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Role of Aspartate-1 in Cu(II) Binding to the Amyloid-β Peptide of Alzheimer's Disease

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Alzheimer's disease (AD) is the leading cause of dementia in the elderly, affecting almost 15 million people.[1] A defining feature of AD is the post-mortem observation of extracellular proteinaceous plaques composed predominantly of the amyloid-beta (Aβ) peptide. In addition to Aβ, the redox-active metal ions iron and copper are found in AD plaques,[2,3] suggesting that these metal ions are involved in AD etiology.[4] Copper is particularly significant because it is implicated in other amyloidosis[5] and its misregulation results in neuropathology associated with Menkes and Wilson's diseases.[6] Because the coordination environment is an important determinant of copper reactivity, work to establish the copper binding site in Aβ and the reactivity of Cu/Aβ complexes[7] are central to understanding copper's role in AD.

Initial studies of Cu(II) with Aβ identified a square planar coordination site dominated by ligands with nitrogen donor atoms.[2] The identities of the ligands involved in Cu(II) binding is controversial,[8−12] but previous in vitro experimental evidence at pH 7.0−7.4 points to nitrogen ligation from histidine residues and/or the amino terminus.[8−12] The full-length peptide (Aβ40) and C-terminal truncated versions (Aβ28 and Aβ16) all bind a high-affinity Cu(II) ion in the same coordination environment.[9,10] However, unlike Aβ40 and Aβ28, Aβ16 does not fibrillize, making it a useful model for high-resolution spectroscopic work on the Cu(II) coordination environment.

We have shown previously that N-terminal deletions to the human Aβ peptide disrupt the native high-affinity Cu(II) binding site.[9] Here, we present low-temperature EPR spectra of Cu(II) bound to N-terminal mutants of human Aβ16 as a means to assess the role these amino acids play in creating the Cu(II) binding site. Figure 1 shows EPR spectra of Cu(II) bound to human Aβ16 and the mutant Aβ16D1N. The EPR spectrum of Cu(II) bound to the Aβ16E3Q mutant (Supporting Information, Figure S1) is the same as that of Cu(II) bound to Aβ40,[9,13] showing that the E3Q mutation has no effect on the Cu(II) binding site. Unlike the E3Q mutant, the EPR spectrum of Cu(II) bound to the D1N mutant is drastically different from Cu(II) bound to wild-type peptide (Figures 1 and S1). At pH 7.2, the EPR spectrum of Cu(II) bound to Aβ16D1N shows two distinct sets of hyperfine peaks indicating the presence of two copper species (components I and II). Component II has $A_{\|}$ and $g_{\|}$ values of 156 $\pm$ 1 G and 2.226, respectively; component I has $A_{\|}$ and $g_{\|}$ values of 170 $\pm$ 2 G and 2.264. The parameters of the two components in the EPR spectrum of Cu(II) bound to Aβ16D1N are identical to those for Cu(II) bound to the Aβ2-16 mutant,[9,14] suggesting that the same two species are present. These results further underscore the assertion that D1 plays an important role in creating the native Cu(II) binding site.[9,10,15]

The pH dependence of Cu(II) bound to Aβ16 or Aβ16D1N was investigated to determine if component II in the D1N mutant spectrum was sensitive to pH (Figure 1).[16] The Cu(II) EPR spectra for Aβ16 are very similar at all of these pH values (Figure 1A) and the dominant species is the same at the highest and lowest pH

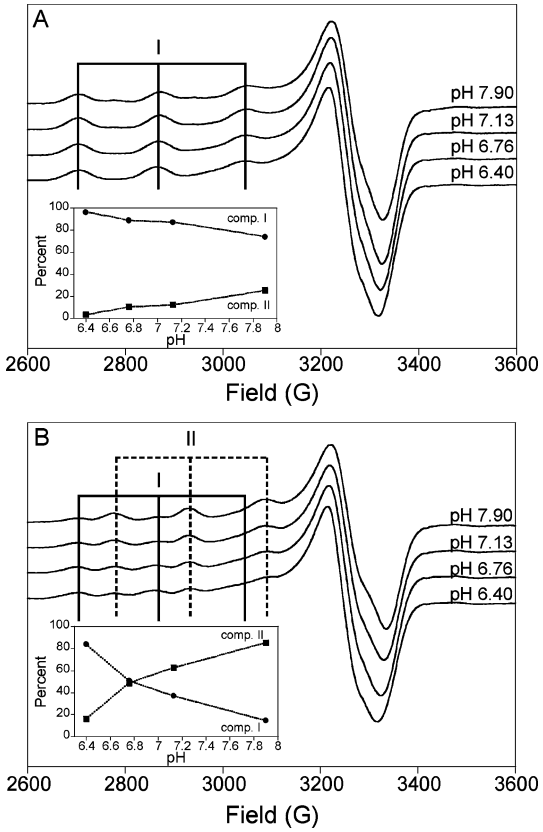

Figure 1. pH dependence of low-temperature EPR spectra of 100 μM Cu(II) bound to 100 μM (A) human Aβ16 [DAEFRHDSGYEVHHQK] and (B) Aβ16D1N. Samples are in 50 mM NaPi, 75 mM NaCl pH 7.2 buffer with 50% glycerol (v/v). EPR conditions: temperature = 20 K, frequency = 9.38 GHz, field center = 3100 G, scan width = 1000 G, modulation amplitude = 10 G, modulation frequency = 100 kHz, microwave power = 0.5 mW, number of scans = 8. Solid and dashed lines show the alignment of the hyperfine peaks. Insets are the relative amounts of simulated component spectra (see Supporting Information).

values (Figure 1A inset). In contrast, spectra of Cu(II) bound to Aβ16D1N are much more pH dependent (Figure 1B). As the pH increases, component II dominates (Figure 1B inset).

Amino acids participate in creating a metal ion binding site by either directly ligating the metal ion or participating in hydrogen bonding interactions at the site.[17] We propose that the carboxylate side chain of D1 participates in a hydrogen-bond that stabilizes the component I coordination mode of Cu(II) at physiological pH (Figure 2). This role for D1 has not been proposed previously. In our model, the two component EPR spectrum observed with the Aβ16D1N mutant (or the Aβ2-16 peptide)[14] represents two Cu(II) species, one with the hydrogen bonding interaction intact (component I) and the other in which it has been changed or removed entirely (component II). A shift in the pK_a of the relevant proton

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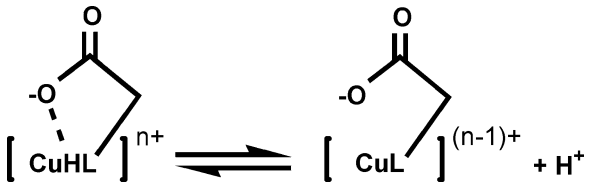

Figure 2. Role of the carboxylate group of D1 in Cu(II) binding to Aβ. CuHL and CuL are the dominant forms of Aβ16 at pH $\approx$ 7.0 and 8.0, respectively.[10] In wild-type peptide, only the CuHL form is observed at pH 7.2. When the D1 carboxylate is removed or mutated, the pK_a of CuHL decreases, leading to our observation of both CuHL and CuL forms.

explains the pH dependences of the Cu(II) EPR signals for the wild-type and mutant peptides. In the wild-type peptide, which has the hydrogen-bonding interaction intact, the pK_a is higher, which leads to very little change in the spectrum over the pH range examined here. When the D1 carboxylate side chain is missing or mutated, the pK_a decreases, resulting in observation of both the protonated and deprotonated forms (CuHL and CuL in Figure 2) in the EPR spectrum at pH 7.2.

This model is supported by several pieces of experimental evidence. First, either mutation or removal[14] of D1 affords the same two-component EPR spectrum. Component I is observed in Cu(II) EPR spectra of both the wild-type and D1N peptides. Thus, D1 removal/mutation does not disrupt the wild-type Cu(II) equatorial coordination sphere. Second, component II, observed in the Cu(II) spectra of the mutants, has parameters identical to those assigned to the CuL species of Aβ16 at pH $\approx$ 8.0.[10] In other words, both components are present in wild-type and D1 mutant peptide Cu(II) spectra; however, the relative amounts differ as a function of pH.

What is hydrogen bonded to the D1 carboxylate? One possibility is an axial water molecule. This model would be like the prion protein octarepeat region (HGGGW) in which a W residue is hydrogen-bonded to an axially bound water on Cu(II).[18] Deletion of W from the octarepeat sequence results in the observation of a mixture of Cu(II)-peptide species.[19] If D1 in Aβ is hydrogen bonded to an axial water on Cu(II), components I and II in our spectra would correspond to the aqua and hydroxo forms, respectively. Another candidate is a backbone amide so that the relevant equilibrium is between the protonated and deprotonated amide. This second scenario is attractive because it is consistent with the observation of amide-N$^-$ $\rightarrow$ Cu(II) charge-transfer bands in solutions of Aβ16 with Cu(II) at pH $\approx$ 8.0.[10,11,20] For both of these scenarios, the change in EPR parameters between the two forms could be explained by either a change in equatorial ligand sphere or a change in complex geometry (without an accompanying change in equatorial coordination sphere). Most changes in Cu(II) EPR parameters are interpreted to mean a change in equatorial donor atoms,[21] but a decrease in $A_{\parallel}$ with no (or little) change in $g_{\parallel}$ also can be attributed to a shift from square planar to a more distorted geometry.[22]

These results and our interpretation have an impact on models for how Cu(II) binds to Aβ and potentially affect other work on copper-binding peptides. It is accepted in the literature that one of the equatorial ligands to Cu(II) in Aβ is an O-atom donor ligand, perhaps tyrosine[8,20] or a carboxylate.[23] There is compelling evidence that tyrosine is not the O-atom donor.[9−11,15] We find that the carboxylates of E3, D7, and E11 also are not involved in Cu(II) binding.[14] On the other hand, there is agreement that D1 participates in creating the native Cu(II) binding site on Aβ.[9,10,15] Our data are consistent with a model in which D1 is involved through hydrogen-bonding interactions to stabilize the native binding site and not via direct equatorial ligation to Cu(II) in Aβ. Many models of Cu(II) with peptides do not include hydrogen-bonding interactions as critical components of the copper binding site.[7] In our view, models that neglect secondary coordination sphere effects have the potential to misdirect work on the chemical role(s) of metal ions in vivo.

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Supporting Information Available: Low-temperature Cu(II) EPR spectra of Aβ mutants, simulations and fits of experimental EPR spectra, and the titration curve to determine pH. This material is available free of charge via the Internet at http://pubs.acs.org.

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