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for

Amyloid Fibrillar Meshwork Formation of Iron-Induced Oligomeric Species of Aβ with Phthalocyanine Tetrasulfonate and Its Toxic Consequences

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Aß40 amyloid fibril formation in the presence of various metals and PcTS

The Aβ40 aggregates were examined with atomic force microscope (AFM; XE-150, PSIA) by placing an aliquot (5 µL) onto freshly cleaved mica (thickness 0.3 mm). Following adsorption of the aggregates (1-2 min), the droplet was displaced with Millipore-filtered water (10 µL). After removing excess water with a filter paper, the aggregates were observed with AFM. The Aβ40 aggregates were also examined with EF-TEM (LIBRA 120, Carl Zeiss). Aliquot (5 µL) of the aggregates was adsorbed onto a carbon-coated copper grid (200 mesh) and air-dried for 5 min. After negative staining with 2% uranyl acetate for another 30 s, the aggregates were observed with EF-TEM.

Structural transition of Aß40 was monitored with circular dichroism spectroscopy (JASCO J-715) in the presence of $FeCl_3$ and PcTS. The Fe^{III}-induced oligomeric species of Aβ40 and the PcTS-induced amyloid fibrillar meshwork were obtained following 3 h incubation with $FeCl_3$ for the monomeric Aβ40 and an additional incubation

for 27 h with PcTS, respectively. Spectra were recorded in a 0.1 mm path-length quartz cell from 195 nm to 250 nm with a step resolution of 1.0 nm, band width of 1.0 nm, and scan speed of 20 nm/min. For all the spectra, an average of five separate scans was taken.

Assay for hydroxyl radical production

Hydroxyl radical formation was assayed with terephthalic acid (TPA) with slight modifications of the previously reported procedure.[S1] Metal-induced Aß40 oligomeric structure and PcTS-induced amyloid mesh were used in this assay. Metal-induced Aß40 oligomeric structure was obtained from Aß40 incubated with $FeCl_3$ (50 µM) in Tris-Cl (20 mM, pH 7.5) containing NaCl (100 mM), for 3 h at 37°C. PcTS-induced amyloid mesh was obtained from PcTS (50 µM) added to metal-induced Aß40 oligomeric structure and incubated for additional 27 h at 37°C. TPA (in DMSO, 10 mM, 7.14 µL), H_2O_2 (in Tris-Cl (20 mM, pH 7.5) containing NaCl (100 mM), 10 mM, 7.14 µL) and reducing agent (glutathione or ascorbic acid in Tris-Cl (20 mM, pH 7.5) containing NaCl (100 mM), 10 mM, 7.14 µL) were mixed with each samples (50 µL). The reaction mixture was incubated at 37°C for 30 min. At the end of incubation, the mixture was filtered through the prewashed filter (centrifugal membrane filter, YM-30) at 14 000 *g* during 20 min, to remove the Aß40 related structures. Excitation and emission wavelengths of fluorescence detector were set at 326 and 432 nm, respectively. The fluorescence intensity was measured with s luminescence spectrometer (LS-55, Perkin-Elmer).

Cell culture and trypan blue staining

Human dopaminergic neuroblastoma cells (SH-SY5Y) were grown in DMEM supplemented with 10% fetal bovine serum in 5% CO_2 at 37°C. The cells were plated in a 96-well plate at 5.0 x 10^4 cells/well. After the cell growth reached to 80-90% confluence, the metal-induced Aß40 oligomeric species and the PcTS-induced amyloid meshwork were separately added to the cells at 0.22 mg/mL, and incubated for 3 h in 5% CO_2 at 37°C. Since live cells are impermeable to trypan blue, cell death would be assessed with trypan blue (TB) staining. Those treated cells were stained with TB solution (in PBS buffer, 100 µL) for 20 s. The cells were directly observed with an inverted microscope in the absence and presence of TB treatment.

References

[S1] E. B. Yan, J. K. Unthank, M. Castillo-Melendez, S. L. Miller, S. J. Langford, D. W. Walker, *J. Appl. Physiol.* **2005**, *98*, 2304-2310.