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Supporting Information

Myricetin: A Naturally Occurring Regulator of Metal-Induced Amyloid-β Aggregation and Neurotoxicity

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Experimental

Materials and Procedures

All reagents were purchased from commercial suppliers and used as received unless stated otherwise. $A\beta_{1-40}$ peptide was purchased from AnaSpec (Fremont, CA, USA). The amino acid sequence for the $A\beta_{1-40}$ peptide is DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV. Myricetin was purchased from AvaChem Scientific (San Antonio, TX, USA) and was used without further purification. Optical spectra were collected on an Agilent 8453 UV-visible spectrophotometer. Transmission electron microscopy (TEM) images were taken using a Philips CM-100 transmission electron microscope. A SpectraMax M5 microplate reader (Molecular Devices) was used for measurements of absorbance for the cell viability assay.

UV-vis Studies. To understand metal binding properties of myricetin, optical spectra were obtained for the solutions of myricetin (25 μM in 20 mM HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid), pH 7.4, 150 mM NaCl) and myricetin incubated with $CuCl_2$ or $ZnCl_2$ (25 μM) for 5 min (room temperature), respectively (Figure S1). To verify if metal chelation occurs by myricetin in the presence of Aβ, optical studies were carried out on samples of Aβ (25 μM) and $CuCl_2$ or $ZnCl_2$ (25 μM, 2 min incubation, room temperature) in the absence and presence of myricetin (25 μM, incubation for 5 min, Figure S1).

Amyloid-β (Aβ) Peptide Experiments. Aβ samples were prepared according to the previously reported procedures.[1-4] All solutions were treated thoroughly with Chelex prior to use (Sigma Aldrich) to remove trace metal ion contamination. The Aβ peptide was dissolved in 1% NH_4OH (w/v, aq), aliquoted to 10 samples, lyophilized, and stored at –80 °C. Stock solutions of Aβ were prepared prior to the experiments by redissolving the lyophilized Aβ with 1% NH_4OH (w/v, aq, 10 μL) and diluting with ddH_2O. Solutions for inhibition and disaggregation studies were prepared in a buffered solution (20 μM HEPES, pH 7.4, 150 μM NaCl). For the inhibition studies, fresh Aβ (25 μM) was incubated with either $CuCl_2$ or $ZnCl_2$ (25 μM) for 2 min at room temperature

followed by addition of myricetin (50 μM). The solutions were incubated for 24 h at 37 °C with constant agitation. For the disaggregation studies, metal-Aβ aggregates were prepared by treating fresh Aβ (25 μM) with $CuCl_2$ or $ZnCl_2$ (25 μM) and incubating for 24 h at 37 °C with constant agitation. Afterwards, myricetin (50 μM) was added and the resulting solutions were incubated for 4 h at 37 °C with continuous agitation. Metal-free Aβ samples were prepared at the same condition in the absence of metal chloride salts. The degree of Aβ aggregation and the morphology of Aβ species samples from the inhibition and disaggregation studies were analyzed by native gel electrophoresis using Western blotting with an anti-Aβ antibody 6E10 (Covance, Princeton, NJ, USA) and by TEM.[1-3]

Native Gel Electrophoresis and Western Blotting. The reactions described above (Aβ Peptide Experiments) and in Figures 1 – 3 were visualized by native gel electrophoresis followed by Western blotting using an anti-Aβ antibody (6E10).[2-4] Each sample (25 μM Aβ, 10 μL) was separated on a 10 – 20% gradient Tris-tricine gel (Invitrogen). The gel was transferred onto a nitrocellulose membrane, blocked with bovine serum albumin (BSA, 3% w/v, Sigma) in Tris-buffered saline (TBS, Fisher) containing 0.1% Tween-20 (TBS-T, Sigma) for 3 h at room temperature, and incubated with an anti-Aβ antibody 6E10 (1:2,000) in 2% BSA in TBS-T for 3 h at room temperature. The membrane was probed with the horseradish peroxidase-conjugated goat anti-mouse antibody (1:10,000; Cayman Chemical, Ann Arbor, MI, USA) in 2% BSA in TBS-T for 1 h at room temperature. The Thermo Scientific Supersignal West Pico Chemiluminescent Substrate was used to visualize protein bands.

Transmission Electron Microscopy (TEM). Samples for TEM were prepared by following the previously reported method.[1-4] Glow-discharged grids (Formar/Carbon 300-mesh, Electron Microscopy Sciences) were treated with samples from the inhibition or disaggregation experiments (5 μL) for 2 min at room temperature. Excess sample solution was removed using filter paper followed by washing three times with ddH_2O. Each grid was incubated with 1% uranyl acetate (ddH_2O, 5 μL) for 1 min. Extra uranyl acetate was removed and the grids were dried for 15 min at room temperature. Images

from each sample were taken by a Philips CM-100 transmission electron microscope (80 kV, 25,000x magnification).

Cell Viability (MTT Assay). Human neuroblastoma SK-N-BE(2)-M17 (M17) cells were purchased from the American Type Culture Collection. The M17 cells were maintained in 1:1 Minimum Essential Media (MEM, GIBCO) and Ham's F12K Kaighn's Modification Media (F12K, Cellgro), containing 10% (v/v) fetal bovine serum (FBS, Atlanta Biologicals), 100 U/mL penicillin, and 100 mg/mL streptomycin (Invitrogen). The cells were grown at 37 °C in a humidified atmosphere of 5% CO_2. Cell viability was measured using the MTT (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich) assay.[2,3] Cells were seeded in a 96 well plate (1,600 cells in 100 μL per well) and treated with Aβ (10 μM) and $CuCl_2$ or $ZnCl_2$ (10 μM) immediately followed by myricetin (20 μM, final 1% DMSO (v/v)). After 24 h incubation of the cells at 37 °C, the cells were treated with 25 μL MTT (5 mg/mL in phosphate buffered saline (PBS), pH 7.4) for 4 h at 37 °C. The cells were lysed in a buffered solution containing *N*,*N*-dimethylformamide (pH 4.5, 50% (v/v, aq)) and sodium dodecyl sulfate (SDS, 20% (w/v)) overnight at room temperature in the dark. The absorbance (A_{600}) was measured using a microplate reader and cell viability (%) was calculated relative to that of cells containing only 1% DMSO (v/v, control cells).

References

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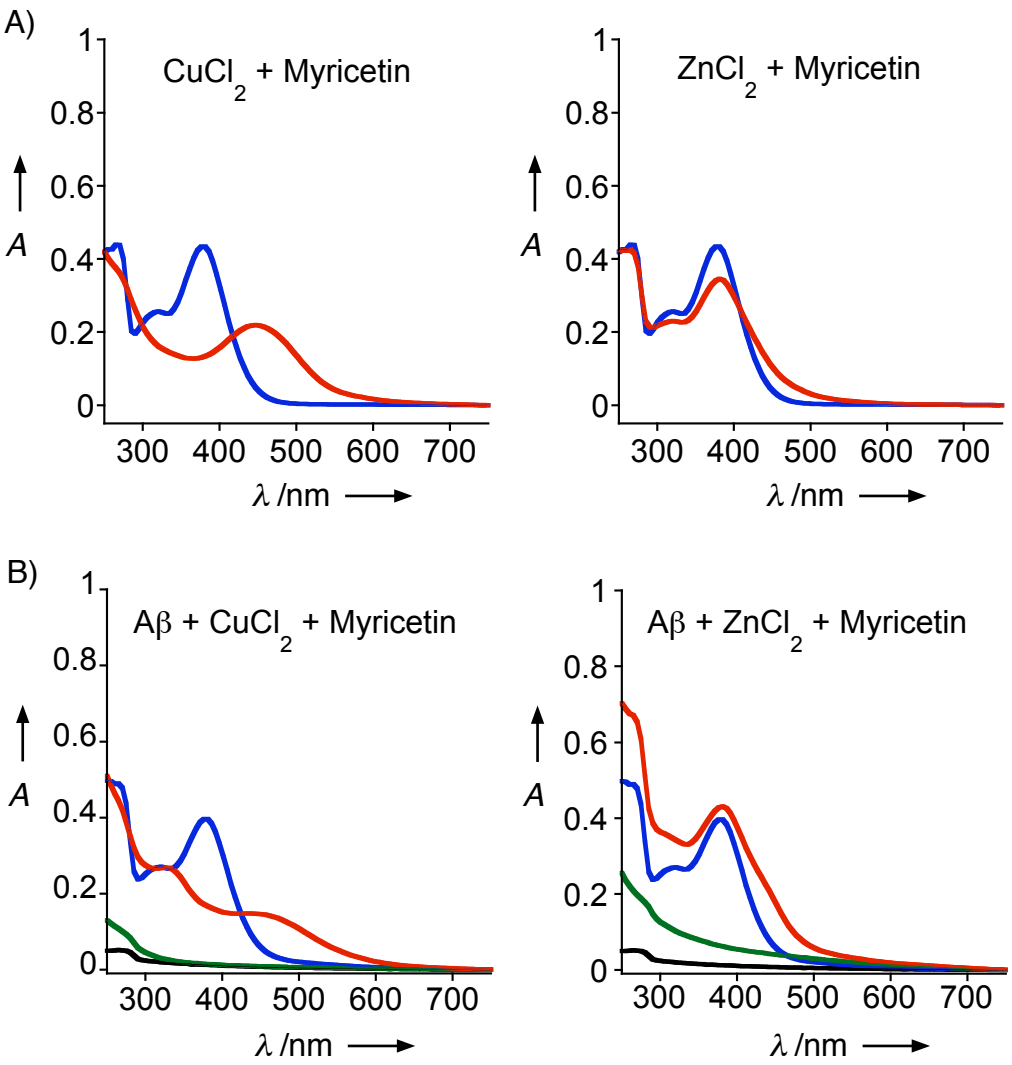

Figure S1. UV-vis studies showing the interaction of myricetin with metal ions in the presence of Aβ. A) The spectra of myricetin in the absence (blue) and presence (red) of $CuCl_2$ or $ZnCl_2$ following 5 min incubation. B) The spectra of the samples containing Aβ, metal ions, and/or myricetin. To Aβ (black), $CuCl_2$ or $ZnCl_2$ was added immediately and incubated for 2 min (green). The solution containing Aβ and $CuCl_2$ or $ZnCl_2$ was treated with myricetin for 5 min (red). The spectrum of metal-free Aβ and myricetin is shown in blue. Experimental conditions: [Aβ] = 25 μM, [$CuCl_2$ or $ZnCl_2$] = 25 μM, [myricetin] = 25 μM, 20 mM HEPES, pH 7.4, 150 mM NaCl, room temperature.