A Mechanism for Copper Inhibition of Infectious Prion Conversion

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ABSTRACT We employ ab initio electronic structure calculations to obtain two structural models for copper bound in the strongest binding site of the noninfectious form of the prion protein. The models are compatible with available experimental constraints from electron spin resonance data. The bending of the peptide backbone attendant with the copper binding is not compatible with the requisite straight β-strand backbone structure for the same sequence contained in two recently proposed models of the prion protein structure in its infectious form. We hypothesize that copper binding at this site is protective against conversion to the infectious form, discuss experimental data that appear to support and conflict with our hypothesis, and propose tests using recombinant prion protein, genetically modified cultured neurons, and transgenic mice.

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The functional role of the normal cellular prion protein (PrP^C) may well be related to copper binding, potentially protecting against oxidative damage in the synaptic region by sequestering divalent copper (1). This suggestion is supported by data for transgenic knockout mice devoid of the gene for expressing PrP^C; these mice appear to suffer late stage oxidative degradation in the neuronal synaptic regions where surface bound prion protein is preferentially concentrated in wild-type mice (2,3). The strongest copper binding site is in the protein region that converts to β-sheet structure in the infectious (PrP^{Sc}) form (1). We use electronic structure calculations to study two possible geometries for the bound copper-PrP^C complex; we find that these geometries are incompatible with recently proposed models (4,5) for PrP^{Sc} oligomers, and we thus hypothesize a mechanism for inhibition of PrP^C-to-PrP^{Sc} conversion via copper binding.

Copper binds principally to mammalian PrP^C at octarepeat sites of highly conserved form (though not number) in a now well-understood pyramidal geometry with binding to the peptide backbone, histidine side chains, and axial waters (6). These octarepeats (residues 60–91 in humans) are not essential to PrP^{Sc}, which remains infectious even after proteinase exposure that leaves residues 92–230 intact. One strong copper binding site is present in the PrP^{Sc} core region, containing the sequence 92–96 GGGTH for humans. Electron spin resonance data suggest there is binding to the H96 (H95 in mice) side chain and the G94 amide group (1). It is not known whether the primary peptide coordination is to four nitrogens (NNNN) or to three nitrogens and an oxygen (NNON), although the former structure has been conjectured to be more likely (1).

We have studied this problem computationally with the SIESTA local orbital-based density functional theory code using conjugate gradient-based energy minimization to examine possible GGGTH-Cu(II) geometries (7,8). We have considered the NNNN structure and an NNON structure compatible with the ESR data (1) and our results are shown in the upper two panels of Fig. 1 for the mouse prion. We built initial candidate structures of the form Ac-Cu$(H_2O)_6$ (GGGTH)-NH_2 using ChemSite Pro (ChemSW, Fairfield, CA) and VMD (9). For each geometry, the lowest energy sampled provides our model bound copper-prion complex. We used double-zeta (DZ) basis sets for light atoms (H, C, N, O) and double-zeta polarized orbitals for copper. We used the Troullier-Martins norm-conserving pseudopotential. We employed the Perdew-Becke-Ernzerhof-based generalized gradient approximation exchange correlation energy functional. Our energy cutoff for matrix element integration was 120 Rydberg. All calculations were carried out in a periodic unit cell size: $30.7 \times 30.7 \times 30.7$ Å. Geometry minimization was carried out to a force tolerance of 32 pN.

Our main result is inferred from comparing the two possible GGGTH-Cu(II) geometries to recently proposed β-helical PrP^{Sc} trimer models (4,5). We see that the backbone bending induced by copper binding is not compatible with formation of β-strands in the left-handed helices. (It is unclear if another recently proposed model has the same characteristics (10).) Hence, copper in the nonoctarepeat sequence GGGTH can protect against PrP^C-to-PrP^{Sc} conversion; removal of this copper by pH reduction associated with synaptic fluctuations or endocytosis may be a key step in the conversion pathway. This picture is supported by the observation that only the GGTH sequence is required for binding, and eutherian prion sequences reveal high conservation of either GGTH or the very similar GGSH sequence in the converting region (11).

Removal of this copper by pH reduction may be a key step in the PrP^C-to-PrP^{Sc} conversion pathway because it is known that acidic conditions favor detachment of the copper (12). We note two routes for such a pH reduction with accompanying conversion of PrP^C. First, upon endocytosis into the cell, the pH is reduced. Assuming that some PrP^{Sc} is endo-

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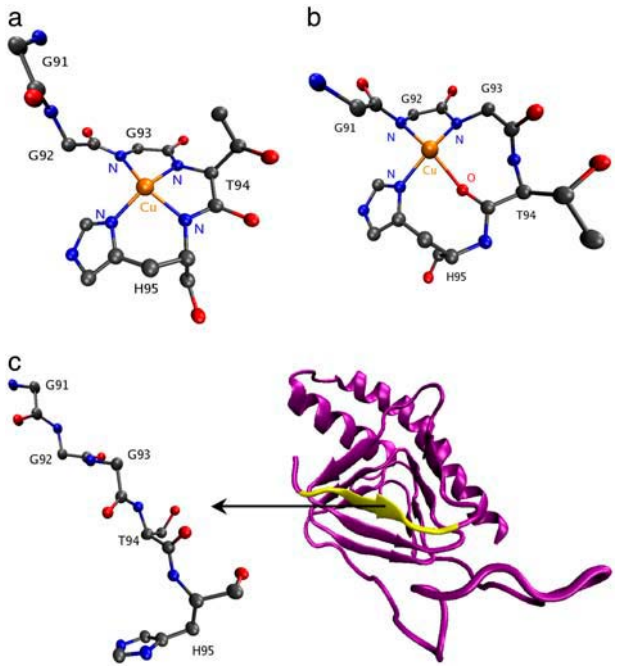

FIGURE 1 Potential copper binding motifs in the converting region of the normal (PrP^{C}) mouse prion protein, which are consistent with ESR data (1) are shown in panels *a* and *b*. The corresponding copper-free stretch of the left-handed β-helix model of the infectious (PrP^{Sc}) protein from Govaerts et al. (4) is shown in panel *c*.

cytosed as well as PrP^{C}, then conversion can take place within the endocytosed compartments after the copper is expelled. Second, given that PrP^{C} is preferentially concentrated near the synapse, then from signal bearing vesicles released near or at the synapse (13) can reduce the pH locally and facilitate copper expulsion.

Three experimental results support our proposed mechanism indirectly: 1), Postmortem studies show copper depletion in regions of infection, with 80–90% reduction of copper content in the corresponding prion proteins (14). This result is consistent with our mechanism, since this GGGTH binds Cu(II) first with potential cooperative enhancement of subsequent binding to other sites. If infection promotes removal of copper from this site, then it is more likely that copper will not be retained at the other sites assuming near-equilibrium conditions. 2), Copper uptake in infected cultured neurons is suppressed ~10-fold compared to control cells (15). Again, this is consistent with the hypothesis, although this requires an understanding of how the prion, which is easily shown to contain <1% of cellular copper, can shut down copper transport. 3), Copper in solution inhibits in vitro growth of amyloid fibrils (16,17). This is a more direct corollary to our hypothesized protective role of copper.

Other experiments provide mixed support for our mechanism. The copper chelator D-penicillamine inoculated in vivo delays disease onset, which is at apparent odds with our hypothesis (18). The chelator cuprizone, however, is known to induce a spongiosis (vacuolation of brain tissue) very similar to that of prion disease although it also induces demyelination of neurons and no transmissibility (19,20). Moreover, it is also known that copper confers proteinase resistance to the cellular prion protein in the weaker binding octarepeat regions (21). This is significant for two reasons. First, proteinase resistance is one of the generic hallmarks of the infectious form of the disease (although not all infectious prion protein is in fact proteinase resistant). Second, the octarepeat deletion does not remove infectivity from PrP^{Sc}. However, in transgenic mice without octarepeats, disease incubation upon direct intercerebral inoculation is slowed (22). Hence, these octarepeats are not necessary for disease, but i), they can impart structure in the presence of copper that confers proteinase resistance to the prion protein, and ii), their presence enhances disease incubation. Indeed, it is known that the octarepeat region adopts a unique structural motif when copper is bound (23). We can rationalize these data by assuming the following affinity ranking: cuprizone > nonoctarepeat site > D-PEN > octarepeat site. This ranking is plausible given the hexadentate chelation of cuprizone versus at most tetradentate coordination of D-PEN. With this assumption, D-PEN lifts the proteinase resistant structure associated with copper binding to octarepeats, and this slows conversion to the infectious form. These assumptions may be tested by performing competitive binding experiments in vitro with D-PEN (18), cuprizone (19), and the relevant copper binding segments of the prion protein. We note that the high affinity of cuprizone for divalent copper apparently leads to stripping of copper from other proteins so that the in vivo phenomena need not be limited to prions; if our assumptions are correct, these nonprion related effects must lead to more rapid degeneration of neurons than conversion to PrP^{Sc}.

To further test the mechanism, it is desirable to perform mutation studies on the stretch GGSH or GGTH. The key idea is to mutate out the H for, e.g., A,Y,G or other amino acids which should severely mitigate copper binding. We propose first that recombinant mutant PrP^{C} from residues 90–230 (with, e.g., H96A in the human form) be allowed to aggregate in copper-full and copper-free environments. Using the reduced length prion will avoid the confounding effects from the octarepeat regions discussed above. If the mechanism is correct, the fibril formation rate should be approximately the same as wild-type fibrils grown in copper-free solutions. Second, one can transfect cultured neurons with the mutated DNA construct and look for increased susceptibility to infection. Third, assuming positive results from the fibril growth and cultured neuron experiments, transgenic mice with the H95 mutated away; these mice should prove to have shorter incubation times for a given dose than wild-type mice. Finally, fibrillization experiments on recombinant marsupial PrP^{C} with and without copper should be informative since the H is replaced with Y24; our prediction based upon the present hypothesis would be that

the copper should have reduced inhibition of fibril formation relative to growth of recombinant human prion protein.

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