Supporting Information

Exploring the Differences between Mouse mAβ$_{1-42}$ and Human hAβ$_{1-42}$ in Alzheimer Disease Related Property and Neuronal Cytotoxicity

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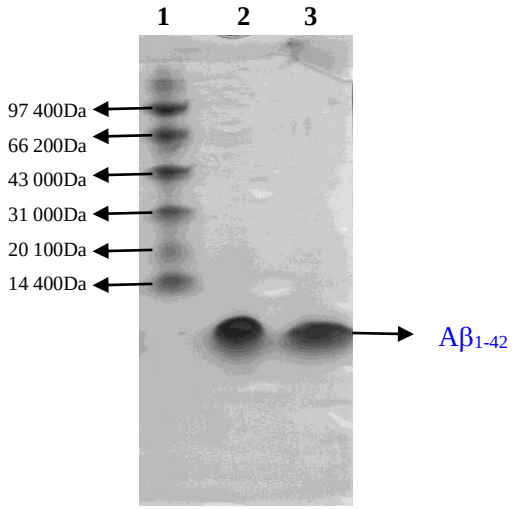
1. Peptide sequence of hAβ$_{1-42}$ and mAβ$_{1-42}$

5 10 13

hAβ$_{1-42}$: DAEF**R**HDSG**Y** EV**H**HQKLVFF AEDVGSNKGA IIGLMVGGVV IA

mAβ$_{1-42}$: DAEF**G**HDSG**F** EV**R**HQKLVFF AEDVGSNKGA IIGLMVGGVV IA

2. Tricine SDS page of hAβ$_{1-42}$ and mAβ$_{1-42}$



igure S1. Tricine SDS page of hAβ$_{1-42}$ and mAβ$_{1-42}$. 1, Marker; 2, hAβ$_{1-42}$; 3, mAβ$_{1-42}$

3. Mass spectrum of hAβ$_{1-42}$ and mAβ$_{1-42}$

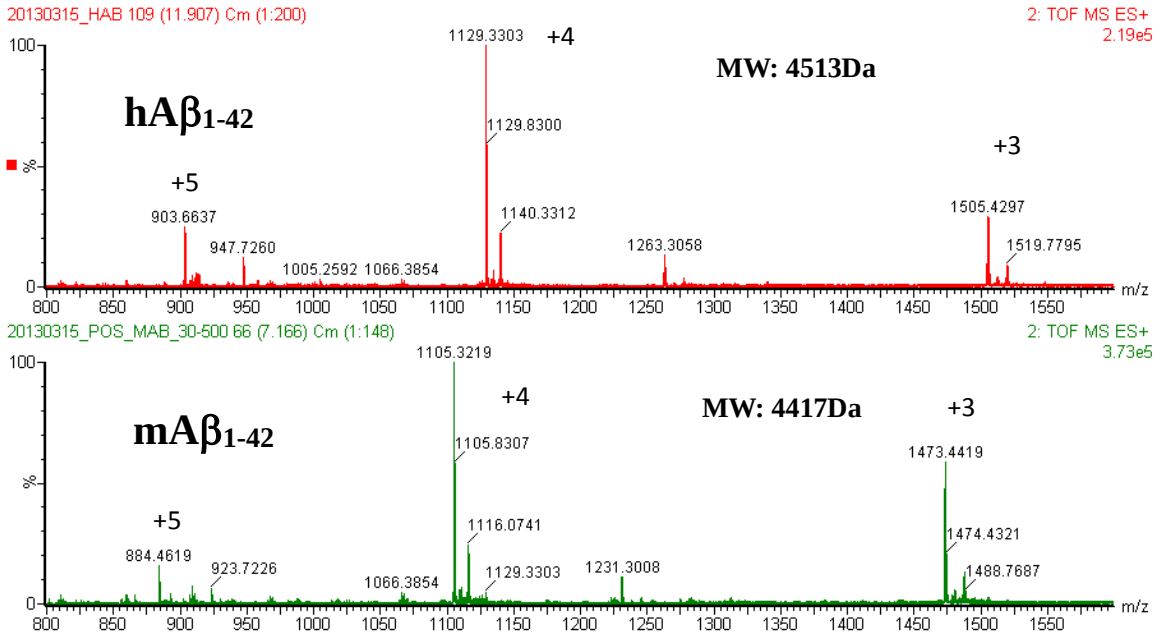


Figure S2. Mass spectrum of hAβ$_{1-42}$ and mAβ$_{1-42}$.

MW(hAβ$_{1-42}$): 4513Da, Compute MW (hAβ$_{1-42}$): 4514.10Da; MW(mAβ$_{1-42}$): 4417Da, Compute MW (mAβ$_{1-42}$): 4418.10Da.

4. Materials and Methods

Materials

The SH-SY5Y human neuroblastoma cell strain was offered by National Neurobiology Laboratory of Fudan University. ThT (thioflavin T) was purchased from Shanghai Source Leaf Biological Technology Co., LTD. ANS (8-anilino-1-naphthalene sulfonate), Uranyl acetate, Heme, Hepes, TMB (3, 3', 5, 5'-tetramethylbenzidine) were purchased from Sigma. DMEM (Dulbecco's modified Eagle's medium) were purchased from GIBCO-BRL (Gaithersburg, MD, USA). CCK-8 was from Shanghai R&S Biotechnology Co., Ltd. The ROS assay kit (2',7'-dichlorodihydrofluorescein diacetate, DCFH-DA) was purchased from Applygen Technologies Inc. The other chemical reagents were of analytical grade.

Mutagenesis, expression and purification of mAβ$_{1-42}$

The plasmid Smt3-Aβ$_{1-42}$ was constructed as described [1]. The mAβ$_{1-42}$ was constructed using plasmid

Smt3-Aβ_{1-42} as template, and residues Arg5, Tyr10, and His13 were successively changed into Gly5, Phe10, and Arg13 with the KOD-Plus Mutagenesis Kit (TOYOBO, Japan) using primer pair P1 as follows:

Forward: 5′-TTTGAAGTGCGTCATCAGAAACTGGTGTTTTTTGCG-3′,

Reverse: 5′- GCCAGAATCATGGCCAAATTCCGCATCACCACCAAT-3′

The mutated plasmid was isolated and sequenced by Shanghai JieLi biology Co. Ltd.

The expression and purification of human Aβ_{1-42} (hAβ_{1-42}) and mAβ_{1-42} were carried out according to the reported methods[1]. The pure hAβ_{1-42} and mAβ_{1-42} were lyophilized into powder and the stock solutions were prepared by dissolving the lyophilized peptide in buffer (pH 7.4, 20 mM HEPES and 100 mM sodium chloride). For the purpose of minimizing the aggregates and undissolved peptide, the Aβ_{1-42} solutions were sonicated at 4°C for 3 min, centrifuged at 12, 000 rpm for 30 min and filtered through the 0.22 μm filter before use.

Circular Dichroism Spectroscopy

The CD spectra were measured with a JASCO (Tokyo, Japan) J-715 instrument, using a 1-mm cuvette, with a scan range of 190-240 nm and at a scanning speed of 100 nm min^{-1}. The resolution was 0.2 nm, with a response time of 0.25 s. Each sample was scanned three times and the average value was obtained.

ThT Fluorescence Spectroscopy Assay

The ThT (thioflavin T) specifically binds to the fibril structure of β-aggregation of amyloid beta[2]. ThT was added to hAβ_{1-42} and mAβ_{1-42} (4 μM), respectively, to a final concentration of 10 μM. Emission spectra were collected from 460 nm to 540 nm with an excitation at 442 nm using Fluorescence Cary Eclipse (Varian company, USA).

ANS Binding Assay

ANS (1-Anilinonaphthaleine-8-Sulphonic Acid), as a stock solution of 10 mM in water, was filtrated through 0.22μm filter and its concentration was determined[3] by measuring the absorption at 350 nm using the extinction coefficient of 5000 $M^{-1}cm^{-1}$. The final concentration of Aβ was 10μM and the ANS was 50μM in the solution. Emission spectra were collect from 400 nm to 650 nm with an

excitation at 350 nm using Fluoromax-4(HORIBA scientific, USA).

Transmission Electron Microscopy

The TEM assays were conducted according to the methods reported by Reinke with marginal changes[4].10 μl of 50 μM different Aβ samples were incubated at 37°C for 72 hours. After that, 5 μl of amyloid peptides were put on the carbon 300-meshcopper grids, then kept for 2 min at room temperature. The extra peptides solution was blot up by filter paper. The copper grids were washed twice with double distilled water. The peptides were negatively stained with 5μl 2% uranyl acetate for 1 min at room temperature. The copper grids were kept for 15 min at room temperature so that the copper grids were blot up. The samples were examined with a TEM (CM200FEG, EM430, PHILIPS, Holland) at an accelerating voltage of 160kV.

Cell Viability Assay

Cell viability was quantitively determined using CCK-8(Cell Counting Kit-8). SH-SY5Y human neuroblastoma cells were cultured in DMEM medium containing 10% horse serum at 37°C, 5% CO_2, 95% O_2. The SH-SY5Y cells were inoculated into 96-well plates at a concentration of 1000 cells per well. Then cells were incubated at 37°C under 5% CO_2 for 24 hours. After that, the cells were treated with different samples for 24 hours at 37°C. Then 10 μl CCK-8 was add to each 96 well-plate and incubated for another 2 hours. Each well was detected at 450 nm by ELISA.

Reactive Oxygen Species Assay

The ROS assay was performed according to the methods with a slightly modification[5]. The original dye without fluorescence, could diffuses quickly into cells and oxidized by the reactive oxygen species. The oxidized product DCF (dichlorofluorescein) has a strong green fluorescence at 530 nm when given an excitation at 502 nm. The fluorescence intensity is in direct proportion with reactive oxygen species in cells. Cells were digested and put in 96-well plates at a concentration of about 1000 cells per well. The cells were incubated at 37°C under 5% CO_2 for 24 hours, and different samples were added and incubated for another 24 hours. The reactive oxygen species assay kit, containing DCFH-DA, were added to the final concentration of 10 μM. The cells were incubated for 1 hour and the old medium was discarded. The cells were washed with PBS buffer for three times. Then the cells were examined with a

fluorescence microscope (TE2000-U, Nikon, Japan).

UV/Vis spectra of binding with heme

Heme was prepared according to the described method[6]. The Aβ was mixed with heme in a 1:1 ration to a final concentration of 30 μM, and kept for 1h at 37°C. The UV/Vis absorption spectra were recorded using a HP8453 instrument (Hewlett-Packard, Palo Alto, CA, USA).

Peroxidase Activity Assay

The substrate TMB (3,3',5,5'-tetramethylbenzidine), and H_2O_2 were used to detect the peroxidase activity[7]. The final concentration of TMB was 0.416 μM mixed with Aβ-heme complex (0.75 μM). The substrate TMB was oxidized quickly by H_2O_2, which was catalyzed by the heme–$A\beta_{1-42}$ complex. The absorption at 652 nm of the reaction mixture was monitored as a time course upon the addition of the heme–$A\beta_{1-42}$ complex. A control experiment without the heme–$A\beta_{1-42}$ complex was performed.

Reference

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