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NMR Reveals Anomalous Copper(II) Binding to the Amyloid Aβ Peptide of Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by the aggregation of the Aβ peptide as insoluble amyloid plaques (amyloidosis), rich in cross-β-sheet structure.[1] The Aβ peptide is a normally secreted and soluble physiological component, ubiquitous in biological fluids. The biological function, if any, of the Aβ peptide remains unknown, even though concentrations vary with inflammation, trauma, and disease state. Increased concentrations occur with head injuries and oxidative stress, suggesting that the Aβ peptide may be neuroprotective,[2] whereas other age-related microenvironmental changes in the AD brain promote amyloidosis that leads to cell injury and death. The amyloid deposits contain high concentrations of redox active metals, such as copper, zinc, and iron,[3] plus the in vitro binding of the Aβ peptide with the metal ions (Cu^{2+}, Zn^{2+}, and Fe^{3+}) can promote[4,5] or hinder amyloidosis.[6−8] Accordingly, it is thought that these binding events in vivo could trigger or prevent amyloidosis in the AD brain,[9] which is supported by encouraging results from clinical trials of drugs with metal chelating properties.[10] Detailed knowledge of the metal−Aβ coordination environment could aid in the development of compounds with more effective and specific metal chelating properties as eventual treatments in AD.

Here, we report that NMR uncovers unique binding properties between copper and the Aβ peptide in solution at pH 7.3. Shown in Figure 1 are HSQC spectra of uniformly ^{15}N-labeled Aβ(1−40) peptide alone and with 1 molar equiv of Cu^{2+}. The HSQC spectra, which detect ^{1}H atoms attached to ^{15}N,[11] show that with Cu^{2+} many Aβ(1−40) signals are too weak for detection. The missing NH signals correspond to the backbone atoms of E3-V18 and the side chain atoms of R5 and Q15. Further inspection at lower contour levels established that the missing signals were buried in the noise. The signals for F19 and F20 became weaker but were still detectable in the contour plot (Figure 1, right). The lack of chemical shift or line width changes for M35[11] demonstrates that reported M35 → M35^{ox} oxidation together with concomitant Cu^{2+} to Cu^{1+} reduction and H_2O_2 production did not take place under the conditions of the experiment.[12−15]

Because the signal disappearance could be from the paramagnetic properties of Cu^{2+}, NMR studies were conducted with the diamagnetic Zn^{2+}. Nearly identical NMR results were seen with Zn^{2+}, suggesting that the signal loss is not from paramagnetic line broadening, but possibly metal-induced amide deprotonation.[16] Supplementing the Aβ/Cu^{2+}or Aβ/Zn^{2+} solutions with 2.0 molar equiv of EDTA resulted in the reappearance of the missing signals. These results establish that the signal disappearance is the direct result of metal binding.

The binding process was further explored by conducting titration experiments, which involved repeated HSQC measurements after incremental Cu^{2+} additions. The amide NH peak intensities (after normalization to a control sample without Cu^{2+}) decreased during the titration (~4 h total), with the NHs belonging to R5-L17 showing the most pronounced intensity reduction (Figure 2A). The hydrophobic region encompassing G29-V40 showed significantly less intensity loss, which is due to an overall loss in signal/noise

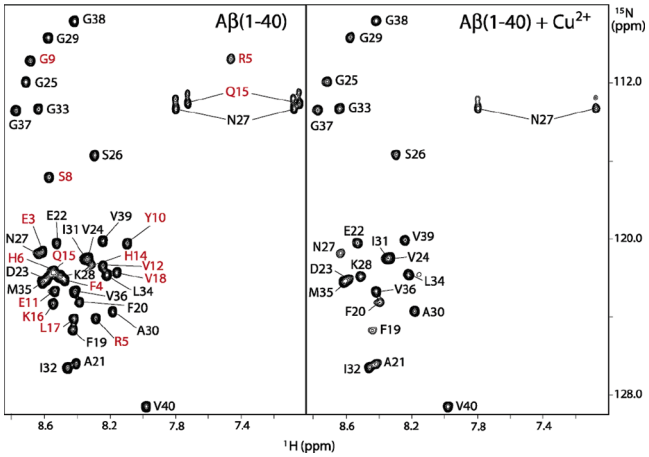

Figure 1. The ^{1}H−^{15}N HSQC spectra of the Aβ(1−40) peptide (130 μM) in aqueous solution containing potassium phosphate buffer (5 mM, pH 7.3, 5 °C). The NMR assignments and sample preparation protocol were described previously,[11] and the Aβ(1−40) was monomeric at the start of each experiment. The two samples were taken from an identical peptide stock solution, in which the control sample (left) contained a 10 μL aliquot of buffer without metal, while the other (right) contained a 10 μL aliquot of buffer with 1 molar equiv of $CuCl_2$. The peaks labeled in red (left) correspond to those that were not detected with the Cu^{2+}.

and not from its proximity to the Cu^{2+}. Additional experiments using native/PAGE gel, Thioflavin-T, and atomic force microscopy demonstrate that, under the experimental conditions, the metals bind to monomeric Aβ(1−40) and prevent β-sheet aggregation and amyloid fibril formation (Supporting Information), in accordance with previous studies.[6−8] The NMR peak loss for Arg5, Val12, and Leu17 yielded a binding constant (K_d) of 1.6 μM (Supporting Information), identical to a previously reported K_d value[17] that was based on a 1:1 binding stoichiometry and fluorescence intensity changes.

At low Cu^{2+} concentrations (5%, 0.05 molar equiv), careful inspection of HSQC spectra showed an approximate 2−8% drop in signal intensity for the NH backbone of R5, S8, K16, and L17, while 1D ^{1}H NMR showed upfield shift movements for the side chain aromatic signals of the three histidines (H6, H13, and H14) (Figure 2B). The lack of ^{1}H NMR chemical shift movements of the N-terminus D1 and the Y10 side chains demonstrates that they are not involved in Cu^{2+} binding. Thus, the Y10 does not undergo oxidative modification,[18,19] consistent with other biophysical studies of the Aβ(1−40).[6−8,20,21]

The present NMR studies reveal unique features about the Aβ/metal coordination environment, notably that a large peptide region is affected by the binding. Previous structural models implied a more precise binding mode and were based on NMR data from smaller peptide fragments, such as the Aβ(1−16) and Aβ(1−28).[22−26] The present NMR data with the native (longer) Aβ(1−40) support a revised interpretation, in which the metal binding is less precise and does not induce formation of typical amyloid

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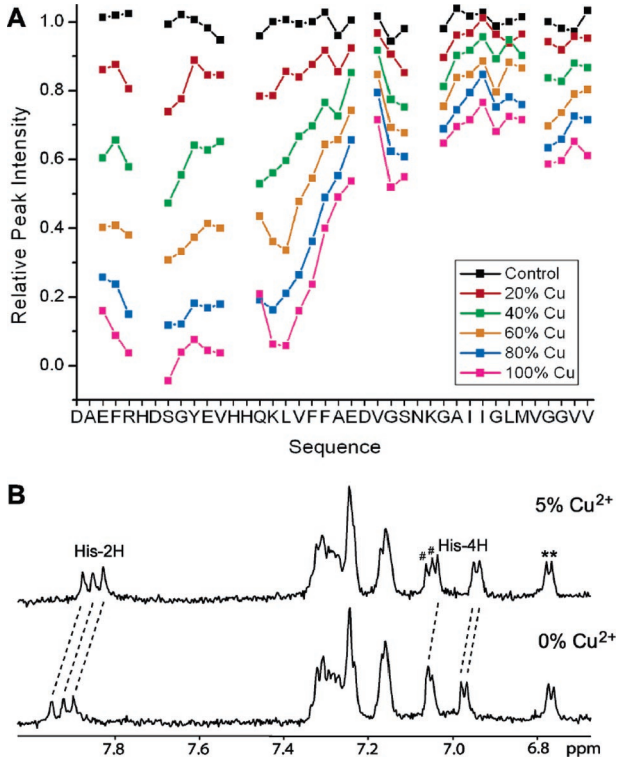

Figure 2. (A) Ratios of NH peak intensities obtained from HSQC spectra with and without different amounts of Cu^{2+}. Higher ratios or peak intensities indicate less effect by Cu^{2+}, whereas lower values are larger effects. The percents in the legend correspond to molar equivalents relative to the Aβ-(1−40) concentration (130 μM); that is, 20% is 26 μM Cu^{2+}. The intensities for D1, H6, D7, H13, H14, D23, N27, K28, and V36 are omitted due to spectral overlap or rapid exchange with solvent. For the sample preparation, a single peptide solution was split into two equal parts: one sample was used for the Cu^{2+} titration studies, and the other kept as a control sample. Both samples were aged under identical conditions (4 h, 5 °C), and the peak intensities were divided by those of the fresh control sample. For each Cu^{2+} addition, the signal loss remained constant over 24 h, suggesting that the samples come to equilibrium after each addition of metal ion. Modest intensity reductions (less than 5%) were seen with the control sample after 4 h aging (black trace), consistent with previous studies.[11] (B) One-dimensional ^{1}H NMR spectra of the Aβ(1−40) peptide with 5% Cu^{2+} (0.05 molar equiv) (upper) and without Cu^{2+} (lower). The upfield shifts of the His6, His13, and His14 are indicated by the dashed lines. The Y10 signals (labeled with $*$) do not move with Cu^{2+}.

aggregates or folding into a well-defined conformation amenable to NMR structure determination. In our revised model, the histidine side chains first anchor Cu^{2+} binding to the Aβ monomer (fast exchange rate), followed by deprotonation and/or severe line broadening of the backbone amide NH for E3-V18 (intermediate exchange rate).[7,8] With Raman spectroscopy, comparable binding was proposed earlier for the Aβ(1−40)/Cu^{2+},[6] along with NMR studies of β_2-microglobulin[27] and other peptides.[28] By contrast, Cu^{2+} binding to soluble Aβ aggregates leads to rapid aggregation and nonfibrillar amorphous structures.[4,5] Without metal, the Aβ can undergo the normal time-dependent aggregation, eventually producing more ordered, late stage-parallel β-sheet structures.[29] These anomalous (rare) binding events may account for some of the unique properties associated with the Aβ, such as its proposed "dual role", where sequestration of metal ions by the monomer is neuroprotective, while that by β-aggregates generates oxygen radicals and causes neuronal death.[30]

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Supporting Information Available: Additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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