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One Octarepeate Expansion to the Human Prion Protein Alters Both the Zn^{2+} and Cu^{2+} Coordination Environments within the Octarepeate Domain

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The influence of a single octarepeat expansion on the Cu^{II} and Zn^{II} coordination environments 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 (four 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 one imidazole residue. Expansion of the octarepeat region by one repeat (five PHGGGWGQ repeats) yields a three-histidine $(N/O)_6$ coordination environment for Zn^{II} and a two-histidine $(N/O)_4$ coordination environment for Cu^{II} at low copper concentrations. This $Cu^{II}[(N/O)_2\text{-histidine}_2]$ coordination motif is redox-active and capable of generating H_2O_2 under reducing aerobic conditions.

Transmissible spongiform encephalopathies (TSEs) 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 6 equiv 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 four 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 the Cu^{2+} concentration; different intraprotein histidine-based coordination modes can be observed as a function of the 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 a 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., four 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 two- to five-repeat fragments (Chart 1) were prepared by a standard solid-phase peptide synthesis, as was previously described.[10,11] Metallopeptides were prepared by adding freshly prepared solutions of $CuCl_2$ and/or $ZnCl_2$ to

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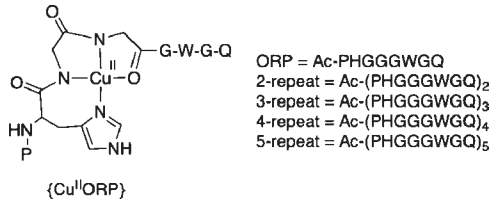
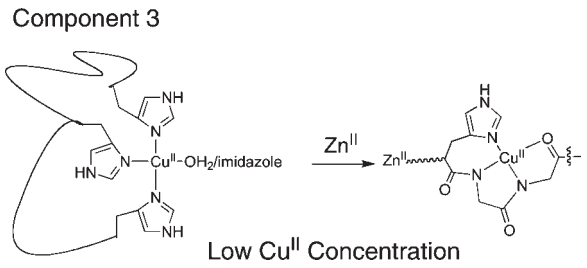
Chart 1



Scheme 1



peptide solutions at room temperature. All studies were performed using a solution pH of 7.4 (maintained by a 50 mM *N*-ethylmorpholine buffer). For all X-ray absorption spectroscopy (XAS) experiments, we used 1:1 solutions of the metallopeptide solutions and glycerol (1) to 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. Because of the sample requirements for XAS experiments, we utilized concentrations on the order of 0.5 mM (in peptide). We note that gel permeation chromatography indicated that there was no interprotein 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 (see the Supporting Information). When 1 equiv of Cu^{2+} is complexed to either the four- or five-repeat fragments and ascorbate is added to the 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 1 equiv each of both Zn^{2+} and Cu^{2+} are coordinated to the four-repeat fragment, 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 1 equiv each of both Zn^{2+} and Cu^{2+} were added to the five-repeat fragment. Fully loading all of the octarepeats with Cu^{2+} in both the four- and five-repeat fragments reduced H_2O_2 production by 93−98%. It thus seems reasonable to suggest that (1) within the four-repeat fragment Zn^{2+} coordination is indeed modulating the Cu^{2+} redox chemistry, and thus its coordination environment, and (2) expansion of the octarepeat domain by *one octarepeat segment* (i.e., the five-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 Cu and Zn K-edge XAS. From our extended X-ray absorption fine structure (EXAFS) analysis, we find that the addition of 1 equiv of $CuCl_2$ to the four-repeat fragment

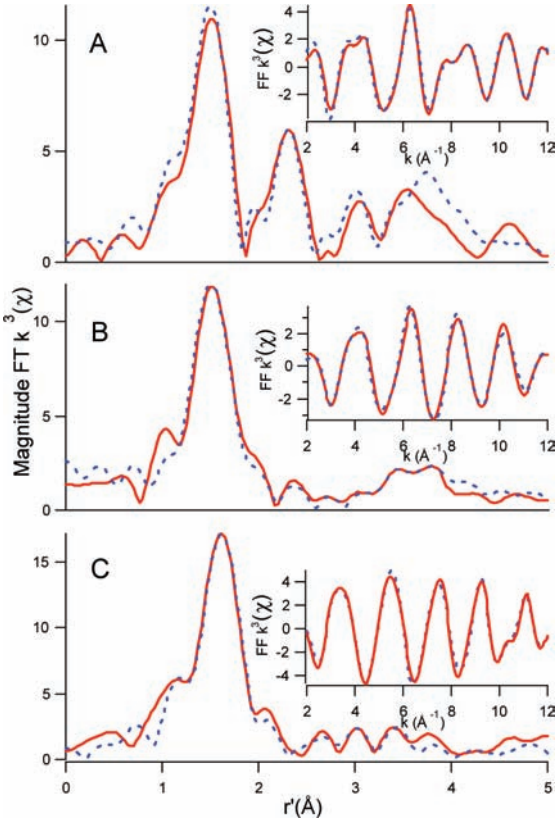


Figure 1. Magnitude FT $k^3(\chi)$ and FF $k^3(\chi)$ (insets) for (A) a four-repeat fragment with 1 equiv of Cu^{2+} (Cu K-edge), (B) a four-repeat fragment with 1 equiv of Cu^{2+} and 1 equiv of Zn^{2+} (Cu K-edge), and (C) a four-repeat fragment with 1 equiv of Cu^{2+} and 1 equiv of Zn^{2+} (Zn K-edge). The refinement details can be found in the Supporting Information.

yielded a square-planar $(N/O)_4$ copper coordination geometry with three to four histidine imidazole groups ligated to the copper center at a distance of ~2 Å (Figure 1a and the Supporting Information). Such a copper center should be capable of undergoing redox cycling and produce reactive oxygen species (ROS), which is what is observed. The addition of 1 equiv of Zn^{2+} to this solution leads to a dramatic change in the Cu^{2+} coordination environment; the copper center remains a four-coordinate $Cu(N/O)_4$ complex but is now only ligated by one imidazole (r_{im} = 2.01 Å; Figure 1b). As such, the coordination environment is nearly identical with that observed in the case of {Cu^{II}ORP}, as supported by circular dichroism (CD) studies (see the 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 equiv of Cu^{2+} (4 total) to the Cu^{2+}/Zn^{2+}-ligated four-repeat fragment yields an average Cu^{2+} coordination environment that is identical with that of {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 high-performance liquid chromatography and liquid chromatography−mass spectrometry can be used to identify metal-coordinated histidines. The addition of 4 equiv of Cu^{2+} to the four-repeat fragment eliminates the labeling of histidine residues, indicating that all four are coordinated to the metal center. When only 1 equiv of Cu^{2+} is added to the four-repeat fragment, a large degree of nonspecific mono- and bis-imidazole labeling is observed, indicating a highly fluxional coordination motif. The addition of excess Zn^{2+} to this solution dramatically suppresses all bislabeling and many of the monolabeling events, but a large degree of monohistidine labeling is still observed. This suggests that one of the histidines remains uncoordinated per four-repeat fragment when Zn^{II} and Cu^{II} are coordinated to the PrP fragment.

The addition of 1 equiv of Cu^{2+} to the five-repeat fragment produces a mixture of copper coordination environments, with the square-planar three/four-repeat histidine environment being the major contributor (r_{im} = 2.09 and 1.97 Å). Unlike the addition of 1 equiv of Zn^{2+} to the four-repeat fragment, *the addition of 1 equiv of* Zn^{2+} *to the four-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_{im} = 1.97 and 2.03 Å; Figure 2a). Similar to the four-repeat fragment, we see complete loading of the octarepeat domain when 5 equiv of Cu^{2+} is added to the five-repeat fragment (i.e., the $\{Cu^{II}ORP\}$ coordination motif is observed). DECP labeling supports these results (see the Supporting Information).

The Zn^{2+} ion also appears to be in a different coordination environment in the five- versus four-repeat fragments. When the Zn^{2+} ion is ligated to the five-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 five-repeat fragment containing 1 equiv of Cu^{2+} will suppress the labeling of all five imidazole groups. As with the four-repeat fragment, when 5 equiv of Cu^{2+} is added to the Zn^{2+}-containing five-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 two- and three-repeat fragments suggests that the two histidines used for Cu^{2+} coordination in the five-repeat fragment are not adjacent to one another. We find good agreement between the Cu-EXAFS data when 1 equiv of Cu^{2+} is added to the three-repeat fragment and the Zn^{2+}/Cu^{2+}-ligated five-repeat fragment. In contrast, 1 (and 2) equiv of Cu^{2+} added to the two-repeat fragment produce(s) a coordination environment reminiscent of $\{Cu^{II}(ORP)\}$.

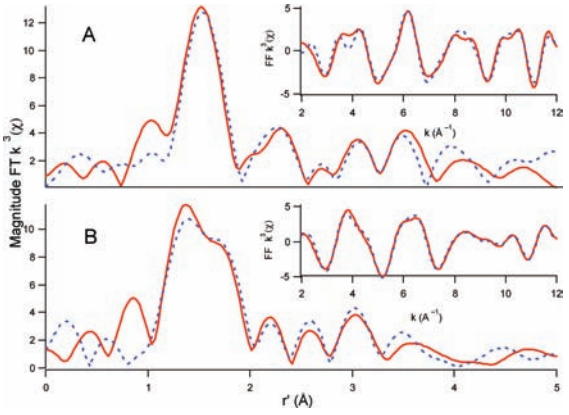


Figure 2. Magnitude FT $k^3(\chi)$ and FF $k^3(\chi)$ (insets) for the five-repeat fragment with 1 equiv each of both Cu^{II} and Zn^{II} at the (A) Cu K-edge and (B) Zn K-edge. Refinement details can be found in the Supporting Information.

These findings support and expand upon Millhauser's previous assertions concerning octarepeat expansions and PrPs.[6−9] Zn^{2+} aids in the modulation of the Cu^{2+} coordination environment within the "normal" four-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 that 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 provides Cu^{2+} with a redox-active bis-histidine coordination environment. This opens a means for neurodegeneration through the production of ROS in these PrP octarepeat expansion mutants. Studies probing this supposition are underway.

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Supporting Information Available: Experimental details, additional XAS spectra, CD spectra, ROS production results, and DEPC labeling assay results. This material is available free of charge via the Internet at http://pubs.acs.org.

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