Supplementary Information

Novel Purification Process for Amyloid Beta Peptide(1-40)

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S1 Materials and Methods

S1.1. Fmoc Peptide Synthesis of Aβ(1-40) Using Wang Resin

Aβ(1-40) was synthesized using Wang-PEG Resin (Watanabe Chemical Industries, Ltd., Hiroshima, Japan) by Fmoc solid-phase synthesis [S1] using the (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) (HBTU)–1-hydroxy benzotriazole monohydrate (HOBt) method (10 eq., double coupling). The DIPCI (N,N′-Diisopropylcarbodiimide)—DMAP (N,N-dimethyl-4-aminopyridine) method was used for the first residue (valine). The side-chain-protecting groups used were t-butyloxy carbonyl (Boc) for Lys; 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for Arg; t-butyl (tBu) for Ser, Tyr, Asp, and Glu; and trityl (Trt) for His, Asn, and Gln.

S1.2. Deprotection and Cleavage of Aβ(1-40) Using Wang Resin

The side-chain-protecting groups on the resins were removed by incubating the peptide-resin for 2 h in deprotection and cleavage solution (trifluoroacetic acid (TFA, Watanabe Chemical Industries)/triisopropylsilane (Wako Pure Chemical Industries, Tokyo, Japan)/water (90/5/5, v/v)). The peptides were precipitated by the addition of cold diethyl ether and collected by centrifugation.

S1.3. Monomerizaiton and HPLC Analysis of Aβ(1-40) Cleaved from Wang Resin

Before the HPLC, the crude peptide cleaved from Wang resin was dissolved in GdnHCl containing phosphate buffer (50 mM sodium phosphate, 8 M GuHCl, pH 7.5). In addition, the sample was incubated at 37 °C for 1 day in order to monomerize the sample completely. Then, the HPLC was performed on the GL7410 pump and GL7450 detector system (GL Sciences) using 220 nm absorbance. The peptide was analyzed on a Shodex Asahipak ODS-50 column (10.0 × 250 mm, Showa Denko K.K., Tokyo, Japan) using an isocratic condition with 100% of A solvent over 5 min and then a linear gradient from 0% to 30% of B solvent (90% acetonitrile, 10% Milli-Q ,and 0.1% NH_4OH) over 30 min at a flow rate of 1.0 mL/min.

References

S1. Chan W. C.; White, P. D. Fmoc solid-phase peptide synthesis; Oxford University Press: New York, **2000**.

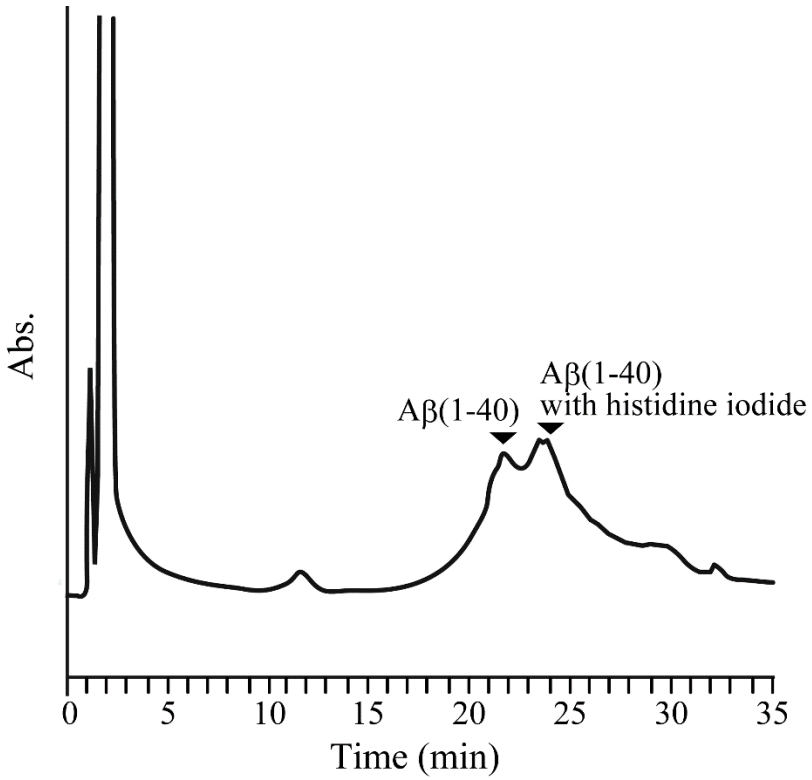

Figure S1. HPLC chart of Aβ(1-40) cleaved from HMBA resin after the reduction process without mercaptoacetic acid. The peaks were characterized by MALDI-TOF MS: Aβ(1-40) with histidine iodide, m/z 4453.2 ($(M+H)^+$ calcd. 4456.5); Aβ(1-40), m/z 4329.4 ($(M+H)^+$ calcd. 4330.6).

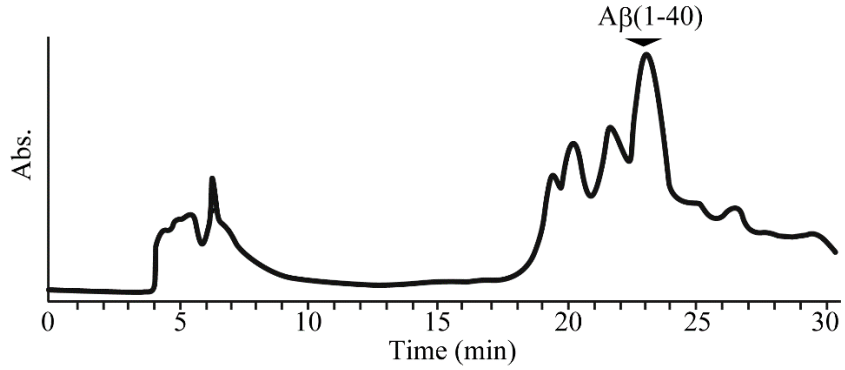

Figure S2. HPLC chart of Aβ(1-40) by conventional peptide synthesis using Wang resin. The peak was characterized by MALDI-TOF MS.