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Mass Spectral Studies Reveal the Structure of Aβ1−16−Cu^{2+} Complex Resembling ATCUN Motif

Yamini P. Ginotra,[†] Shefali N. Ramteke,[†] Rapole Srikanth,[§] and Prasad P. Kulkarni*[,†]

[†]Biometry and Nutrition Group, Agharkar Research Institute, Pune-411004, India
[§]National Centre for Cell Science, Pune University Campus, Pune-411007, India

Ⓢ *Supporting Information*

ABSTRACT: In Alzheimer's disease, copper binds to amyloid beta (Aβ) peptide and generates oxidative stress. The coordination of histidine (His) residues to Cu^{2+} is still uncertain. We studied Cu^{2+} binding to Aβ1−16 peptide using the diethyl pyrocarbonate (DEPC) assay and mass spectrometry. Our results show that only one His is involved in Cu^{2+} coordination, which is identified as His6 using mass spectral studies. Novel nickel displacement studies have further supported the proposal that the Cu^{2+} binding site of Aβ1−16 peptide resembles the ATCUN motif of human serum albumin.

Copper is an essential trace element required by all living organisms, and excess accumulation of copper is associated with various neurological disorders such as Wilson's disease, Alzheimer's disease (AD), and prion disease.[1,2] In AD, copper is believed to be responsible for generating oxidative stress via redox cycling between +2 and +1 oxidation states.[3] Copper, in both Cu^{2+} and Cu^{+} forms, is known to bind Aβ peptide with high affinity in a 1:1 ratio.[4,5] In vivo, copper binds Aβ peptide in the +1 oxidation state and is responsible for neuroprotection.[6] Findings also suggest that linear coordination of His13 and His14 residues with Cu^{+} is imperative in governing the electrochemical properties of the Aβ−Cu^{+} complex and the production of reactive oxygen species[7,8] (Scheme 1A). Despite this knowledge, binding of Aβ peptide to Cu^{2+} remains inconclusive due to flexibility of the coordination sphere around Cu^{2+} in terms of the number of coordinating atoms and the variety of geometries possible for Cu^{2+} complexes.[9] Various techniques such as EPR, ESSEM, XAS, IR, CD, and potentiometry have proposed that in solution the Aβ−Cu^{2+} complex exists as multiple species in equilibrium; however, an equatorial binding site with a 3N1O coordination environment around Cu^{2+} is accepted by and large.[8,10−13] These proposed structures vary mostly by the number and positions of His residues bound to Cu^{2+}, and the structures also depend on the pH of the solution and the metal/peptide ratio.[14] At a pH value of around 7.0, two species have been reported, and yet the structure of major species of the Aβ−Cu^{2+} complex and the number of His residues involved in Cu^{2+} binding remain elusive[13,15] (Scheme 1B). Therefore, we studied Cu^{2+} binding to the Aβ peptide using the DEPC assay and mass spectrometry to reveal the number and position of His residues involved in Cu^{2+} binding. The DEPC assay is very specific for the binding of Cu^{2+} to histidine both as free amino acids or as His residues in the protein over other metals such as Zn^{2+} and Ni^{2+}.[16−18] The covalent modification of histidine at imidazole nitrogen by the carbethoxy group can be quantified using $\varepsilon = 3200$ M^{-1} cm^{-1} at 240 nm, and the His residue of the peptide involved in Cu^{2+} binding can be identified using mass spectrometry.

Initially, to discover the number of histidine residues involved in Cu^{2+} binding, we carried out a DEPC assay on the Aβ1−16 peptide in the presence of various Cu^{2+} concentrations (see the Supporting Information (SI)). The spectrophotometric assay results are depicted in Figure 1. These results show that Cu^{2+} protects the Aβ1−16 peptide from DEPC modification in a concentration dependent manner such that when the Cu^{2+}/Aβ1−16 peptide ratio is equal to 1,

Scheme 1. (A) Structure of the Aβ−Cu^{+} Complex and (B) Proposed Structures for Two Major Species of the Aβ−Cu^{2+} Complex
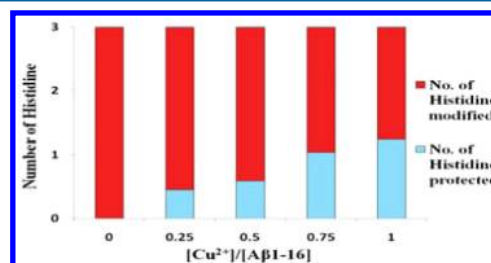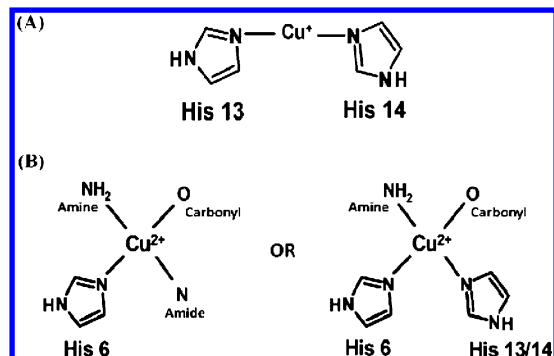

Figure 1. DEPC assay of 40 μM Aβ1−16 peptide in the presence of various Cu^{2+} concentrations (10, 20, 30, 40, 80, and 120 μM).

only one His residue is protected from DEPC modification. This data suggest that Cu^{2+} is bound to only one His residue of the Aβ1−16 peptide. To check if the remaining His residues were amenable for Cu^{2+} binding, additional equivalents of copper were added. We found that a maximum of two His residues are protected when the Cu^{2+}/Aβ1−16 peptide ratio is equal to 3. The Aβ1−16 peptide is known to bind Cu^{2+} in a 1:1 ratio with high affinity, and the increase in protection upon the addition of two or more equivalents of Cu^{2+} may be attributed to atypical binding of Cu^{2+} to the Aβ1−16 peptide. The concentration of Cu^{2+} in the AD brain is 3 times higher compared to that in a normal human brain.[19] Therefore, it is likely that the copper/Aβ peptide ratio may be greater than 1 under special conditions, and under these circumstances Aβ peptide may bind more than one copper.

Further, we monitored DEPC modification of the Aβ1−16 peptide in the absence and presence of 1 equivalent of Cu^{2+} using mass spectrometry to identify the His residues involved in Cu^{2+} binding (see SI). The mass spectrum of the DEPC-modified Aβ1−16 peptide shows peaks corresponding to four carbethoxy modifications at m/z 2027, 2099, 2171, and 2243 along with a peak for the unmodified peptide at m/z 1955 (Figure 2A).
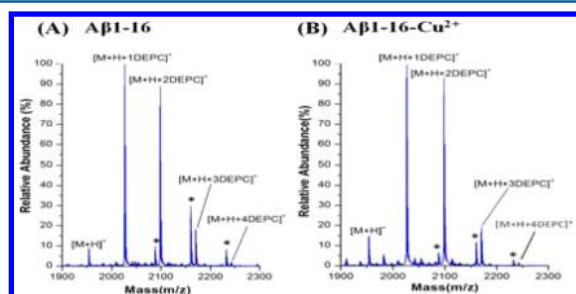

Figure 2. Mass spectra of the DEPC-modified (A) Aβ1−16 peptide and (B) the Aβ1−16−Cu^{2+} complex. The peaks labeled with an asterisk (∗) are Bamberger cleavage peaks formed by the loss of 10 Da of carbethoxy modified product.

Out of the four carbethoxy modifications observed, three correspond to adducts formed at His residues, while the fourth adduct can be ascribed to the modification of the amino group at the N-terminus.[17] Additionally, the mass spectrum shows peaks for Bamberger cleavage at 2089, 2161, and 2233 which arise from the loss of 10 Da from carbethoxy modification. Thus, the intensity of these modification peaks corresponds to the addition of the carbethoxy modified and the corresponding Bamberger cleavage peaks. In the presence of Cu^{2+}, the mass spectrum of the DEPC-modified Aβ1−16 peptide shows three prominent peaks, while the fourth peak at m/z 2243 is almost negligible (Figure 2B). The intensities of the peaks at m/z 2099 and m/z 2171 are significantly decreased, while the intensity of the peak at m/z 1955 is increased. These results authenticate our earlier assay results, asserting the involvement of only one His residue of Aβ1−16 peptide in Cu^{2+} binding. The product ion at m/z 2027, corresponding to single carbethoxy modification, may be comprised of a mixture of peptides modified either at one of the four positions mentioned above.

The MS/MS analysis of the m/z 2027 product ion will therefore decipher the position of the His residue involved in Cu^{2+} binding. The MS/MS spectrum of the m/z 2027 ion of the Aβ1−16 peptide shows a most prominent peak at m/z 943

corresponding to the *b7 ion, a carbethoxy modified b7 ion (Figure 3A).
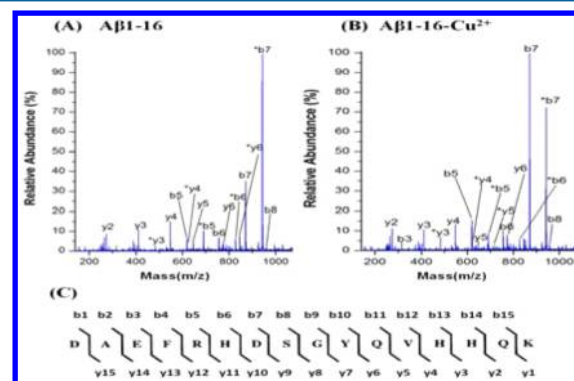
Figure 3. MS/MS spectra of the m/z 2027 ion corresponding to single carbethoxy modification for (A) the Aβ1−16 peptide and (B) the Aβ1−16−Cu^{2+}complex. The ions labeled with an asterisk (∗) are carbethoxy modified ions having +72 mass compared to that of the parent ion. (C) Fragmentation scheme for the Aβ1−16 peptide.

The intensity of the peak for the *b7 ion is higher compared to that of the b7 ion due to carbethoxy modification of the Aβ1−16 peptide. This peak, therefore, can serve as a diagnostic feature as it separates His13 and His14 residues from His6 and the rest of the amino terminal Cu^{2+} binding site. The intensity of *b7 is reduced significantly compared to the parent b7 ion in the case of the DEPC-modified Aβ1−16−Cu^{2+} complex. This result clearly shows that residues 1−7 are involved in Cu^{2+} binding (Figure 3B). Besides this, the intensities of the peaks for *b5 and *b6 that encompasses the His6 residue are also reduced. On the other hand, no major changes in the intensities of the *y3 and *y4 ion are observed, suggesting that His13 and His14 are not involved in Cu^{2+} binding. In this case, the structure of the 3N1O coordinated Aβ−Cu^{2+}complex can be easily deduced on the basis of the literature data.[8,10−13] Besides His6, the other ligands involved in Cu^{2+} binding could be amino nitrogen of the N-terminal amino group and deprotonated amide nitrogen adjacent to Asp1−Ala2 residues along with a carbonyl group adjacent to Ala2−Glu3 completing the 3N1O coordination sphere (Figure 4). This structure of the
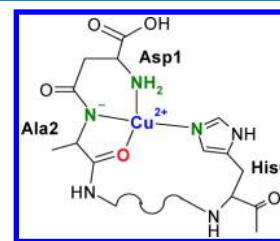

Figure 4. Possible structure of the Aβ−Cu^{2+}complex.

Aβ1−16−Cu^{2+} complex resembles the ATCUN motif of Human Serum Albumin (HSA), except that His is at the sixth position in the Aβ peptide, while in the ATCUN motif His is at the third position from the N-terminus.[20] Binding of His6 to Cu^{2+} along with other residues located at the N-terminus will be more favorable than that of His13 and His14 in a linear Aβ1−16 peptide. Moreover, His6 can easily come in close proximity to the Cu^{2+} bound at the N-terminus because of the well-known phenomenon observed in cyclic hexapeptides.

Cyclic hexapeptides assume a close cyclic conformation with two β-turns, and because of this they are relatively easy to synthesize.[21,22]

We exploited the fact that the structure of the Aβ1−16−Cu^{2+} complex resembles the ATCUN motif to further confirm its structure. We hypothesize that if the structure of the Aβ1−16−Cu^{2+} complex resembles the ATCUN motif, then Ni^{2+} should compete with Cu^{2+} for binding to Aβ1−16. Since DEPC is specific for Cu^{2+} only, the addition of Ni^{2+} to the Aβ1−16−Cu^{2+}complex should result in DEPC modification of the His6 residue. Accordingly, the DEPC assay was performed on the 100 μM Aβ1−16−Cu^{2+} complex in the presence of 100 μM Ni^{2+}, and the mass spectrum was recorded (see SI). The MS/MS spectrum of the product ion m/z 2027 shows a reversal of the intensities of peaks for *b5, *b6, and *b7 peaks (Figure 5).
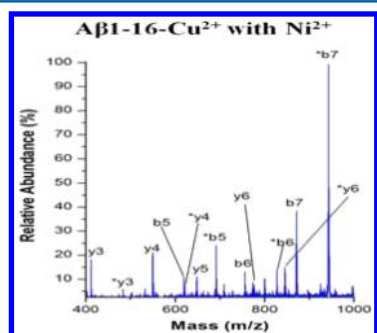
Figure 5. MS/MS spectra of the product ion m/z 2027 for the Aβ1−16−Cu^{2+} complex in the presence of Ni^{2+}. The ions labeled with an asterisk (∗) are the carbethoxy modified ion having +72 mass compared to that of the parent ion.

The intensity of the peak for *b7 is increased in the presence of Ni^{2+} relative to that of the peak for b7. This suggests that Ni^{2+} does compete with Cu^{2+} for binding to Aβ1−16. Additionally, the results also confirm that His6 is indeed participating in Cu^{2+} coordination as a major species.

In summary, we have for the first time performed a DEPC assay and mass spectroscopic studies on the Aβ1−16−Cu^{2+} complex and showed that only His6 participates in Cu^{2+} coordination, forming the major species. Our results indicate that the structure of the Aβ1−16−Cu^{2+} complex resembles the ATCUN motif of HSA. The role of the ATCUN motif in copper transport in human blood is well studied and has been used to develop therapies for Wilson's and Menkes diseases.[23] Similar structural properties for the copper binding site of the Aβ peptide would expedite the chelation therapies for Alzheimer's disease, except that the chelators need to be modified to access the copper across the brain−blood barrier.

ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, full scale mass spectra, and MS/MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*E-mail: kulkarniari@gmail.com.
Notes
The authors declare no competing financial interest.

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REFERENCES

(1) Millhauser, G. *Acc. Chem. Res.* **2004**, *37*, 79−85.
(2) Kulkarni, P.; She, Y.; Smith, S.; Roberts, E.; Sarkar, B. *Chem.—Eur. J.* **2006**, *14*, 2410−2422.
(3) Smith, D.; Cappai, R.; Barnham, K. *Biochim. Biophys. Acta* **2007**, *1768*, 1976−1990.
(4) Gaggelli, E.; Kozlowski, H.; Valensin, D.; Valensin, G. *Chem. Rev.* **2006**, *106*, 1995−2044.
(5) Drew, S.; Barnham, K. *Acc. Chem. Res.* **2011**, *44*, 1146−1155.
(6) Zou, K.; Gong, J. S.; Yanagisawa, K.; Michikawa, M. *J. Neurosci.* **2002**, *22*, 4833−4841.
(7) Himes, R.; Park, G.; Siluvai, G.; Blackburn, N.; Karlin, D. *Angew. Chem., Int. Ed.* **2008**, *47*, 9084−9087.
(8) Shearer, J.; Szalai, V. *J. Am. Chem. Soc.* **2008**, *130*, 17826−17835.
(9) Solomon, E.; Sundaram, U.; Machonkin, T. *Chem. Rev.* **1996**, *96*, 2563−2605.
(10) Dorlet, P.; Gambarelli, S.; Faller, P.; Hureau, C. *Angew. Chem., Int. Ed.* **2009**, *48*, 9522−9525.
(11) Drew, S.; Masters, C.; Barnham, K. *J. Am. Chem. Soc.* **2009**, *131*, 8760−8761.
(12) Drew, S.; Noble, C.; Masters, C.; Hanson, G.; Barnham, K. *J. Am. Chem. Soc.* **2009**, *131*, 1195−1207.
(13) Alies, B.; Eury, H.; Bijani, C.; Rechignat, L.; Faller, P.; Hureau, C. *Inorg. Chem.* **2011**, *50*, 11192−11201.
(14) Damante, C.; Osz, K.; Nagy, Z.; Pappalardo, G.; Grasso, G.; Impellizzeri, G.; Rizzarelli, E.; Sovago, I. *Inorg. Chem.* **2008**, *47*, 9669−9683.
(15) Hong, L.; Carducci, T.; Bush, W.; Dudzik, G.; Millhauser, G.; Simon, J. *J. Phys. Chem. B* **2010**, *114*, 11261−11271.
(16) Ginotra, Y.; Kulkarni, P. *Inorg. Chem.* **2009**, *48*, 7000−7002.
(17) Narindrasorasak, S.; Kulkarni, P.; Deschamps, P.; She, Y.; Sarkar, B. *Biochemistry* **2007**, *46*, 3116−3128.
(18) Qin, K.; Yang, Y.; Mastrangelo, P.; Westaway, D. *J. Biol. Chem.* **2002**, *277*, 1981−1990.
(19) Lovell, A.; Robertson, D.; Teesdale, J.; Campbell, L.; Markesbery, R. *J. Neurol. Sci.* **1998**, *158*, 47−52.
(20) Harford, C.; Sarkar., B. *Acc. Chem. Res.* **1997**, *30*, 123−130.
(21) He, Y.; Huang, Z.; Raynor, K.; Reisine, T.; Goodman, M. *J. Am. Chem. Soc.* **1993**, *115*, 8066−8072.
(22) Kessler, H.; Gratias, R.; Hessler, G.; Gurrath, M.; Miiller, G. *Pure Appl. Chem.* **1996**, *68*, 1201−1205.
(23) Sarkar, B. *Chem. Rev.* **1999**, *99*, 2535−2544.