as compared to age-matched controls, have been reported [47]. Metal concentrations are significantly increased specifically within Aβ plaques with copper (390 μM), zinc (1055 μM) and iron (940 μM) all elevated (although iron is found primarily complexed with ferritin in plaque-associated neuritic processes and within

neurons and NFTs) as compared to the normal age-mathched neuropil (Copper (70 μM), Zinc (350 μM) and Iron (340 μM)) [46,47].

Human beta-amyloid peptides are able to bind metal ions such as copper, zinc and iron, and a redox chemistry-based pathogenic model of neurodegenerative diseases has been proposed [48]. Rat Aβ-28 differs from human Aβ by three mutated residues, with Arg^{5}, Tyr^{10}, and His^{13} of human Aβ substituted by Gly^{5}, Phe^{10}, and Arg^{13} [49]. It has been shown that, *in vitro*, rat Aβ binds transient metals weakly, unlike human Aβ [48, 50, 51]. In this case the coordination of Cu^{2+} or Fe^{3+} does not induce redox chemical reactions, thus reducing the amount of reactive oxygen species generated [46,48]. This could explain the resistance in mice to develop Alzheimer's disease despite the high sequence identity (97%) of mouse Aβ to its human homologue [49, 52]. According to this pathogenic model, the lack of these key residues of mouse Aβ, compared with the human counterpart, results in different metal ions coordination; it may explain why mice do not suffer from AD.

From a chemical point of view, a metal can be bound to a specific protein binding site but, depending on pH, concentration of metals and metal to ligand ratios, different complex species can form with different coordination environments. This could explain also the contradictory data reported for the role of the metal ions in different neurodegenerative diseases. Therefore, it appears necessary to obtain data on copper ions binding, complex species, affinity and coordination mode, in order to have valuable grounding to explain the so-called “metal paradox”.

As shown in the previous example of rat and human Aβ, a useful approach consists in making comparative studies on proteins and peptides of different organisms. This approach allows us also to obtain information on the possible biological role of the proteins. Currently another common aspect of several proteins or peptides involved in such diseases is that their biological function remains unknown.

PrP^C is a highly conserved protein in mammals, but genes encoding homologous prion proteins have been reported in different species such as avians/birds, turtles, amphibians and fish [53-59]. It is important to point out that prion diseases are observed only in mammals, appearing to be precluded to the other species. Thus the comparison between prion proteins of different species can reveal functional evolutionary trends related to certain aspects of prion pathogenesis.

In this review we deal with the different behaviour of avian and mammal prion protein, also reporting our latest data on SOD-like activity of N-terminal repeats region, which is useful in settling some of the disputed functions of the prion protein.

2. The prion protein and its role on the cellular metabolism

Prion protein (PrP^C) is a ubiquitous anchored cell surface glycoprotein, mainly expressed in the central nervous system [60-62].

The conversion of normal, soluble α-helix rich protein into insoluble β-sheet enriched pathogenic conformer (PrP^{Sc}), is thought to be the central event of prion disease [18, 19, 63, 64]. Despite uncertainties about the transmission of the disease, there is strong evidence that normal prion expression and the Scrapie isoform conversion are essential for the neurodegeneration. In fact, it has been observed that mice with knockout neurons for the PrP^C gene are resistant to PrP^{Sc} neurotoxicity, despite being more exposed to oxidative stress [65, 66]. Notably, the two prion isoforms show some intriguing features. It was observed, in fact, that while PrP^C is entirely degraded by proteases, PrP^{Sc} is degraded into a stable fragment [67]. Therefore, the protein can be incorporated into an aggregate, growing up continuously forming plaques, like the amyloids, in the infected brain.

Although the physiological role of the prion protein is still an open question, it has been suggested that PrP^C is involved in oxidative stress protection [68], in apoptosis [69], in cellular signalling [70], in membrane excitability and synaptic transmission [71], in transport and copper metabolism [72],

but it is still unclear how these functions can be all carried out by the same protein. The PrP^C was found on the cellular surface and it is believed that the endocytosis may influence its physiological function, although the mechanism of prion endocytosis is not, so far, clear [73].

3. The avian prion and its difference from mammal analogue

The chicken prion protein ($chPrP^C$) was the first non mammalian prion protein isolated and characterized, being initially described as having acetylcholine receptor-inducing activity [55]. The analysis of 27 mammalian and 9 avian PrPs revealed high conservation of the flexible regions of prion proteins, encompassing the N-terminal part characterized by the presence of peptide repeats [53].

Interestingly, even if the presence of internal repeats occur in 14% of all known proteins, eukaryotic proteins are three times more likely to have these repeats than prokaryotic ones. It has been proposed that proteins containing repetitive sequences may evolve more quickly, allowing them a faster adaptation to new environments [74]. From fish to humans, the repeat units within one molecule show reduced degeneracy, but their size increase reaching a maximum of eight amino acids in mammals (see Fig. (**1**)).

Despite the divergence between the primary sequences, the comparison among prions from different species shows the presence of some conserved protein domain structures [75]. NMR measurements have established the structure of mammal, chicken, turtle and frog prion proteins, showing closely similar global folds. The C-terminal domain contains three α-helices, one short 3_{10}-helix and a short antiparallel β-sheet, while the N-terminal domain is supposed to be flexible and unordered [75].

In chicken prion proteins many mammalian prion essential features are observed (see Fig. (**2**)). In particular both proteins possess: *i)* multiple N-glycosylated sites; *ii)* an amino-terminal signal sequence that is removed in the mature protein; *iii)* a carboxy-terminal signal that is eliminated

when the mature protein is linked to Glycosyl Phosphatidyl Inositol (GPI), and *iv)* an N-terminal domain featured by tandem amino acid repeats, octameric in mammalian prion, and hexameric in the avian one, with the following sequences (PHGGGWGQ) and (PHNPGY) for mammal and avian, respectively.

The N-terminal region, made up of polypeptide repeats, has been analyzed by proteolysis [76]. Differently from mammalian homologues, the digestion of chicken prion protein with trypsine or proteinase K, produces peptide fragments stable to further proteolysis [76], suggesting that they adopt a different structure than that of mammalian prion tandem repeats.

A closer examination of the avian hexarepeats reveals the presence of PXXP motif, known to interact with SH3 domains present in different proteins [77]. Another PXXP motif has been identified within the N-terminal region of mammalian as well as avian prion proteins and it corresponds to region 101-104 in mouse PrP^{C} and 107-111 in chicken PrP^{C} [78]. It has been supposed that these sequences may be the recognition sites of the C-terminal SH3 domain of the murine growth factor receptor-bound protein 2 (Grb2), suggesting a role in signal transduction for the prion protein of different species [78].

Other experimental studies indicate that the N-terminal region of PrP^{C} plays a regulatory role in the PrP^{C}-PrP^{Sc} conversion [79, 80]. Furthermore, the N-terminal domain of chicken prion protein is essential for anterograde axonal transport [81] and has been described to drive also clathrin-coated pit endocytosis [82], probably due to the abundance of glycines, prolines and the formation of turn motifs. Recent NMR and simulation studies gave support to this hypothesis showing the abundance of turn structures inside the chicken hexarepeat domain [83, 84], which can lower the flexibility of the avian hexarepeats. In particular, a type I β-turn structure was found in the NPGY region [83], a conformation adopted also by the NPXY internalization signal of the low-density lipoprotein (LDL) receptor [85]; tyrosine-containing motifs, essential for coated pit-mediated endocytosis, have been found to adopt an i-i+3 turn conformation [86]. Moreover, an interesting dependence of the peptide

shape on the tyrosine residue deprotonation was also detected; the deprotonation causes a shortening of the distance between the phenolate oxygen of tyrosine and the amide side chain hydrogens of asparagine thus tilting the glycine residue. This appears to be the driving force for the increase of unordered structures at basic pH explaining the relative blue shift in the CD spectra found in a previous study [87].

The analysis of the more extended hexarepeat domain, $(PHNPGY)_4$, from CD and molecular dynamics simulations [84], reveals a conformational dependence on the protonation states of histidine and tyrosine residues (Fig. (**3**)).

In particular, the turn formation is pH driven and the pivotal role of histidine and tyrosine residues is observed. These residues are able to stabilize turn regions in the peptide chain, giving rise to a very compact bent structure of backbone, upon forming a hydrogen bond. This condition occurs at physiological pH, when histidine 8 is deprotonated and tyrosine 18 is protonated.

The differences found on secondary structures between the chicken and mammalian tandem, can be associated to the different ratio between Gly and Pro residues. The mammalian octarepeat peptides contain 50% of glycine and 12% of proline residues, respectively, while the chicken hexarepeats encompass 16% of glycine and 33% of proline residues. The greater flexibility conferred by glycine residues might explain why the mammalian prion protein N-terminal tandem amino acid repeats is unordered. On the contrary the high number of proline residues can explain the tendency of avian hexarepeats to form turn structure. Moreover, tyrosine residues, stabilizing turn structure, introduce another important difference between mammalian prion protein and that of avians, reptiles and amphibians. The non mammalian proteins display the conserved sequence PXYP in the repeats region [88], whereas mammal octarepeat not only has no PXXP motif but it is also lacking in tyrosine residues, essential, as above reported, for coated pit-mediated endocytosis [86].

These data on N-terminal domain peptide fragments provide useful information and indications to a better understanding of the entire protein structure.

Molecular dynamics simulations of the globular core of two mammalian and two non mammalian prions, respectively human, Syrian hamster, chicken and turtle, underlined that a higher mechanical force is necessary to unfold mammalian prions [89], but another MD simulation showed the same thermostability for human and chicken prion proteins [90]. Interestingly, the structural role played by water molecules was analyzed by a molecular dynamics approach [91], highlighting that the bound and buried water that stabilizes a β-bulge between one edge of the sheet and helix 3, could prevent the avian PrP^C aggregation. This finding was also supported by a 130 ns long molecular dynamics simulation of the full avian prion protein chPrP1-267, which finds again the latter bulge and a water molecule tightly bound to the Valine 168 [92]. Moreover, a rigid domain in the hexarepeat region was found, revealing a hydrogen bond mainly between the imidazole nitrogen of histidine 72 and the phenolic hydrogen of tyrosine 64, found also in the tetra-hexarepeat fragment [84]. In addition, the α-helix 2 of the $chPrP^C$ was found to be rigid, while the corresponding α-helix 2 of the human PrP^C was reported to be rather flexible [93, 94]. Such latter stiffness is also connected to the presence of the first proline, which, being in the first position, protects the helix from non-native interaction [95]. Moreover, β-sheet was found to be stable during dynamics, while in the mouse prion D178N pathogenic mutant, the β-sheet structure undergoes disruption [96].

All these results reinforce the hypothesis that the chicken prion protein possessing more rigid domains than those of mammal analogues tends to prevent aggregation, which may in turn be the cause for its high resistance to proteases at physiological pH.

4. Prion and copper

Copper is an essential element for living systems, being a cofactor for many key enzymes that catalyze redox reactions [97-99] but, at the same time, the redox cycling between Cu(II) and Cu(I) oxidation states, may be harmful for the formation of radicals such as reactive oxygen species (ROS). These highly reactive radicals can determine oxidative damage to cellular components, and

these processes have been also related, directly or indirectly, to protein misfolding diseases [100-103]. Copper ions have been reported to alter the biochemical properties of cellular prion proteins and to promote aggregation [104-107]. On the other hand, much experimental evidence suggests that mammalian PrP^C is a copper-binding protein and that its biological role is strictly connected with copper homeostasis [108,109].

Brain extracts from PrP-knockout mice have copper content lower than wild type [72]. Noteworthy, within the central nervous system, PrP^C is concentrated at presynaptic membranes a region of high copper localization and flux [71, 109]. All these studies suggest that prion protein may act as a receptor for cellular uptake or efflux of copper ions by cells [110]. Actually, micromolar concentrations of copper rapidly stimulate endocytosis of cell-surface mammalian PrP, via clathrin-coated pit [111,112]. Interestingly, the first evidence about copper, prion and endocytosis was reported on chicken prion protein [113]. It has been supposed that the binding of copper(II) ion to the $chPrP^C$ induces a change in conformation, but how this could be a signal for the internalization is unclear [113].

PrP^C expression and the amount of copper content bound to it increase the cellular resistance to oxidative stress. The protein may act as a copper chelating agent, when extracellular copper reaches high concentrations peaks (15-300 μM) e.g. during synaptic transmission and depolarization [110].

Another hypothesis is that the binding of copper to prion could act directly to detoxify oxygen reactive species, performing superoxide dismutase-like activity (SOD-like) [114-116]; the SOD-like activity appears to be a general property of PrP^C since it is displayed by both mouse and chicken prion proteins [115]. It has been established that copper binding to the octarepeat is necessary for the observed SOD-like activity of mammalian prion protein, therefore it has been postulated that the same can be occurring for the corresponding chicken hexarepeat region [115].

In general, many studies and efforts have been dedicated to understanding copper interaction with mammal PrP^C and less for prion expressed in other species.

The first measurements on the copper(II) specificity of peptide fragments belonging to the N-terminal chicken prion domain, was carried out by means of MALDI TOF MS and CD [117,118]. Marcotte and Heisenberg ruled out the ability of chicken prion protein to bind copper ions, although copper(II) addition strongly destabilized PrP^C structure as indicated by the lowering of the melting temperature [76]. Just the observed destabilization would result from the metal binding to the unfolded form of the prion protein.

In light of these information, comparative studies on copper(II) binding sites and coordination environment with chicken PrP^C may be useful in understanding the potential biological role associated to copper interactions with the protein.

4.1 Prion and copper(II) coordination features: mammal vs avian

It is widely accepted that in mammalian protein the main domain for the copper binding is the N-terminal region, and namely that encompassing the tetraoctarepeats (amino acid residues 60-91) [119-123]. This region can bind from one up to four copper ions, while a fifth binding site has been located within residues 91-111 even if conflicting results have been reported for copper(II) binding features in this region [124-127].

Studies carried out on peptide fragments allowed us to obtain more detailed information on the copper(II) coordination environment.

The histidine-containing octarepeats bind copper(II) at physiological pH with three different coordination modes, depending on the copper peptide molar ratio [128]. At substoichiometric copper(II) concentration, the predominant species consists of three or four imidazole nitrogen atoms bound to the metal center. Increasing the copper amount, the copper(II) is bound to each octa-repeat by means of one imidazole nitrogen, two amide nitrogens from the two glycine residues and one carbonyl oxygen [128-130]. These copper(II) complex species have different K_d values, showing a negative cooperative effect [131]. The affinity for the copper decreases as a function of increasing

metal ion concentration, starting from a K_d of 0.12 nM, when only one copper ion is bound to the tetraoctarepeat, to a K_d of 7-12 μM when the same peptide binds four copper ions [130]. These data suggest that PrP^C function related with copper ion can change as a function of extracellular copper levels.

Differently from mammalian prion proteins, no detailed results have been reported, as far as we know, for the entire chicken prion protein. However, studies have been carried out on peptide fragments encompassing the hexarepeat region [87,132-134] and the sequence encompassing fragments 119-139, which is the well-conserved part corresponding to the neurotoxic 106-126 region of mammalian protein [135-136].

Although it has been reported that the tetrahexarepeat peptide can bind up to four copper atoms, being in this similar to human PrP^C even if with a lower affinity [133], other results questioned this finding [87, 132, 137]. UV-vis and CD studies carried out on chicken hexameric peptide fragments containing two histidine residues indicate that this peptide binds only one copper(II), evidencing the significant role of Gly residues in copper coordination for the mammalian octameric region [132]. X-ray absorption spectroscopy analyses have been performed on Cu(II) complexes with the corresponding repeat sequences of both mammal and avian prion protein at pH 6.5 [137]. The results confirm that single hexarepeat of chicken display a copper(II) coordination environment different from the mammalian octarepeat; avian prion tetrahexapeptide fragments show the same coordination features of mammalian tetraoctarepeats only in the presence of a sub-stoichiometric amount of copper ions [137].

The most important difference in copper(II) coordination can be assigned to the role played by the higher number of proline residues in chicken hexarepeats.

Comparative analysis carried out on copper(II) complex species formed by different hexapeptide sequences PHNPGY, HNPGYP and NPGYPH, reveal a different behaviour with respect to an octarepeat sequence [87]. There is no formation of an analogous $CuLH_{-2}$ species in which the

copper ion is involved in a 3N1O coordination environment. Clearly the proline residues encompassed in the sequence act as break-points in the metal coordination [138].

At physiological pH, the peptide fragments containing two hexarepeats $(PHNPGY)_2$ show the tendency to bind copper through an inter-repeat mode with the involvement of two imidazole nitrogens (Fig. (**4a**)).

Interestingly the bishexarepeat binds one copper ion more tightly than the corresponding human $(PHGGGWQG)_2$ peptide. This finding is in agreement with the lesser number of residues involved in the avian macrochelate complexes and with the greater number of proline residues.

On increasing the copper(II) equivalents, the $(PHNPGY)_2$ peptide is able to bind two copper ions in an intra-repeat mode but with the direct involvement of phenolic group of tyrosine side chain (Fig. (**4b**)). This is indicated by the appearance of a charge transfer (CT) band around 390 nm in the UV-vis and CD spectra, that can be assigned to $TyrO^- \rightarrow Cu(II)$ ligand-metal transition. Moreover the CT band is not observed in the copper(II) complexes with analogous peptides in which tyrosine residues have been substituted by phenylalanine [87].

It is also interesting to note that the involvement of tyrosine in copper binding has been invoked for the copper coordination with Aβ and this has been related also with the formation of tyrosine cross-linking and consequently with the starting of aggregation processes [139].

The study of copper interaction with the tetrahexarepeat $(PHNPGY)_4$ confirms the difference with mammalian $(PHGGGWGQ)_4$ [140, 141]. Differently from that reported for the human $(PHGGGWQG)_4$, the copper(II) complex species of chicken $(PHNPGY)_4$, where the copper ion is bound to four imidazole nitrogens, results predominant in a wide range of pH; this occurs when copper to ligand ratio increases and not only with sub-stoichiometric amount of metal. Such a copper(II) complex species with the avian tetrahexapeptide shows the same stability constant value of the analogous complex with mammalian tetraoctarepeat [140, 141]; similar hamiltonian parameters for the two metal complexes have also been found [140, 128]. These magnetic

parameters indicate that the four imidazole nitrogen atoms are in a planar arrangement, forming a macrochelate (Fig. (**5**)).

The $(PHNPGY)_4$ is able to bind no more than two copper ions and always with the involvement of tyrosine side chain [140]. The addition of more than two equivalents of copper ions favours precipitation phenomena as reported for the entire protein.

4.2 Copper binding sites outside the repeat region

Substantial evidence suggests the presence of a fifth copper(II) binding site outside the octameric region of mammal PrP^C [124-127]. Two histidine residues have been considered the potential anchoring site His96 and His111. Even if contrasting results have been reported, the His111 appears to be the most likely site, displaying a high affinity for the copper(II) [126-127]. The His111 is included in the neurotoxic region 106-126 which is considered critical for the conformational transition for $PrP^C \rightarrow PrP^{Sc}$ [142]. Copper binding to this region has been shown to promote β-sheet formation and to enhance its neurotoxicity [143-146].

Interestingly, this region is perfectly conserved in avian prion protein (see Fig. (**1**)).

The comparison between human and chicken peptide fragments showed similar coordination features. The only remarkable difference is due to a slight contribution of Met sulphur atom for the species 3N1O in which copper is bound to histidine imidazole and two amides of His and Lys residues [135]. The copper(II) coordination to chicken peptide induces the same conformational effects observed in human peptide. The metal ion drives a change from a random coil towards a structured bent conformation, an effect not observed on analogous scrambled peptide with a different primary sequence [135].

Notwithstanding this, no studies have been carried out both on the potential neurotoxicity of chicken 119-139 peptide and on whether this can be affected by metal ions binding as reported for the mammal species.

4.3 The SOD-like activity of copper(II) bound to PrP^C N-terminal region

As reported above there is a lot of evidence to support a direct SOD-like activity of prion protein. However, the SOD activity of recombinant PrP^C protein, measured by means of indirect methods, resulted significantly less than that of native cytosolic Cu-Zn Superoxide Dismutase enzyme (SOD-1), amounting to 30% and 15% for mouse and chicken PrP^C, respectively [115].

The zinc ion, which has a structural role in the Cu-Zn SOD, does not seem to be involved in the prion SOD-like function because the refolding in presence of both copper and zinc does not show any increase of SOD activity, although a recent paper indicates that zinc ion regulates the copper coordination in the octa-repeat region [147].

The prion SOD-like function in mammals is supported by the fact that in the synapses, where the concentration of PrP^C is very high, the presence of Cu-Zn SOD was not observed, therefore PrP^C may be the main, if not the only protein capable to carry on SOD-like activity. Moreover, prion protein-deficient cells show altered response to oxidative stress due to the decrease of SOD-1 activity [148], and immunodepletion of PrP^C from the brain extracts involves a reduction of SOD-1 activity within the extract [116].

In this context, conformational change caused by the binding of copper(II) to the PrP^C could be a switch for the expression of SOD activity. Consequently, an intriguing hypothesis could be that the increased levels of PrP^{Sc} may modify the ROS metabolism, causing the disease [105]. Therefore, a study aimed at identifying the domains which could carry out SOD-like activity, the species formed with copper(II) and their coordination features with respect to the scavenger activity against O_2 should clarify the correlation between PrP^C and SOD activity. The N-terminal region role has been invoked because a mouse prion protein mutant, lacking this octapeptide-repeat region retained the 60% of copper but it was SOD inactive [115]. In addition, the SOD activity of mouse PrP^C mutants with none, one, two, three octarepeats was tested and compared to wild type. The mutants containing one or no octarepeat did not show SOD-1 activity. The mutant PrP^C with two and three

octarepeats displays an activity very similar each other and however lower than that of the wild type protein [149]. Therefore, the presence of a copper(II) coordination with three or four imidazoles in the octarepeats region can favour SOD-like activity for this complex species. A SOD activity has been reported for copper(II) complexes with tetraocta and tetrahexarepeats by means of indirect assays, while longer chicken peptide fragments did not show any activity [150-151].

In contrast with these results, other studies have questioned PrP^C SOD-1 activity [152-153], *in vivo and in vitro*. In particular the experimental approach (indirect assay based on xanthine/xanthine oxidase system) has been challenged [153]. Consequently, the use of a direct method, such as the pulse radiolysis, to determine SOD-like activity is more valuable than indirect assays [140].

Studies carried out by means of pulse radiolysis [140] show that: i) copper complexes with mammalian tetrarepeats, $(PHGGGWGQ)_4$, show a very low SOD-like activity, two orders of magnitude less than SOD enzyme, and less than other copper(II) complexes with small molecules such as amino acids and peptides [154-156]; ii) the analogous complex species with chicken repeats, $(PHNPGY)_4$, display no SOD activity and further, the superoxide radical reacts with $(PHNPGY)_4$ in no catalytic fashion, involving tyrosine residues. Recently, the prion protein-associated SOD-1 activity in Pichia pastoris has been studied [157]. Different test systems showed that prion protein gained a SOD-like activity only in the presence of additional copper in the medium. To explain the conflicting results on SOD-like activity of Prp^C *in vitro and in vivo*, it was proposed that hyperphysiological concentrations of copper are needed for prion protein to act as SOD-1 mimics. Interestingly, the failure of copper complex species with synthetic peptide encompassing the four repeats of the mammalian sequence to show SOD activity was explained invoking the need of the involvement also of the histidines 96, 111 or 187 in the metal binding for the SOD-like activity of PrP^C [157].

Copper complex species with chPrP^{C} peptide fragments show binding constant values [84, 87, 133-135, 140] lower than those found in SOD-1 enzymes. This appears in contrast with the biological significance of a true SOD-like enzyme *in vivo*, which has to bind strongly the metal ion.

The conflicting results here reported stress the need to have more valuable data on key questions concerning the affinity constants of the different copper complex species formed with prion protein, their redox properties and the dual role of copper ion, protective or toxic cofactor in prion diseases [158].

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References

1. Dobson CM. Protein aggregation and its consequences for human disease. Protein Pept Lett 13: 219-227 (2006).

2. Westermark P. Aspects on human amyloid forms and their fibril polypeptides. FEBS J 272: 5942-5949 (2005).

3. Pepys MB. Amyloidosis. Annu Rev Med 57:223-241(2006).

4. Bellotti V, Nuvolone M, Giorgetti S, Obici L, Palladini G, Russo P, Lavatelli F, Perfetti V, and Merlini G. The workings of the amyloid diseases. Ann Med 39: 200-207 (2007).

5. Fandrich M. On the structural definition of amyloid fibrils and other polypeptide aggregates. Cell Mol Life Sci 64: 2066-2078 (2007).

6. Glabe CG and Kayed R. Common structure and toxic function of amyloid oligomers implies a common mechanism of pathogenesis. Neurology 66 (2 Suppl 1): S74-78 (2006).

7. Dobson CM. Principles of protein folding, misfolding and aggregation. Semin Cell Dev Biol 15: 3-16 (2004).

8. Harrison RS, Sharpe PC, Singh Y and Fairlie DP. Amyloid peptides and proteins in review. Rev Physiol Biochem Pharmacol 159: 1-77 (2007).

9. AigelsreiterA, Janig E, Stumptner C, Fuchsbichler A, Zatloukal K and Denk H. How a cell deals with abnormal proteins. Pathogenetic mechanisms in protein aggregation diseases. Pathobiology 74: 145-158 (2007).

10. Khare SD and Dokholyan NV. Molecular mechanisms of polypeptide aggregation in human diseases. Curr Protein Pept Sci 8: 573-579 (2007).

11. Ross CA and Poirier MA. Protein aggregation and neurodegenerative disease. Nat Med 10 Suppl: S10-S17 (2004).

12. Skovronsky DM, Lee VM and Trojanowski JQ. Neurodegenerative diseases: new concepts of pathogenesis and their therapeutic implications. Annu Rev Pathol 1: 151-170 (2006).

13. Finder VH and Glockshuber R. Amyloid-beta aggregation. Neurodegener Dis 4: 13-27 (2007).

14. Soto C, Estrada L and Castilla J. Amyloids, prions and the inherent infectious nature of misfolded protein aggregates. Trends Biochem Sci 31: 150-155 (2006).

15. Beyer K. Mechanistic aspects of Parkinson's disease: alpha-synuclein and the biomembrane. Cell Biochem Biophys 47: 285-299 (2007).

16. Shaw BF and Valentine JS. How do ALS-associated mutations in superoxide dismutase 1 promote aggregation of the protein?. Trends Biochem Sci 32: 78-85 (2007).

17. Stack EC and Ferrante RJ. Huntington's disease: progress and potential in the field. Expert Opin Investig Drugs 16: 1933-1953 (2007).

18. Aguzzi A, Heikenwalser M and Polymenidou M. Insights into prion strains and neurotoxicity. Nat Rev Mol Cell Biol 8: 552-561 (2007).

19. Bertram L and Tanzi RE. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell 120: 545-555 (2005).

20. Ward M. Biomarkers for Alzheimer's disease. Expert Rev Mol Diagn 7: 635-646 (2007).

21. Collinge J. Molecular neurology of prion disease. J Neurol Neurosurg Psychiatry 76: 906-919 (2005).

22. Colucci M, Moleres FJ, Xie ZL, Ray-Chaudhury A, Gutti S, Butefish CM, Cervenakova L, Wang W, Goldfarb LG, Kong Q, Ghetti B, Chen SG and Gambetti P. Gerstmann-Straussler-Scheinker: a new phenotype wit'curly' PrP deposits. J Neuropathol Exp Neurol 65: 642-651 (2006).

23. Windl O, Dempster M, Estiberio JP, Lathe R, de Silva R, Esmonde T, Will R, Springbett A, Campbell TA, Sidle KC, palmer MS and Collinge J. Genetic basis of Creutzfeldt-Jakob disease in the United Kingdom: a systematic analysis of predisposing mutations and allelic variation in the PRNP gene. Hum Genet 98: 259-264 (1996).

24. Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCardle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H and Bostock CJ. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 389: 498-501 (1997).

25. Caughey B and Baron GS. Prions and their partners in crime. Nature 443: 803-810 (2006).

26. Brown RC, Lockwood AH and Sonawane BR. Neurodegenerative diseases: an overview of environmental risk factors. Environ Health Perspect 113: 1250-1256 (2005).

27. Prusiner SB. Molecular biology and pathogenesis of prion diseases. Trends Biochem Sci 21: 482-487 (1996).

28. Pasternak SH, Callahan JW and Mahuran DJ. The role of the endosomal/lysosomal system in amyloid-beta production and the pathophysiology of Alzheimer's disease: reexamining the spatial paradox from a lysosomal perspective. J Alzheimers Dis 6: 53-65 (2004).

29. DeMarco ML and Daggett V. Local environmental effects on the structure of the prion protein. CR Biologies 328: 847-862 (2005).

30. La Mendola D, Magrì A, Milardi D, Pappalardo G and Rizzarelli E. Environmental effects on conformational diseases. Reactive oxygen species and membrane interactions with prion, amylin and their peptide fragments. In: 'Recent Development in Bioinorganic Chemistry: Metal Complexes of Bioactive Molecules' (Ed. Saviano M.) Transworld Research Network, Kerala, p. 85-110 (2006).

31. Picotti P, De Franceschi G, Frare E, Spolaore B, Zambonin M, Chiti F, de Laureto PP and Fontana A. Amyloid fibril formation and disaggregation of fragment 1-29 of apomyoglobin: insights into the effect of pH on protein fibrillogenesis. J Mol Biol 367: 1237-1245 (2007).

32. Gaeta A and Hider RC. The crucial role of metal ions in neurodegeneration: the basis for a promising therapeutic strategy Br J Pharmacol 146: 1041-1059 (2005).

33. Mrak RE and Griffin WS. Common inflammatory mechanisms in Lewy body disease and Alzheimer disease. J Neuropathol Exp Neurol 66: 683-686 (2007).

34. Molina-Holgado F, Hider RC, Gaeta A, Williams R and Francis P. Metals ions and neurodegeneration. Biometals 20: 639-654 (2007).

35. Chiti F and Dobson CM. Protein misfolding and diseases. Ann Rev Biochem 75: 333-366 (2006).

36. Glabe CG. Common mechanism of amyloid oligomer pathogenesis in degenerative disease. Neurobiol Aging 27: 570-575 (2006).

37. Ferreira ST, Vieira MN and De Felice FG. Soluble protein oligomers as emerging toxins in Alzheimer's and other amyloid diseases. IUBMB Life 59: 332-345 (2007).

38. Liu G, Huang W, Moir RD, Vanderburg CR, Lai B, Peng Z, Tanzi RE, Rogers JT and Huang X. Metal exposure and Alzheimer's pathogenesis. J Struct Biol 155: 45-51 (2006).

39. Huang X, Atwood CS, Moir RD, Hartshorn MA, Tanzi RE and Bush AI. Trace metal contamination initiates the apparent auto-aggregation, amyloidosis, and oligomerization of Alzheimer's Abeta peptides. J Biol Inorg Chem 9: 954-960 (2004).

40. Zatta P and Frank A. Copper deficiency and neurological disorders in man and animals. Brain Res Rev 54: 19-33 (2007).

41. Ong WY and Farooqui AA. Iron, neuroinflammation, and Alzheimer's disease. J Alzheimers Dis 8: 183-200 (2005).

42. Mocchegiani E, Bertoni-Freddari C, Marcellini F and Malavolta M. Brain, aging and neurodegeneration: role of zinc ion avalaibility. Prog Neurobiol 75: 367-390 (2005).

43. Tarohda T, Yamamoto M and Amamo R. Regional distribution of manganese, iron, copper, and zinc in the rat brain during development. Anal Bioanal Chem 380: 240-246 (2004).

44. Jackson B., Harper S, Smith L and Flinn J. Elemental mapping and quantitative analysis of Cu, Zn and Fe in rat brain sections by laser ablation ICP-MS. Anal Bioanal Chem 384: 951-957 (2006).

45. Maynard CJ, Cappai R, Volitakis I, Cherny RA, White AR, Beyreuther K, Masters CL, Bush AI and Li QX. Overexpression of Alzheimer's disease amyloid-beta opposes the age-dependent elevations of brain copper and iron. J Biol Chem 277: 44670-44676 (2002).

46. Adlard PA and Bush AI. Metals and Alzheimer's disease. J Alzheimers Dis 10: 145-163 (2006).

47. Lovell MA, Robertson JD, Teesdale WJ, Campbell JL and Markesbery WR. Copper, iron and zinc in Alzheimer's disease senile plaques. J Neurol Sci 158: 47-52 (1998).

48. Smith DG, Cappai R and Barnham KJ. The redox chemistry of the Alzheimer's disease amyloid beta peptide. Biochim Biophys Acta 1768: 1976-1990 (2007).

49. Shivers BD, Hilbich C, Multhaup G, Salbaum M, Beyreuther K and Seeburg PH. Alzheimer's disease amyloidogenic glycoprotein: expression pattern in rat brain suggests a role in cell contact. EMBO J 7: 1365-1370 (1988).

50. Atwood CS, Moir RD, Huang X, Scarpa RC, Bacarra NM, Romano DM, Hartshorn MA, Tanzi RE and Bush AI. Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. J Biol Chem 273: 12817-12826 (1998).

51. Liu ST, Howlett G and Barrow CJ. Histidine-13 is a crucial residue in the zinc ion-induced aggregation of the Abeta peptide of Alzheimer's disease. Biochemistry 38: 9373-9378 (1999).

52. Shen L and Ji HF. Rat's trick to escape Alzheimer's disease. J Biomol Struct Dyn 25: 271-274 (2007).

53. Wopfner F, Weidenhofer G, Schneider R, von Brunn A, Gilch S, Schwarz T F, Werner T and Schatzl H M. Analysis of 27 mammalian and 9 avian PrPs reveals high conservation of flexible regions of the prion protein. J Mol Biol 289: 1163-1178 (1999).

54. van Rheede T, Smolenaars MMW, Madsen O and de Jong WW. Molecular evolution of the mammalian prion protein. Mol Biol Evol 20: 111-121 (2003)

55. Harris DA, Falls DL, Johnson FA and Fischbach GD. A prion-like protein from chicken brain copurifies with an acetylcholine receptor-inducing activity. Proc Natl Acad Sci USA 88: 7664-7668 (1991).

56. Gabriel JM, Oesch B, Kretzschamer H, Scott M and Prusiner SB. Molecular cloning of a candidate chicken prion protein. Proc Natl Acad Sci USA 89: 9097-90101 (1992).

57. Simonic T, Duga S, Strumbo B, Asselta R, Ceciliani F and Ronchi S. cDNA cloning of turtle prion protein. FEBS Lett 469: 33-38 (2000).

58. Strumbo B, Ronchi S, Bolis LC and Simonic T. Molecular cloning of the cDNA coding for Xenopus laevis prion protein. FEBS Lett 508: 170-174 (2001).

59. Suzuki T, Kurokawa T, Hashimoto H and Sugiyama M. cDNA sequence and tissue expression of Fugu rubripes prion protein-like: a candidate for the teleost orthologue of tetrapod PrPs. Biochem Biophys Res Commun 294: 912-917 (2002).

60. Stahl N and Prusiner SB. Prions and prion proteins. FASEB J 5: 2799-2807 (1991).

61. Prusiner SB. Prions, Nobel Lecture. Proc Natl Acad Sci USA 95: 13363-13383 (1998).

62. Horiuchi M, Yamazaki N, Ikeda T, Ishiguro N and Shinagawa M. A cellular form of prion protein (PrP^{C}) exists in many non-neuronal tissues of sheep. J Gen Virol 76: 2583-2587 (1995).

63. Stahl N, Baldwin MA, Teplow DB, Hood L, Gibson BW, Burlingame AL and Prusiner SB. Structural studies of the scrapie prion protein using mass spectrometry and amino acid sequencing. Biochemistry 32: 1991-2002 (1993).

64. Zou WQ and Gambetti P. Prion: the chameleon protein. Cell Mol Life Sci 64: 3266-3270 (2007).

65. Bueler H, Fisher M, Lang Y, Bluethmann H, Lipp HP, DeArmond SJ, Prusiner SB, Aguet M and Weissmann C. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature 356: 577-582 (1992).

66. Brandner S, Isenmann S, Raeber A, Fischer M, Sailer A, Kobayashi Y, Marino S, Weissmann C and Aguzzi A. Normal host prion protein necessary for scrapie-induced neurotoxicity. Nature 379: 339-343 (1996).

67. Rochet J C and Lansbury P Jr. Amyloid fibrillogenesis: themes and variations. Curr Opin Struct Biol 10: 60-68 (2000).

68. Brown DR, Clive C and Haswell SJ. Antioxidant activity related to copper binding of native prion protein. J Neurochem 76: 69-76 (2001).

69. Chiesa R, Drisaldi B, Quaglio E, Migheli A, Piccardo P, Ghetti B and Harris D. Accumulation of protease-resistant prion protein (PrP) and apoptosis of cerebellar granule cells in transgenic mice expressing a PrP insertional mutation. Proc Natl Acad Sci USA 97: 5574-5579 (2000).

70. Mouillet-Richard S, Ermonval M, Chebassier C, Laplanche JL, Lehmann S, Launay JM and Kellermann O. Signal transduction through prion protein. Science 289: 1925-1928 (2000).

71. Herms J, Tings T, Gall S, Madlung A, Giese A, Siebert H, Schurmann P, Windl O, Brose N and Kretzschmar H. Evidence of presynaptic location and function of the prion protein. J Neurosci 19: 8866-8875 (1999).

72. Brown DR, Qin K, Herms JW, Madlung A, Manson J, Strome R, Fraser PE, Kruc T, von Bohlen A, Schultz Schaeffer W, Giese A, Westaway D and Kretzschmar H. The cellular prion protein binds copper in vivo. Nature 390: 684-687 (1997).

73. Prado MA, Alves-Silva J, Magalhães AC, Prado VF, Linden R, Martins VR and Brentani RR. PrP^{C} on the road: trafficking of the cellular prion protein. J Neurochem 88: 769-781 (2004).

74. Marcotte, EM, Pellegrini, M, Yeates TO and Eisenberg D. A census of protein repeats. J Mol Biol 293: 151-160 (1998).

75. Calzolai L, Lysek DA, Perez DR, Guntert P and Wüthrich K. Prion protein NMR structures of chickens, turtles, and frogs. Proc Natl Acad Sci USA 102: 651-655 (2005).

76. Marcotte EM and Eisemberg D. Chicken prion tandem repeats form a stable, protease resistant domain. Biochemistry 38: 667-676 (1999).

77. Ravi Chandra B, Gowthaman R, Raj Akhouri R, Gupta D and Sharma A. Distribution of proline-rich (PxxP) motifs in distinct proteomes: functional and therapeutic implications for malaria and tuberculosis. Protein Eng Des Sel 17: 175-182 (2004).

78. Lysek, DA and Wuthrich, K. Prion protein interaction with the C-terminal SH3 domain of Grb2 studied using NMR and optical spectroscopy. Biochemistry 43: 10393-10399 (2004).

79. Nunziante M, Gilch S and Schätzl HM. Essential role of the prion protein N-terminus in subcellular trafficking and half-life of cellular prion protein. J Biol Chem 278: 3726-3734 (2003).

80. Frankenfield KN, Powers ET and Kelly JW. Influence of the N-terminal domain on the aggregation properties of the prion protein. Protein Sci 14: 2154-2166 (2005).

81. Butowt R, Davies P and Brown DR. Anterograde axonal transport of chicken cellular prion protein (PrPc) in vivo requires its N-terminal part. J Neurosci Res 85: 2567-2579 (2007).

82. Shyng SL, Moulder KL, Lesko A and Harris DA. The N-terminal domain of a glycolipid-anchored prion protein is essential for its endocytosis via clathrin-coated pits. J Biol Chem 270: 14793-14800 (1995).

83. Pietropaolo A, Raiola L, Muccioli L, Tiberio G, Zannoni C, Fattorusso R, Isernia C, La Mendola D, Pappalardo G and Rizzarelli E. An NMR and Molecular Dynamics investigation of the avian prion hexarepeat conformational features in solution. Chem Phys Letters 442: 110-118 (2007).

84. Pietropaolo A, Muccioli L, Zannoni C, La Mendola D, Maccarrone G, Pappalardo G and Rizzarelli E. Unveiling the role of histidine and tyrosine residues on the conformation of the avian prion hexarepeat domain. J Phys Chem B 112: 5182-5188 (2008).

85. Bansal A and Gierasch LM. The NPXY internalization signal of the LDL receptor adopts a reverse-turn conformation. Cell 67: 1195-1201 (1991).

86. Paccaud JP, Reith W, Johansson B, Magnusson KE, Mach B and Carpenter JL. Clathrin-coated pit-mediated receptor internalization. Role of internalization signals and receptor mobility. J Biol Chem 268: 23191-23196 (1993).

87. La Mendola D, Bonomo RP, Impellizzeri G, Maccarrone G, Pappalardo G, Rizzarelli E, Pietropaolo A and Zito V. Copper(II) complexes with chicken prion repeats: influence of proline and tyrosine residues on the coordination features. J Biol Inorg Chem 10: 463-475 (2005).

88. Rivera-Milla E, Stuermer CA and Malaga-Trillo E. An evolutionary basis for scrapie disease: identification of a fish prion mRNA. Trends Genet 19: 72-75 (2003).

89. Pappalardo M, Milardi D, Grasso D and La Rosa C. Steered molecular dynamics studies reveal different unfolding pathways of prions from mammalian and non-mammalian species.javascript:submit_form() New J Chem 31: 901-905 (2007).

90. Ji HF and Zhang HY. A comparative molecular dynamics study on thermostability of human and chicken prion proteins. Biochem Biophys Res Comm 359: 790-794 (2007).

91. De Simone A, Dodson GG, Zagari A and Fraternali F. Water molecules as structural determinants among prions of low sequence identity. FEBS Lett 580: 2488-2494 (2006).

92. Pietropaolo A, Muccioli L, Zannoni C and Rizzarelli E. Conformational stiffness of the full chicken Prion Protein in solution and its differencies with respect to mammals. submitted (2008).

93. Dima RI and Thirumalai D. Exploring the Propensities of Helices in PrP^C to fom β-Sheet Using NMR Structures and Sequence Alignments Biochem J 83: 1268-1280 (2002).

94. Pappalardo M, Milardi D, La Rosa C, Zannoni C, Grasso D and Rizzarelli E. Molecular dynamics study on the conformational stability of 180-193 Prion second helix fragment.Chem Phys Lett 390: 511-516 (2004).

95. George RA and Heringa J. An analysis of protein domain linkers: their classification and role in protein folding. Protein Eng 15: 871-879 (2003).

96. Barducci A, Chelli R, Procacci P, Schettino V, Gervasio F L and Parrinello M. Metadynamics simulation of prion protein: beta-structure stability and the early stages of misfolding. J Am Chem Soc 128: 2705-2710 (2006).

97. Crichton RR and Pierre JL. Old iron, young copper: from Mars to Venus. Biometals 14: 99-112 (2001).

98. MacPherson IS and Murphy ME, Type-2 copper-containing enzymes. Cell Mol Life Sci 64: 2887-2899 (2007).

99. Bertinato J and L'Abbe MR, Maintaining copper homeostasis: regulation of copper-trafficking proteins in response to copper deficiency or overload. J Nutr Biochem 15: 316-322 (2004).

100. Bayer TA, Schafer S, Breyhan H, Wirths O, Treiber C and Multhaup G. A vicious circle: role of oxidative stress, intraneuronal Abeta and Cu in Alzheimer's disease. Clin Neuropathol 25: 163-171 (2006).

101. Brown DR. Neurodegeneration and oxidative stress: prion disease results from loss of antioxidant defence. Folia Neuropathol 43: 229-243 (2005).

102. Milhavet O and Lehmann S. Oxidative stress and the prion protein in transmissible spongiform encephalopathies. Brain Res Brain Res Rev 38: 328-339 (2002).

103. Kim JI, Choi SI, Kim NH, Jin JK, Choi EK, Carp RI and Kim Ys. Oxidative stress and neurodegeneration in prion diseases. Ann N Y Acad Sci 928: 182-186 (2001).

104. Quaglio E, Chiesa R and Harris DA. Copper converts the cellular prion protein into a protease-resistant species that is distinct from the scrapie isoform. J Biol Chem 276: 11432-11438 (2001).

105. Brown DR. Copper and prion disease. Brain Res Bull 55: 165-173 (2001).

106. Westergard L, Christensen HM and Harris DA. The cellular prion protein (PrP(C)): its physiological function and role in disease. Biochim Biophys Acta 1772: 629-644 (2007).

107. Choi CJ, Kanthasamy A, Anantharam V and Kanthasamy AG. Interaction of metals with prion protein: possible role of divalent cations in the pathogenesis of prion diseases. Neurotoxicology 27: 777-787 (2006).

108. Brown DR and Sasson J. Copper-dependent functions for the prion protein. Mol Biotechnol 22: 165-178 (2002).

109. Vassallo N and Herms J. Cellular prion protein function in copper homeostasis and redox signalling at the synapse. J Neurochem 86: 538-544 (2003).

110. Brown DR. Trends Neurosci 24: 85-90 (2001).

111. Perera WS and Hooper NM. Ablation of the metal ion-induced endocytosis of the prion protein by disease-associated mutation of the octarepeat region. Curr Biol 11: 519–523 (2001).

112. Taylor DR, Watt NT, Perera WS and Hooper NM. Assigning functions to distinct regions of the N-terminus of the prion protein that are involved in its copper-stimulated, clathrin-dependent endocytosis. J Cell Sci 118: 5141–5153 (2005).

113. Pauly P C and Harris D A. Copper stimulates endocytosis of the prion protein. J Biol Chem 273: 33107-33110 (1998).

114. Brown DR and Besinger A. Prion protein expression and superoxide dismutase activity. Biochem J 334: 423-429 (1998).

115. Brown DR, Wong BS, Hafiz F, Clive C, Haswell SJ and Jones IM. Normal prion protein has an activity like that of superoxide dismutase. Biochem J 344: 1-5 (1999).

116. Wong BS, Pan T, Liu T, Li R, Gambetti P and Sy MS. Differential contribution of superoxide dismutase activity by prion protein in vivo. Biochem Biophys Res Commun 273: 136-139 (2000).

117. Hornshaw MP, McDermott JR and Candy JM. Copper binding to the N-terminal tandem repeat regions of mammalian and avian prion protein. Biochem Biophys Res Commun 207: 621-629 (1995).

118. Hornshaw MP, McDermott JR, Candy JM and Lakey JH. Copper binding to the N-terminal tandem repeat region of mammalian and avian prion protein: structural studies using synthetic peptides. Biochem Biophys Res Commun 214: 993-999 (1995).

119. Millhauser, GL. Copper binding in the prion protein. Acc Chem Res 37: 79-85 (2004).

120. Whittal RM, Ball HL, Cohen FE, Burlingame AL, Prusiner SB and Baldwin MA. Copper binding to octarepeat peptides of the prion protein monitored by mass spectrometry. Protein Sci 9: 332-343 (2000).

121. Qin K, Yang Y, Mastrangelo P and Westaway D. Mapping Cu(II) binding sites in prion proteins by diethyl pyrocarbonate modification and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometric footprinting. J Biol Chem 277: 1981-1990 (2002).

122. Burns CS, Aronoff-Spencer E, Legname G, Prusiner SB, Antholine WE, Gerfen GJ, Peisach J and Millhauser GL. Copper coordination in the full-length, recombinant prion protein. Biochemistry 42: 6794-6803 (2003).

123. Bonomo RP, La Mendola D, Pappalardo G, Rizzarelli E and Sovago I. Coordination features of prion protein domains. In “Recent Development in Bioinorganic Chemistry: Metal Complexes of Bioactive Molecules” (Ed. Saviano M.) Transworld Research Network, Kerala, p. 133-160 (2006).

124. Thompsett AR, Abdelraheim SR, Daniels M and Brown DR. High affinity binding between copper and full-length prion protein identified by two different techniques. J Biol Chem 280: 42750-42758 (2005).

125. Jackson GS, Murray I, Hosszu LL, Gibbs N, Waltho JP, Clarke AR and Collinge J. Location and properties of metal-binding sites on the human prion protein. Proc Natl Acad Sci USA 98: 8531-85355 (2001

126. Klewpatinond M and Viles JH. Fragment length influences affinity for Cu^{2+} and Ni^{2+} binding to His96 or His111 of the prion protein and spectroscopic evidence for a multiple histidine binding only at low pH. Biochem J 404: 393-402 (2007).

127. Osz K, Nagy Z, Pappalardo G, Di Natale G, Sanna D, Micera G, Rizzarelli E and Sovago I. Copper(II) interaction with prion peptide fragments encompassing histidine residues within and outside the octarepeat domain: speciation, stability constants and binding details. Chemistry Eur J 13: 7129-7143 (2007).

128. Chattopadhyay M, Walter ED, Newell, D J, Jackson, P J, Aronoff-Spencer E, Peisach J, Gerfen G J, Bennett B, Antholine W E, and Millhauser G L. The octarepeat domain of the prion protein binds Cu(II) with three distinct coordination modes at pH 7.4. J Am Chem Soc 127: 12647-12656 (2005).

129. Burns CS, Aronoff-Spencer E, Dunham CM, Lario P, Avdievich NI, Olmstead MM; Vrielink A, Gerfen GJ, Peisach J, Scott WG and Millhauser GL. Molecular features of the copper binding sites in the octarepeat domain of the prion protein. Biochemistry 41: 3991-4001 (2002).

130. Bonomo RP, Cucinotta V, Giuffrida A, Impellizzeri G, Magrì A, Pappalardo G, Rizzarelli E, Santoro AM, Tabbì G and Vagliasindi LI. A re-investigation of copper coordination in the octa-repeats region of the prion protein. Dalton Trans 150-158 (2005).

131. Walter ED, Chattopadhyay M and Millhauser GL. The affinity of copper binding to the prion protein octarepeat domain: evidence for negative cooperativity. Biochemistry 45: 13083-13092 (2006).

132. Garnett AP and Viles JH. Copper binding to the octarepeats of the prion protein. Affinity, specificity, folding, and cooperativity: insights from circular dichroism. J Biol Chem 278: 6795-6802 (2003).

133. Stanczak P, Luczkowski M, Juszczyk P, Grzonka Z and Kozlowski H. Interactions of Cu^{2+} ions with chicken prion tandem repeats.Dalton Trans 2102-2107 (2004).

134. Stanczak P, Valensin D, Juszczyk P, Grzonka Z, Migliorini C, Molteni E, Valensin G, Gaggelli E, Kozlowski H. Structure and stability of the Cu(II) complexes with tandem repeats of the chicken prion. Biochemistry 44: 12940-12954 (2005).

135. Di Natale G, Grasso G, Impellizzeri G, La Mendola D, Micera G, Mihala N, Nagy Z, Osz K, Pappalardo G, Rigo V, Rizzarelli E, Sanna D and Sovago I.http://www.ncbi.nlm.nih.gov/pubmed/16180886?ordinalpos=74&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum Copper(II) interaction with unstructured prion domain outside the octarepeat region: speciation, stability, and binding details of copper(II) complexes with PrP106-126 peptides. Inorg Chem 44: 7214-7225 (2005).

136. Joszai V, Nagy Z, Osz K, Sanna D, Di Natale G, La Mendola D, Pappalardo G, Rizzarelli E and Sovago I. Transition metal complexes of terminally protected peptides containing histidyl residues. J Inorg Biochem 100: 1399-1409 (2006).

137. Redecke L, Meyer-Klaucke W, Koker M, Clos J, Georgieva D, Genov N, Echner H, Kalbacher H, Perbandt M, Bredehorst R, Voelter W and Betzel C. Comparative analysis of the human and chicken prion protein copper binding regions at pH 6.5. J Biol Chem 280:13987-13992 (2005).

138. Bataille M, Formicka-Kozlowska K, Kozlowski H, Pettit LD and Steel I. The L-proline residue as a break-point in the co-ordination of metal-peptide systems. J Chem Soc Chem Commun 4: 231-234 (1984)

139. Atwood CS, Perry G, Zeng H, Kato Y, Jones WD, Ling KQ, Huang X, Moir RD, Wang D, SayreLM, Smith MA, Chen SG and Bush AI. Copper mediates dityrosine cross-linking of Alzheimer's amyloid-beta. Biochemistry 43: 560-568 (2004).

140. La Mendola D, Bonomo RP, Carminati S, Di Natale G, Emmi SS, Hansson O, Maccarrone G, Pappalardo G, Pietropaolo A and Rizzarelli E. Copper(II) complexes with avian prion N-terminal region and their potential SOD-like activity. submitted (2008)

141. Valensin D, Luczkowski M, Mancini FM, Legowska A, Gaggelli E, Valensin G, Rolka K and Kozlowski H. The dimeric and tetrameric octarepeat fragments of prion protein behave differently to its monomeric unit. Dalton Trans 1284-1293 (2004).

142. Fioriti L, Quaglio E, Massignan T, Colombo L, Stewart RS, Salmona M, Harris DA, Forloni G and Chiesa R. The neurotoxicity of prion protein (PrP) peptide 106-126 is independent of the expression level of PrP and is not mediated by abnormal PrP species. Mol Cell Neurosci 28: 165-176 (2005).

143. Jobling MF, Huang X, Stewart LR, Barnham KJ, Curtain C, Volitakis I, Perugini M, White AR, Cherny RA, Masters CL, Barrow CJ, Collins SJ, Bush AI, and Cappai R. Copper and zinc binding modulates the aggregation and neurotoxic properties of the prion peptide PrP106-126. Biochemistry 40: 8073-8084 (2001).

144. Kourie JI, Kenna BL, Tew D, Jobling MF, Curtain CC, Masters CL, Barnham KJ and Cappai R. Copper modulation of ion channels of PrP[106-126] mutant prion peptide fragments. J Membr Biol 193: 35-45 (2003).

145. Grasso D, Milardi D, la Rosa C and Rizzarelli E. The different role of Cu^{2+} and Zn^{2+} ions in affecting the interaction of prion peptide PrP106-126 with model membranes. Chem Commun 246-247 (2004)

146. Jones CE, Abdelraheim SR, Brown DR and Viles JH. Preferential Cu^{2+} coordination by His96 and His111 induces β-sheet formation in the unstructured amyloidogenic region of the prion protein. J Biol Chem 279: 32018-32027 (2004).

147. Walter ED, Stevens DJ, Visconte MP and Millhauser GL. The prion protein is a combined zinc and copper binding protein: Zn^{2+} alters the distribution of Cu^{2+} coordination modes. J Am Chem Soc 129: 15440-15441 (2007).

148. Brown, DR, Schulz-Schaeffer WJ, Schmidt B and Kretzschmar HA, Prion protein-deficient cells show altered response to oxidative stress due to decreased SOD-1 activity. Exp Neurol 146:104-112 (1997).

149. Brown DR, Hafiz F, Glasssmith LL, Wong BS, Jones IM, Clive C and Haswell SJ. Consequences of manganese replacement of copper for prion protein function and proteinase resistance. EMBO J 19: 1180-1186 (2000).

150. Stanczak P and Kozlowski H. Can chicken and human PrPs possess SOD-like activity after beta-cleavage? Biochem Biophys Res Commun 352: 198-202 (2007).

151. Stanczak P, Juszczyk P, Grzonka Z and Kozlowski H. The whole hexapeptide repeats domain from avian PrP displays untypical hallmarks in aspect of the Cu^{2+} complexes formation. FEBS Lett 581: 4544-4548 (2007).

152. Hutter G, Heppner FL and Aguzzi A. No superoxide dismutase activity of cellular prion protein in vivo. Biol Chem. 384: 1279-1285 (2003).

153. Jones S, Batchelor M, Bhelt D, Clarke AR, Collinge J and Jackson GS. Recombinant prion protein does not possess SOD-1 activity. Biochem J 392: 309-312 (2005).

154. Weinstein J and Bielski BHJ. Reaction of superoxide radicals with copper(II)-histidine complexes. J Am Chem Soc 102: 4916-4919 (1980).

155. Goldstein S, Czapski G and Meyerstein D. A mechanistic study of the copper(II)-peptide catalyzed superoxide dismutation. A pulse radiolysis study. J Am Chem Soc 112: 6489-6492 (1990).

156. Bonomo RP, Bruno V, Conte E, De Guidi G, La Mendola D, Maccarrone G, Nicoletti F, Rizzarelli E, Sortino S and Vecchio G. Potentiometric, spectroscopic and biological activity studies of SOD mimics containing carnosine. Dalton Trans 4406-4415 (2003).

157. Treiber C, Pipkorn R, Weise C, Holland G and Multhaup G. Copper is required for prion protein-associated superoxide dismutase-1 activity in Pichia pastoris. FEBS J 274: 1304-1317 (2007).

158. Davies P and Brown DR. Biochem J 410: 237-244 (2008).

Figure Captions

Figure 1. Sequence alignment of prion proteins from different species.

Figure 2. Primary structure of chicken prion protein ($chPrP^{C}$).

Figure 3. Tetra-hexarepeat domain conformations as a function of pH (acidic LH_8^{4+}, neutral LH_4^{1};LH_4^{2} and basic L^{4-}) obtained from MD simulations ref [84]. The different structures are shown according to a colour code: blue for turn, violet for 3_{10} helix and gray for coil regions. The phenolate hydrogens and oxygens of tyrosines 6, 12, 18 and 24 are shown respectively in white and red, the imidazole nitrogens of histidines 2, 8,14, 20 are shown in silver blue and the amide hydrogens of asparagine 21 are shown in white. The side chains hydrogen bonds are circled in blue. N and C termini are shown respectively from left to right.

Figure 4. a) Major complex species 2N(Imidazole)2O(water); b) Minor complex species 2N (Imidazole, amide)2O(tyrosine,water).

Figure 5. Copper coordination mode of metal ion complex with the tetra-hexarepeat domain suggested from EPR parameters ($g_{\parallel} = 2.257$; $A_{\parallel} = 187 \times 10^{-4}$ cm^{-1}).


Chicken MARLLTTCCLLALLLAACTDVALSKKGKGKPSGGGWGAGSHRQPSYPRQPGYPHNPGYPH 60
turtle MGRYRLTCWIVVLLVVMWSDVSFSKKGKGK-GGGGGNTGSNRNPNYPSNPGYPQNPGYPR 59
mouse --MANLGYWLLALFVTTCTDVGLCKK--RPKPGG-WNTGGSRYPGQGSPGGNRYPPQSGG 55
human --MANLGCWMLVLFVATWSDLGLCKK--RPKPGG-WNTGGSRYPGQGSPGGNRYPPQGGG 55
cattle MVKSHIGSWILVLFVAMWSDVGLCKK--RPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGG 58
Xenopus -MPQSLWTCLVLISLICTLTVSSKKSGGGKSKTGGWNTGSNRNPNYPGG----YPGNTGG 55
chicken NPGYPHNPGYPHNPGYPQNPGYPHNPGYP----------GWGQGYNPSSGGS-YHNQ 106
turtle NPSYPHNPAYPPNPAYPPNPGYPHNPSYPRNPSYPQNPGYPGGGGQHYNPAGGGTNFKNQ 119
mouse TWGQPHGGGWGQPHGG--GWGQPHG----------GGWGQPHGGGWSQGGGTHN 97
human GWGQPHGGGWGQPHGG--GWGQPHG----------GGWGQPHGGGWGQGGGTHS 97
cattle GWGQPHGGGWGQPHGG--GWGQPHG----------GGWGQPHGGGGWGQGGSHS 100
Xenopus SWGQ----------QPYNPS------GYN 68
chicken KPWKP--PKTNFKHVAGAAAAGAVVGGLGGYAMGRVMSGMNYHFDRPDEYRWWSENSARY 164
turtle KPWKPDKPKTNMKAMAGAAAAGAVVGGLGGYALGSAMSGMRMNFDRPEERQWWNENSNRY 179
mouse QWNKPSKPKTNLKHVAGAAAAGAVVGGLGGYMLGSAMSRPMLHFGNDWEDRYYRENMYRY 157
human QWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHFGSDYEDRYYRENMHRY 157
cattle QWNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYYRENMYRY 160
Xenopus KQWKPPKSKTNMKSVAIGAAAGAIG----GYMLGNAVGRMSYQFNNPMESRYYNDYYNQM 124
chicken PNRVYYRDYSS--PVPQDVFVADCFNITVTEYSIGPAAKKNTSEAVAAANQTEVEMENKV 222
turtle PNQVYYKEYNDR-SVPEGRFVRDCLNNTVTEYKIDPNE----------NQNVTQVEVRV 227
mouse PNQVYYRPVDQY-SNQNN-FVHDCVNITIKQHTVTTTTK----------GENFTETDVKM 205
human PNQVYYRPMDEY-SNQNN-FVHDCVNITIKQHTVTTTTK----------GENFTETDVKM 205
cattle PNQVYYRPVDQY-SNQNN-FVHDCVNITVKQHTVTTTTK----------GENFTETDIKI 208
Xenopus PNRVYRPMYRGEEYVSEDRFVRDCYNMSVTEYIIKPTEGKN--------NSELNQLDTTV 176
chicken VTKVIREMCVQQYREYR------LASGIQLHPADTWLAVLLLLLTTLFAMH 267
turtle MKQVIQEMCMQQYQQYQ------LASGVKLLSDPSLMLIIMLVIFFVMH-- 270
mouse MERVVEQMCVTQYQKESQAYYDGRRSSAVLFSSPPVILLISFLIFLIVG-- 254
human MERVVEQMCITQYERESQAYYQ-RGSSMVLFSSPPVILLISFLIFLIVG-- 253
cattle MERVVEQMCITQYQRESQAYYQ-RGASVILFSSPPVILLISFLIFLIVG-- 256
Xenopus KSQIIREMCITEYRRGS---------GFKVLSNPWLILTITLFVYFVIE-- 216

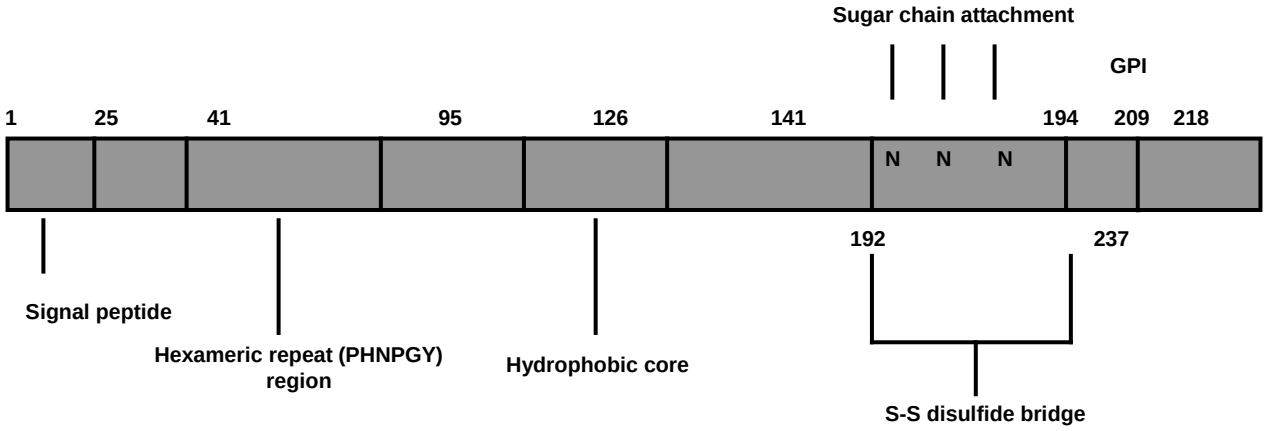


Figure 2. Primary structure of chicken prion protein chPrP^{C}

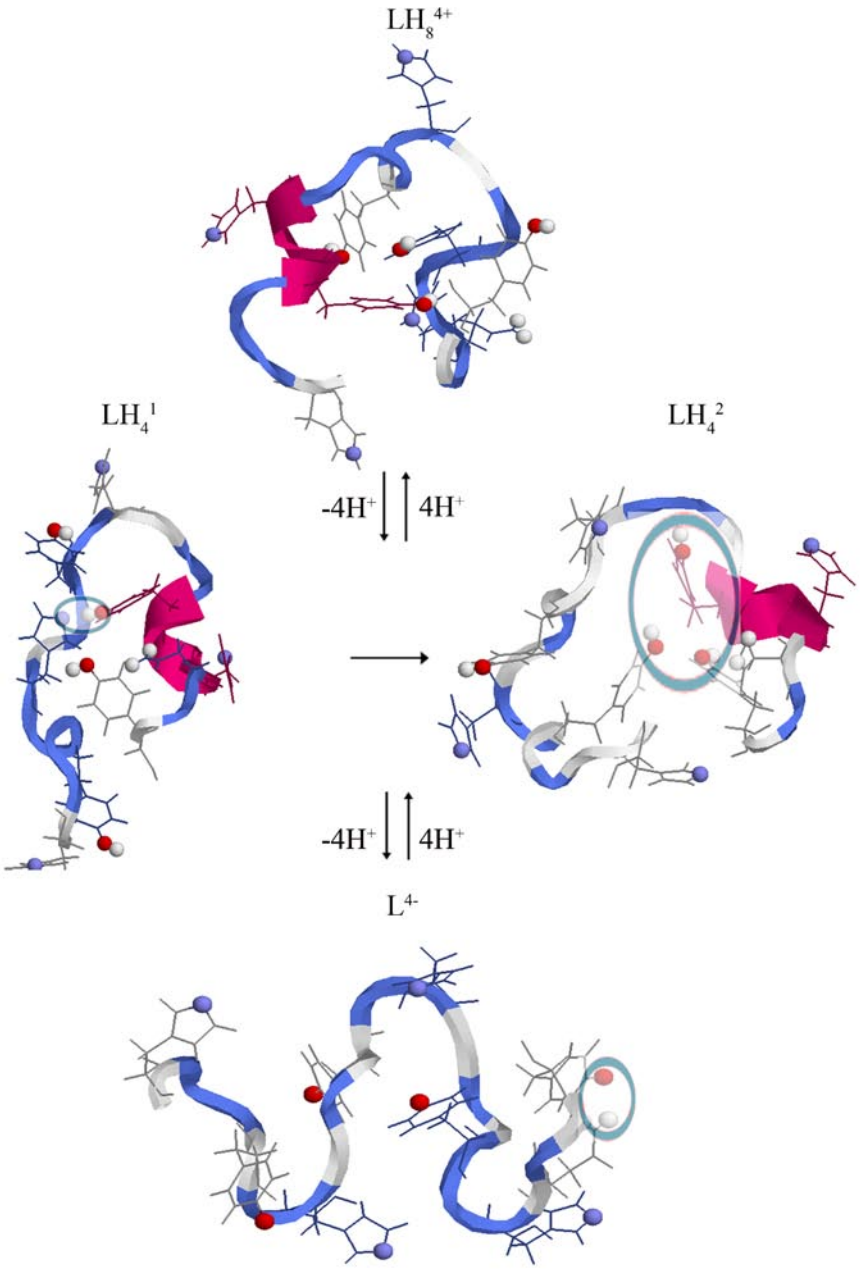
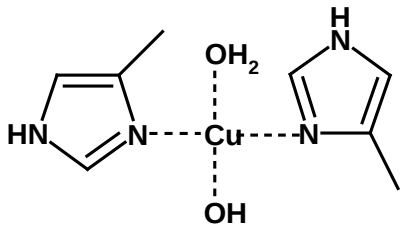


Figure 3: Tetra-hexarepeat domain conformations as a function of pH (acidic LH_8^{4+}, neutral LH_4^1;LH_4^2 and basic L^{4-}) obtained from MD simulations of ref [84]. It is worth noting the strong conformational dependence on pH: At acidic pH the histidine 8 is involved in a 3_{10} helix. At neutral pH, upon the deprotonation of the four histidines, this latter helix is disrupted because of the interaction between the imidazole nitrogen of histidine 8 and the phenol hydrogen of tyrosine 18 (LH_4^1). Such a conformation leads to the formation of another one in which tyrosines 6, 18, 24 are involved in a hydrogen bond network, as shown in LH_4^2. At basic pH, when the four tyrosines are deprotonated (L^{4-}, bottom) , a new interaction between the phenolate oxygen of tyrosine 24 and the side chain amide hydrogens of asparagine 21 causes a tilt of the peptide backbone, bringing all phelolate residues towards the water solvent (not shown for clarity). The different structures are shown according to a colour code: blue for turn, violet for 3_{10} helix and gray for coil regions. The phenolate hydrogens and oxygens of tyrosines 6, 12, 18 and 24 are shown respectively in white and red, the imidazole nitrogens of histidines 2, 8,14, 20 are shown in silver blue and the amide hydrogens of asparagine 21 are shown in white. The side chains hydrogen bonds are circled in blue. N and C termini are shown respectively from left to right.

a)



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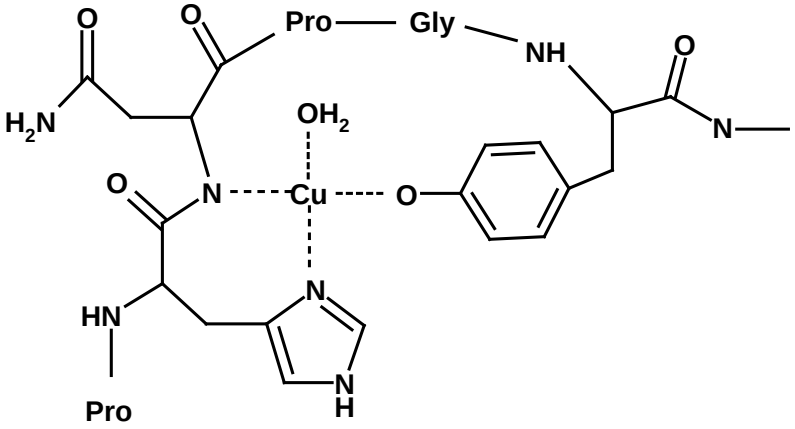

Figure 4: a) Major complex species 2N(Imidazole)2O(water); b) Minor complex species 2N (Imidazole, amide)2O(tyrosine,water)

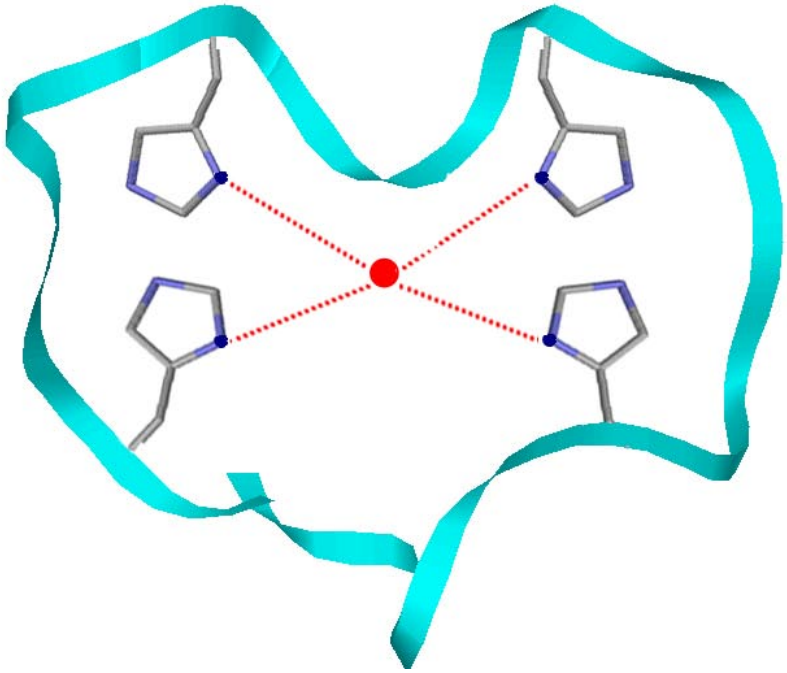

Figure 5: Copper coordination inside the tetra-hexarepeat domain suggested from EPR parameters (g=; A//=). A square planar geometry involving the four histidines is adopted at physiological pH.