REVIEW ARTICLE
The chemistry of copper binding to PrP: is there sufficient evidence to elucidate a role for copper in protein function?

Paul DAVIES and David R. BROWN

Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, U.K.

There has been an enormous body of literature published in the last 10 years concerning copper and PrP (prion protein). Despite this, there is still no generally accepted role for copper in the function of PrP or any real consensus as to how and to what affinity copper associates with the protein. The present review attempts to look at all the evidence for the chemistry, co-ordination and affinity of copper binding to PrP, and then looks at what effect this has on the protein. We then connect this evidence with possible roles for PrP when bound to copper. No clear conclusions can be made from the available data, but it is clear from the present review what aspects of copper association with PrP need to be re-investigated.

Key words: binding affinity, co-ordination, copper, octapeptide, prion protein (PrP), protein stability.

INTRODUCTION
PrP (prion protein) is a cell-surface glycoprotein that has been directly implicated in the pathogenesis of a range of neurodegenerative disorders collectively named the TSEs (transmissible spongiform encephalopathies). These conditions include CJD (Creutzfeldt–Jakob disease) in humans, BSE (bovine spongiform encephalopathy) in cows and CWD (chronic wasting disease) in deer [1,2]. The protein itself exists in two distinct forms, PrPc (cellular PrP) and PrPsc (pathogenic or scrapie form of PrP). PrPsc is derived from PrPc through a process yet to be fully elucidated, but evidence suggests that PrPsc itself is able to act as a template for the conversion [2]. This conversion is entirely conformational and involves no amino acid substitutions or deletions.

Although the role of PrPsc in disease is very well characterized, the physiological role of PrPc remains unclear. It is now fairly well accepted, however, that PrPc binds copper in vivo [3–5]. Several possible functions have been suggested that tie in with this observation including copper sequestration and internalization [4,5], protection against oxidants [6], and direct SOD (superoxide dismutase) activity [3,7–10]. In addition, there have been some studies that suggest copper-independent roles in cell signalling [11,12] and neuron growth [13]. However, these non-copper-related functions have usually been suggested after studies that monitored changes in cellular activity following manipulation of the protein, rather than observation of protein activity.

In the present review, we first look at the chemistry of copper binding to PrP and the structural implications this has for the protein. We then link this evidence to the various proposed roles of PrP in an attempt to elucidate the cellular function of this elusive protein.

THE BIOLOGY OF COPPER
The average human body contains approx. 110 mg of copper, with the brain, kidney and liver containing the most copper per gram of tissue [14]. The majority of this copper is utilized as a cofactor for a variety of key enzymes, most notably SOD and the terminal enzyme in the respiratory chain cytochrome c oxidase [14]. The main function of cofactored copper is in redox chemistry.

In humans, approx. half of the 0.6–1.6 mg of copper ingested per day is absorbed across the intestine and enters the blood [15]. This copper, bound mainly to albumin, travels to the liver and kidney where it then re-emerges in plasma associated mainly with ceruloplasmin. The chelated copper then travels to the various tissues where it is transferred to internal cellular processes, most probably by cell-surface receptors. There is mixed opinion as to the involvement of amino acids or other chelators in this transport process. Certainly the proteins that have been associated with copper transport have a high affinity for the Cu2+, with dissociation constants in the region of 10–100 pM [16], and so would successfully compete with many of the other potential carriers. Additionally, experiments following radioactive copper tracers in rats showed that the majority of copper is bound to high-molecular-mass blood components, suggesting that proteins are responsible for copper transport as opposed to low-molecular-mass amino acids or metal chelators [17]. However, this assumption is complicated by the fact that copper transport is unaffected or even enhanced by the absence of albumin [18]. Furthermore, absence of ceruloplasmin does not prohibit the uptake of copper by cells [19]. This contradicting evidence indicates a level of redundancy in the copper transport system.

PrP AND COPPER BINDING
The octarepeat region: co-ordination
Figure 1 shows a schematic of the primary structure of mouse PrP. The mature PrP, when cleaved from its signal sequence and GPI (glycosylphosphatidylinositol) motif, is approx. 209 amino acids long with a structured C-terminus and, in the absence of copper at least, an unstructured N-terminus [20]. It is within this unstructured N-terminal region that the primary copper-binding domain is found within residues 60–91, known as the octarepeat...
can bind up to four copper atoms. Most mammals also have an incomplete repeat prior to this.

Figure 2 Models of the three equatorial co-ordination modes of copper binding to the octarepeat region

This protein is anchored to the cell membrane by a GPI anchor. The signal peptide for entry into the endoplasmic reticulum and the GPI signal peptide are cleaved off before the protein reaches the cell surface. Glycosylation can occur on one, two or none of the asparagine residues indicated. A hydrophobic region envelopes a cleavage point where the protein is cleaved during normal metabolic breakdown. A disulfide bridge links two regions of the protein which form separate α-helices in the three-dimensional structure of the protein. The complete octarepeats can bind up to four copper atoms. Most mammals also have an incomplete repeat prior to this.

region [21–23], and more controversially within residues 91–111 or the so-called ‘fifth site’ [23–25]. The octarepeat region in the human protein is composed of a sequence of eight amino acids (PHGGGWWGQ) repeated four times, each repeat containing a histidine residue which is generally accepted to be the primary residue responsible for copper co-ordination [2,24,27]. The first real hard evidence for the association of copper with PrP came from the work of Brown et al. [28] in 1997. By carrying out equilibrium dialysis on the recombinant N-terminal region (amino acids 23–98), they were able to show that between 5 and 6 atoms of copper could bind per receptor peptide fragment. Further detailed studies involving EPR imaging and X-ray crystallography on recombinant peptide fragments have demonstrated that a single copper atom is co-ordinated by each octarepeat region in a pentaco-ordinate complex involving residues HGGGW of the peptide only [24,27]. The study by Aronoff-Spencer et al. [27] revealed an equatorial co-ordination involving the imidazole group of the histidine residue, deprotonated amide groups from the adjacent two glycine residues and a deprotonated carbonyl group from the last glycine. A water molecule was also identified as allowing one of its oxygen molecules to co-ordinate axially, forming a bridge to the NH of the indole group on the tryptophan residue. Further work by this group, however, has revealed a more complex binding system. In 2005, the same group demonstrated that the co-ordination of copper was dependent on the degree of copper occupancy on the protein [29]. They revealed three distinct co-ordination modes, clearly divisible at different relative concentrations of copper. Using X-band and S-band EPR and ESEEM (electron spin-echo envelope modulation) to analyse a library of modified peptides, they showed a multiple histidine residue co-ordination mode at low copper occupancy, moving through a transitional co-ordination to maximal occupancy at a physiological pH (pH 7.4). Figure 2 shows the proposed co-ordination modes from their results. Component 1 co-ordination dominates at high copper occupancy of 2 copper equivalents and above. As the group revealed previously, a 3N1O arrangement co-ordinated a single copper per HGGGW motif. Figure 3 shows a three-dimensional model of this mode for clarity. EPR also revealed evidence of dipolar copper–copper centres in approx. 20% of the spectra, suggesting a close copper–copper proximity of between 3.5 Å (1 Å = 0.1 nm) and 6 Å, close enough for van der Waals interactions. These interactions may be responsible for driving a hydrophobic collapse and consequent N-terminal structural organization at full copper occupancy. The possible implications of this structural change are discussed in a later section of this review. Component 2 co-ordination is present only as a transition between low copper occupancy of 1 atom or less and full copper occupancy. The precise co-ordination mode of this component was extremely difficult to accurately characterize, as the authors found it to be mixed with other modes in all of the conditions tested. However, by methylating the second glycine residue in each octarepeat, they were able to successfully block component 1 formation and resolve the co-ordination mode for component 2. The results suggest a 2N2O arrangement at the expected neutral charge state and is illustrated in Figure 2. These findings suggest an intermediate co-ordination involving the histidine residue imidazole group and its exocyclic nitrogen. Further contributions appear to stem from the oxygens of two water molecules within the equatorial arrangement. Component 3, present at low copper occupancy only, is likely to provide the highest-affinity copper binding within the octarepeat as a result of its co-ordination of multiple histidine residues. This co-ordination mode involves either a 3N1O or 4N arrangement and is only available above pH 6.5 and when multiple histidine residues are present. The combined results from this group point towards a mechanism for dramatic structural change within the N-terminus of PrP that is entirely dependent on the amount of copper bound to the protein.

Recent work by Weiss et al. [30] employed EXAFS, EPR, ENDOR (electron nuclear double resonance) and molecular modelling to resolve the copper co-ordination to PrP. In contrast with many previous studies [24,27,29,31,32], they concentrated on spectra from full-length recombinant human PrP. Although the authors report multiple configurations dependent on copper occupancy, they only identified two distinct modes of binding, models of which are shown in Figure 4. As found previously by Mentler et al. [33], a co-ordination identified as species 1 was evident which utilized a 3N1O configuration. Contributions are from the nitrogen on the imidazole group, two glycine amide...
has led to an interest in finding copper analogues to help elucidate NMR when paramagnetic elements, such as copper, are present. Work by Jones et al. [25,37] highlighted these copper-binding regions as His\textsuperscript{96} and His\textsuperscript{111} in the human protein. They also identified the minimum sequence necessary for copper binding to this region of PrP are amino acids 92–96 and 107–111. Recently, Klewpatinond and Viles [42] utilized NMR and Vis-CD (visible-CD) to fully elucidate the co-ordination of copper to this so-called fifth site. Interestingly, they found that the co-ordination of copper changed dramatically depending on both the chain length and pH. The Vis-CD spectra for amino acids 90–126 was strikingly different from amino acids 91–115 of PrP, despite only two co-ordination sites being present on both fragments. By studying each individual site on each fragment by replacing His\textsuperscript{96} or His\textsuperscript{111} with an alanine residue and comparing the spectra of these mutant fragments with that obtained from the original fragments, they determined that this change in spectra was not caused by differences in co-ordination. They concluded that the spectra differences were caused by a change in the relative affinity of His\textsuperscript{96} and His\textsuperscript{111} for copper. Although His\textsuperscript{111} seems to display the highest affinity for copper, His\textsuperscript{96} affinity increases dramatically on the addition of the 11-amino-acid hydrophobic segment. Interestingly, the relative affinity of these two sites is reversed for nickel, with His\textsuperscript{96} demonstrating the tightest binding. They also discovered a multi-co-ordination mode that was strongly influenced by pH. Figure 5 illustrates these three possible co-ordination modes. Although all display a square planar geometry, the differences are clear. At pH 7.5 and above, a 4N complex dominates, which is also illustrated as a three-dimensional model in Figure 6. At pH 6, a ligand rearrangement shifts the co-ordination to a 3N1O configuration. At low pH, a multi-histidine residue 2N2O co-ordination dominates. By combining this EPR data with Vis-CD, the authors conclude that His\textsuperscript{96} and His\textsuperscript{111} bind copper independently, except at low pH, where it appears that both histidine residues are involved in the co-ordination of a single copper atom. Analysis by \textsuperscript{1}H-NMR using nickel as a probe further confirms these findings. A study by Shearer and Soh [43] also reported the key binding site as being present between amino acid residues 106 and 114.

In stark contrast with the finding that His\textsuperscript{111} is most important for copper binding, another group reported that His\textsuperscript{96} was the key site involved [44]. Using real time SPR (surface plasmon resonance) analysis on synthetic peptides and recombinant protein, they also discovered a binding site for manganese within amino acids 91–126. Clearly, in order to understand the physiological importance of copper binding to PrP, it is necessary to know the affinity of the metal for the protein. With copper so tightly regulated in the body [14], any functional copper-binding protein must be able to chelate copper and keep hold of it through its functional life. There have been a great number of attempts made to calculate the dissociation

The chemistry of copper binding to PrP

A very interesting article utilizing \textit{ab initio} simulations [34] produced some co-ordination models that were very similar to those proposed experimentally. The study supported the idea of multi-histidine residue co-ordination under certain conditions. Interestingly, however, the authors suggested that the axial water molecule thought to be involved in pentaco-ordination was actually only bound to the indole group of the tryptophan residue and not directly involved. The authors went on to verify this and confirmed that the copper–water interaction was extremely weak.

The relative difficulty of resolving structures accurately by NMR when paramagnetic elements, such as copper, are present has led to an interest in finding copper analogues to help elucidate the copper–octarepeat environment. One group investigated Ni\textsuperscript{2+}, Pd\textsuperscript{2+}, Pr\textsuperscript{2+} and Au\textsuperscript{3+} ions for their suitability as a diamagnetic probe of Cu\textsuperscript{2+} binding [35]. They found that Pd\textsuperscript{2+} would form a square planar complex (the other ions would not) but in a different co-ordination from that seen for copper. Hence they concluded that, for the octarepeat at least, there are no suitable analogues. Work by Shearer and Soh [36] also showed Ni\textsuperscript{2+} was an unsuitable analogue.

The fifth site: co-ordination

A large body of literature now exists that demonstrates that copper is able to bind outside of the octarepeat region of PrP [37–41]. Work by Jones et al. [25,37] highlighted these copper-binding regions as His\textsuperscript{96} and His\textsuperscript{111} in the human protein. They also identified the minimum sequence necessary for copper binding to this region of PrP are amino acids 92–96 and 107–111. Recently, Klewpatinond and Viles [42] utilized NMR and Vis-CD (visible-CD) to fully elucidate the co-ordination of copper to this so-called fifth site. Interestingly, they found that the co-ordination of copper changed dramatically depending on both the chain length and pH. The Vis-CD spectra for amino acids 90–126 was strikingly different from amino acids 91–115 of PrP, despite only two co-ordination sites being present on both fragments. By studying each individual site on each fragment by replacing His\textsuperscript{96} or His\textsuperscript{111} with an alanine residue and comparing the spectra of these mutant fragments with that obtained from the original fragments, they determined that this change in spectra was not caused by differences in co-ordination. They concluded that the spectra differences were caused by a change in the relative affinity of His\textsuperscript{96} and His\textsuperscript{111} for copper. Although His\textsuperscript{111} seems to display the highest affinity for copper, His\textsuperscript{96} affinity increases dramatically on the addition of the 11-amino-acid hydrophobic segment. Interestingly, the relative affinity of these two sites is reversed for nickel, with His\textsuperscript{96} demonstrating the tightest binding. They also discovered a multi-co-ordination mode that was strongly influenced by pH. Figure 5 illustrates these three possible co-ordination modes. Although all display a square planar geometry, the differences are clear. At pH 7.5 and above, a 4N complex dominates, which is also illustrated as a three-dimensional model in Figure 6. At pH 6, a ligand rearrangement shifts the co-ordination to a 3N1O configuration. At low pH, a multi-histidine residue 2N2O co-ordination dominates. By combining this EPR data with Vis-CD, the authors conclude that His\textsuperscript{96} and His\textsuperscript{111} bind copper independently, except at low pH, where it appears that both histidine residues are involved in the co-ordination of a single copper atom. Analysis by \textsuperscript{1}H-NMR using nickel as a probe further confirms these findings. A study by Shearer and Soh [43] also reported the key binding site as being present between amino acid residues 106 and 114.

In stark contrast with the finding that His\textsuperscript{111} is most important for copper binding, another group reported that His\textsuperscript{96} was the key site involved [44]. Using real time SPR (surface plasmon resonance) analysis on synthetic peptides and recombinant protein, they also discovered a binding site for manganese within amino acids 91–231.

The affinity of copper for PrP

Clearly, in order to understand the physiological importance of copper binding to PrP, it is necessary to know the affinity of the metal for the protein. With copper so tightly regulated in the body [14], any functional copper-binding protein must be able to chelate copper and keep hold of it through its functional life. There have been a great number of attempts made to calculate the dissociation

Figure 3 Three-dimensional model representing component 1 of the equatorial co-ordination mode of copper binding to the octarepeat region

Bond lengths shown are in angstroms (1 Å = 0.1 nm).

Figure 4 Models of the two Cu\textsuperscript{2+} co-ordination modes

(a) Species 1 and (b) species 2 [30].
Figure 5 Models of the Cu²⁺ co-ordination modes for the fifth site

Square planar metal-binding sites at His⁹⁶ and His¹¹¹ in PrP (90–126). Z represents Thr⁹⁵ or Lys¹¹⁰. X represents Gly⁹⁴ or Met¹⁰⁹. (a) Component 1 is a 4N complex that dominates at pH 7.5 and above, (b) component 2 is a 3N1O complex that exists at pH 6 and (c) component 3 is a 2N2O complex that may exist at pH 5.5 [42]. C term, C-terminus; N term, N-terminus.

Figure 6 Three-dimensional model of component 1 of the co-ordination mode of copper at the fifth-site region

Bond lengths are in angstroms (1 Å = 0.1 nm).

THE IMPLICATIONS OF COPPER BINDING

A key factor in the physiological relevance of copper binding to PrP is the effect that copper has when bound. There is an enormous body of evidence reporting on the ability of PrP to aggregate, form amyloid, alter its resistance to proteases, carry out electrochemical activities and transport metals through membranes. The next section of the present review, therefore, looks at the evidence for the effects of copper on PrP.

Protein stability

Metals have been implicated in the tendency of PrP to aggregate and form amyloid fibrils in disease. The first real evidence for an effect of copper on PrP stability was established in 1996...
This group detected an increase in α-helix content in the presence of copper, and therefore a decrease in the β-sheet content which is evident in disease. In 2004, Geise et al. [51] showed that copper was able to block de novo aggregation that had been induced by exposure to manganese. To quantitate and characterize PrP aggregates, they used fluorescently labelled PrP and applied FCS (fluorescence cross-correlation spectroscopy) analysis as well as SIFT (scanning for intensely fluorescent targets) in a confocal single-molecule detection system. Another group showed in 2005 that copper inhibited the in vitro conversion of PrP into its abnormal form [52]. Using a variety of techniques including ThT (thioflavin T) assay and FTIR (Fourier-transform infrared) spectroscopy, they carried out cell-free assays on recombinant human PrP which showed that, at physiological pH, copper was more effective than other metals tested at preventing amyloid formation. Interestingly, they showed that although less efficient, this inhibition still occurred even in the absence of the octarepeat region. They also demonstrated that, despite this apparent protective effect, copper was also able to stabilize pre-formed PrPres (non-amyloidogenic protease-resistant PrP) and copper could also stabilize existing fibrils. Ricchelli et al. [53] also utilized ThT assays to show that copper prevented fibril formation by human PrP, whereas other metals, such as aluminium and zinc, induced aggregation.

Several studies have used PMCA (protein misfolding cyclic amplification) to investigate the effect of copper on the ability of PrP to generate more misfolded protein [54,55]. Both of these studies showed that copper was able to weakly facilitate the process, but less so than other divalent cations.

In contrast, another study in 2006 demonstrated that copper could actually induce amyloid formation in sheep carrying certain allelic variants [56]. This idea is certainly not in isolation, however. For example, Qin et al. [57] showed that copper could increase the proportion of β-sheet content, increase protease resistance and cause aggregation, but only in the aged protein, where Asn107 is converted into aspartic acid by covalent modification. This finding was also supported by work carried out by another group [37,42]. Jobling et al. [58] demonstrated that copper-bound protein was more likely to aggregate than metal-free protein, and also demonstrated increases in β-sheet content as a result of the presence of the metal.

**Protein electrochemistry**

There have been studies performed on the electrochemistry of the PrP when bound to copper. It has been shown that copper is reduced from Cu²⁺ to Cu⁺ on binding to PrP [59]. Brown et al. [7] first suggested that PrP displayed SOD-like activity by carrying out assays with both native and recombinant mouse PrP. By modifying sections of the protein they demonstrated that it was the copper-bound octarepeat region that was responsible for the enzymatic activity of PrP. They further demonstrated this in 2001 with native protein and showed that it was able to protect against oxidative stress [3]. A study in the same year showed that PrP from scrapie-infected mice demonstrated a dramatic reduction in this antioxidant activity [8]. Also in 2001, a study demonstrated that PrP-knockout mice demonstrated an increase in markers of oxidative stress, such as lipid and protein oxidation [60]. Another study produced evidence that the fifth site was redox active by employing cyclic voltammetry on copper-bound PrP fragments encompassing the fifth-site region [43]. Their results clearly demonstrate a quasi-reversible reaction where the fifth site is able to cycle electrons without becoming permanently oxidized or reduced. A separate study also demonstrated a quasi-reversible reaction for copper-bound peptides corresponding to the octarepeat region [61]. Together, these studies suggest that all copper centres on the protein are able to undergo cyclic redox chemistry. In line with this evidence, an independent study showed that PrP does not redox-silence copper [62]. They found that the protein was able to dramatically reduce the amount of hydroxyl radicals present in a Cu²⁺/ascorbate/oxygen system without affecting hydrogen peroxide levels. The conclusion is clearly that the protein is quenching these radicals by a method other than Fenton chemistry, and is doing so sacrificially. Interestingly, they also showed that the octarepeat region was protective against residue oxidation within the fifth site, suggesting that a peptide fragment containing amino acids 90–231 may be more susceptible to oxidative damage.

One recent study has focused on the ability of PrP to generate the free radical superoxide [63]. The study provides compelling evidence that copper-bound PrP is able to catalyse the formation of superoxide and become damaged irreversibly by the radical. It suggests that these oxidative effects are able to produce PrPdx, an intermediate form of PrP, which is then able to undergo conversion into the disease form. This evidence for electrochemical activity involving PrP is supported by numerous other articles (for example, see [64–73]).

In contrast with this rather compelling evidence for the electrochemical properties of PrP, two other studies have cast serious doubt on the ability of PrP to act as a SOD, demonstrating that neither the in vivo protein [74] nor the recombinant protein [75] possess any SOD-like activity.

**Protein behaviour and turnover**

There have been several publications that have investigated the physical behaviour of PrP when exposed to copper. Pally and Harris [4] showed that exposure to copper at the cell surface increased the rate of PrP internalization. They also proved that the N-terminal region of the protein was important for the rate increase of internalization. This finding was later supported by other work [76,77]. Additionally, another area of the protein located at the far N-terminal region consisting of basic residues was also identified as key to the internalization process [76,78,79]. These findings were supported by a study that showed that copper-induced PrP internalization was abolished in the disease state [80]. Brown and Harris [81] showed that copper accelerated the internalization of PrP to transferrin-containing early endosomes and Golgi compartments. Work in 1999 by Brown [5] showed that PrP expression aided cellular copper uptake and further work in 2004 supported this by showing that PrP expression influenced cellular uptake in astrocytes [82]. Further work by our group discovered that physiological levels of copper were sufficient to drive the internalization process and that no other metal could stimulate it [83].

In contrast with this seemingly overwhelming evidence, work in 2000 by Waggoner et al. [84] suggested that both brain copper levels and cuproenzyme activity were unaffected by PrP expression levels, calling into question the importance of any sequestration role by PrP. Additionally, Rachidi et al. [73] also showed that PrP expression did not affect copper delivery.

**COPPER AND THE FUNCTION OF PrP**

**Antioxidant role**

There can be little doubt that PrP does not stifle the ability of copper to undergo redox cycling. Recent studies have clearly demonstrated that copper-bound PrP is fully able to accept electrons and donate electrons cyclically [43,61]. The evidence showing
that PrP does not redox-silence copper [62] is supportive of an electrochemical role for the protein. There is an enormous body of literature supporting a role for PrP directly acting as a SOD [3,7,8,60,68–73]. This evidence, however, really only links PrP to having an effect on oxidative stress, rather than a direct observation of antioxidant activity. It is likely that any protein that bound significant amounts of copper would have some effect on oxidant levels or provide some protection from oxidative stress, if only by chelating a metal capable of producing oxidants itself. A clear piece of evidence that would discount or allow for PrP as an enzymatic antioxidant is the affinity for copper. Unfortunately, the reported affinity values range from micromolar [32] to femtomolar affinity [39]. However, the majority view is that there is at least one nanomolar binding event [44,47,48]. Even a nanomolar dissociation constant, however, does not fit with what is known that a protein with SOD activity needs [38]. The variation in affinity values means a SOD-like role cannot be ruled out. What seems more likely is that PrP protects against oxidative damage by simply chelating redox-active copper or possibly acting as a sacrificial quencher of ROS (reactive oxygen species) [62].

Copper sequestration/buffering/sensing

The extensive work on the metal binding characteristics of both re-combinate and native PrP [24,27,29–31,41,85] all suggest that PrP has multiple metal-binding sites with a high specificity for copper. However, the question remains as to whether the affinity of PrP for copper is high enough to directly chelate copper from copper serum transporters with dissociation constants in the region of 10–100 µM [16]. The evidence for the affinity of PrP for copper is simply too variable to make a reasoned assessment. The highest values given of femtomolar affinity [39,49] would allow for a sequestration role, whereas values in the micromolar range [32] or nanomolar range [47,48] would not. Additionally, does the protein become loaded with copper inside the cells, transporting it to the outside for a protective or catalytic purpose [87] or is the protein presented to the extracellular environment without copper loaded or partially loaded so as to chelate copper from the outside of the cell? These concepts are illustrated in Figure 7 and are key to understanding the role of PrP. It would seem plausible, given the multiple reports of a negative co-operation and multiple dissociation constants within the octarepeat-binding region [39,47–49], that there would be at least some available copper-binding sites free on presentation of the protein to the extracellular environment. This would leave open the interesting possibility that the dramatic restructuring seen in the N-terminus of the protein during maximum copper occupancy [29,30] could be a trigger for internalization of the protein. Clearly there is much direct evidence showing that copper increases the rate of internalization [4] and that the N-terminus is key to this process [76,77]. Additionally, it is now known that physiologically relevant amounts of copper are sufficient to drive the internalization process [83]. This may suggest that PrP is able to function as a concentration-sensitive sequester of copper, activated when extracellular copper reaches peak concentrations during synaptic transmission and depolarization of between 15 µM and 300 µM [88,89]. This copper may then be transferred back into the cells for recycling.

CONCLUSIONS

Despite over 10 years of substantial research on PrP, there is still too many inconsistencies in the available data to draw any final conclusions regarding the role of copper in the function of PrP. Key questions surrounding the affinity of the protein for copper, the redox activity and conformational shift on binding still need to be established with a higher degree of consistency. Clearly, a copper-related function cannot be assigned to a protein that may have an affinity for copper in the micromolar or femtomolar range. One intriguing possibility, however, is that this variability of affinity for copper may actually be reflective of its function as a copper sensor. Clearly, yet more work is still necessary.

We thank Dr Yves Chaperon (Alpine Institute of Environmental Dynamics, La Terrasse, France) and Dr Jean van den Elsen (Department of Biology and Biochemistry, University of Bath, Bath, U.K.) for the molecular modelling in the present review.

REFERENCES


© The Authors. Journal compilation © 2008 Biochemical Society
26 Reference deleted
41 Kim, N. H., Choi, J. K., Jeong, B. H., Kim, J. I., Jeon, M. S., Carp, R. I. and Kim, Y. S. (2005) Effect of transition metals (Mn, Cu, Fe) and dioxygenic acid (DA) on the conversion of PrPC to PrPres. FASEB J. 19, 783–785


Reference deleted

