Electronic Supplementary Information for Metallomics

Supplementary Information

Zinc release of Zn_7-metallothionein-3 induces fibrillar type amyloid-β aggregates

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Materials and Methods

Zn_7MT-3 sample: Zn_7MT-3 was produced recombinantly as described [1]. The stock solutions were prepared by dissolving Zn_7MT-3 in water. For all experiments, the peptide solutions were buffered at pH 7.4 in 20 mM 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES), containing 20 mM NaCl. Zn_7MT-3 samples were stored at -20°C. The Zn_7MT-3 concentration was determined by absorption spectroscopy, by using the extinction coefficient of the peptide bond at 220 nm (ε =53'000 $M^{-1}cm^{-1}$ at pH 2 in 10 mM HCl and at room temperature).[2] The sample concentrations were verified by measuring the reduced Cys concentration, via the 2,2'-dithiopyridine (DTP) test [3]. Stock solution of 3mM DTP containing 100 mM EDTA at pH 4 was mixed with the samples containing 20µM MT-3 (buffer : Hepes 20mM, NaCl 20mM, pH 7,4) with H_2O to achieve a final concentration of 0.4 µM MT-3 and 300 µM DTP. The pH was adjusted to pH 7.4 with 1M NaOH. Then absorbance at 343 nm, corresponding to the absorbance of the characteristic final product 2-pyridinethiolate (ε = 7600 $M^{-1}.cm^{-1}$), was measured after five minutes of sample incubation at room temperature.

Aβ peptide sample: The amyloid-β(1-40) peptide ($A\beta_{40}$) was synthesized by standard F-MOC chemistry (*Genscript*). The stock solutions were prepared by dissolving the peptides in water and centrifuged to remove aggregates. The supernatant was recovered and its peptide concentration determined by absorption spectroscopy using the molar extinction coefficient of single tyrosine at 276 nm (ε =1410 $M^{-1}cm^{-1}$, pH = 7.4, room temperature)[4].

Absorption spectroscopy: UV/Visible absorption spectra were obtained with an *Agilent 8453 Hewlett Packard* spectrometer, at room temperature, with use of a 1 cm pathlength quartz cuvette. Turbidity was measured at 300 nm.

Zincon measurements: Zn^{2+} release from Zn_7MT-3 was measured by using the complexing dye zincon. Upon Zn^{2+} binding the Zn^{2+}-zincon complex shows a band at 620 nm with an extinction coefficient of 23'200 $M^{-1}cm^{-1}$ [5]. Zn^{2+} concentration was estimated by using the difference absorption at 620 nm between the Zn^{2+}-zincon complex and zincon. In the time course of Zn^{2+} release from Zn_7MT-3, the difference absorption between 620 and 800 nm of the buffer was used to account for baseline shifts over time.

Mass spectrometry: Positive mode ESI-MS spectra were recorded on an *API-365* quadrupole mass spectrometer (Perkin-Elmer Sciex) from 800 – 2500 Da. The Zn_7MT-3 samples were prepared at a concentration of 40 μM and incubated with 0, 40 or 200 μM H_2O_2 for 1 day at room temperature. Then, the samples were diluted by a factor of two with H_2O/acetonitrile (50% v/v) containing 0.1% formic acid. At the resulting pH 3, Zn^{2+} is released from Zn_7MT-3 and the apoprotein generated. Normally the peaks according to the 4+, 5+ and 6+ charged apo-MT-3 were well detected. Data acquisition and processing were performed with the *Analyst* software.

Size-exclusion chromatography: 20 μM Zn_7MT-3 peptide solutions were incubated with 0, 20, 100 or 200 μM H_2O_2 for one day and centrifuged. Different forms of ZnMT-3 obtained in the supernatant were separated by an AKTA Instrument (Amersham) using an Amersham Biosciences Superdex 75 10/300 GL size exclusion column (300 x 10 mm) eluted under isocratic conditions, at room temperature. ZnMT-3 elution was monitored at 220 nm and protein samples collected. At the same time, a 20μM solution of ZnMT-3 without H_2O_2 was also analyzed as a control.

ThT fluorescence: ThT binding assays were performed with a Safas Monaco SP 2000 spectrofluorimeter using a ninetysix-well plate. The final volume of 200 μl containing 30 μM $A\beta_{40}$, 20 μM Zn_7MT-3 and 0 or 100 μM H_2O_2 was incubated for one day. ThT (1 mM stock solution in water) was added to reach a final concentration of 10 μM. The fluorescence

spectra of ThT were obtained at room temperature employing excitation wavelength of 435 nm and emission wavelength of 482 nm. As a control, an aqueous solution of $ZnSO_4$ was used for Zn^{2+} addition.

Transmission electron microscopy: Solution of 30 μM Aβ were added to 20 μM Zn_7MT-3 and incubated with 0 or 100 μM of H_2O_2 for one day at room temperature. Samples (5 μL) were deposited on electron microscopy grids, washed with 5 μL of Milli-Q water, and negatively stained with an aqueous solution (5 μL) of uranyl acetate (1% w/w). Samples were air-dried and examined with a JEOL 1011 transmission electron microscope operating at an accelerating voltage of 100 kV. Numeric pictures acquisition was realized by the *ITEM* program. Samples were Abate; Aβ with $ZnSO_4$; Aβ (30 μM) with Zn_7MT-3; and Aβ, Zn_7MT-3 and H_2O_2. Concentrations Aβ (30 μM), $ZnSO_4$ (30 μM), Zn_7MT-3 (20 μM) and H_2O_2 (100 μM).

References

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