Published in final edited form as:

Chem Commun (Camb). 2013 May 25; 49(42): 4797–4799. doi:10.1039/c3cc40326b.

Copper Ligation to Soluble Oligomers of the English Mutant of the Amyloid- β Peptide Yields a Linear Cu(I) Site That is Resistant to O₂ Oxidation[†]

Kristy L. Peck^a, Heather S. Clewett^a, Jennifer C. Schmitt^a, and Jason Shearer^{a,*}

^aDepartment of Chemistry, University of Nevada, Reno, NV 89557 USA

Abstract

Copper coordination to soluble oligomers of the English (A β H(6)R) mutant of the amyloid- β peptide is probed. Cu(II) coordination yields a square planar (N/O)₄ coordination environment, while reduction yields an O₂ inert linear bis-His Cu(I) centre.

Alzheimer's disease (AD) is a fatal neurodegenerative disorder that is currently the most common cause of senile dementia.¹ It appears that a key step in AD is the formation of amyloid- β (A β) peptides from the processing of the neuronal membrane amyloid precursor protein (APP), a ubiquitous protein of unknown function.² Depending on the manner in which the APP is cleaved different A β peptides can be produced, of which A β (42) (DAEFRHDSGY EVHHQ KLVFF AEDVG SNKGA IIGLM VGGVV IA) appears to be the most neurotoxic. Once formed, A β (42) monomers tend to aggregate rapidly forming soluble low molecular weight oligomers, which subsequently form insoluble proteinaceous plaques; all three of these forms have a high affinity for copper ions.

Although the proteinaceous plaques are a hallmark of AD, it appears that the soluble oligomers are the most neurotoxic form of A β (42).³ Isolated A β -dimers from the human brain have been shown to be synaptotoxic, as are synthetically prepared A β -dimers.^{4,5} However, in a recent study it was shown that freshly prepared synthetic A β -dimers are not highly synaptotoxic requiring an incubation time to become toxic.⁶ This suggests that A β -dimers may be templates for the formation of larger soluble neurotoxic A β -oligomers.

AD is mostly thought of as a geriatric disorder, however there is a small but significant number of individuals who begin displaying symptoms of AD in their early 40s.⁷ Early onset AD has been linked to a number of different mutations of the APP and the subsequent A β peptides. One of the mutants that has been linked to early onset AD is the so-called English mutant (A β (42)H(6)R).⁸ Herein we explore the Cu(II) and Cu(I) coordination environments of soluble A β (42)H(6)R oligomers. It will be shown that the Cu(I) coordination environment and redox properties of A β (42)H(6)R soluble oligomers at physiological pH are altered from wild-type A β (42) soluble oligomers.⁹

[†]Electronic Supplementary Information (ESI) available from DOI: 10.1039/b000000x/

© The Royal Society of Chemistry [year]

*shearer@unr.edu.

Soluble non-fibril preglobulomers (soluble oligomers) of A β (42)H(6)R peptides (30 kDa) stabilised by a small concentration of SDS were prepared according to previously described protocols.^{10,11} This allows for the generation of a well defined oligomeric state to probe potentially biologically relevant coordination chemistries. Preparation and handling of the soluble oligomers of A β (42)H(6)R peptides are more difficult than wild type A β (42) peptides due to their propensity to form insoluble fibrils. However, we can readily prepare both copper-coordinated and metal-free soluble oligomers of A β (42)H(6)R oligomers in concentrations of at least 300 μ M as long as the samples are maintained at low temperature (2 °C) and not excessively agitated. This was confirmed by GPC, FT-IR and Thioflavin-T dye-binding assays.^{11,12}

Cu(II) coordination to soluble oligomers of A β (42)H(6)R coordination Thioflavin-T peptides was first probed by X-band EPR spectroscopy (Fig. 1). Previously it was shown that soluble oligomers of wild-type {Cu^{II}(A β (42))} consist of one major species in solution with $g_{\parallel} = 2.274$ ($A_{\parallel} = 169(3) \times 10^{-4} \text{ cm}^{-1}$).⁹ This was tentatively identified as a 4-coordinate Cu(II)-His₃(N/O) centre. The EPR spectrum of {Cu^{II}(A β (42)H(6)R)} oligomers can be contrasted to this; spectral deconvolution yields two distinct Cu(II) coordination environments.¹¹ The first copper site (component I) displays a $g_{\parallel} = 2.260(2)$ ($A_{\parallel} = 163(2) \times 10^{-4} \text{ cm}^{-1}$) and the second copper site has a $g_{\parallel} = 2.216(2)$ ($A_{\parallel} = 151(2) \times 10^{-4} \text{ cm}^{-1}$). Deconvolution yields a component I:II ratio of 1:1.5. As the components are in equilibrium different ratios of components I:II may be observed under slightly different sample conditions.

Based on modified EPR “truth tables” of Peisach and Blumberg,^{11,13} and Cu(II)-A β studies we suggest that component I ($g_{\parallel} = 2.260(2)$, $A_{\parallel} = 163(2) \times 10^{-4} \text{ cm}^{-1}$) is consistent with a Cu(II)His₂(N/O)₂ centre, while component II ($g_{\parallel} = 2.216(2)$, $A_{\parallel} = 151(2) \times 10^{-4} \text{ cm}^{-1}$) is consistent with a Cu(II)HisN⁻₂(N/O) coordination environment (Scheme 1). Caution should be taken in considering the above EPR based structural assignments as they are based on empirical structure/spectroscopic correlations. EPR studies by Hureau and coworkers on monomeric {Cu^{II} A β (16)H(6)R}, which contains the copper binding N-terminus but does not undergo aggregation, displays two components ($g_{\parallel} = 2.26$, $A_{\parallel} = 180 \times 10^{-4} \text{ cm}^{-1}$ and $g_{\parallel} = 2.22$, $A_{\parallel} = 162 \times 10^{-4} \text{ cm}^{-1}$).¹⁴ These were assigned as a Cu^{II}His₂O^{carbonyl}N^{amine} and Cu^{II}HisO^{carbonyl}N^{amide}N^{amine} coordination motifs. Thus, the Cu(II) coordination environment between A β (42)H(6)R monomers and oligomers may be distinct.

The Cu(II) centre can be effectively reduced by the addition of 1 eq. of ascorbate at room temperature as noted by the complete disappearance of the EPR signal. Previously, Cu(II) reduction to a coordinated Cu(I) ion in wild-type {Cu^{II}(A β (42))} soluble oligomers and monomers was noted. In the case of wild-type oligomers a highly O₂ reactive tetrahedral Cu(I) species is formed that is rapidly oxidized back to {Cu^{II}(A β (42))}.⁹ In contrast, the Cu(I) centres of reduced {Cu^I(A β (42)H(6)R)} soluble oligomers are not oxidized upon O₂ exposure to {Cu^{II} (A β (42)H(6)R)}. This is reminiscent of how *monomers* of {Cu^I(A β)} behave.¹⁵ In the case of {Cu^I(A β)} monomers a linear bis-His Cu(I) coordination environment is formed,¹¹ which is reasonably O₂ stable taking ~20 hours to reoxidize back to {Cu^{II}(A β)} with subsequent peptide-based oxidative damage noted by HPLC.^{15c} The soluble oligomers of {Cu^{II}(A β (42)H(6)R)} are especially resistant to O₂ induced oxidation;

EPR spectroscopy shows no signal for $\{\text{Cu}^{\text{II}}(\text{A}\beta(42)\text{H}(6)\text{R})\}$, even after 48 hours of air exposure, and HPLC shows no peptide-based modification events.¹¹ We note that the above studies ignore possible ternary complexes formed between $\{\text{Cu}^{\text{II}}(\text{A}\beta(42)\text{H}(6)\text{R})\}$ and exogenous ligands found in the extracellular fluid, which may be important in AD.

The Cu K-edge X-ray absorption spectra of Cu(I) and Cu(II) coordinated oligomers of $\{\text{Cu}(\text{A}\beta(42)\text{H}(6)\text{R})\}$ can be contrasted to those of soluble oligomers of $\{\text{Cu}(\text{A}\beta(42))\}$.⁹ Unlike the single well defined 4-coordinate $\text{His}_3(\text{N/O})$ Cu(II) structure of $\{\text{Cu}^{\text{II}}(\text{A}\beta(42))\}$ soluble oligomers, the Cu(II) coordination environment of $\{\text{Cu}^{\text{II}}(\text{A}\beta(42)\text{H}(6)\text{R})\}$ is consistent with a mixture of four coordinate Cu(II)-coordination motifs. The data was best modelled with 1 imidazole scatterers in one shell, 0.5 imidazole scatterers in a second and 2.5 N/O scatterers in a third shell, suggestive of a mixed coordination environment as noted by EPR. Reduction of the Cu(II) centre to Cu(I) yields a linear two coordinate Cu(I) centre as evidenced by the XANES region of the X-ray absorption spectrum; there is a prominent peak at 8984.2(1) eV in the pre-edge region of the XANES of soluble oligomers of $\{\text{Cu}^{\text{I}}(\text{A}\beta(42)\text{H}(6)\text{R})\}$ (Fig. 2), which is characteristic of linear two-coordinate Cu(I).¹⁶ Analysis of the EXAFS region is also consistent with a linear Cu(I) centre. The best fit to the data indicates a $\text{His-Cu}^{\text{I}}\text{-His}$ motif with a N-Cu-N bond angle of $175(5)^\circ$ (φ) and Cu-N bond lengths of 1.88 Å.¹¹ This is consistent with what was previously observed with $\{\text{Cu}^{\text{I}}(\text{A}\beta)\}$ monomers. We note that prolonged O_2 exposure (36 hours) yields no noticeable change in the X-ray absorption spectrum. It therefore appears that the linear Cu(I) centre in $\{\text{Cu}^{\text{I}}(\text{A}\beta(42)\text{H}(6)\text{R})\}$ soluble oligomers is especially inert towards O_2 oxidation events (Scheme 1).

To gain insight into the differential coordination environment and reactivity of $\{\text{Cu}^{\text{I}}(\text{A}\beta(42)\text{H}(6)\text{R})\}$ vs. $\{\text{Cu}^{\text{I}}(\text{A}\beta(42))\}$ soluble oligomers we employed molecular dynamics (MD) simulations of $\text{A}\beta(42)\text{H}(6)\text{R}$ vs. $\text{A}\beta(42)$ hexamers.¹¹ The hexameric form of $\text{A}\beta$ was chosen as these are of a similar molecular weight as the soluble oligomers. MD simulations were performed at 310 K over a period of 350 ns. For wild-type $\text{A}\beta(42)$ hexamers equilibrium energies were reached within ~40 ns, while equilibrium energies were reached within ~55 ns for $\text{A}\beta(42)\text{H}(6)\text{R}$. Examination of equilibrium structures demonstrate a clear difference between the two hexameric structures (Fig. 3). Consistent with experimental data,¹⁸ the wild-type $\text{A}\beta(42)$ hexamer displays high β -sheet content that is well ordered. The β -sheet structure persists from the C-terminus towards the copper-binding N-terminus (for the sake of this discussion we will define the N-terminus as being between residues 1 – 18); the β -sheets extend well into the N-terminal region ending between Y(10) – S(8) depending on whether the $\text{A}\beta$ peptide is an interior or exterior peptide. Over the course of the simulations little change in the structure was observed and the snapshot in Fig 3 remains virtually static. We note that the wild-type hexamer is dramatically different from how wild-type monomers behave, which appears to form rapidly interchanging α -helix/coil structures.¹⁸

This can be contrasted with the $\text{A}\beta(42)\text{H}(6)\text{R}$ mutant. Although this possess a high β -sheet content, there is far more flexibility in the N-terminal domain. Going towards the N-terminus, the β -sheet ends at approximately residue Q(15). Beyond Q(15) the N-terminus behaves similarly to how the N-terminus behaves in $\text{A}\beta$ monomers; the N-terminus displays

rapidly interconverting coil/helical structures. This is due primarily to the large degree of electrostatic repulsion between R(5) and R(6) on opposing A β (42)H(6)R strands. Absent are the R(5) and H(6) H-bonding interactions on opposing A β (42) strands that stabilises the wild-type hexamer. One might therefore expect that the A β (42)H(6)R hexamer will coordinate Cu(I) like a monomer, *which is what is observed*.

In summary, we have demonstrated the differential copper coordination environment of A β (42)H(6)R SDS stabilised soluble oligomers vs. A β (42) SDS stabilised soluble oligomers. Most importantly, a redox inactive linear bis-His Cu(I) coordination environment is found in soluble oligomers of {Cu^I(A β (42)H(6)R)}, which is identical to what is observed in monomers of {Cu^IA β (42)}. This is likely due to a more “monomer-like” oligomeric *N*-terminus in hexamers of A β (42)H(6)R vs. A β (42). It is also possible that the lack of H(6) as a ligand may be altering the Cu(I) coordination environment in the A β (42)H(6)R vs. A β (42) oligomers.

Some have assumed that redox active copper-bound A β is responsible for oxidative stress and subsequent neuronal damage in the O₂ rich reducing environment of the cerebral spinal fluid.¹⁹ These data on the A β (42)H(6)R mutant, which *promotes* early onset AD, demonstrates that oligomers of {Cu^I(A β (42)H(6)R)} are both inert towards O₂ and cannot induce oxidative damage. The implication is that either copper-A β based O₂/reductant redox cycling is not responsible for the initial stages of AD, or that multiple AD mechanisms are occurring. For example, considering the high affinity of Cu(I) for A β monomers²⁰ it could be that A β oligomers are disrupting cellular copper homeostasis. In addition to biological studies, further detailed investigations of the fundamental coordination chemistries of these peptides are needed to sort out such issues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the NSF (CHE-0844234 and CHE-0840429). Beamtime for XAS measurements at the NSLS was supported by the DOE (contract no. DE-AC02-98CH10886) and NIH (P30-EB-009998). We thank Dr. Veronika Szalai (National Institutes of Standards and Technology, Gaithersburg MD, USA) for valuable advice.

References

- 1). Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A, Mathers C, Menezes PR, Rimmer E, Scazufca M. *Lancet*. 2005; 35:352.
- 2). Holtzman DM, Morris JC, Goate AM. *Sci. Transl. Med.* 2011; 3:77sr1. [PubMed: 21471435]
- 3). Ono K, Condron MM, Teplow DB. *Proc. Natl. Acad. Sci. USA*. 2009; 106:14745. [PubMed: 19706468]
- 4). Shankar GM, Li SM, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ. *Nature Med.* 2008; 14:837. [PubMed: 18568035]
- 5). Kok WM, Scanlon DB, Karas JA, Miles LA, Tew DJ, Parker MW, Barnham KJ, Hutton CA. *Chem. Comm.* 2009:6228. [PubMed: 19826677]

- 6). O'Nuallain B, Freir DB, Nicoll AJ, Risse E, Ferguson N, Herron CE, Collinge J, Walsh DM. J. Neurosci. 2010; 30:14411. [PubMed: 20980598]
- 7). Wu L, Rosa-Neto P, Hsiung GY, Sadovnick AD, Masellis M, Black SE, Jia J, Gauthire S. Can. J. Neurol. Sci. 2012; 39:436. [PubMed: 22728850]
- 8). Janssen JC, Beck JA, Campbell TA, Dickinson A, Fox NC, Harvey RJ, Houlden H, Rossor MN, Collinge J. Neurology. 2003; 60:235. [PubMed: 12552037]
- 9). Shearer J, Callan PC, Tran T, Szalai VA. Chem. Comm. 2010; 46:9137. [PubMed: 21060917]
- 10). Yu L, Edalji R, Harlan JE, Holzman TF, Lopez AP, Labovsky B, Hillen H, Barghorn S, Ebert U, Richardson PL, Miesbauer L, Solomon L, Bartley D, Walter K, Johnson RW, Hajduk PJ, Olejniczak ET. Biochemistry. 2009; 48:1870. [PubMed: 19216516]
- 11). See supporting information.
- 12). Barghorn S, Nimmrich V, Striebinger A, Krantz C, Keller P, Janson B, Bahr M, Schmidt M, Bitner RS, Harlan J, Barlow E, Ebert U, Hillen HJ. J. Neurochem. 2005; 95:834. [PubMed: 16135089]
- 13). Peisach J, Blumberg WE. Arch. Biochem. Biophys. 1974; 165:691. [PubMed: 4374138]
- 14). Alies B, Eury H, Bijani C, Rechinat L, Faller P, Hureau C. Inorg. Chem. 2011; 50:11192. [PubMed: 21980910]
- 15). a) Himes RA, Park GY, Barry AN, Blackburn NJ, Karlin KD. J. Am. Chem. Soc. 2007; 129:5352. [PubMed: 17411054] b) Himes RA, Park GY, Siluvai GS, Blackburn NJ, Karlin KD. Angew. Chem. Int. Ed. 2008; 47:9084. c) Shearer J, Szalai VA. J. Am. Chem. Soc. 2008; 130:17826. [PubMed: 19035781]
- 16). Kau L-S, Spira-Solomon DJ, Penner-Hahn JE, Hodgson KO, Solomon EI. J. Am. Chem. Soc. 1987; 109:6433.
- 17). Hamley IW. Chem. Rev. 2012; 112:5147. [PubMed: 22813427]
- 18). Baumketner A, Bernstein SL, Wyttenbach T, Bitan G, Teplow DB, Bowers MT, Shea J-E. Protein Sci. 2006; 15:420. [PubMed: 16501222]
- 19). Adlard PA, Bush AI. J. Alzheimers Dis. 2006; 10:145. [PubMed: 17119284]
- 20). Feaga HA, Maduka RC, Foster MN, Szalai VA. Inorg. Chem. 2011; 50:1614. [PubMed: 21280585]

Fig. 1.

X-band EPR spectrum of $\{\text{Cu}^{\text{II}}(\text{A}\beta(42)\text{H}(6)\text{R})\}$ soluble oligomers (solid line) in 1:1 50 mM NEM buffer (pH 7.4):glycerol (85 K, $\nu = 9.11$ GHz, amplitude modulation = 5.0 G, microwave power = 20 mW). The dashed line is the simulated spectrum (Component I: $g_{\parallel} = 2.260$, $g_{\perp} = 2.040$, $A_{\parallel} = 163 \times 10^{-4} \text{ cm}^{-1}$, $A_{\perp} = 9 \times 10^{-4} \text{ cm}^{-1}$; Component II: $g_{\parallel} = 2.216$, $g_{\perp} = 2.039$, $A_{\parallel} = 151 \times 10^{-4} \text{ cm}^{-1}$, $A_{\perp} = 6 \times 10^{-4} \text{ cm}^{-1}$; I: II = 1.00:1.5114).¹¹

Fig 2.

Cu K-edge X-ray absorption spectrum of $\{\text{Cu}^{\text{I}}(\text{A}\beta(42))\text{H}(6)\text{R}\}$ displaying (top) the XANES region, (bottom) the magnitude FT-EXAFS (FT from $k = 2.0 - 12.0 \text{ \AA}^{-1}$) and (inset) the FF-EXAFS (back-transformed from $r = 1.0 - 4.0 \text{ \AA}$). For the EXAFS plots, the solid line represent the experimental data, the thick dashed line represents the modelled data and the thin dotted line are the difference spectra. Cu-His₂ shell: $n = 1$; $r = 1.882(6) \text{ \AA}$; $\sigma^2 = 0.0031(4) \text{ \AA}^2$; $\varphi = 175(5)^\circ$; $\theta = 32.6(22)^\circ$; $E_o = 8985.4(2) \text{ eV}$; GOF = 0.37.¹¹

Fig 3.

Snapshots of the Aβ(42)H(6)R (top) and wild-type Aβ(42) (bottom) hexamers corresponding to lowest-energy structures from the MD simulation. H(13), H(14) and R(6) (or H(6)) have been highlighted.

Scheme 1.

Reduction of $\{\text{Cu}^{\text{II}}(\text{A}\beta(42))\text{H}(6)\text{R}\}$ to $\{\text{Cu}^{\text{I}}(\text{A}\beta(42))\text{H}(6)\text{R}\}$. The orientation of the ligands about the $\text{Cu}(\text{II})$ centres are not meant to indicate the absolute ligand stereochemistry.