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Title: Resistance of Cu(Aβ4-16) to copper capture by metallothionein-3 supports a function of Aβ4-42 peptide as synaptic CuII scavenger

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Resistance of Cu(Aβ4-16) to copper capture by metallothionein-3 supports a function of Aβ4-42 peptide as synaptic Cu^{II} scavenger

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Abstract: Aβ4-42 is a major species of Aβ peptide in brains of both healthy individuals and those affected by Alzheimer's disease. It has recently been demonstrated to bind Cu^{II} with an affinity ca. 3000 times higher from commonly studied Aβ1-42 and Aβ1-40 peptides, which are implicated in Alzheimer's disease pathogenesis. Metallothionein-3, a protein considered to orchestrate copper and zinc metabolism in the brain and provide antioxidant protection was shown to extract Cu^{II} from Aβ1-40 acting in its native Zn_7MT-3 form. This reaction is assumed to underlie the neuroprotective effect of Zn_7MT-3 against Aβ toxicity. In this study, using truncated model peptides Aβ1-16 and Aβ4-16, we demontratall5(s)-6( )-howe es use del

In this study, we sought to reinforce the concept of Aβ4-42 as a physiological Cu^{II} binding peptide, by establishing that the N-terminal Cu^{II}(Aβ4-16) complex is fully resistant to copper/zinc swap with Zn_7MT-3. In our experiments we used recombinant human MT-3, overproduced in *E. coli*, purified as apoprotein and reconstituted with $ZnSO_4$ as Zn_7MT-3. We began by monitoring with UV-Vis spectroscopy whether Zn_7MT from our preparation was able to react with Cu^{II} ions. Figure 1 presents the Cu^{II} titration of a 5 μM Zn_7-MT3 sample in a 20 mM Tris-HCl/100 mM NaCl buffer, pH 7.4. The titration revealed a roughly biphasic character of the copper/zinc swap at MT-3. The isosbestic point at 231 nm was maintained up to ca. 6.3 molar equivalents (mol eq.) of added Cu^{II}. The subtraction of the initial Zn_7-MT3 spectrum from titration spectra (Figure 1B) revealed the increase of absorption at 256 nm, assigned to the S-Cu^{I} charge transfer (CT) band, and a corresponding decrease of the band at 213 nm, which can be assigned to the S-Zn^{II} CT band.[27] The analysis of the titration curve (Figure 1C) indicated that 6.3 Cu^{II} equivalents were incorporated into nearly equivalent binding sites in MT-3, as evidenced by a linear increase of the absorption of the S-Cu^{I} CT band (but a slight shift of this band maximum seen in difference spectra indicates some interactions between these sites). Our results are fully consistent with previous studies on the interaction between Cu(II) and Zn_7MT-3, confirming the correctness of our approach.[23] Further Cu^{II} equivalents were incorporated into MT-3 in a clearly different fashion. The band at 213 nm ceased to decrease, resulting in the loss of the isosbestic point. Also, a more complicated pattern of bands in the S-Cu^{I} CT region emerged. These results suggest that the first 6-7 Cu^{II} ions displaced Zn^{II} from MT-3. Cu^{II} ions had to be reduced to Cu^{I} in order to be incorporated into MT3, and the thiolates are the only clear reductant in our experimental system. The reduction of 6.3 mol eq. Cu^{II} requires the same number of thiolates to be oxidized to disulfides. This leaves ca. 13 thiolates per MT-3 molecule, the number about right for the formation of ca. 6.3 S-Cu-S binding sites, known from yeast MT studies to be sufficient for the efficient Cu^{I} coordination.[28,29]

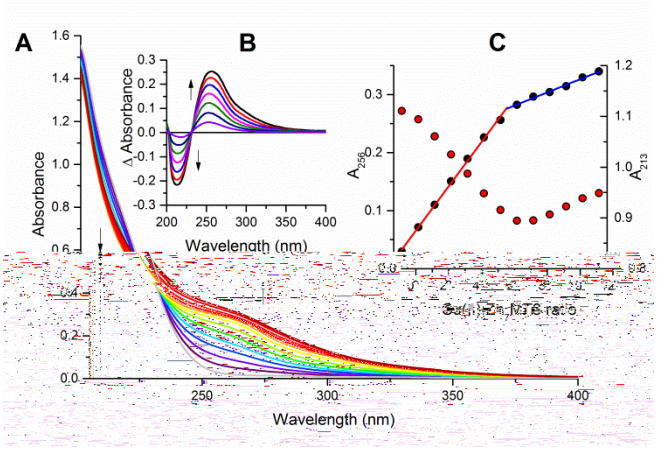

Figure 1. A. The titration of Zn_7MT-3 (5 μM in 20 mM Tris-HCl and 100 mM NaCl, pH 7.4) with $CuCl_2$ from 0 to 12 mol eq. **B.** Difference spectra for the first seven mol eq. **C.** Black spheres: titration curve generated by absorbance at 256 nm. with linear fits for two branches (0 to 6 and 7 to 12 mol eq.); red spheres: absorption at 213 nm.

The reaction of Cu^{II} ions with Zn_7MT-3 was also checked by ESI-MS. The results (Supporting Information Figures S1-S4) corroborated the conclusions from UV-Vis spectra. At the 1:1 Cu^{II} to MT-3 ratio, two similarly intense peaks were detected: one was the intact Zn_7MT-3, another contained two Cu^{I} ions swapped for one Zn^{II}. At the 4:1 ratio the major peak contained four Cu^{I} ions swapped for four Zn^{II} ions, with ca. 2-4 disulfide bridges formed. At the 8:1 ratio a series of additional peaks were detected, containing a higher number, up to nine, of Cu^{I} ions. No MT-3 oligomers were detected in the spectra. A more thorough analysis of ESI-MS results was hampered by overlaps with peaks of adducts with NH_4^+ ions derived from the ammonium carbonate buffer used in these experiments.

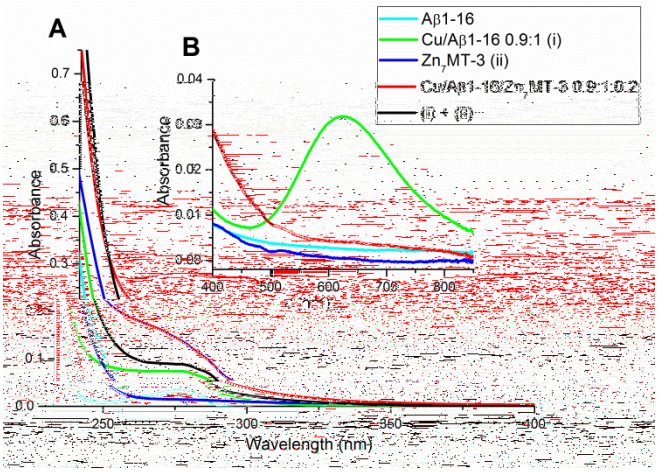

Figure 2. The reactions of Cu(Aβ1-16) complex with Zn_7MT-3 in 20 mM Tris-HCl and 100 mM NaCl, pH 7.4 observed (A) in the UV region for 23.6 μM Aβ1-16 with 21.2 μM Cu^{II} (the Cu(Aβ1-16) complex), and 5 μM Zn_7MT-3, and (B) for d-d bands for 446 μM Aβ1-16 with 401 μM Cu^{II} (the Cu(Aβ1-16) complex, and 80.2 μM Zn_7MT-3. For the UV region, the spectrum of the reaction product, Cu/Aβ1-16/Zn_7MT-3 0.9:1:0.2 was compared to the mathematical sum of Cu(Aβ1-16) complex and Zn_7-MT3 spectra, black line.

Having established the conditions of the copper/zinc swap at Zn_7MT-3, we performed experiments with Cu^{II} complexes of Aβ peptides under the identical pH/buffer conditions. The Zn_7MT-3 portions were added to respective complexes, at peptide-to-Cu^{II} molar ratios of 0.9 and 1.8, to avoid oversaturation of peptide binding sites. The final concentrations of Zn_7-MT3 and the peptides were 5.0 μM and 23.6 μM, respectively. The course of reactions was controlled by recording whole spectra at 5 min intervals over the 30 minute period. The key results of UV-Vis experiments with one mol eq. of Cu^{II} are presented in Figure 2 for Aβ1-16, and Figure 3 for Aβ4-16. All spectra are provided in Supporting Information, Figures S5 and S6. Separate experiments with both Aβ peptides, performed under identical conditions, were monitored using circular dichroism. The CD spectra are presented in Supporting Information, Figures S7 and S8. The copper/zinc swap reactions were fast, with more than 95% of the transfer occurring in all cases within the dead time of sample mixing and recording the first spectrum (ca. 2.5 min).

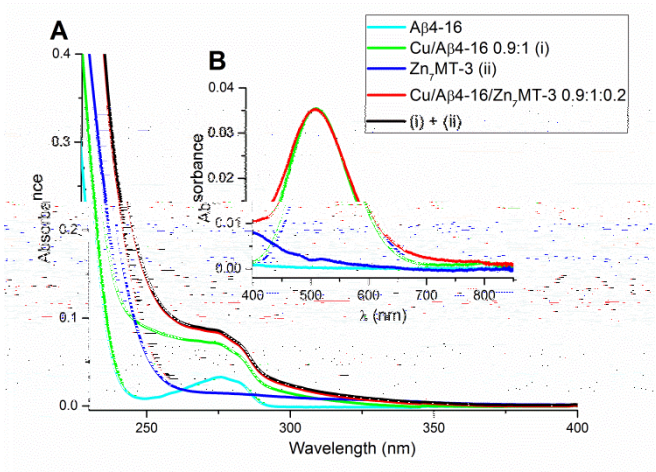

Figure 3. The reactions of Cu(Aβ4-16) complex with Zn_7-MT3 in 20 mM Tris-HCl and 100 mM NaCl, pH 7.4 observed (A) in the UV region for 23.6 μM Aβ4-16 with 21.2 μM Cu^{II} (the Cu(Aβ4-16) complex), and 5 μM Zn_7MT-3, and (B) for the Vis region for 475 μM Aβ4-16 with 367 μM Cu^{II} (the Cu(Aβ4-16) complex, and 73.4 μM Zn_7MT-3. For the UV region, the spectrum of the reaction product, Cu/Aβ4-16/Zn_7MT-3 0.9:1:0.2 was compared to the mathematical sum of Cu(Aβ4-16) complex and Zn_7MT-3 spectra, black line.

As expected on the basis of previous studies,[23-25] Cu^{II} ions bound to Aβ1-16 reacted readily with Zn_7MT-3. The evidence of the complete copper/zinc swap was provided by the occurrence of a CT band at 256 nm and a loss of Cu^{II} d-d band (Figure 2), Zn_7MT-3 was also able to extract both Cu^{II} ions from Cu_2(Aβ1-16) (Figures S5 and S7). The behavior of the first Cu^{II} site in the Aβ4-16 peptide was remarkably different. As shown in Figures 3, S6 and S8, it was totally unreactive during a 30 min incubation with Zn_7MT-3, while the 2nd mol eq. of Cu^{II} was swapped readily (Figures S6 and S8). This was evidenced by the accurate reconstruction of the experimental spectra of these reaction mixtures. We assumed that the (partially) Cu^{I} loaded MT-3 and the Aβ apopeptide were the only species remaining in solution after the copper/zinc swap reaction. Consequently, we summed up the spectra of Zn_7MT-3 reacted with corresponding amounts of Cu^{II} ions (presented in Figure 1) and those of respective forms of Aβ peptides remaining in solution. For Aβ1-16 samples, the reconstruction was successful when the spectrum of the Aβ1-16 apopeptide was used, while the spectrum of the Cu(Aβ4-16) complex had to be used instead for the reaction of Cu_2(Aβ4-16).

We also performed additional control experiments. A reverse swap experiment, where apo-Aβ4-16 was added to the pre-formed (Cu/Zn)MT-3 complex did not yield Cu^{II} transfer from metallothionein to the peptide (Figure S9), but addition of Cu^{II} ions to a mixture of Zn_7MT-3 and apo-Aβ4-16 resulted in a partition of copper between these biomolecules, with ca. 30% as Cu(Aβ4-16) (Figure S10). The addition of physiological amounts of ascorbate or hydrogen peroxide did not facilitate the swap, but very high, non-physiological concentrations of these redox agents resulted in the transfer of copper from Aβ4-16 or to Aβ4-16 (ascorbate or H_2O_2, respectively, Figure S11). Collectively, these experiments indicate the kinetic as well as thermodynamic basis for the resistance of Cu(Aβ4-16) to copper/zinc swap with Zn_7MT-3.

These results correlate with redox properties of Cu(Aβ4-16), presented in our recent work.[26] The voltammetric experiments on the N-terminal Cu^{II} complex of Aβ4-16 revealed that this complex could be oxidized irreversibly at a high potential, but could not be reduced to Cu^{I}. Analogous behavior was observed for the Cu^{II} complex with the peptide modeling the Cu^{II} site in human serum albumin (HSA), which has a similar stability,[30] but the much weaker Cu^{II} site in the full-length albumin could be reduced to Cu^{I}.[31] The secondary Cu^{II} complex of Aβ4-16, which supported the Cu^{I}/Cu^{II} redox pair in voltammetric experiments, readily released copper to metallothionein.

The resistance of Cu(Aβ4-16) to Zn_7MT3 reactivity indicates that the analogous complex of the full-length peptide, Cu(Aβ4-42) will not yield copper to MT-3 in the brain extracellular space as well. The copper/zinc swap was postulated in the literature as key mechanism of control of toxicity of copper-Aβ complexes by MT-3.[23-25] This fact, combined with a very high stability of the Cu(Aβ4-42) complex, the ability of its binding site to extract Cu^{II} ions from the binding site of Aβ1-x peptides and its lack of ROS production, strongly supports the physiological role of Aβ4-42 as a Cu^{II} scavenger in the synaptic cleft, postulated in our recent paper.[26] We can speculate that Aβ4-42 and MT-3 may play parallel roles in a synaptic copper clearance: the former handling copper under more oxidizing conditions, and the latter in the more reducing environments. Noteworthy, Aβ4-42 will not impair the antioxidant function of Zn_7MTs.[32]

Other truncated ATCUN-type Aβ peptides may have properties similar to those of Aβ4-42. A recent paper reported a 34 fM Cu^{II} affinity of such peptide, Aβ11-42.[33] Nevertheless, the majority of species truncated at residue Glu11 exist in the pyroglutamate form,[7,34] which blocks ATCUN coordination. Moreover, it remains to be determined whether the ATCUN site of Aβ11-x peptides can redox cycle and generate ROS.

Acknowledgements

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Keywords: Amyloid-beta peptides • copper • metalloproteins • metallothionein • zinc

[1] C. L. Masters, D. J. Selkoe, *Cold Spring Harbor Perspect. Med.* **2012**, *2*, a006262.

[2] C. Haass, C. Kaether, G. Thinakaran, S. Sisodia, *Cold Spring Harb. Perspect. Med.* **2012**, *2*, a006270

[3] C. R. Jack Jr, D. S Knopman, W. J. Jagust, R. C. Petersen, M. W. Weiner, P. S. Aisen, L. M. Shaw, P. Vemuri, H. J. Wiste, S. D. Weigand, T. G Lesnick, V. S. Pankratz, M. C. Donohue, J. Q. Trojanowski, *Lancet Neurol* **2013**, *12*, 207–216.

[4] C. L. Masters, G. Simms, N. A. Weinman, G. Multhaup, B. L. McDonald, K. Beyreuther, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 4245–4249.

[5] C. L. Masters, G. Multhaup, G. Simms, J. Pottgiesser, R. N. Martins, K. Beyreuther, *EMBO J.* **1985**, *4*, 2757–2763.

[6] H. Lewis, D. Beher, N. Cookson, A. Oakley, M. Piggott, C. M. Morris, E. Jaros, R. Perry, P. Ince, R. A. Kenny, C. G. Ballard, M. S. Shearman, R. N. Kalaria, *Neuropathol. Appl. Neurobiol.* **2006**, *32*, 103–118.

[7] E. Portelius, N. Bogdanovic, M. K. Gustavsson, I. Volkmann, G. Brinkmalm, H. Zetterberg, B. Winblad, K. Blennow, *Acta Neuropathol.* **2010**, *120*, 185–193.

[8] J. Kardos, I. Kovacs, F. Hajos, M. Kalman, M. Simonyi, *Neurosci Lett*. **1989**, 103, 139-144.

[9] B. Alies, E. Renaglia, M. Rózga, W. Bal, P. Faller, C. Hureau, *Anal. Chem.* **2013**, *85*, 1501–1508.

[10] T. R. Young, A. Kirchner, A. G. Wedd, Z. Xiao Z, *Metallomics* **2014**, *6*, 505–517.

[11] S. C. Drew, K. J. Barnham, *Acc. Chem. Res.* **2011**, *44*, 1146–1155.

[12] P. Faller, C. Hureau, G. La Penna, *Acc. Chem. Res.* **2014**, *47*, 2252–2259.

[13] V. B. Kenche, K. J. Barnham, *Br. J. Pharmacol.* **2011**, *163*, 211–219.

[14] X. Huang, R. D. Moir, R. E. Tanzi, A. I. Bush, T. J. Rogers, *Ann. N. Y. Acad. Sci.* **2004**, *1012*, 153–163.

[15] K. Reybier, S. Ayala, B. Alies, J. V. Rodrigues, S. Bustos Rodriguez, G. La Penna, F. Collin, C. M. Gomes, C. Hureau, P. Faller, *Angew. Chem. Int. Ed.* **2016**, *55*, 1085–1089.

[16] A. K. West, J. Y. K. Leung, R. Chung, *J. Biol. Inorg. Chem.* **2011**, *16*, 1115−1122.

[17] S. Atrian, M. Capdevila, *Biomol. Concepts* **2013**, *4*, 143−160.

[18] A. Krężel, W. Maret, *J. Am. Chem. Soc.* **2007**, *129*, 10911–10921.

[19] Y. Irie, W. M. Keung, *Biochem. Biophys. Res. Commun.* **2001**, *282***,** 416–420.

[20] E. Artells, O. Palacios, M. Capdevila S. Atrian, *FEBS J.* **2014**, *281*, 1659–1678.

[21] G. Meloni, M. Vašák, *Free. Radic. Biol. Med.* **2011***, 50*, 1471−1479.

[22] G. Meloni, A. Crameri, G. Fritz, P. Davies, D. R. Brown, P. M. Kroneck, M. Vašák, *ChemBioChem* **2012**, *13*, 1261−1265.

[23] G. Meloni, P. Faller, M. Vašák, *J. Biol. Chem.* **2007**, 282, 16068–16078.

[24] G. Meloni, V. Sonois, T. Delaine, L. Guilloreau, A. Gillet, J. Teissie, P. Faller, M. Vašák, *Nat. Chem. Biol.* **2008**, *4*, 366–372.

[25] J. T. Pedersen, C. Hureau, L. Hemmingsen, N. H. Heegaard, J. Østergaard, M. Vašák, P. Faller, *Biochemistry* **2012**, *51*, 1697−1706.

[26] M. Mital, N. E. Wezynfeld, T. Frączyk, M. Z. Wiloch, U. E. Wawrzyniak, A. Bonna, C. Tumpach, C. L. Haigh, K. J. Barnham, W. Bal, S. C. Drew, *Angew. Chem. Int. Ed.* **2015**, *54*, 10460–10464.

[27] M. Vašák, J. H. Kägi, H. A. Hill, *Biochemistry* **1981**, *20*, 2852–2856.

[28] V. Calderone, B. Dolderer, H.-J. Hartmann, H. Echner, C. Luchinat, C. Del Bianco, S. Mangani, U. Weser, *Proc. Natl Acad. Sci. USA* **2005**,*102*, 51–56.

[29] C. W. Peterson, S. S. Narula, I. M. Armitage, *FEBS Lett.* **1996**, *379*, 85–93.

[30] L. Perrone, E. Mothes, M. Vignes, A. Mockel, C. Figueroa, M.-C. Miquel, M.-L. Maddelein, P. Faller, *ChemBioChem* **2010**, *11*, 110–118.

[31] A. I. Ivanov, J. A. Parkinson, E. Cossins, J. Woodrow, P. J. Sadler, *J. Biol. Inorg. Chem.* **2000**, *5*, 102−109.

[32] H. Gonzalez-Iglesias, L. Alvarez, M. Garcıa, C. Petrash, A. Sanz-Medel, M. Coca-Prados, *Metallomics* **2014**, *6*, 201–208.

[33] J. D. Barritt, J. H. Viles, *J. Biol. Chem.* **2015**, *290*, 27791−27802.

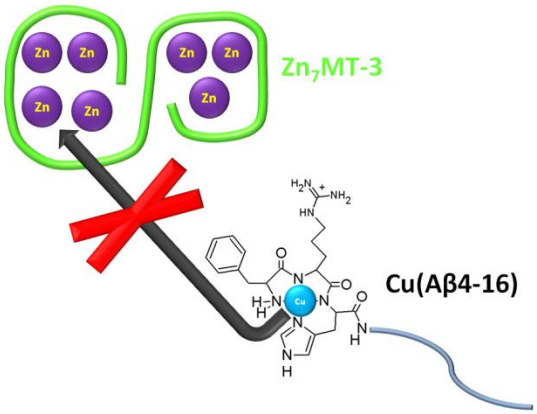
[34] J. Naslund, A. Schierhorn, U. Hellman, L. Lannfelt, A. D. Roses, L. 0. Tjernberg, J. Silberring, S. E. Gandy, B. Winblad, P. Greengard, C. Nordstedt, L. Terenius, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 8378−8382.

Entry for the Table of Contents (Please choose one layout)

Layout 1:

COMMUNICATION

Zinc-metallothionein-3 (Zn_7MT3) is not able to capture copper from the high affinity Cu^{II} complex of Aβ4-16. This finding supports the role of Aβ4-42 as a Cu^{II} scavenger in the synaptic cleft.



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Resistance of Cu(Aβ4-16) to copper capture by metallothionein 3 supports a function of Aβ4-42 peptide as synaptic Cu^{II} scavenger