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Can copper binding to the prion protein generate a misfolded form of the protein?

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Abstract The native prion protein (PrP) has a two domain structure, with a globular folded α-helical C-terminal domain and a flexible extended N-terminal region. The latter can selectively bind Cu^{2+} via four His residues in the octarepeat (OR) region, as well as two sites (His96 and His111) outside this region. In the disease state, the folded C-terminal domain of PrP undergoes a conformational change, forming amorphous aggregates high in β-sheet content. Cu^{2+} bound to the ORs can be redox active and has been shown to induce cleavage within the OR region, a process requiring conserved Trp residues. Using computational modeling, we have observed that electron transfer from Trp residues to copper can be favorable. These models also reveal that an indole-based radical cation or Cu^{+} can initiate reactions leading to protein backbone cleavage. We have also demonstrated, by molecular dynamics simulations, that Cu^{2+} binding to the His96 and His111 residues in the remaining PrP N-terminal fragment can induce localized β-sheet structure, allowing us to suggest a potential mechanism for the initiation of β-sheet misfolding in the C-terminal domain by Cu^{2+}.

Keywords Prion protein · Copper binding · β-cleavage · Protein misfolding · Oxidative damage · Molecular dynamics · Density functional theory

Abbreviations

CD	Circular dichroism
DFT	Density functional theory
MD	Molecular dynamics
OR	Octarepeat
PrP	Prion protein
PrP^{C}	Cellular form of the prion protein
PrP^{Sc}	Scrapie isoform of the prion protein
ROS	Reactive oxygen species

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Introduction

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a family of rare fatal neurodegenerative diseases caused by a misfolded (and infectious) form of the host-encoded prion protein (PrP) (Prusiner 1991). PrP is best known

through its role in mad cow disease, which is officially known as bovine spongiform encephalopathy (BSE). The infectious form of the protein is commonly referred to as PrP^{Sc}, after it was shown to play a role in scrapie disease in sheep. In humans, the most common form of prion disease is called Creutzfeldt–Jakob disease (CJD) which occurs spontaneously in one in 1 million individuals. In North America, chronic wasting disease (CWD) in farmed and free-ranging deer and elk populations is now starting to emerge as a serious threat (Sigurdson and Aguzzi 2007). TSEs may arise sporadically due to random disease-predisposing gene mutations within the *prnp* gene (which encodes PrP), through familial inheritance of such mutations, or through exposure to infectious prion material (Prusiner 1991). Routes for the transmission of prion diseases have been attributed to the consumption of prion-containing foodstuffs, such as BSE-tainted beef, as well as iatrogenic transmission. Consumption of infected beef products leads to variant vCJD; it is presently unclear if consumption of scrapie-infected sheep material or CWD-infected deer and elk products are transmissible to humans as well.

The native cellular form of PrP (PrP^{C}, Fig. 1) is a 231-residue membrane-bound protein that is anchored to the exterior cell surface by a glycosylphosphatidylinosotol (GPI) lipid anchor. Although PrP is expressed in most mammalian tissues, the highest levels of expression occurs in neuronal cells where it is found in high abundance on the exterior surface of presynaptic membranes (Herms et al. 1999). PrP contains two dissimilar domains, a folded C-terminal domain rich in α-helical structure, and a disordered N-terminal domain (Fig. 1).

Prion disease is understood to be initiated by misfolding of PrP, whereby the protein undergoes a

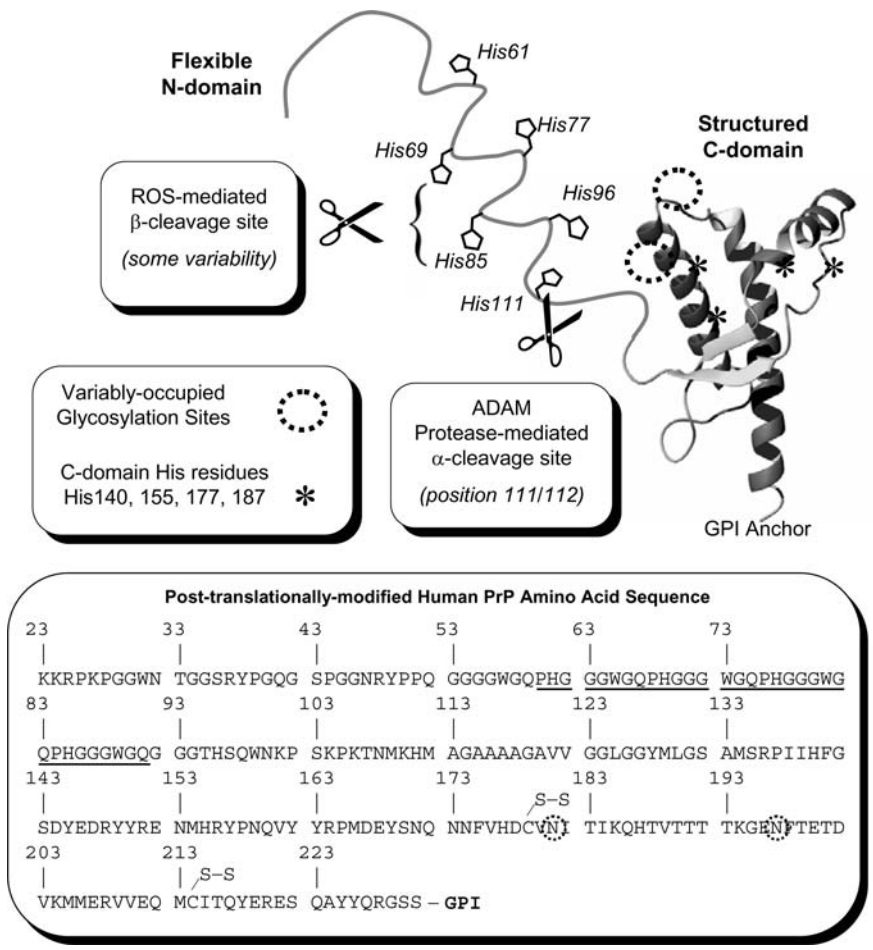


Fig. 1 Human PrP (rendered from the solution NMR structure, PDB code: 1HJM) as well as the sequence of the protein, following post-translational modification. The Cu^{2+}-binding OR residues are underlined. Cys residues denoted with S–S indicate these residues are linked by a disulfide bond. The C-domain His residues do not demonstrate significant interactions with copper and as such are not explicitly represented; their positions are denoted by an *asterisk*

conformational change to one with increased β-sheet secondary structure and reduced α-helical structure. These misfolded PrP monomers oligomerize and become insoluble. The resulting aggregates of PrP are protease resistant and persist within the host. Propagation of disease results from continued aggregation of misfolded PrP molecules through a template-mediated misfolding process, whereby PrP^C is recruited to the misfolded template—a process conceptually similar to crystallization. The template-driven misfolding of PrP is key to the transmissibility of prion diseases and constitutes the infectious particles associated with iatrogenic transmission and consumption of contaminated foods. Mice, in which the *prnp* gene has been deleted, have a very mild phenotype (Weissmann and Flechsig 2003); however, recent studies have demonstrated that PrP can significantly attenuate T lymphocyte-mediated neuroinflammation (Tsutsui et al. 2008). The pathogenesis of TSEs as well as the initial steps of PrP misfolding (in the absence of a prion template) remains elusive. A number of gene mutations which give rise to single amino acid changes or insertions of multiple ORs within the N-terminal domain can predispose individuals to disease.

Our studies have focused on copper coordination to the N-domain of PrP (Pushie and Rauk 2003; Pushie et al. 2007; Pushie and Vogel 2007, 2008, 2009). In the following, we will briefly summarize our current knowledge regarding copper-binding to the prion protein.

The flexible N-domain of mammalian PrP contains a sequence of eight amino acids which is repeated four or more times (four in human PrP), in tandem, with the repeating sequence PHGGGWGQ (residues 60–91, underlined in Fig. 1). This portion of the N-domain is termed the octarepeat (OR) region, and it is one of the most highly conserved regions of the protein, suggesting a functional significance (Wopfner et al. 1999). The N-domain, as well as the isolated ORs, selectively bind Cu^{2+} with an affinity spanning the nM to μM range (Hornshaw et al. 1995; Stöckel et al. 1998), conceptually represented in Fig. 2. Synapses use copper in signaling events, where the local [Cu] in the synaptic cleft can range from 10 μM in a resting state to transiently as high as 0.3 mM (Hartter and Barnea 1988; Watt and Hooper 2005). Whether these numbers represent the amount of free or total

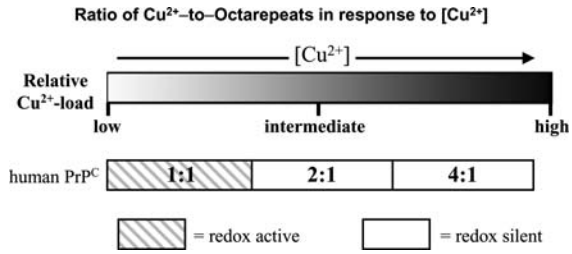


Fig. 2 Representation of the response of the OR region to increasing copper concentration

copper present is not totally clear (Millhauser 2007). Binding of copper to the ORs induces endocytosis (Pauly and Harris 1998) and an OR-deletion mutant has demonstrated that without the OR region PrP fails to undergo copper-mediated endocytosis (Perera and Hooper 2001). Furthermore, PrP mutants with additional repeats have been shown to have increased susceptibility to oxidative damage (Yin et al. 2006).

The flexible N-domain has been proposed to play a key role during early events that initiate misfolding of PrP (Cordeiro et al. 2005; Leliveld et al. 2006, 2008). A conserved hydrophobic stretch of residues spanning 106–126 (Fig. 1) is thought to be mostly responsible for conferring PrP with its neurotoxic and amyloidogenic properties (Selvaggini et al. 1993; De Gioia et al. 1994); however, the copper-binding ORs have also been implicated in misfolding. It has been clearly demonstrated that extra copies of the repeats, from one to nine additional inserts, can trigger spontaneous disease in humans (Collinge et al. 1989, 1992; Owen et al. 1989, 1991; Goldfarb et al. 1991; Laplanche et al. 1995, 1999; van Gool et al. 1995; Campbell et al. 1996; Cochran et al. 1996; Windl et al. 1999). In transgenic mouse experiments, OR-deletion mutants of PrP are still susceptible to disease, but the duration until disease signs appear is increased (Flechsig et al. 2000). Taken together these experiments suggest that the N-domain can modulate the misfolding process.

PrP^C has two regions where cleavage of the protein backbone can take place in vivo: within the flexible N-domain, as well as a third site at the C-terminus where the GPI anchor is attached. The first cleavage site in the N-domain is within the conserved hydrophobic region that confers upon PrP its neurotoxic and amyloidogenic properties. Normal proteolytic processing of PrP is termed α-cleavage

and cuts within this region (Fig. 1; Chen et al. 1995; Checler and Vincent 2002; Mangé et al. 2004). The α-cleavage site, along with cleavage of the GPI anchor at the C-terminal end of PrP (which results in a soluble form of PrP), are the sites of normal proteolytic processing of PrP^C by members of the ADAMs family of extracellular proteases (Chen et al. 1995; Mangé et al. 2004).

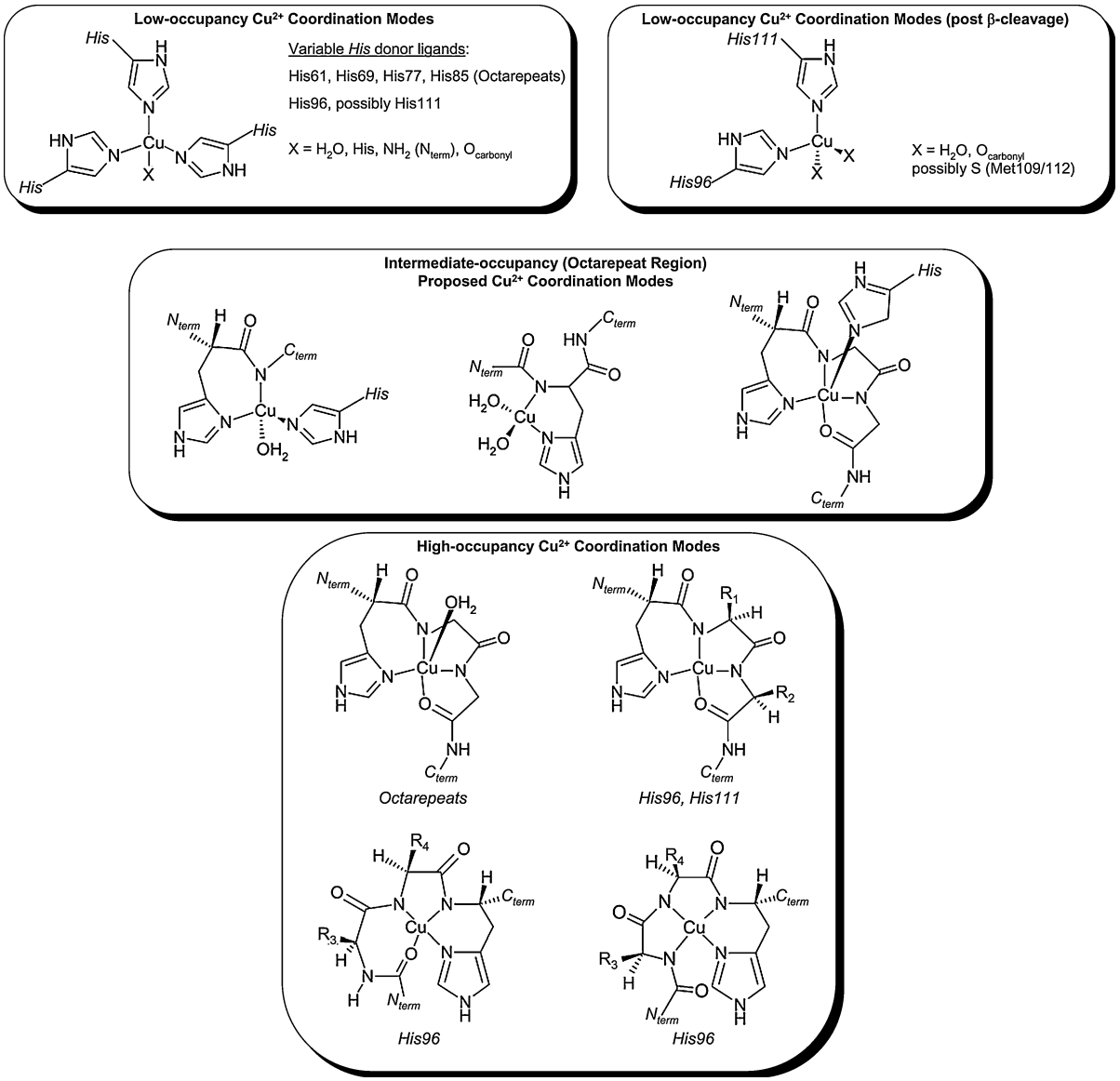
An alternate cleavage site in the N-domain is termed β-cleavage (Mangé et al. 2004) and occurs with some variability in the site of cleavage—approximately within the OR region, but before His96. β-Cleavage appears to occur as a result of exposure to ROS as opposed to cleavage by proteases (McMahon et al. 2001) and is dependent on Cu^{2+} and the Trp residues within the repeats (Ruiz et al. 2000; Opazo et al. 2003; Miura et al. 2005).

Interestingly, PrP isolated from prion plaques is not necessarily the full-length protein, but a truncated form of PrP has been found, where much of the N-domain is missing (Chen et al. 1995; Jiménez-Huete et al. 1998). These truncated PrP molecules are of a mass similar to the fragment which results from β-cleavage of PrP. This may indicate that β-cleavage of PrP is an important process in the accumulation of prion plaques. The remaining PrP fragment still contains the residues C-terminal to the OR region, including His96 and His111 and the amyloidogenic portion. This demonstrates that the ORs are neither required for disease propagation nor are they likely to be directly involved in the initiation of misfolding events. PrP isoforms that are truncated at residue 82 and 97 have also been reported in isolates from uninfected brain tissues (Yuan et al. 2006, 2008) and may be indicative of a base level of such cleavage events.

The N-domain of PrP selectively binds Cu^{2+} over other biologically relevant and surrogate divalent cations—although the coordination of other metal ions has been extensively studied, including Mn^{2+} (Zhu et al. 2008), Zn^{2+} (Walter et al. 2007), Ni^{2+} and Pd^{2+} (Garnett et al. 2006; Pushie et al. 2007), as well as Pt^{2+} and Co^{3+} (Pushie et al. 2007). Low occupancy (1:1) coordination of Cu^{2+} to the N-terminal domain involves His residues within an exchangeable environment, possibly completed by exchangeable coordination of the N-terminal amino group ($:NH_2$), additional His residues, or O-atom donors such as H_2O or backbone carbonyls (Scheme 1). With increasing equivalents of Cu^{2+}, coordination takes place between His96 and His111, followed by continued coordination by the OR region (Klewpatinond et al. 2008). High-Cu^{2+}-occupancy conditions results in coordination of up to 6-equivalents of Cu^{2+}, one for each of the His residues, in coordination modes involving deprotonated backbone amide N-atoms (Scheme 1). The OR region, specifically, has been extensively studied as it displays a wide range of coordination forms in response to Cu^{2+} concentration, similar to the full-length N-domain, and are discussed below. The complexes depicted in Scheme 1 are representative of typical coordination modes which form near physiological pH (~6.5–8.0). Below we will provide a brief description, but for more detailed discussions regarding copper coordination modes in the N-terminal domain the reader could consult (for example, Burns et al. 2002; Chattopadhyay et al. 2005; Wells et al. 2006a, b; Osz et al. 2007; Millhauser 2007; Klewpatinond et al. 2008; Srikanth et al. 2008).

The OR region has been shown to act as a sensitive indicator of the local copper concentration within the synaptic environment. The normal cellular form of human PrP, for example, is capable of binding Cu^{2+} in three distinct coordination modes at physiological pH within the OR region (Fig. 2; Chattopadhyay et al. 2005). At low [Cu^{2+}] as many as four ORs act cooperatively to bind a single Cu^{2+} ion; herein this form is referred to as the low-occupancy form of the OR region or (1:1). In the full-length N-domain, the low-occupancy form also contains isomers where His96 is additionally coordinated (Srikanth et al. 2008). Chattopadhyay et al. (2005) have observed that OR_3-and OR_4-containing peptide fragments yield a similar EPR spectrum as Cu^{2+} in the presence of a 50-fold excess of imidazole. This indicates either N3O1 or N4 coordination to Cu^{2+} and possible coordination by four OR histidine residues in the full-length OR region (Scheme 1) (Wells et al. 2006b). Thus, Cu^{2+} binding under low Cu^{2+} occupancy conditions (and also observed at low pH, Aronoff-Spencer et al. 2000; Miura et al. 2005) is comprised of one copper bound to the full-length OR region, containing four repeats (Fig. 1). The low occupancy form of the OR region binds Cu^{2+} in the nM range (Wells et al. 2006b).

At intermediate [Cu^{2+}] four ORs can bind 2-equivalents of Cu^{2+} which bridges two ORs. Each Cu^{2+}-binding site is comprised of two histidine



Scheme 1 Overview of copper coordination modes demonstrated by the N-terminal domain, in response to increasing copper concentration. The low-occupancy form can accommodate either square planar or tetrahedral coordination of a single copper atom. R-groups for the high-occupancy form correspond to the side chains of the amino acids: R_1 = Ser (His96), Met (His111); R_2 = Gln (His96), Ala (His111); R_3 = Gly, and R_4 = Thr. Apical interaction of Met112(S:) not illustrated for the high-occupancy complex with His111

imidazole N-donor atoms, a backbone amide N-atom, likely adjacent to one of the bound histidines, and an O-atom donor from solvent or the protein. This Cu^{2+} coordination form has a K_d of $\sim$200 nM (Wells et al. 2006b). The precise coordination structure is yet to be conclusively determined—examples of potential coordination structures are illustrated in Scheme 1.

Under conditions of high [Cu^{2+}], each OR can bind 1-equivalent of Cu^{2+}. X-ray crystallographic studies of Cu^{2+} bound to the OR peptide fragment Ac-HGGGW-NH_2 reveal that coordination is via the histidine imidazole ring, two deprotonated amide N-atoms from the adjacent glycine residues and the backbone carbonyl O-atom from the second glycine,

with a solvent molecule occupying the apical position above the peptide coordination plane (Scheme 1; Burns et al. 2002; see also Pushie and Rauk 2003). The K_d for this site is $\sim$1 μM (Wells et al. 2006b).

At low [Cu^{2+}], the 1:1 mode of binding is redox active (Fig. 2) and facilitates reduction of Cu^{2+} to Cu^{+} (Ruiz et al. 2000). Free Cu^{+} in solution can readily partake in Fenton-type reactions (Halliwell 2006), which give rise to reactive oxygen species (ROS) that cause oxidative stress and cell damage. The binding of Cu^{2+} in a 2:1- or 4:1-binding mode (Fig. 2) with PrP under intermediate-to-high copper-loads results in stabilization of the Cu(II) oxidation state. The binding of Cu^{2+} in redox inactive forms may be a neuroprotective mechanism guarding against high levels of free aqueous Cu^{2+}, such as following high synaptic activity or synaptic depolarization (Hartter and Barnea 1988). In situations where the local [Cu] is high occupancy of Cu^{+} would lead to ROS formation. In the high-occupancy form, the Cu^{2+} binding environment has been shown to be redox silent (demonstrating an unfavorable reduction potential, Bonomo et al. 2000) and molecular dynamics simulations have revealed that the individual Cu^{2+}-loaded ORs collapse together, further shielding the bound Cu^{2+} ions from solvent access (Pushie and Vogel 2007).

The Cu^{2+} affinity of the His96 and His111 region is relevant because it can form low-occupancy complexes in conjunction with the ORs and high occupancy complexes in isolation (Scheme 1). Additionally, following β-cleavage and loss of the OR region, these residues remain exposed at the cell surface and remain available to bind Cu^{2+}. The low Cu^{2+} occupancy form of the His96−His111 region (where copper bridges the two His residues) has been reported to be redox active (Nadal et al. 2007) and ROS formation can modulate the folding and neurotoxic properties of this region (Requena et al. 2001; Canello et al. 2008). Circular dichroism (CD) spectroscopy has been used to demonstrate that low occupancy coordination of Cu^{2+} to a PrP(91–115) synthetic peptide, which is initially unstructured in the absence of copper, can induce the formation of β-sheet secondary structure (Jones et al. 2004). This copper binding locale is adjacent to the region necessary for conferring PrP with its neurotoxic and amyloidogenic properties and could be one mechanism by which misfolding is initiated in vivo.

One of the most enigmatic questions surrounding prions revolves around the pathogenesis of spontaneous disease. In the absence of infectious PrP^{Sc}, events must conspire to convert PrP^{C} into its misfolded isoform. We have used computational approaches—directly supported by experimental results—to examine how copper can initiate cleavage and induce β-sheet formation in PrP. PrP appears to have a high degree of conformational plasticity and there are likely multiple misfolding routes which result in infectious β-sheet-containing isoforms. The possibility of multiple PrP^{Sc} isoforms is a likely source for the apparent strain specificity in animal models of prion disease (Caughey 2003).

Here, we present a potential two-stage mechanism for the role of copper in sporadic prion disease pathogenesis. First, we demonstrate how the low-occupancy copper coordination mode within the OR region can promote β-cleavage of the PrP N-terminal domain, thereby shifting the accessible coordination forms of membrane-anchored PrP to the remaining His96 and His111 sites. Second, copper coordination to the His96/His111 residues in the remaining N-terminal fragment can induce localized β-sheet formation which may be sufficient to induce further misfolding in the structured C-terminal domain. We also speculate how sporadic gene mutations which give rise to additional copies of the N-terminal ORs may predispose PrP to misfold through its interactions with copper. The mechanisms presented here are applicable to the formation of spontaneous prion disease and the early events in the pathogenesis of disease, oxidative damage, and neurodegeneration. Similar events play a role in the formation of the protein amyloid plaques that accompany the onset of Alzheimer's disease (for a review, see Rauk 2008).

Computational methods

Structure calculations and thermodynamics

Density functional theory (DFT) has been used to model copper coordination environments and assess stabilities of coordination geometries and ligand combinations. This method also affords a means of calculating thermodynamic parameters and solvation energies for the determination of free energies of reactions (ΔG^{Calc}). Copper and ligand species are modeled and calculated according to previously

published methods. Briefly, geometries and harmonic frequencies are calculated using the Becke 3-parameter hybrid density functional method, B3LYP, with the 6-31G(d) basis set, without geometry or symmetry constraints. Single point energies for all optimized structures are then calculated using the larger 6-311+G(2df,2p) basis set for subsequent free energy calculations. Solvation of each optimized species is calculated at the B3LYP/6-31G(d) level using the self-consistent reaction field method IEFPCM with united atom radii. The dielectric of the continuum value (ε) that is used for solvation by water is 78.39. Alternatively, in some instances, solvation is modeled using a lower dielectric value of 4.00, to represent species buried within a proteinaceous environment. More complete descriptions of the DFT methods, generation of thermodynamic data, and calculation of free energies ($\Delta G_{(aq)}$) can be found in Pushie and Rauk (2003) and Pushie and Vogel (2007, 2008).

Molecular dynamics simulations

Molecular dynamics (MD) simulations, which use only an implicit representation of electrons in atoms and molecules, allows modeling of much larger systems, such as proteins and protein fragments. We use the OPLS all-atom force field for MD simulations as it is built on DFT-calculated structures and properties. The same computational procedure used to derive parameters and values for OPLS is used in our DFT calculations. As such, our DFT models are directly portable to OPLS force field-based MD simulations. The MD simulation methods follow previously published methods. For more detailed descriptions of the MD simulation procedures and analysis, see Pushie and Vogel (2007, 2008, 2009).

Results and discussion

Models of the low occupancy form give clues to Cu^{2+} reduction

DFT models of His coordination to Cu^{2+} (modeled using 4-methylimidazole as the ligand) reveal that the aqueous free energy for coordination by four imidazoles, each via the Nε2-atom, is favored over Nδ1 coordination or the coordination of water molecules. The 4-coordinate Cu^{2+} center in the 1:1 complex is relatively solvent exposed, as the favored geometry is pseudo square-planar. Additional solvent interactions are best modeled by bulk solvation rather than inclusion of explicit axial solvent molecules.

Previous MD simulations of a copper-free OR_2 model demonstrated that although the peptide was inherently flexible it can form stable local structures, as shown in Fig. 3 (Pushie and Vogel 2007). Based on these simulations, the average ϕ and ψ backbone angles of the PHGG and GWGQ regions were used in the construction of an OR_4 model (Pushie and Vogel 2008). The assembled model reveals clustering of the four His residues, indicating that a suitable coordination site for one Cu^{2+} may pre-exist in the apo form of the OR region. This pre-folded conformation would make subsequent coordination of Cu^{2+} highly entropically favorable.

MD simulations of Cu^{2+}, bound via the His Nε2-atoms to the full-length OR region (Fig. 4), reveals that the remaining residues of the OR region are flexible enough to accommodate both square planar and tetrahedral coordination, making it compatible with Cu^{2+} and Cu^{+} (data not shown). Our MD simulations of Cu^{2+} bound to the OR region also reveal close approach of Trp81, Trp89 (within $\sim$10 Å, shown in Fig. 4) as well as the carbonyl

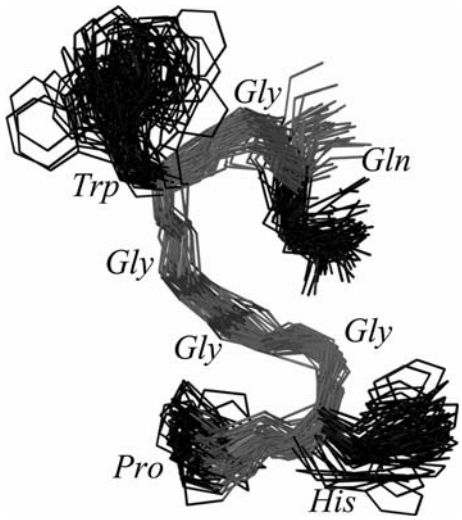


Fig. 3 Overlay of the protein backbone of a single copper-free OR from MD simulations. The PHGG region of the sequence forms flexible loops, while the GWGQ portion forms bends and turns—some turns of which are further stabilized by the formation of H-bonding (not explicitly shown)

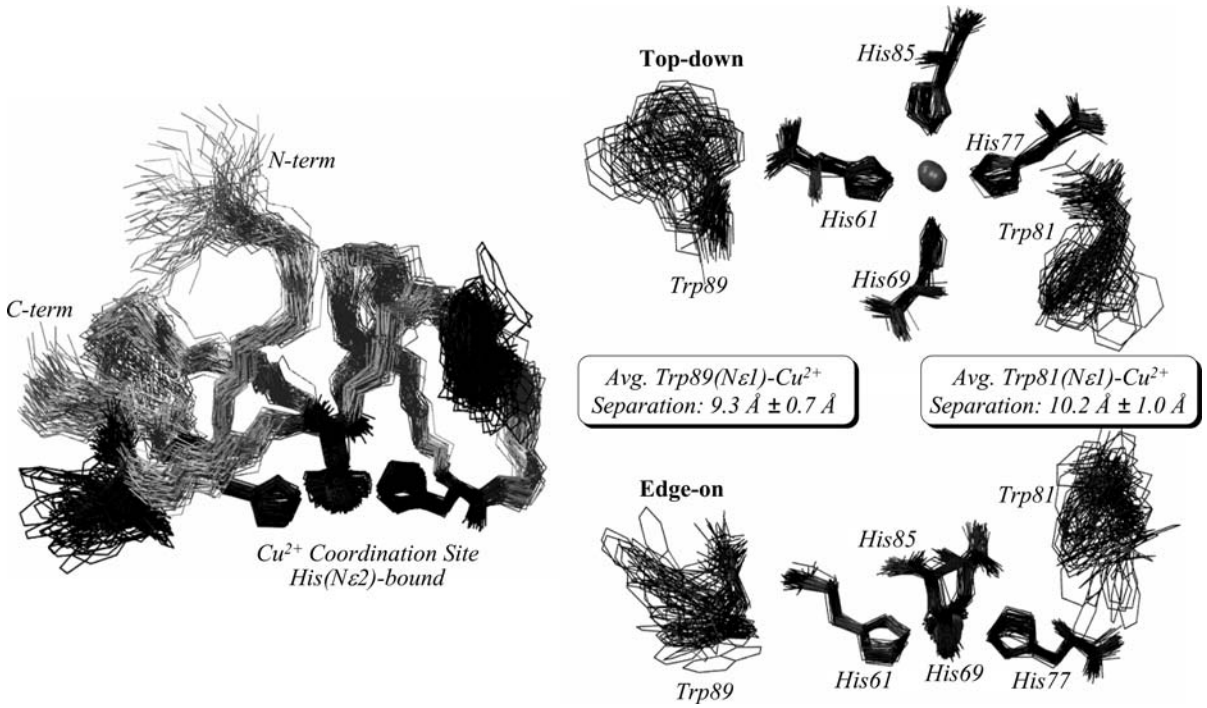


Fig. 4 Overlay of the protein backbone from MD simulations, showing multiple conformations of the low-occupancy form of the isolated OR region (Cu_1:OR_4), with coordination via the His Nε2-atoms. Trp81 and Trp89 are observed to make frequent close approaches to the copper coordination site, their average distances are shown

O-atom of Gly 90 toward the Cu^{2+} center (not shown). We propose that such conformations may predominate when additional OR insertion copies are presented, even at elevated [Cu^{2+}].

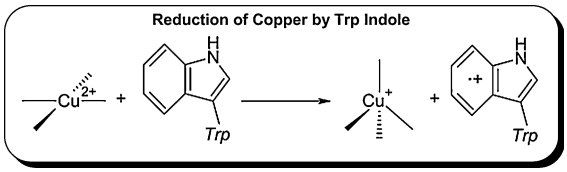
Electron transfer from Trp indole to Cu^{2+}

Following observations from Cu^{2+}:OR_4 MD simulations that Trp81 and Trp89 make frequent close approaches to the Cu^{2+} coordination site, we evaluated the thermodynamics of electron transfer from the Trp indole moiety to the copper center using DFT. Formation of Cu^{+} and an indole-based radical cation (Scheme 2) was found to be strongly favorable (-66 kJ mol^{-1}) if the reactants and product species are shielded from solvent within a protein-like environment (modeled using a lower dielectric continuum value of 4). The Cu^{2+}:OR_4 simulations reveal that the Cu^{2+} coordination site is only partially solvent-exposed while the Trp residues remain fully solvent-exposed (Pushie and Vogel 2008), and taking these two dissimilar environments into account leads to a slightly more favorable ΔG for the reaction of -74 kJ mol^{-1}. Modeling all species in an aqueous-like continuum makes the electron transfer reaction slightly endothermic: $+34$ kJ mol^{-1}.[1] The products shown in Scheme 2 are each discussed separately below for their potential roles in PrP oxidative damage and subsequent β-cleavage of the protein backbone.

Redox reactions and β-cleavage

The reduction of Cu^{2+} within the OR region, with concomitant formation of a Trp indole-based radical cation and β-cleavage of PrP (Fig. 1), has been linked to the copper-mediated formation of ROS. This process has been clearly demonstrated to rely on the OR region, Cu^{2+}, as well as the presence of the

[1] The listed free energies in this section take into account a $+20$ kJ mol^{-1} energetic penalty to first exchange a His ligand for H_2O from the Cu^{2+} coordination sphere prior to reduction to Cu^{+}.



Scheme 2 Reduction of Cu^{2+} by the Trp side chain indole—applicable to the low-occupancy form of the OR region and likely following close approach of Trp to the copper binding site. Cu^{+} and an indole-based radical cation are produced, either of which may contribute to a subsequent β-cleavage reaction. Note that a change of a square planar (Cu^{2+}) to a tetrahedral (Cu^{+}) coordination is also indicated

Trp residues within the OR region (Ruiz et al. 2000; Opazo et al. 2003; Miura et al. 2005). The formation of a solvent-accessible and exchangeable reduced copper species within the extracellular environment would be problematic due to its propensity to react with H_2O_2, forming $HO^{\bullet}$, the most potent ROS. $HO^{\bullet}$ can abstract H-atoms (H^{+} along with an associated electron, e^{-}) from essentially any amino acid (Huang and Rauk 2004; Wood et al. 2006); however, aliphatic sites are particularly susceptible to attack. Protein backbone cleavage reactions by radical species can be divided into three types: N_i—$C\alpha_i$, $C\alpha_i$—$C(O)_i$, or $C(O)_i$—N_{i+1} cleavage, where i is the ith residue in the sequence. The Cα of Gly is a particularly attractive target for H-atom abstraction because the resulting $C\alpha^{\bullet}$ is initially stabilized by the flanking peptide bonds (Rauk et al. 1999). Below we present two potential mechanisms for H-atom abstraction from Gly by $HO^{\bullet}$ and a Trp-based radical species.

Hydroxyl radical-directed β-cleavage

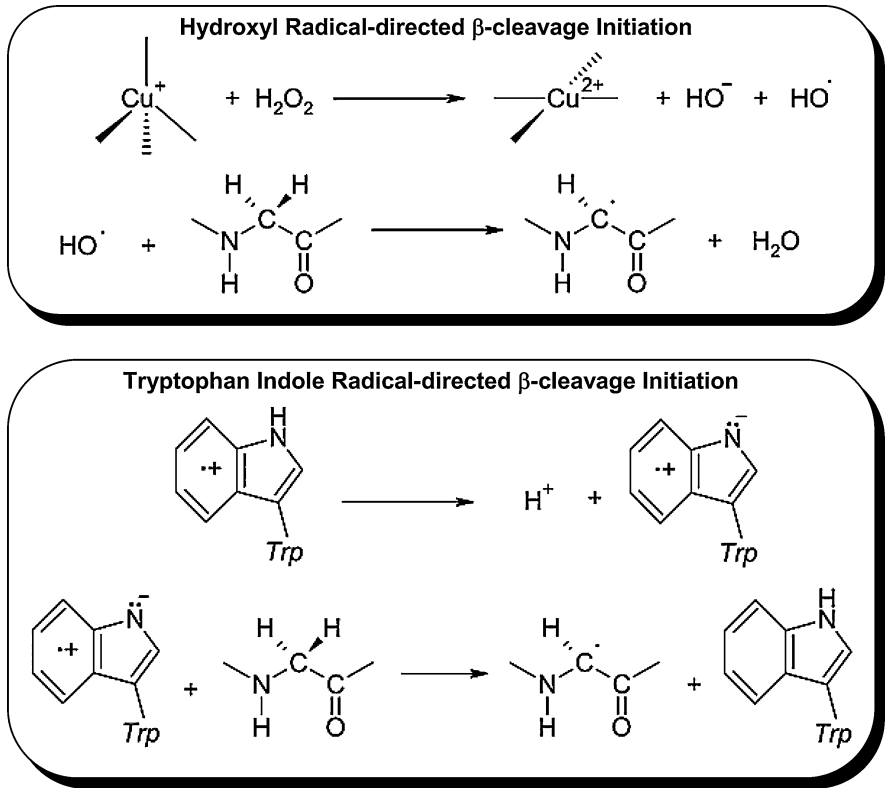
The formation of a solvent-accessible reduced copper site affords a direct mechanism for the production of ROS through Fenton chemistry (Scheme 3). The transfer of an electron from Cu^{+} to H_2O_2 results in the formation of Cu^{2+}, HO^{-}, and $HO^{\bullet}$ (Scheme 3). The calculated $\Delta G_{(aq)}$ for this reaction within the OR region reveals that the products and reactants are essentially isoenergetic ($\Delta G_{(aq)} = -0.7$ kJ mol^{-1}) and is likely to occur readily. Hydroxyl radicals are highly reactive and are likely to react at, or near, their site of formation. It is plausible that $HO^{\bullet}$ would result in H-atom abstraction with any of the Gly residues in the OR region, albeit non-specifically. Our calculated $\Delta G_{(aq)}$ for the reaction of $HO^{\bullet}$ with Ac-Gly-NH_2 (as the protein model), producing H_2O and Ac-Gly($C\alpha^{\bullet}$-H)-NH_2 is -140 kJ mol^{-1}. Due in part to the high reactivity of $HO^{\bullet}$, the site of H-atom abstraction is not expected to be site-specific, and $HO^{\bullet}$ would also result in further oxidative damage to PrP, such as Met and His oxidation—events which are consistent with prion disease.

Tryptophan indole radical-directed β-cleavage

An alternative mechanism to ROS-mediated attack on an OR Gly residue is direct attack by the oxidized Trp residue within the OR region. The formed Trp indole radical cation (Trp^{+}) can readily undergo deprotonation at physiological pH ($pK_a = 4.3$ (Evleth et al. 1977), our calculated pK_a is 6.7, due to a difference in the $\Delta G_{(aq)}$ of $\sim$14 kJ mol^{-1}) to produce the neutral indole-based radical. The neutral radical can then abstract a H-atom from one of its adjacent Gly Cα-centers, thereby re-generating the Trp indole moiety (re-protonated at the Nε1 position) and generating the Gly($C\alpha$)$^{\bullet}$ radical (Scheme 3). Modeling the H-atom abstraction reaction by 3-methylindole neutral radical (deprotonated at Nε1) from Ac-Gly-NH_2, the calculated $\Delta G_{(aq)}$ is -13 kJ mol^{-1}. Although this reaction is not as exothermic as the hydroxyl radical-mediated reaction above, each Trp residue within the OR region is flanked by a Gly and this mechanism potentially affords a means of directing H-atom abstraction from these Gly residues in a site specific manner and would not require H_2O_2 formation in vivo.

Proposed mechanism of β-cleavage at glycine

Once a Gly Cα radical forms it has a relatively long lifetime due to its stabilization from the adjacent peptide bonds (Rauk et al. 1999) and the most probable fate of this species is C–C bond cleavage (Scheme 4). The brain is rich in dissolved O_2 and will react with the Gly($C\alpha$) radical, forming a neutral peroxy radical. Addition of two protons and two electrons results in the loss of H_2O, producing an oxo radical. This radical species can ultimately undergo C–C bond homolysis, forming an aldehyde on the N-terminal side and a carboxy radical on the C-terminal side.



Scheme 3 Possible routes of H-atom abstraction from a Gly residue within the OR region from the product species generated in Scheme 2. The first mechanism is directed by a hydroxyl radical, formed through Fenton chemistry. The resulting $HO^{\bullet}$ is highly reactive but non-site-specific. The second mechanism is directed by the indole radical, following deprotonation of the Nε1-position ($pK_a = 4.3$). The neutral indole radical is also capable of abstracting a H-atom from the protein backbone and would likely result in site-specific attack on one of the glycines adjacent to the OR Trp residues

Cu^{2+} binding to the H96 and H111 region can promote β-sheet formation

With the loss of the N-terminal OR region His96 and His111 are the only copper-binding ligands available to membrane-anchored PrP with which to bind Cu^{2+}. This likely has the dual effect of loss of protective function afforded by the OR region as well as altering the Cu^{2+} coordination modes and local protein conformation in the His96–His111 region. We have examined two dissimilar Cu^{2+} coordination modes accessible by the His96 and His111 sites when the OR region is absent: low-occupancy coordination, where Cu^{2+} bridges both His residues, as well as high-occupancy coordination, where Cu^{2+} coordinates into the backbone from the anchoring His residue. β-sheet formation in the low occupancy coordination form of this region has been experimentally observed by CD spectroscopy (Jones et al. 2004).

MD simulations of Cu^{2+} bound in the low occupancy form (summarized in Fig 4) reveal that this form generates a β-hairpin structure, with several hydrogen bonds stabilizing the antiparallel β-sheet structure.

We have also modeled the high occupancy form of Cu^{2+} coordination at His96 and His111, bound via the protein backbone in the reverse direction from the anchoring His residue (Scheme 1). Depending on the local protein backbone geometry about the Cu^{2+} center, the binding-induced turn can also result in β-sheet formation (data not shown). As this type of copper-binding induced effect has not been observed experimentally under high occupancy conditions (Jones et al. 2004), we presently favor the low-Cu^{2+}-occupancy bridging model (Fig. 5) as the most likely route to local β-sheet formation. Be that as it may, either of the copper-occupancy routes to β-sheet formation is capable of promoting localized β-sheet formation and such an event might serve as a nucleation site for driving further misfolding through interaction with the C-terminal domain.

His96 is a dominant Cu^{2+}-binding ligand under low occupancy conditions, with coordination completed by the OR His residues (Srikanth et al. 2008). This coordination form would shift Cu^{2+} coordination away from His111 and would abrogate the possibility of localized β-sheet formation. However, under conditions where the OR region has been

Scheme 4 Proposed mechanism of β-cleavage, following H-atom abstraction at one of the Gly residues within the OR region

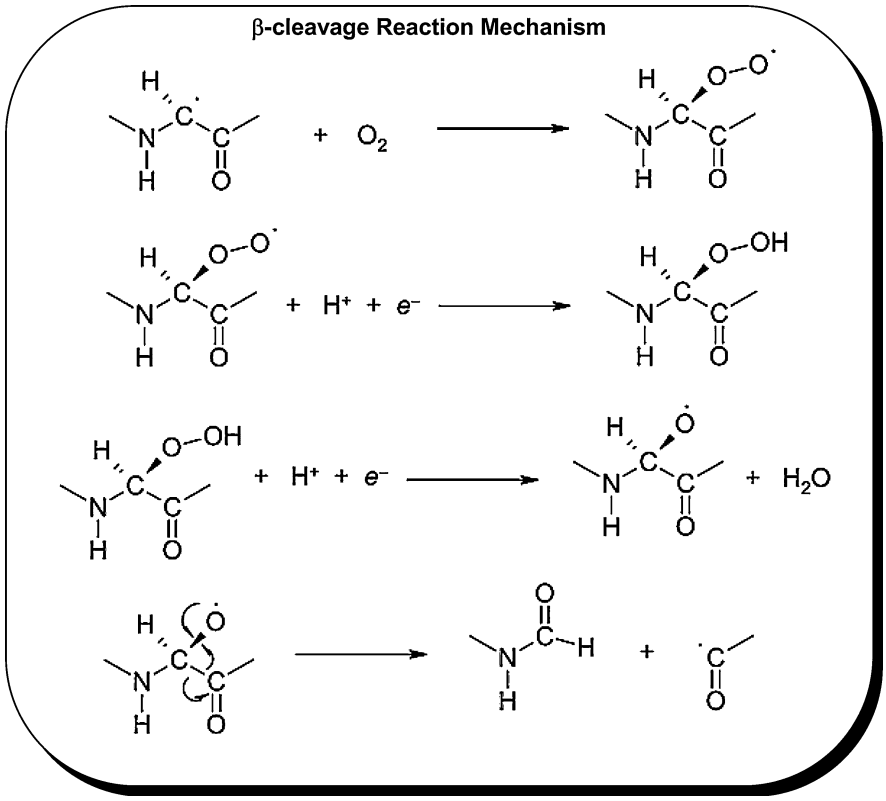
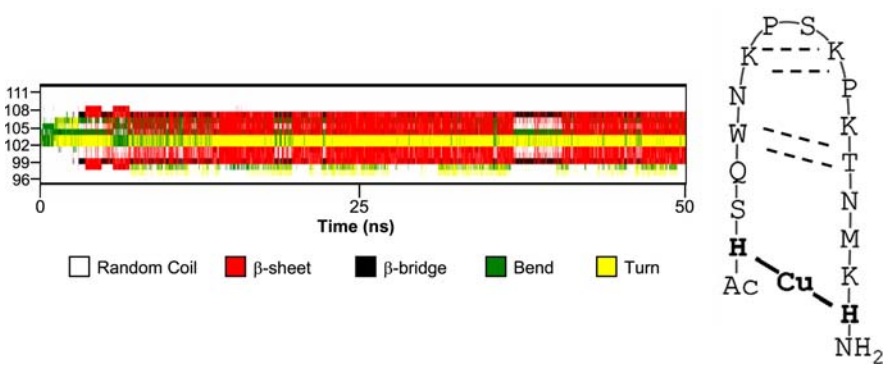


Fig. 5 Secondary structure plot from a representative MD simulation of Cu^{2+} bridging His96 and His111, showing the formation of flexibly disordered and β-sheet-like conformations



deleted by β-cleavage, the low occupancy form comprised of His96 and His111 is likely to be significantly populated.

Contribution from Cu^{+}/Cu^{2+} toxicity?

The reduction of Cu^{2+} to Cu^{+} by PrP affords a direct mechanism for the production of ROS in the sensitive environment of the synaptic junction and initially appears problematic from a functional perspective. Most copper chaperones that are involved in trafficking specifically bind Cu^{+} via deprotonated cysteine S^{-} ligands (Davis and O'Halloran 2008), which are good ligands for Cu^{+} but not Cu^{2+}. The function of PrP, under low copper-loads may be to reduce the metal to the Cu^{+} state in preparation for trafficking, following endocytosis. Coordination of Cu^{2+} via the side chain S-atoms of Met109 or Met112 in the isolated amyloidogenic fragment of PrP has also been reported (Shearer et al. 2008) and may signal an important binding environment form for copper reduction.

The low-Cu^{2+}-occupancy form of the His96–His111 region is also redox competent and can generate ROS which will oxidatively modify PrP, a feature which alters the toxicity and folding of the local region (Shearer and Soh 2007). Redox reactions with the N-domain trigger increased oxidative modification of the N-terminal domain and are a hallmark of prion disease (Bergström et al. 2007; Pamplona et al. 2008).

The loss of much of the N-terminal domain, including the OR region, leaves the membrane-anchored PrP without the copper-binding and neuroprotective capacity afforded by the OR region. Disruption in copper homeostasis is likely to result in peripheral oxidative stress which is associated with prion diseases and TSEs. Sporadic prion diseases arise later in life, similar to Alzheimer's disease, when the antioxidant capacity is diminished (Hallwell 2006). Although there are mechanisms in place to protect against oxidative stress and copper dyshomeostasis, oxidative damage in prion diseases appear to be an example of exceeding the capacity of the host system.

Conclusions

Any excess Cu^{2+} in the circulatory system would quickly be bound in a redox-inactive form by serum albumin (Harford and Sarkar 1997). However, the concentration of albumin in the brain is substantially lower and this may perhaps provide an increased need for PrP^{C}—acting as a protective agent for Cu^{2+}. Part of the normal functioning of PrP is relegated to trafficking copper from outside of the cell to the inside under high synaptic copper loads (Vassallo and Herms 2003). Under low-occupancy conditions bound copper is redox active and can promote ROS formation. MD simulations of an isomeric form of the low occupancy state of the N-domain (Fig. 2) have demonstrated that specific Trp and Gly residues can come into close association with the bound Cu^{2+} ion (Pushie and Vogel 2008). Such Cu^{2+} coordination modes could lead to reduction of Cu^{2+} to Cu^{+} by Trp, subsequently resulting in β-cleavage of PrP. Under normal conditions, the OR region is proposed to offer a level of neuroprotection (Mitteregger et al. 2007), possibly by acting as a sacrificial quencher of ROS (Nadal et al. 2007).

The β-cleavage of the N-terminal domain of PrP removes the N-terminal OR region from the remaining membrane-anchored PrP molecule. This results in a loss-of-function for cell-anchored PrP, as it is no longer able to bind multiple equivalents of Cu^{2+} in redox inactive forms under high copper concentration conditions. Without the OR region, oxidative damage to residues in the vicinity of His96 and His111 has been shown to alter the neurotoxicity of this region (Bergström et al. 2007). Mutations in the *prnp* gene giving rise to additional OR copies are also likely to contribute to a loss-of-neuroprotective-function, as the additional repeats will continue to bind further equivalents of Cu^{2+} in a redox competent form. These additional redox active copper centers will contribute to the production of additional ROS and are also likely to trigger more extensive β-cleavage.

Following loss of the OR region, the available residues for binding Cu^{2+} in the remaining flexible N-terminal fragment of PrP are His96 and His111. The coordination of a single Cu^{2+} atom by these residues generates a redox competent center and can promote the formation of localized β-sheet secondary structure. The Cu^{2+}-induced β-sheet formation is adjacent to the highly conserved hydrophobic stretch of residues implicated as the pathogenic region of PrP. Nucleation of β-sheet structure in this region, and subsequent propagation into the C-domain, may be an important conformational transition in the pathogenesis of prion disease.

In addition to PrP^{Sc}-containing plaques and fibrils, increased oxidative stress is also a hallmark of prion disease. Increases in localized ROS formation, due to a loss-of-neuroprotective-function from PrP likely plays a role in the early stages of prion disease, prior to apoptosis and disruption of neurochemical homeostasis (Vassallo and Herms 2003).

The OR region has been shown to influence interactions between PrP molecules (Leliveld et al. 2006) and alternate misfolding pathways may be accessible to OR-interacting oligomers (Leliveld et al. 2008). Single amino acids mutations can also predispose PrP to misfold and initiate sporadic instances of disease; however, these are likely to be separate mechanisms from those incorporating copper, described herein.

Figure 6 provides a synopsis of the role Cu^{2+} can play in β-cleavage and misfolding of PrP. Figure 6 highlights how, following β-cleavage, the remaining

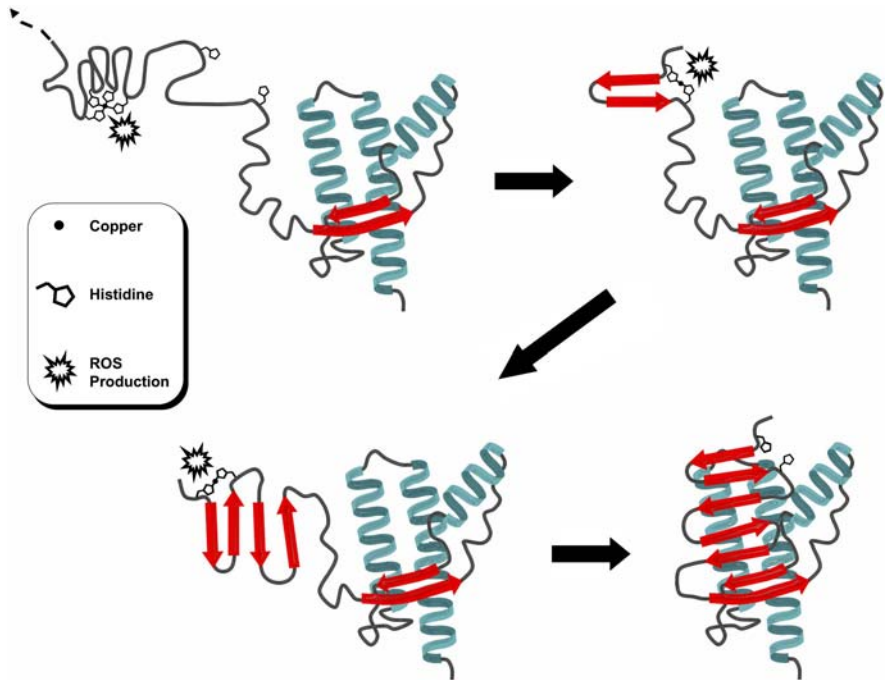


Fig. 6 Proposed mechanism of copper-induced cleavage and β-sheet initiation regions of secondary structure are shown for illustrative purposes only and do not indicate any specific conformation for the misfolded isoform in oligomers or mature PrP plaques

His96 and His111 residues can bind Cu^{2+}, inducing the local formation of β-sheet structure. Such structural changes are less likely to be accessible under normal low occupancy conditions because in the intact protein His96 participates with the ORs in coordinating Cu^{2+} (Srikanth et al. 2008). Upon nucleation of β-sheet structure in the N-domain fragment, the structure can ultimately propagate into the C-domain (Fig. 6), altering the C-domain secondary structure content and providing a seed template to initiate oligomerization.

An infectious PrP template—whose conformation was initially seeded by a Cu^{2+}-induced β-sheet structure—may be but one example of a possible underlying route to misfolding. In this model, the final prion plaques would contain an underlying structure related to the initial copper-induced conformation, which may differ from host-to-host or from prion diseases initiated by other mechanisms. Caughey (2003) has similarly proposed that alternate misfolded PrP conformers may account for the observed species barrier and strain specificities between animals. Doppel is structurally homologous to PrP and is also expressed in mammalian tissues, but lacks the corresponding N-domain and has not been demonstrated to participate in the propagation of PrP^{Sc}. However, recently Erlich et al. (2008) have demonstrated that a chimeric form of Doppel, which includes the N-terminal domain of PrP and the Doppel C-domain, is capable of undergoing the requisite conformational transition to a β-sheet-containing structure and forms oligomers which are partially resistant to proteolysis.

Our proposed mechanism does not require copper for the propagation of PrP^{Sc}, only its initial formation. For this reason, copper is not expected to be found enriched in prion plaques, unlike the plaques generated by related diseases such as Alzheimer's disease (Huang et al. 2004). A similar mechanism has been proposed for β-2-microglobulin amyloid formation which also requires Cu^{2+} for initiation, but does not involve Cu^{2+} accumulation in the microfibrillar amyloids (Antwi et al. 2008). The mechanism of copper-mediated β-cleavage, copper-contributed oxidative modification of PrP and peripheral copper-mediated oxidative damage may also explain why the copper chelator, penicillamine, has been found to delay onset of signs of disease (Sigurdsson et al. 2003). In combination with these effects, a disruption in brain copper homeostasis and speciation, due to the loss-of-function of PrP, associated with disease is expected.

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References

Antwi K, Mahar M, Srikanth R, Olbris MR, Tyson JF, Vachet RW (2008) Cu(II) organizes beta-2-microglobulin oligomers but is released upon amyloid formation. Protein Sci 17:748–759. doi:10.1110/ps.073249008

Aronoff-Spencer E, Burns CS, Avdievich NI, Gerfen GJ, Peisach J, Antholine WE, Ball HL, Cohen FE, Prusiner SB, Millhauser GL (2000) Identification of the Cu^{2+} binding sites in the N-terminal domain of the prion protein by EPR and CD spectroscopy. Biochemistry 39:13760–13771. doi:10.1021/bi001472t

Bergström A-L, Chabry J, Bastholm L, Heegaard PMH (2007) Oxidation reduces the fibrillation but not the neurotoxicity of the prion peptide PrP106–126. Biochim Biophys Acta 1774:1118–1127

Bonomo RP, Imperllizzeri G, Pappalardo G, Rizzarelli E, Tabbì G (2000) Copper(II) binding modes in the prion octapeptide PHGGGWGQ: a spectroscopic and voltammetric study. Chemistry (Easton) 6:4195–4202

Burns CS, Aronoff-Spencer E, Dunham CM, Lario P, Avdievich NI, Antholine WE, Olmstead MM, Vrielink A, Gerfen GJ, Peisach J, Scott WG, Millhauser GL (2002) Molecular features of the copper binding sites in the octarepeat domain of the prion protein. Biochemistry 41:3991–4001. doi:10.1021/bi011922x

Campbell TA, Palmer MS, Will RG, Gibb WR, Luthert PJ, Collinge J (1996) A prion disease with a novel 96-base pair insertional mutation in the prion protein gene. Neurology 46:761–766

Canello T, Engelstein R, Moshel O, Xanthopoulos K, Juanes ME, Langeveld J, Sklaviadis T, Gasset M, Gabizon R (2008) Methionine sulfoxides on PrP^{Sc}: a prion-specific covalent signature. Biochemistry 47:8866–8873. doi:10.1021/bi800801f

Caughey B (2003) Prion protein conversions: insight into mechanisms, TSE transmission barriers and strains. Br Med Bull 66:109–120. doi:10.1093/bmb/66.1.109

Chattopadhyay M, Walter ED, Newell DJ, Jackson PJ, Aronoff-Spencer E, Peisach J, Gerfen GJ, Bennett B, Antholine WE, Millhauser GL (2005) 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. doi:10.1021/ja053254z

Checler F, Vincent B (2002) Alzheimer's and prion diseases: distinct pathologies, common proteolytic denominators. Trends Neurosci 25:616–620. doi:10.1016/S0166-2236(02)02263-4

Chen SG, Teplow DB, Parchi P, Teller JK, Gambetti P, Autilio-Gambetti L (1995) Truncated forms of the human prion protein in normal brain and in prion diseases. J Biol Chem 270:19173–19180. doi:10.1074/jbc.270.32.19173

Cochran EJ, Bennett DA, Cervenakova L, Kenney K, Bernard B, Foster NL, Benson DF, Goldfarb LG, Brown P (1996) Familial Creutzfeldt-Jakob disease with a five-repeat octapeptide insert mutation. Neurology 47:727–733

Collinge J, Harding AE, Owen F, Poulter M, Lofthouse R, Boughey AM, Shah T, Crow TJ (1989) Diagnosis of Gerstmann–Sträussler syndrome in familial dementia with prion protein gene analysis. Lancet 2:15–17. doi:10.1016/S0140-6736(89)90256-0

Collinge J, Brown J, Hardy J, Mullan M, Rossor MN, Baker H, Crow TJ, Lofthouse R, Poulter M, Ridley R, Owen F, Bennett C, Dunn G, Harding AE, Quinn N, Doshi B, Roberts GW, Honavar M, Janota I, Lantos PL (1992) Inherited prion disease with 144 base pair gene insertion. 2. Clinical and pathological features. Brain 115:687–710. doi:10.1093/brain/115.3.687

Cordeiro Y, Kraineva J, Gomes MP, Lopes MH, Martins VR, Lima LM, Foguel D, Winter R, Silva JL (2005) The amino-terminal PrP domain is crucial to modulate prion misfolding and aggregation. Biophys J 89:2667–2676. doi:10.1529/biophysj.105.067603

Davis AV, O'Halloran TV (2008) A place for thioether chemistry in cellular copper ion recognition and trafficking. Nat Chem Biol 4:148–151. doi:10.1038/nchembio0308-148

De Gioia L, Selvaggini C, Ghibaudi E, Diomede L, Bugiani O, Forloni G, Tagliavini F, Salmona M (1994) Conformational polymorphism of the amyloidogenic and neurotoxic peptide homologous to residues 106–126 of the prion protein. J Biol Chem 269:7859–7862

Erlich P, Cesbron JY, Lemaire-Vieille C, Curt A, Andrieu JP, Schoehn G, Jamin M, Gagnon J (2008) PrP N-terminal domain triggers PrP(Sc)-like aggregation of Dpl. Biochem Biophys Res Commun 2365:478–483. doi:10.1016/j.bbrc.2007.10.202

Evleth EM, Chalvet O, Bamlère P (1977) Theoretical analysis of the photophysical properties of indole, the ondolyl radical, and the indole radical cation. J Phys Chem 81:1913–1917. doi:10.1021/j100535a006

Flechsig E, Shmerling D, Hegyi I, Raeber AJ, Fischer M, Cozzio A, von Mering C, Aguzzi A, Weissmann C (2000) Prion protein devoid of the octapeptide repeat region restores susceptibility to scrapie in PrP knockout mice. Neuron 27:399–408. doi:10.1016/S0896-6273(00)00046-5

Garnett AP, Jones CE, Viles JH (2006) A survey of diamagnetic probes for copper2+ binding to the prion protein. 1H NMR solution structure of the palladium2+ bound single octarepeat. J Chem Soc Dalton Trans 509–518. doi:10.1039/b511553a

Goldfarb LG, Brown P, McCombie WR, Goldgaber D, Swergold GD, Wills PR, Cervenakova L, Baron H, Gibbs CJ Jr, Gajdusek DC (1991) Transmissible familial Creutzfeldt–Jakob disease associated with five, seven, and eight extra octapeptide coding repeats in the PRNP gene. Proc Natl

Acad Sci USA 88:10926–10930. doi:10.1073/pnas.88.23.10926

Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? J Neurochem 97:1634–1658. doi:10.1111/j.1471-4159.2006.03907.x

Harford C, Sarkar B (1997) Amino terminal Cu(II)- and Ni(II)-binding (ATCUN) motif of proteins and peptides: metal binding, DNA cleavage, and other properties. Acc Chem Res 30:123–130. doi:10.1021/ar9501535

Hartter DE, Barnea A (1988) Evidence for release of copper in the brain: depolarization-induced release of newly taken-up 67copper. Synapse 2:412–415. doi:10.1002/syn.890020408

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

Hornshaw MP, McDermott JR, Candy JM (1995) Copper binding to the N-terminal tandem repeat regions of mammalian and avian prion protein. Biochem Biophys Res Commun 207:621–629. doi:10.1006/bbrc.1995.1233

Huang ML, Rauk A (2004) Structure and reactions of the peroxy radicals of glycine and alanine in peptides: an ab initio study. J Phys Org Chem 17:777–786. doi:10.1002/poc.794

Huang X, Moir RD, Tanzi RE, Bush AI, Rogers JT (2004) Redox-active metals, oxidative stress, and Alzheimer's disease pathology. Ann N Y Acad Sci 1012:153–163. doi:10.1196/annals.1306.012

Jiménez-Huete A, Lievens PM, Vidal R, Piccardo P, Ghetti B, Tagliavini F, Frangione B, Prelli F (1998) Endogenous proteolytic cleavage of normal and disease-associated isoforms of the human prion protein in neural and non-neural tissues. Am J Pathol 153:1561–1572

Jones CE, Abdelraheim SR, Brown DR, Viles JH (2004) Preferential Cu^{2+} coordination by His96 and His111 induces beta-sheet formation in the unstructured amyloidogenic region of the prion protein. J Biol Chem 279:32018–32027. doi:10.1074/jbc.M403467200

Klewpatinond M, Davies P, Bowen S, Brown DR, Viles JH (2008) Deconvoluting the Cu^{2+} binding modes of full-length prion protein. J Biol Chem 283:1870–1881

Laplanche JL, Delasnerie-Laupretre N, Brandel JP, Dussaucy M, Chatelain J, Launay JM (1995) Two novel insertions in the prion protein gene in patients with late onset dementia. Hum Mol Genet 4:1109–1111. doi:10.1093/hmg/4.6.1109

Laplanche JL, Hachimi KH, Durieux I, Thuillet P, Defebvre L, Delasnerie-Laupretre N, Peoc'h K, Foncin JF, Destee A (1999) Prominent psychiatric features and early onset in an inherited prion disease with a new insertional mutation in the prion protein gene. Brain 122:2375–2386. doi:10.1093/brain/122.12.2375

Leliveld SR, Dame RT, Wuite GJ, Stitz L, Korth C (2006) The expanded octarepeat domain selectively binds prions and disrupts homomeric prion protein interactions. J Biol Chem 281:3268–3275. doi:10.1074/jbc.M510606200

Leliveld SR, Stitz L, Korth C (2008) Expansion of the octarepeat domain alters the misfolding pathway but not the folding pathway of the prion protein. Biochemistry 47:6267–6278. doi:10.1021/bi800253c

Mangé A, Béranger F, Peoc'h K, Onodera T, Frobert Y, Lehmann S (2004) Alpha- and beta-cleavages of the amino-terminus of the cellular prion protein. Biol Cell 96:125–132. doi:10.1016/j.biolcel.2003.11.007

McMahon HE, Mange A, Nishida N, Creminon C, Casanova D, Lehmann S (2001) Cleavage of the amino terminus of the prion protein by reactive oxygen species. J Biol Chem 276:2286–2291. doi:10.1074/jbc.M007243200

Millhauser GL (2007) Copper and the prion protein: methods, structures, function, and disease. Annu Rev Phys Chem 58:299–320. doi:10.1146/annurev.physchem.58.032806.104657

Mitteregger G, Vosko M, Krebs B, Xiang W, Kohlmannsperger V, Nölting S, Hamann GF, Kretzschmar HA (2007) The role of the octarepeat region in neuroprotective function of the cellular prion protein. Brain Pathol 17:174–183. doi:10.1111/j.1750-3639.2007.00061.x

Miura T, Sasaki S, Toyama A, Takeuchi H (2005) Copper reduction by the octapeptide repeat region of prion protein: pH dependence and implications in cellular copper uptake. Biochemistry 44:8712–8720. doi:10.1021/bi0501784

Nadal RC, Abdelraheim SR, Brazier MW, Rigby SE, Brown DR, Viles JH (2007) Prion protein does not redox-silence Cu^{2+}, but is a sacrificial quencher of hydroxyl radicals. Free Radic Biol Med 42:79–89. doi:10.1016/j.freeradbiomed.2006.09.019

Opazo C, Barría MI, Ruiz FH, Inestrosa NC (2003) Copper reduction by copper binding proteins and its relation to neurodegenerative diseases. Biometals 16:91–98. doi:10.1023/A:1020795422185

Osz K, Nagy Z, Pappalardo G, Di Natale G, Sanna D, Micera G, Rizzarelli E, Sóvágó I (2007) Copper(II) interaction with prion peptide fragments encompassing histidine residues within and outside the octarepeat domain: speciation, stability constants and binding details. Chemistry 13:7129–7143. doi:10.1002/chem.200601568

Owen F, Poulter M, Lofthouse R, Collinge J, Crow TJ, Risby D, Baker HF, Ridley RM, Hsiao K, Prusiner SB (1989) Insertion in prion protein gene in familial Creutzfeldt–Jakob disease. Lancet 1:51–52. doi:10.1016/S0140-6736(89)91713-3

Owen F, Poulter M, Collinge J, Leach M, Shah T, Lofthouse R, Chen YF, Crow TJ, Harding AE, Hardy J, Rossor MN (1991) Insertions in the prion protein gene in atypical dementias. Exp Neurol 112:240–242. doi:10.1016/0014-4886(91)90075-N

Pamplona R, Naudí A, Gavín R, Pastrana MA, Sajnani G, Ilieva EV, Del Río JA, Portero-Otín M, Ferrer I, Requena JR (2008) Increased oxidation, glycoxidation, and lipoxidation of brain proteins in prion disease. Free Radic Biol Med 45:1159–1166. doi:10.1016/j.freeradbiomed.2008.07.009

Pauly PC, Harris DA (1998) Copper stimulates endocytosis of the prion protein. J Biol Chem 273:33107–33110. doi:10.1074/jbc.273.50.33107

Perera WS, Hooper NM (2001) Ablation of the metal ion-induced endocytosis of the prion protein by disease-associated mutation of the octarepeat region. Curr Biol 11:519–523. doi:10.1016/S0960-9822(01)00147-6

Prusiner SB (1991) Molecular biology of prion diseases. Science 252:1515–1522. doi:10.1126/science.1675487

Pushie MJ, Rauk A (2003) Computational studies of Cu(II)[peptide] binding motifs: Cu[HGGG] and Cu[HG] as models for Cu(II) binding to the prion protein octarepeat region. J Biol Inorg Chem 8:53–65. doi:10.1007/s00775-002-0386-7

Pushie MJ, Vogel HJ (2007) Molecular dynamics simulations of two tandem octarepeats from the mammalian prion protein: fully Cu^{2+}-bound and metal-free forms. Biophys J 93:3762–3774. doi:10.1529/biophysj.107.109512

Pushie MJ, Vogel HJ (2008) Modeling by assembly and molecular dynamics simulations of the low Cu^{2+} occupancy form of the mammalian prion protein octarepeat region: gaining insight into Cu^{2+}-mediated β-cleavage. Biophys J 95:5084–5091. doi:10.1529/biophysj.108.139568

Pushie MJ, Vogel HJ (2009) A potential mechanism for Cu^{2+} reduction, β-cleavage and β-sheet initiation within the N-terminal domain of the prion protein: insights from density functional theory and molecular dynamics calculations. J Toxicol Environ Health A (in press)

Pushie MJ, Ross ARS, Vogel HJ (2007) Mass spectrometric determination of the coordination geometry of potential copper(II) surrogates for the mammalian prion protein octarepeat region. Anal Chem 79:5659–6767. doi:10.1021/ac070312l

Rauk A (2008) Why is the amyloid beta peptide of Alzheimer's disease neurotoxic? J Chem Soc Dalton Trans 1273–1282. doi:10.1039/b718601k

Rauk A, Yu D, Taylor J, Shustov GV, Block DA, Armstrong DA (1999) Effects of structure on αC-H bond enthalpies of amino acid residues: relevance to H transfers in enzyme mechanisms and in protein oxidation. Biochemistry 38:9089–9096

Requena JR, Groth D, Legname G, Stadtman ER, Prusiner SB, Levine RL (2001) Copper-catalyzed oxidation of the recombinant SHa(29–231) prion protein. Proc Natl Acad Sci USA 98:7170–7175. doi:10.1073/pnas.121190898

Ruiz FH, Silva E, Inestrosa NC (2000) The N-terminal tandem repeat region of human prion protein reduces copper: role of tryptophan residues. Biochem Biophys Res Commun 269:491–495. doi:10.1006/bbrc.2000.2270

Selvaggini C, De Gioia L, Cantù L, Ghibaudi E, Diomede L, Passerini F, Forloni G, Bugiani O, Tagliavini F, Salmona M (1993) Molecular characteristics of a protease-resistant, amyloidogenic and neurotoxic peptide homologous to residues 106–126 of the prion protein. Biochem Biophys Res Commun 194:1380–1386. doi:10.1006/bbrc.1993.1977

Shearer J, Soh P (2007) The copper(II) adduct of the unstructured region of the amyloidogenic fragment derived from the human prion protein is redox-active at physiological pH. Inorg Chem 46:710–719. doi:10.1021/ic061236s

Shearer J, Soh P, Lentz S (2008) Both Met(109) and Met(112) are utilized for Cu(II) coordination by the amyloidogenic fragment of the human prion protein at physiological pH. J Inorg Biochem 102:2103–2113. doi:10.1016/j.jinorgbio.2008.07.016

Sigurdson CJ, Aguzzi A (2007) Chronic wasting disease. Biochim Biophys Acta 1772:610–618

Sigurdsson EM, Brown DR, Alim MA, Scholtzova H, Carp R, Meeker HC, Prelli F, Frangione B, Wisniewski T (2003) Copper chelation delays the onset of prion disease. J Biol Chem 278:46199–46202. doi:10.1074/jbc.C300303200

Srikanth R, Wilson J, Burns CS, Vachet RW (2008) Identification of the copper(II) coordinating residues in the prion protein by metal-catalyzed oxidation mass spectrometry: evidence for multiple isomers at low copper(II) loadings. Biochemistry 47:9258–9268. doi:10.1021/bi800970m

Stöckel J, Safar J, Wallace AC, Cohen FE, Prusiner SB (1998) Prion protein selectively binds copper(II) ions. Biochemistry 37:7185–7193. doi:10.1021/bi972827k

Tsutsui S, Hahn JN, Johnson TA, Ali Z, Jirik FR (2008) Absence of the cellular prion protein exacerbates and prolongs neuroinflammation in experimental autoimmune encephalomyelitis. Am J Pathol 173:1029–1041. doi:10.2353/ajpath.2008.071062

van Gool WA, Hensels GW, Hoogerwaard EM, Wiezer JH, Wesseling P, Bolhuis PA (1995) Hypokinesia and presenile dementia in a Dutch family with a novel insertion in the prion protein gene. Brain 118:1565–1571. doi:10.1093/brain/118.6.1565

Vassallo N, Herms J (2003) Cellular prion protein function in copper homeostasis and redox signalling at the synapse. J Neurochem 86:538–544. doi:10.1046/j.1471-4159.2003.01882.x

Walter ED, Stevens DJ, Visconte MP, Millhauser GL (2007) 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. doi:10.1021/ja077146j

Watt NT, Hooper NM (2005) Reactive oxygen species (ROS)-mediated β-cleavage of the prion protein in the mechanism of the cellular response to oxidative stress. Biochem Soc Trans 33:1123–1125. doi:10.1042/BST20051123

Weissmann C, Flechsig E (2003) PrP knock-out and PrP transgenic mice in prion research. Br Med Bull 66:43–60. doi:10.1093/bmb/66.1.43

Wells MA, Jackson GS, Jones S, Hosszu LLP, Craven CJ, Clarke AR, Collinge J, Waltho JP, Jackson GS (2006a) A reassessment of copper (II) binding in the full-length prion protein. Biochem J 399:435–444. doi:10.1042/BJ20060458

Wells MA, Jelinska C, Hosszu LLP, Craven CJ, Clarke AR, Collinge J, Waltho JP, Jackson GS (2006b) Multiple forms of copper (II) coordination occur throughout the disordered N-terminal region of the prion protein at pH 7.4. Biochem J 400:501–510. doi:10.1042/BJ20060721

Windl O, Giese A, Schulz-Schaeffer W, Zerr I, Skworc K, Arendt S, Oberdieck C, Bodemer M, Poser S, Kretzschmar HA (1999) Molecular genetics of human prion diseases in Germany. Hum Genet 105:244–252. doi:10.1007/s004390051096

Wood GPF, Easton CJ, Rauk A, Davies MJ, Radom L (2006) Effect of side chains on competing pathways for β-scission reactions of peptide-backbone alkoxyl radicals. J Phys Chem A 110:10316–10323. doi:10.1021/jp062916j

Wopfner F, Weidenhöfer G, Schneider R, von Brunn A, Gilch S, Schwarz TF, Werner T, Schätzl HM (1999) Analysis of 27 mammalian and 9 avian PrPs reveals high conservation of flexible regions of the prion protein. J Mol Biol 289:1163–1178. doi:10.1006/jmbi.1999.2831

Yin S, Yu S, Li C, Wong P, Chang B, Xiao F, Kang SC, Yan H, Xiao G, Grassi J, Tien P, Sy MS (2006) Prion proteins with insertion mutations have altered N-terminal conformation and increased ligand binding activity and are more susceptible to oxidative attack. J Biol Chem 281:10698–10705. doi:10.1074/jbc.M511819200

Yuan J, Xiao X, McGeehan J, Dong Z, Cali I, Fujioka H, Kong Q, Kneale G, Gambetti P, Zou WQ (2006) Insoluble aggregates and protease-resistant conformers of prion protein in uninfected human brains. J Biol Chem 281:34848–34858. doi:10.1074/jbc.M602238200

Yuan J, Dong Z, Guo JP, McGeehan J, Xiao X, Wang J, Cali I, McGeer PL, Cashman NR, Bessen R, Surewicz WK, Kneale G, Petersen RB, Gambetti P, Zou WQ (2008) Accessibility of a critical prion protein region involved in strain recognition and its implications for the early detection of prions. Cell Mol Life Sci 65:631–643. doi:10.1007/s00018-007-7478-z

Zhu F, Davies P, Thompsett AR, Kelly SM, Tranter GE, Hecht L, Isaacs NW, Brown DR, Barron LD (2008) Raman optical activity and circular dichroism reveal dramatic differences in the influence of divalent copper and manganese ions on prion protein folding. Biochemistry 47:2510–2517