ISSN 1466-2523

Animal Health Research Reviews 7(1/2); 97–105
DOI: 10.1017/S1466252307001181

Trace elements and prion diseases: a review of the interactions of copper, manganese and zinc with the prion protein

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Received 25 September 2006; Accepted 29 November 2006

Abstract

Transmissible spongiform encephalopathies (TSEs) are a family of neurodegenerative diseases characterized by their long incubation periods, progressive neurological changes, and spongiform appearance in the brain. There is much evidence to show that TSEs are caused by an isoform of the normal cellular surface prion protein PrP^{C}. The normal function of PrP^{C} is still unknown, but it exhibits properties of a cupro-protein, capable of binding up to six copper ions. There are two differing views on copper's role in prion diseases. While one view looks at the PrP^{C} copper-binding as the trigger for conversion to PrP^{Sc}, the opposing viewpoint sees a lack of PrP^{C} copper-binding resulting in the conformational change into the disease causing isoform. Manganese and zinc have been shown to interact with PrP^{C} as well and have been found in abnormal levels in prion diseases. This review addresses the interaction between select trace elements and the PrP^{C}.

Keywords: transmissible spongiform encephalopathy, prion, copper, manganese, zinc, trace elements

Introduction

Transmissible spongiform encephalopathies (TSEs)

The family of diseases classified as TSEs include bovine spongiform encephalopathy (BSE) in cattle; chronic wasting disease (CWD) in elk, deer and moose; scrapie in sheep and goats; transmissible mink encephalopathy (TME) in mink; and both sporadic and new variant Creutzfeldt–Jakob disease (sCJD and vCJD) in humans (Prusiner, 1995). The scientific community has overwhelming evidence that TSEs are caused by an abnormal prion protein (PrP^{Sc}) (Prusiner, 1982). PrP^{C} is the normal cell membrane prion protein and is distributed in virtually every organ, but more heavily concentrated in the central nervous system. Studies have shown that PrP^{C} is converted post-translationally into a partially proteinase K-resistant, infectious isoform, PrP^{Sc} (Bradley and Verwoerd, 2004). The PrP^{Sc} converts surrounding normal prion proteins by promoting them to adopt this abnormal conformation. PrP^{Sc} aggregates build up within the cell and burst out of the cell into the surrounding tissues. As clusters of these cells die, they form the 'spongiform' appearance of these diseases (Prusiner, 1995).

Trace elements

Trace elements, or trace minerals, are so named because of their low concentrations in animal tissues (Underwood and Mertz, 1987). Of the 73 known trace elements, there is unanimous consent that copper, zinc, and selenium are deemed essential to life (Underwood and Mertz, 1987)

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and are essential for the central nervous system function (Prohaska, 1987). An element can be defined as essential when there is a reduction below the 'range of safe and adequate intake' that results in 'impairment of physiological function' (Underwood and Mertz, 1987). An animal suffers from mineral deficiency when the element is below the level needed to maintain the nutrient-dependent function. In the 1930s many nutritional disorders among humans and livestock species were linked to either deficiency or toxicity of ingested trace minerals (Underwood and Mertz, 1987).

Trace elements most often function as catalysts in metabolic processes (Prohaska, 1987; Underwood and Mertz, 1987), but they also play a role in structure of tissues and organs and as constituents of tissues and fluids (Underwood, 1981). When they are a part of a metalloenzyme, the trace element is strongly associated with the protein, with a fixed number of metal atoms per molecule of protein. This metal–protein interaction adds to the stability of the enzyme. These metal atoms should not be able to be replaced by any other metal (Underwood and Mertz, 1987), but there have been instances of metal substitution in the metalloenzymes, resulting in an unstable protein. Concentrations of trace minerals are controlled by homeostatic mechanisms, such as intestinal absorption and the use of chemical sinks that bind otherwise toxic amounts of elements into benign forms. A continued exposure to mineral deficiency or toxicity results in changes in the concentration and functionality of trace elements in the body tissues. When this happens, not only are physiological processes affected, but there can be biochemical defects and structural disorders as well (Underwood and Mertz, 1987).

The interactions of trace elements can be synergistic or antagonistic, depending on the reaction of the metabolic function. A synergistic interaction occurs when one element depends on another element(s) in order to perform its normal metabolic function. An antagonistic interaction occurs when the relative excess of another element(s) impairs one element's normal function and there is competition for the same binding site (Kirchgessner *et al.*, 1982).

Copper

Copper and the PrP^C

Copper binding

The PrP^C N-terminus has been shown to be unstructured, while the C-terminal region is highly structured, having three α-helices and two β-sheets (Jones *et al.*, 2005; Zahn *et al.*, 2000). Studies have shown that PrP^C is a cuproprotein, and it is reasonable to assume that it functions in the metal-bound state (Thompsett and Brown, 2004). In PrP^C there are several octapeptide repeat sequences (PHGGGWGQ) towards the N-terminus of the protein, and PHGGGWGQ is highly conserved between mammals. The histidine-rich sequence (PHG) is similar to other histidine-rich sequences in cupro-proteins (Hornshaw *et al.*, 1995a). It has been shown that one copper ion binds to each of four histidine residues on the octapeptide repeat regions on PrP^C, with a higher affinity over other divalent ions (Brown *et al.*, 1997b; Hornshaw *et al.*, 1995a; Viles *et al.*, 1999), although other metals in sufficient concentration can bind to PrP^C (Gaggelli *et al.*, 2005). However, there are only two high affinity Cu^{2+} binding sites on the PrP^C octapeptide repeat region that have similar binding affinities as superoxide dismutase (SOD) and ceruloplasmin. Burns *et al.* (2002) suggest that there may be a pH-dependent process for PrP^C to detect copper in the extracellular matrix or to release copper in the endosome. At a weakly acidic pH, the Cu^{2+} binding site changes and the Cu^{2+} ion is shared between two histidine residues of different peptide chains. It appears that this pH dependency would regulate the delivery of copper via the endocytosis of PrP^C from the cell surface to the endosome. The researchers feel that the sharing of the copper ion between two peptide chains may be related to the aggregation of the pathogenic prion proteins or the conversion of PrP^C to PrP^{Sc} (Miura *et al.*, 1999; Burns *et al.*, 2002).

Whether copper loading of PrP^C happens internally or externally of the cell is unknown. Copper-loaded PrP^C may act as a copper transport protein or perhaps as a copper chelator, preventing copper from participating in redox reactions that cause reactive oxygen species (ROS) (Thompsett and Brown, 2004). Hornshaw *et al.* (1995b) note that the binding of copper to PrP^C causes conformational altering of the protein structure, possibly for protection from degradation. Copper may act as a catalyst for the protein to perform certain functions, or PrP^C may act as a 'sink' for otherwise toxic copper ions. Copper has also been found to bind in the C-terminal domain, perhaps to the nitrogen atom of one of the three histidines in this region. This finding raises the question of copper's involvement with the conversion of PrP^C to PrP^{Sc} (Cereghetti *et al.*, 2001; Jackson *et al.*, 2001; Van Doorslaer *et al.*, 2001).

Copper, when bound to PrP^C, has been also found to stimulate endocytosis into the endosome and portions of the Golgi (Brown and Harris, 2003). Deletions of portions of the N-terminal domain result in rates of endocytosis that are less efficient (Pauly and Harris, 1998). Recombinant PrP^C, in which the histidine residues in the repeat segments are removed, is poorly endocytosed when bound to copper. This suggests that it is the binding of metal that induces the endocytosis as opposed to another protein that indirectly controls endocytosis (Brown *et al.*, 1999; Jackson *et al.*, 2001). Additions of extra octapeptide repeats, such as found in familial CJD (Goldfarb *et al.*, 1991), caused no endocytosis of the protein. The authors concluded that: the octapeptide repeat region, and not the other copper-binding sites, are critical for

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copper-induced endocytosis of PrP^C; the octapeptide repeats do not independently bind copper ions, but rather cooperatively bind copper ions; and additional copies of the repeat region inhibit the copper-binding endocytosis of PrP^C and may inhibit the other functions or interactions of PrP^C (Sumudhu *et al.*, 2001).

The uptake of copper has been shown to be proportional to PrP^C expression at the synapse (Brown, 1999; Herms *et al.*, 1999). Brown *et al.* (1997b) have found that synaptosomes and endosomes in mice genetically modified not to express PrP^C ($Prnp^{0/0}$) had one-tenth the amount of copper as compared to wild-type mice expressing PrP^C, which is important considering the high levels of synaptic PrP^C. This provides strong evidence that membranes deficient in PrP^c are deficient in copper. The authors conclude that PrP^C is 'either a principal copper-binding protein in brain membrane functions, or that it controls the activity of other membrane-associated copper-binding proteins' (Brown *et al.*, 1997b). Brown *et al.* (1999) also found that a deletion of the octapeptide repeat region of recombinant mouse PrP^C showed a reduction in the level of copper present.

SOD activity

Cu, Zn-SOD reacts with the damaging superoxide ion (O_2^-) to form hydrogen peroxide and oxygen (Thompsett and Brown, 2004). It is speculated that one of the functions of PrP^C may be as a copper chaperone, delivering copper to Cu, Zn-SOD (Thompsett and Brown, 2004). Despite this, recombinant PrP^C has been shown to exhibit SOD activity of its own *in vitro*, at almost 30% that of Cu, Zn-SOD (Brown *et al.*, 1999). Using transgenic mice that over-express PrP^C, Brown and Bessinger (1998) found that increasing the level of PrP^C expression increases Cu, Zn-SOD activity without changing Cu, Zn-SOD. These results show a correlation between PrP^C expression and the incorporation of copper into Cu, Zn-SOD, although the results do not prove that PrP^C bound the copper ions. This SOD activity is found when the protein is refolded in the presence of copper and is not found on mutant prion proteins lacking the octapeptide repeat region, which is highly selective for copper (Stöckel *et al.*, 1998; Brown *et al.*, 1999). Native PrP^C, as compared to recombinant PrP^c, isolated from mouse cells bound up to four atoms of copper per molecule. The amount of copper available closely regulates the antioxidant activity of PrP^C, with only two atoms of copper per molecule needed for SOD-like activity to occur (Brown *et al.*, 2001). Despite these findings, two independent studies reported that *in vivo* there was no relation between brain copper content and PrP^C expression, and there was no indication of PrP^C having SOD-like activity (Waggoner *et al.*, 2000; Hutter *et al.*, 2003).

The expression of PrP^C has been shown to be linked to total SOD activity (Brown *et al.*, 1997c; Wong *et al.*, 2000b), and reduction of SOD activity in $Prnp^{0/0}$ mice was not related to a reduction in Cu, Zn-SOD or Mn-SOD (Wong *et al.*, 2000b). PrP^C expression helps cells fight off oxidative stress. Cells extracted from $Prnp^{0/0}$ mice die more rapidly from increased susceptibility to oxidative stress and are unable to upregulate SOD-1 (Brown *et al.*, 1997a). These cells have increased oxidative damage to lipids and proteins in many structures where PrP^C is expressed and there is an indicator of decreased SOD activity (Klamt *et al.*, 2001). *In vitro* studies have shown that in $Prnp^{0/0}$ mice brains there were elevated levels of oxidative markers, such as protein oxidation and lipid peroxidation (Wong *et al.*, 2001c), and neurons from $Prnp^{0/0}$ mice were up to 60% more susceptible to hydrogen peroxide toxicity than wild-type mice (White *et al.*, 1999). $Prnp^{0/0}$ mice have been shown to exhibit a normal lifespan and behavior (Büeler *et al.*, 1992; Büeler *et al.*, 1993). Despite the evidence of increased oxidative damage in $Prnp^{0/0}$ mouse brain, no clinical signs of neurological oxidative stress in the live mice were discussed (Brown *et al.*, 1997a; White *et al.*, 1999; Wong *et al.*, 2001c). It is possible that these *in vitro* studies may have artificially increased the oxidative stress shown.

PrP^{Sc}-like properties

In regards to its structure, PrP^c is found to contain about 42% α-helices and only 3% β-sheets, while PrP^{Sc} contains about 30% α-helices and 43% β-sheets (Pan *et al.*, 1993). Studies have found that the octapeptide repeat segment is unstructured without copper (Viles *et al.*, 1999). PrP^C adopts a new conformation when bound with copper, which is restricted to the N-terminal region (Wong *et al.*, 2000a). This shows that copper-binding to PrP^C causes a physical change in the protein that is related to its functionality (Brown and Harris, 2002). There are several differing views about conformational changes in PrP^C when it binds to copper. One study found that the binding of Cu^{2+} to the N-terminal domain octapeptide repeat region propagates an α-helical structure (Miura *et al.*, 1996). This is supported by the fact that correct incorporation of copper into recombinant PrP^c stabilizes the protein and makes it more soluble (Daniels and Brown, 2002). On the opposing viewpoint, a study found that fresh recombinant PrP^C fragments had no interaction with Cu^{2+}, but the aged PrP^C fragment in the presence of Cu^{2+} converted Asn-107 to Asp and showed a 30% decrease in α-helices and a 100% increase in β-sheet formation (Qin *et al.*, 2000). The authors suggest that this conversion of Asn-107 to Asp may form PrP^C variants that, in the presence of Cu^{2+}, may generate PrP^{Sc}-like species (Prusiner, 1982; Qin *et al.*, 2000). Another study showed that PrP^C bound with copper tends to shift from an α-helical secondary structure to a β-sheet aggregate (Stöckel *et al.*, 1998). This is supported by the fact that each Cu^{2+}-HGGGW segment on the octapeptide repeat region is separated by a Gly-Gln-Pro link, in which the Gly and Pro molecules are associated with β-turns

https://doi.org/10.1017/S1466252307001181 Published online by Cambridge University Press

(Aronoff-Spencer *et al.*, 2000), and the conformational change that occurs when Cu^{2+} binds to the repeat segment is expected to be a β-turn (Bonomo *et al.*, 2000). It is evident that copper contributes to the conformation of PrP^{C}, but Viles *et al.* (1999) found it was only in turns and structured loops, as opposed to α-helices or β-sheets, as found in the other studies mentioned. These authors feel that these other studies may have had competition between the buffer and the octapeptide repeats for copper binding.

In addition to proposed conformational changes and stabilization in the protein's secondary and tertiary structures (Gustiananda *et al.*, 2002), copper-binding can also cause other PrP^{Sc}-like properties. The histidines in the octapeptide repeat region are highly susceptible to oxidation given that they are involved with copper binding. One study found that the oxidation of recombinant PrP^{c} with bound copper causes aggregation of the protein, similar to that of α-synuclein (Requena *et al.*, 2001), while another study saw that when PrP^{C} is refolded with copper, it decreases its sensitivity to proteinase-K digestion, but reduces the level of aggregation (Wong *et al.*, 2000a). A third study saw the outcome of both of these previous studies. Incubation of recombinant PrP^{C} with Cu^{2+} generated a proteinase-K resistant PrP^{c} that formed aggregates. This transformation to the recombinant PrP^{C} fragment needed no acidic pH, denaturants or reduction agents (Qin *et al.*, 2000). Other studies report that: in the presence of copper, PrP^{Sc} brain renatured abnormally and regained proteinase-K resistance (McKenzie *et al.*, 1998); and PrP106-126 aggregation and fibril formation was restored in the presence of Cu^{2+}, indicating that copper binding to PrP106-126 may cause the peptide to interact with PrP^{C} (Jobling *et al.*, 2001). It should be noted, though, that studies using PrP^{C} extracted from transgenic mice and recombinant PrP^{C} show that copper causes these proteins to adopt a proteinase-K resistant and detergent-insoluble form, similar to, yet structurally different from PrP^{Sc}. This transformation requires only one octapeptide repeat segment on the N-terminal domain (Quaglio *et al.*, 2001) and shows total resistance to proteolytic degradation, while PrP^{Sc} shows only partial resistance to proteolytic degradation (Kuczius *et al.*, 2004).

Copper-binding causes prion diseases

There are differing viewpoints as to copper's role in prion diseases. There are those studies that support the viewpoint that copper plays a role in the origin of the PrP^{Sc} isoform. Metal ions, such as Cu^{2+} and Zn^{2+}, have been shown to interact with two phenotypical strains of PrP^{Sc}. When the metal ion contents are maintained, the authors found that they could interconvert these two forms (Wadsworth *et al.*, 1999); however it did not prove that the metal ion occupancy caused the PrP^{Sc} isoform. Mouse PrP^{C} expressing a valine at codon 128, which corresponds to the human CJD mutation at codon 129, showed a higher β-sheet content in the C-terminus and higher aggregation than the mouse PrP^{C} expressing the normal methionine when refolded with copper ions (Wong *et al.*, 2000c).

Since copper is involved in the production of ROS, it is implicated in several studies in the oxidative stress associated with prion disease. During prion disease, many tissues and organs that express PrP^{C} may become sensitive to ROS injury which leads to multiple tissue and organ damage (Klamt *et al.*, 2001). In the presence of ROS, specifically hydrogen peroxide, there is a copper-dependent cleavage in the N-terminal octapeptide repeat region of PrP^{C}. The authors suggest that, since there have been additional octapeptide repeat regions found in PrP^{C} in CJD patients, there would be additional copper-binding sites on this mutant protein, which could increase the susceptibility of the protein to ROS (McMahon *et al.*, 2001). In prion infected neuronal cells, as compared to non-infected controls, there was an increase in the cells to oxidative stress; there was a decrease in both Cu, Zn-SOD and Mn-SOD activities, as well as a decrease in Cu, Zn-SOD protein expression; and there was a higher rate of rapid cell death, all of which suggest that ROS plays an important role in TSE pathology (Milhavet *et al.*, 2000).

Copper deficiency causes prion diseases

The opposing viewpoint is that a copper deficiency or copper displacement on the PrP^{c} contributes to the pathology of prion disease. Over 30 years ago, Pattison and Jebbett (Pattison and Jebbett, 1971a) found that the copper chelator cuprizone caused toxicity in mice that presented clinical signs similar to that of scrapie. They found astrocyte hypertrophy and bilateral symmetry of the lesions in the cuprizone encephalopathy that is characteristic of scrapie; however, the patterns of extracellular vacuolation differ between the two diseases (Pattison and Jebbett, 1971b). Unlike scrapie, the encephalopathy found in cuprizone toxicity was not infectious to other animals (Pattison and Jebbett, 1973). CWD was first thought to be a disease related to copper deficiency because of the similarities in pathology, but it was subsequently classified as a spongiform encephalopathy (Bahmanyar *et al.*, 1985; Thompsett and Brown, 2004).

Both PrP^{Sc} and PrP106-126 bind PrP^{C} in the same region (AGAAAAGA), inhibiting the cell from taking up copper and preventing the SOD-like activity of recombinant PrP^{C} (Brown, 2000). PrP^{C} binding of copper is significantly reduced in sCJD patient brains, while levels of Mn^{2+} and Zn^{2+} are increased. Cu, Zn-SOD activity was greatly reduced in sCJD patients as compared to non-sCJD patients, for whom Mn-SOD activity was twice as much as that in sCJD patients. The expression of Cu, Zn-SOD and Mn-SOD were not altered and were the same between cases and controls (Wong *et al.*, 2001a). In mice infected with scrapie, the antioxidant activity was greatly reduced

https://doi.org/10.1017/S1466252307001181 Published online by Cambridge University Press

and the copper level was reduced by 60%. There was a marked increase of nitric oxide and superoxide in the TSE brains (Wong *et al.*, 2001b). An additional study using scrapie-infected mice found that copper levels in brain showed significant reduction before the onset of clinical signs, but the liver showed an increase in copper levels.

Since the liver is the main site for copper storage and processing, this suggests that copper was displaced from other tissues (Thackray *et al.*, 2002). Rachidi *et al.* (2003) showed that copper binding was diminished in PrP^{Sc} infected cells that had an increased number of cleaved PrP^{d}. And finally, recombinant PrP^{Sc} folded with manganese and nickel will retain both metals. The manganese-loaded protein showed almost no SOD activity after aging 2 weeks, whereas the copper-loaded protein showed the same SOD activity as fresh material. The aged manganese-refolded PrP^{c} protein showed increased proteinase-K resistance and showed secondary structure changes with greater β-pleated sheets. This is the first study to show the formation of this misfolded protein with PK resistance without interaction with PrP^{Sc}. This supports the thought that a metal imbalance could lead to the formation of the 'rogue' protein (Brown *et al.*, 2000). There is substantial evidence to support the hypothesis that the formation of PrP^{Sc} is associated with a loss of copper binding on the normal PrP^{c}.

Manganese

Manganese, copper and the PrP^{C}

Although manganese is not a true antagonist to copper, its competition with copper for a binding site on PrP^{C} may be more important than any other transition metal in regards to forming PrP^{Sc}. Recombinant PrP^{C} has been shown to bind manganese in place of copper with the same affinity (Brown *et al.*, 2000) or with a weaker affinity on the N-terminal octarepeat segment, and Mn^{2+} will also bind at a second site near His-96 or His-111 (Jackson *et al.*, 2001). After binding, manganese alters the conformation of PrP^{c} into a proteinase-resistant isoform that forms fibrils and has a destabilizing behavior (Brown *et al.*, 2000; Tsenkova *et al.*, 2004). PrP^{C}-Mn fibril formation can be explained by its hydrophobic nature and by an increase in water trimers (Tsenkova *et al.*, 2004). PrP^{C}-Mn shows reduced SOD activity, an increase in proteinase-K resistance, and an increase in β-sheet content over a period of time, without a loss of manganese content and without any interaction with PrP^{Sc} (Brown *et al.*, 2000). On the neurotoxic PrP106-126 fragment there is a strong binding affinity for both Mn^{2+} and Cu^{2+}. Although they bind at separate locations, the metal-binding sites share the imidazole ring of His-111, where there could be competition between manganese and copper (Gaggelli *et al.*, 2005).

In sCJD patients, Wong *et al.* (2001a) found a decrease in the bound copper and an increase in manganese cations in PrP^{Sc} species solutions, while they found that manganese was undetectable in PrP^{C} solutions from controls. With these outcomes, they also found an 85% decline in SOD activity in sCJD cases. In scrapie infected mice, the expression of the Mn-SOD and Cu, Zn-SOD enzymes was unaffected; however, overall SOD activity was significantly reduced, suggesting that PrP^{C} has some form of SOD activity (Wong *et al.*, 2001b). In another study with scrapie-infected mice, changes in trace metal concentrations were assessed post-inoculation. The researchers found significant elevations of manganese first in blood and later in brain and muscle and also a decrease of copper in the brain. An increase in Mn-SOD activity was noted, indicating an increase in oxidative stress, which has been demonstrated in TSE disease (Guentchev *et al.*, 2000; Thackray *et al.*, 2002). It has been suggested that metal imbalances could influence the incidence of prion disease (Brown *et al.*, 2000; Purdey, 2000) and also that prion disease may cause a change in copper and manganese levels, especially in the brain of infected animals (Thackray *et al.*, 2002).

Zinc

Zinc, copper and the PrP^{C}

Zinc and copper have a mutually antagonistic relationship. Animals with a zinc deficiency end up with accumulation of copper in the tissues and signs of copper toxicity, while animals with high dietary concentrations of zinc will have lower concentration of copper in the tissues (Stahl *et al.*, 1989) and clinical and pathological signs of copper deficiency (Gawthorne, 1987). Because of its ability to reduce the amounts of copper in tissues, zinc has been used for treatment of Wilson's disease in humans (Brewer *et al.*, 1993; Friedman and Yarze, 1993). The site of the interaction between zinc and copper is thought to occur in the intestine at the site of absorption (Ogden *et al.*, 1988). In the intestine there may be competition between zinc and copper for binding sites on an absorption-enhancing protein or a transporter (Van Campen and Scaife, 1967; Condomina *et al.*, 2002). Metallothioneins (MTs) are metalloproteins that are synthesized in the presence of certain bivalent cations, such as zinc (Mason *et al.*, 1980). High amounts of zinc cause the synthesis of MTs in the intestine and liver. Copper then binds to the MTs and is unavailable for absorption (Sandstead, 1978; Fischer *et al.*, 1981). MTs are also thought to be involved in the metabolism of both zinc and copper by transferring them to metalloenzymes like Cu, Zn-SOD (Cousins, 1985). Zn^{2+} competes with Cu^{2+} for the Cu^{2+}-binding site on Cu, Zn-SOD, causing Cu^{2+} to be unable to transfer electrons (Sharonova *et al.*, 2000). Zinc appears to act as an antioxidant against

https://doi.org/10.1017/S1466252307001181 Published online by Cambridge University Press

copper. Copper supplementation causes an oxidant effect, as indicated by an increase in SOD activity (Irato and Albergoni, 2005), and oxidative damage to cell membranes is seen when there is a zinc deficiency (Bettger *et al.*, 1978).

Extracellular zinc is associated with the normal PrP^{C}. Similar to copper, Zn leads to endocytosis of PrP^{C} in neuroblastoma cell. This endocytosis is specific for zinc and copper over the other transition metals (Brown and Harris, 2003; Pauly and Harris, 1998). Wong *et al.* (2001) found that human subjects with sCJD had a decreased amount of bound copper and an increased amount of zinc cations in PrP^{C} solution, as well as an increase in markers associated with oxidative damage. The authors speculate that metal ions, such as zinc, replace copper at PrP^{C}-binding sites, leading to the loss of the PrP^{C} SOD-like activity that is found in non-CJD patients. Recombinant PrP^{C} was found to have strong binding properties for Cu^{2+} but was able to substitute Zn^{2+} at that binding site with less affinity (Jackson *et al.*, 2001). Human PrP106-126 is a good model for PrP^{Sc}-mediated cell death because of its expression for neurotoxicity and stabilization of β-sheet aggregation (Jobling *et al.*, 1999). Jobling *et al.* (2001) found that the N-terminal region of PrP106-126 peptide contains a binding site for Cu^{2+} and Zn^{2+}, which changes aggregation and toxicity. Either of the two transition metals is needed for the aggregation to occur. Wadsworth *et al.* (1999) found that strain-specific human PrP^{Sc}, in particular Type-1 sCJD, interacts with both Cu^{2+} and Zn^{2+} ions, indicating that both of these metals are important in prion disease. The PrP^{Sc} conformation was maintained in the presence of Cu^{2+} and Zn^{2+}, while other metal ions had no effect on the conformation. In a study with scrapie-infected mice, liver, muscle, and blood were examined in both cases and controls for changes in trace metal concentrations. In the cases, there were no changes found in zinc levels in the liver, and only a minor increase of zinc levels in the blood up to 160 days post inoculation (Thackray *et al.*, 2002).

Conclusions

Copper has been shown to be an integral part of the normal prion protein's structure and function; however, copper-binding has also been shown to increase the percentage of β-sheets and proteinse-K resistance, which are properties of PrP^{Sc}. Both manganese and zinc have been shown to interact with PrP^{C} as well. When these metals bind to PrP^{C} instead of copper, there is an increase in proteinase-K resistance, fibril formation and aggregation. Increases in the levels of manganese and zinc have been found in tissues of prion-infected animals with a coinciding decrease of copper levels. Although, it is difficult to reach a conclusion on the association between trace elements and prion diseases, the involvement of these trace elements with other neurodegenerative diseases may indicate the need to explore their role. Experimental and observational studies are needed to explore this relationship.

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https://doi.org/10.1017/S1466252307001181 Published online by Cambridge University Press