One Octarepeate Expansion to the Human Prion Protein Alters Both the Zn²⁺ and Cu²⁺ Coordination Environments Within the Octarepeate Domain

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Abstract

The influence of a single octarepeat expansion on the Cu^{II} and Zn^{II} coordination environment within the octarepeat domain of the human prion protein is examined. Using X-ray absorption spectroscopy and diethyl pyrocarbonate labeling studies we find that at low copper concentrations the "normal" octarepeat domain (4 PHGGGWGQ repeats) coordinates Zn^{II} in an $(N/O)_6$ coordination environment with two histidine residues and Cu^{II} in a redox inactive $(N/O)_4$ coordination environment using 1 imidazole residue. Expansion of the octarepeat region by one repeat (5 PHGGGWGQ repeats) yields a 3 histidine $(N/O)_6$ coordination environment for Zn^{II} and a 2 histidine $(N/O)_4$ coordination environment for Cu^{II} at low copper concentrations. This Cu^{II}-$((N/O)_2$-histidine$_2)$ coordination motif is redox active and capable of generating H_2O_2 under reducing aerobic conditions.

Transmissible spongiform encephalopathies (TSE) are a class of neurodegenerative disorders including kuru, bovine spongiform encephalopathy (Mad Cow disease) and Creutzfeldt-Jakob disease.[1] These have been linked to the misfolding/misfunction of the ubiquitous neuronal membrane prion protein (PrP).[1,2] Despite several decades of research, the specifics of the prion disease mechanism(s) or the role that the PrP plays in normal cellular physiology remain unknown. Because of its high affinity for Cu^{2+} it has been speculated that the PrP may be involved in copper trafficking or homeostasis, and that a disruption of this function may lead to TSEs.[3–5]

The PrP contains a disordered *N*-terminal region that comprises roughly half of the protein sequence. It has been shown that this region can normally bind up to six-equivalents of Cu^{2+}.[4–6] The most widely studied copper binding region of the PrP is the so-called octarepeat domain (one octarepeat = -PHGGGWGQ-), which coordinates Cu^{2+} in a redox-inactive square planar coordination geometry ({Cu^{II}ORP} Chart 1). In the normal human PrP there are 4 sequential octarepeats within the octarepeat domain. Recently Millhauser and colleagues demonstrated two interesting aspects concerning the PrP octarepeat domain. One is that the Cu^{2+} coordination environment is dependent upon Cu^{2+} concentration; different intraprotein histidine-based coordination modes can be observed as a function of copper concentration.[7] Some of these copper-sites may lead to a redox active copper ion (e.g. the purported "component 3" motif (Scheme 1)).[7] Another aspect concerning the octarepeat domain is that Zn^{2+} ion can "tie-up" several histidine residues yielding a {Cu^{II}ORP}-like center at low copper concentrations (Scheme 1).[8] Increasing the Cu^{2+} concentration

displaces the Zn^{2+} ion yielding a fully Cu^{2+}-loaded octarepeat domain. These findings suggest that Zn^{2+} may offer neuroprotection by forcing Cu^{2+} to adopt a redox inactive Cu^{2+} coordination geometry under low copper loads. Furthermore, Millhauser has reported on the strong correlation between octarepeat expansions and the onset of Prion diseases.[9]

Herein, we explore the structural basis of these findings. It will be demonstrated that the Zn^{2+} ion does induce Cu^{2+} to adopt a redox inactive coordination geometry for the normal octarepeat domain (i.e. 4 PHGGGWGQ repeats). Additionally we will show that one octarepeat addition to the normal octarepeat domain yields a different and *redox-active* Cu^{2+} coordination geometry upon Zn^{2+} addition.

The ORP, and 2– through 5–repeat fragments (Chart 1) were prepared by standard solid phase peptide synthesis as previously described.[10,11] Metallopeptides were prepared by adding freshly prepared solutions of $CuCl_2$ and/or $ZnCl_2$ to peptide solutions at room temperature. All studies were performed using a solution pH of 7.4 (maintained by 50 mM *N*–ethylmorpholine (NEM) buffer). For all X-ray absorption experiments we used 1:1 solutions of the metallopeptide solutions and glycerol to: 1) avoid sample icing, and 2) to minimize photodegradation of the samples. The addition of glycerol to the solutions did not have any noticeable influence on the Zn^{2+} and Cu^{2+} coordination environments. Due to the sample requirements for X-ray absorption experiments, we utilized concentrations on the order of 0.5 mM (in peptide). We note that gel permeation chromatography indicated that there was no inter-protein metal coordination observed under these conditions.

The ability of these metal-coordinated fragments to produce H_2O_2 was probed using a quantitative peroxide assay (supporting information). When one equivalent of Cu^{2+} is complexed to either the 4- or 5-repeat fragments and ascorbate is added to solution under aerobic conditions we observe the rapid formation of H_2O_2. This is in stark contrast to {$Cu^{II}ORP$}, which does not promote the formation of H_2O_2 under identical conditions. When one equivalent of both Zn^{2+} and Cu^{2+} are coordinated to the 4- repeat there is a reduction in the production of H_2O_2 by 84% relative to a control complex ([Cu(im)$_4$] (ClO$_4$)$_2$). In contrast, we were unable to detect a significant decrease in the formation of H_2O_2 when one equivalent of Zn^{2+} and Cu^{2+} was added to the 5-repeat. Fully loading all of the octarepeats with Cu^{2+} in both the 4- and 5-repeat fragments reduced H_2O_2 production by 93–98%. It thus seems reasonable to suggest that: 1) within the 4-repeat fragment Zn^{2+} coordination is indeed modulating the Cu^{2+} redox chemistry, and thus its coordination environment, and 2) the expansion of the octarepeat domain by *one octarepeat segment* (i.e. the 5-repeat fragment) alters the redox chemistry and coordination environment of at least the Cu^{2+} center at low copper-concentration.

To gain insight into the structural changes induced by the octarepeat expansion in these PrP fragments we utilized both copper and zinc K-edge X-ray absorption spectroscopies (XAS). From our EXAFS analysis, we find that the addition of one equivalent of $CuCl_2$ to the 4-repeat fragment yielded a square-planar (N/O)$_4$ Cu-coordination geometry with 3 to 4 histidine imidazole groups ligated to the copper center at a distance of ~2 Å (Figure 1a; supporting information). Such a copper-center should be capable of undergoing redox cycling and produce reactive oxygen species, which is what is observed. The addition of one equivalent of Zn^{2+} to this solution leads to a dramatic change in the Cu^{2+} coordination environment; the copper center remains a 4-coordinate Cu(N/O)$_4$ complex, but is now only ligated by 1 imidazole (r_{im} = 2.01 Å; Figure 1b). As such the coordination environment is nearly identical to what is observed in the case of {$Cu^{II}ORP$}, as is supported by CD studies (supporting information). The EXAFS region of the Zn K-edge spectrum is best modeled with Zn^{2+} contained in a six coordinate ligand environment with two imidazole ligands (r_{im} = 2.04 and 2.00 Å) and four additional N/O ligands (r = 2.09 Å; Figure 1c). We note that the

observed coordination environment/affinity of Zn^{2+} for the PrP fragment may be influenced by the low temperature (20 K) under which the XAS data were obtained. The addition of 3 equivalents of Cu^{2+} (4 total) to the Cu^{2+}/Zn^{2+} ligated 4-repeat fragment yields an average Cu^{2+} coordination environment that is identical to {$Cu^{II}ORP$}, suggesting that Zn^{2+} is displaced from the peptide, which was confirmed by Zn K-edge XAS.

The above determined Cu^{2+} and Zn^{2+} coordination environments were indirectly supported by employing diethyl pyrocarbonate (DEPC) labeling studies, which identifies uncoordinated histidine residues.[8,12] Histidine imidazoles are readily alkylated by DEPC except when coordinated to a metal-center. Thus, identification of alkylated peptides by HPLC and LC-MS can be used to identify metal-coordinated histidines. The addition of four eq of Cu^{2+} to the 4-repeat fragment eliminates the labeling of histidine residues indicating all four are coordinated to the metal-center. When only one eq. of Cu^{2+} is added to the 4-repeat fragment a large degree of non-specific mono- and bis-imidazole labeling is observed indicting a highly fluxional coordination motif. The addition of excess Zn^{2+} to this solution dramatically suppress all bis- and many of the mono-labeling events, but a large degree of mono-histidine labeling is still observed. This suggests one of the histidines remains uncoordinated per 4-repeat fragment when Zn^{II} and Cu^{II} are coordinated to the PrP-fragment.

The addition of one eq. of Cu^{2+} to the 5-repeat fragment produces a mixture of copper coordination environments, with the square planar 3/4-histidine environment being the major contributor ($r_{imidazole}$ = 2.09 and 1.97 Å). Unlike the addition of one equivalent of Zn^{2+} to the 4-repeat fragment, *the addition of one equivalent of Zn^{2+} to the 5-repeat fragment does not result in a copper coordination environment reminiscent of {$Cu^{II}ORP$}.* Instead, it appears to be a mixture of different $(N/O)_4$ Cu^{2+} coordination structures with a two-histidine coordination complex being the largest contributor ($r_{imidazole}$ = 1.97 and 2.03 Å; Figure 2a). Similar to the 4-repeat we see complete loading of the octarepeat domain when 5 equivalents of Cu^{2+} are added to the 5-repeat (i.e. the {$Cu^{II}ORP$} coordination motif is observed). DECP labeling supports these results (supporting information).

The Zn^{2+} ion also appears to be in a different coordination environment in the 5- vs. 4-repeat fragments. When the Zn^{2+} ion is ligated to the 5-repeat peptide in the presence of Cu^{2+} zinc is contained within a six-coordinate $(N/O)_6$ ligand environment utilizing three histidine residues (one at r = 1.94 Å and two at r = 2.00 Å; Figure 2b) and three other N/O ligands ($r_{N/O}$ = 1.98 Å). Such a situation is confirmed by DEPC labeling studies. The addition of Zn^{2+} to the 5-repeat fragment containing one equivalent of Cu^{2+} will suppressed labeling of all five imidazole groups. As with the 4-repeat fragment, when 5 eq of Cu^{2+} are added to the Zn^{2+} containing 5-repeat peptide fragment Zn^{2+} is released as the aqua-cation and five {$Cu^{II}(ORP)$}-like Cu^{2+} centers are formed.

The use of 2– and 3–repeat fragments suggests that the two-histidines used for Cu^{2+} coordination in the 5-repeat are not adjacent to one another. We find good agreement between the Cu-EXAFS data when one eq of Cu^{2+} is added to the 3–repeat fragment and those of the Zn^{2+}/Cu^{2+} ligated 5-repeat fragment. In contrast, one (and two) equivalent(s) of Cu^{2+} added to the 2–repeat fragment produces a coordination environment reminiscent of {$Cu^{II}(ORP)$}.

These findings support and expand upon Millhauser's previous assertions concerning octarepeat expansions and PrPs.[6–9] Zn^{2+} aids in modulating the Cu^{2+} coordination environment within the "normal" 4-repeat PrP octarepeat domain. Furthermore, Zn^{2+} coordination shuts off ROS production under low-copper loads by forcing Cu^{2+} to adopt a redox inactive {$Cu^{II}(ORP)$} coordination environment, suggesting Zn^{2+} is neuroprotective.

It also appears that octarepeat expansions are potentially neurotoxic. The addition of just one octarepeat expansion completely changes the copper and zinc coordination geometries. Zn^{2+} addition will no longer force the {Cu^{II}(ORP)} coordination geometry, but instead provide Cu^{2+} with a redox-active bis-histidine coordination environment. This opens a means for neurodegeneration through the production of ROSs in these PrP octarepeat expansion mutants. Studies probing this supposition are under-way.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Prusiner SB. Proc Natl Acad Sci USA. 1998; 95:13363–13383. [PubMed: 9811807]
2. Weissman C. Nat Rev Microbiol. 2004; 2:861–871. [PubMed: 15494743]
3. Brown DR, Qin K, Herms JW, Madling A, Manson J, Strome R, Fraser PE, Kruck T, von Bohlen A, Schulz-Schaeffer W, Giese A, Westaway D, Kretzschmar H. Nature. 1997; 390:684–687. [PubMed: 9414160]
4. Millhauser GL. Acc Chem Res. 2004; 37:79–85. [PubMed: 14967054]
5. Gaggelli E, Kozlowski H, Valensin D, Valensin G. Chem Rev. 2006; 106:1995–2044. [PubMed: 16771441]
6. Walter ED, Stevens DJ, Spevacek AR, Visconte MP, Dei Rossi A, Millhauser GL. Curr Protein Pep Sci. 2009; 10:529–535.
7. Walter ED, Chattopadhyay M, Millhauser GL. Biochemistry. 2006; 45:13083–13092. [PubMed: 17059225]
8. Walter ED, Stevens DJ, Visconte MP, Millhauser GL. J Am Chem Soc. 2007; 129:15440–15441. [PubMed: 18034490]
9. Stevens DJ, Walter ED, Rodriguez A, Draper D, Davies P, Brown DR, Millhauser GL. PLoS Pathogens. 2009; 5:e1000390.10.1371/journal.ppat.1000390 [PubMed: 19381258]
10. Aronoff-Soencer E, Burns CS, Avdievich NI, Gerfen GJ, Peisach J, Antholine WE, Ball HL, Cohen FE, Prusiner SB, Millhauser GL. Biochemistry. 2000; 39:13760–13771. [PubMed: 11076515]
11. Shearer J, Soh P. Inorg Chem. 2007; 46:710–719. [PubMed: 17257012]
12. Qin K, Yang Y, Mastrangelo P, Westaway D. J Biol Chem. 2002; 277:1981–90. [PubMed: 11698407]
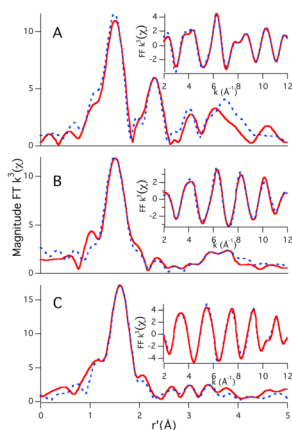
Figure 1.
Magnitude FT $k^3(\chi)$ and FF $k^3(\chi)$ (insets) for (A) 4-repeat with 1 equivalent of Cu^{2+} (Cu-edge), (B) 4-repeat with 1 equivalent of Cu^{2+} and 1 equivalent of Zn^{2+} (Cu-edge) and (C) 4-repeat with 1 equivalent of Cu^{2+} and 1 equivalent of Zn^{2+} (Zn-edge). The refinement details can be found in the supporting information.
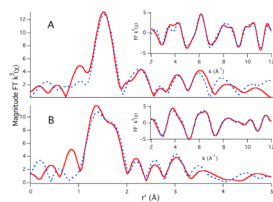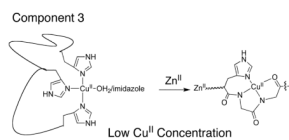
Figure 2.
Magnitude FT $k^3(\chi)$ and FF $k^3(\chi)$ (insets) for 5-repeat with 1 equivalent of Cu^{II} and Zn^{II} at the (A) Cu-edge and (B) Zn-edge. Refinement details can be found in the supporting information.
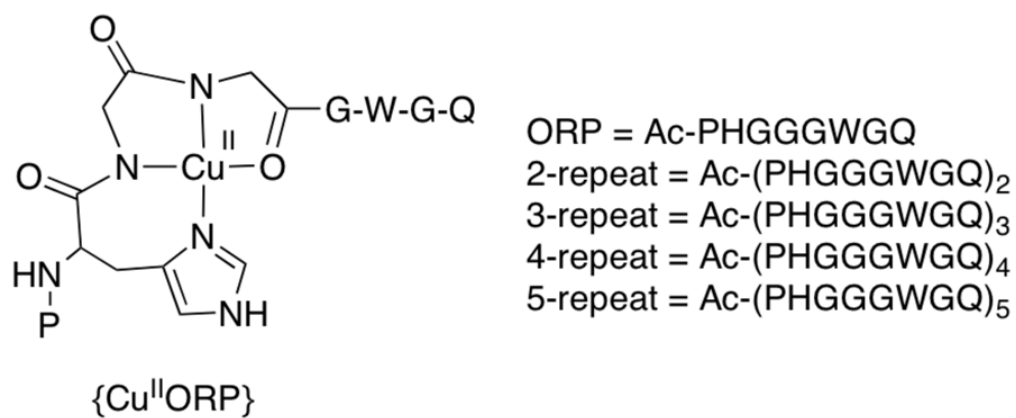
Scheme 1.

ORP = Ac-PHGGGWGQ
2-repeat = Ac-(PHGGGWGQ)$_2$
3-repeat = Ac-(PHGGGWGQ)$_3$
4-repeat = Ac-(PHGGGWGQ)$_4$
5-repeat = Ac-(PHGGGWGQ)$_5$

{Cu^{II}ORP}

Chart 1.