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Protective effects of Aβ-derived tripeptide, $Aβ_{32–34}$, on $Aβ_{1–42}$-induced phosphatidylinositol 4-kinase inhibition and neurotoxicity

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Abstract

We previously reported that the neurotoxicity of pathophysiological concentrations of amyloid β proteins (Aβs, 0.1–10 nM) as assessed by the inhibition of type II phosphatidylinositol 4-kinase (PI4KII) activity and the enhancement of glutamate toxicity was blocked by a short fragment of Aβ, $Aβ_{31–35}$. Such protective effects of shorter fragments derived from $Aβ_{31–35}$ were examined in this study to reach the shortest effective peptide, using recombinant human PI4KII and primary cultured rat hippocampal neurons. Among the peptides tested ($Aβ_{31–34}$, $Aβ_{31–33}$, $Aβ_{31–32}$, $Aβ_{32–35}$, $Aβ_{33–35}$, $Aβ_{34–35}$, $Aβ_{32–34}$, $Aβ_{33–34}$ and $Aβ_{32–33}$), $Aβ_{31–34}$, $Aβ_{32–35}$ and $Aβ_{32–34}$ blocked both the $Aβ_{1–42}$-induced inhibition of PI4KII activity and enhancement of glutamate toxicity on cell viability. The shortest peptide among them, $Aβ_{32–34}$, showed a dose-dependent protective effect with 50% effective concentration near 1 nM, while $Aβ_{34–32}$, with a reverse amino acid sequence for $Aβ_{32–34}$, showed no protective effects. Thus, a tripeptide, $Aβ_{32–34}$ i.e. Ile–Gly–Leu, may be available as a lead compound for designing effective Aβ antagonists.

Keywords: Type II phosphatidylinositol 4-kinase; Amyloid β protein; Tripeptide (Ile–Gly–Leu); Glutamate toxicity; Alzheimer's disease

Amyloid β proteins (Aβs) are pathogenic peptides of Alzheimer's disease (AD), strategies for blocking their toxicity being long searched based on their toxic mechanisms without successful efficacy in human trials [7]. Although relatively high concentrations (>10 μM) of Aβs have been used to analyse their toxicity in previous reports [11,18], pathophysiological concentrations (≤10 nM) of Aβs were demonstrated in our laboratory to induce the enhancement of glutamate toxicity [21–23], resulting in neuronal cell death via the inhibition of type II phosphatidylinositol 4-kinase (PI4KII) activity [20,21], raising a possibility that reagents blocking such effects of Aβs yield candidates for new therapeutics for AD. Most recently, we found that $Aβ_{31–35}$ and $Aβ_{20–29}$ peptides with partial amino acid sequences of toxic $Aβ_{1–42}$ or $Aβ_{25–35}$ peptides recovered such Aβ-induced inhibition of PI4KII activity and enhancement of glutamate toxicity, showing that a peptide as short as $Aβ_{31–35}$ is effective to protect the toxic effects of pathophysiological concentrations of Aβs [21]. In the present study, we tried to determine the $Aβ_{31–35}$-derived shortest peptide fragment interfering with Aβs' effects. Nine shorter peptides with 2–4 amino acid residues derived from $Aβ_{31–35}$ were synthesized and applied to examine their effects on $Aβ_{1–42}$-induced inhibition of recombinant human type IIα phosphatidylinositol 4-kinaseα (PI4KIIα) activity and enhancement of glutamate toxicity in primary cultured rat hippocampal neurons.

$Aβ_{1–42}$, $Aβ_{31–35}$ and nine $Aβ_{31–35}$-derived short peptides ($Aβ_{31–34}$, $Aβ_{31–33}$, $Aβ_{31–32}$, $Aβ_{32–35}$, $Aβ_{33–35}$, $Aβ_{34–35}$, $Aβ_{32–34}$, $Aβ_{33–34}$ and $Aβ_{32–33}$), as well as $Aβ_{34–32}$ with a reverse sequence of $Aβ_{32–34}$, were synthesized in Peptide Institute, Inc., Osaka, Japan. $Aβ_{1–42}$ was synthesized by the solution procedure as reported previously [10]. Aβ short peptides were synthesized with an automatic peptide synthesizer, ABI 433A (Foster City, CA, USA), employing 9-fluoremethoxycarbonyl (Fmoc) chemistry on Wang resin. Stock solutions of $Aβ_{1–42}$ and Aβ short peptides were prepared by dissolving in 10% dimethylsulfoxide at 20 μM and aliquoted before freezing at −80 °C. These peptides were applied to PI4KII assay or culture medium immediately after dilution of the stock

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solution with distilled water without incubation for peptide aggregation.

Recombinant human type II phosphatidylinositol 4-kinaseα was prepared by transfection of the plasmid (pGEX-KG) containing the open reading frame of human type II PI4Kα (PI4KIIα) in *Escherichia coli* DH5α competent cells (Toyobo, Osaka, Japan) as described previously [20]. Type II phosphatidylinositol 4-kinase activity was measured by phosphorylation of exogenous L-α-phosphatidylinositol (PI, Nacalai Tesque, Kyoto, Japan) using 10 mCi/mmol [γ-^{32}P] ATP (Amersham Biosciences, Piscataway, NJ, USA) as a phosphate donor [3]. Briefly, recombinant PI4KIIα bound to glutathione–sepharose 4B (Amersham Pharmacia Biotech, Uppsala, Sweden) was pre-incubated with or without $A\beta_{1–42}$ in the presence or absence of different short peptides for 30 min at room temperature, and then with 150 μM PI for 5 min at room temperature in 50 μL reaction buffer containing 20 mM Tris, pH 7.5, 100 g/L glycerol, 0.1 M NaCl, 10 g/L Triton X-100, 1 mM dithiothreitol and protease inhibitors set (Roche Diagnostics GmbH, Mannheim, Germany). Reactions were initiated by the addition of [γ-^{32}P] ATP and $MgCl_2$ at final concentrations of 0.1 mM and 15 mM, respectively, carried out at 37 °C for 10 min and then terminated by the addition of four volumes of chloroform/methanol/HCl (20:40:1, v/v) followed by the addition of one volume each of chloroform and 0.2 M KCl to extract phospholipids. Extraction and development of phospholipids were performed as described by Andrews and Conn [1]. Labeled PIP was detected by autoradiography at −80 °C using Kodak X-Omat AR film followed by densitometry using a color scanner and a public domain image processing and analysis program (NIH IMAGE; National Institute of Mental Health, Bethesda, MD, USA). Labeled PIP was regarded as a product of kinase activity.

Primary culture of rat hippocampal neurons was prepared as described previously [22]. The animal treatment and experimental procedures were all based on the Guidelines for Animal Care and Use Committee at Kansai Medical University. Hippocampal tissues removed from the brains of 19-day-old Wistar rat embryos were triturated in Ca^{2+}- and Mg^{2+}-free Hank's solution. The cells were suspended in Dulbecco's modified Eagle's medium supplemented with 4 mM L-glutamine, 100 IU/mL penicillin G sulfate, 10% fetal calf serum and 10% horse serum, and then seeded in poly-L-lysine-coated plastic dishes at a density of 2.55×10^5 cells/cm^2. After incubation for 2 days, the cells were exposed to 5 μM adenine-9ß-arabinofuranoside (Ara-A) in modified Eagle's medium (MEM) supplemented with 2 mM L-glutamine and 5% horse serum for 4 days. $A\beta_{1–42}$ and/or short peptides were applied for 2 days from the 8th day of culture. For monitoring glutamate excitotoxicity, the cells were exposed to glutamate (10 μM, 10 min, in serum-free MEM) on the 10th day of culture and assayed for cell viability after another 2-day culture in the same media as used during the 8–10 days of culture. Cell viability was assayed by measuring 2-(2-methoxy-4-nitrophenyl)-3-(4-nitro-phenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-8) reduction reflecting mitochondrial activity using a Cell Counting Kit-8 (Dojindo, Tokyo, Japan) and lactate dehydrogenase (LDH) release from damaged plasma membranes using an LDH-Cytotoxic Test (Wako, Osaka, Japan). Cl^--ATPase activity was measured

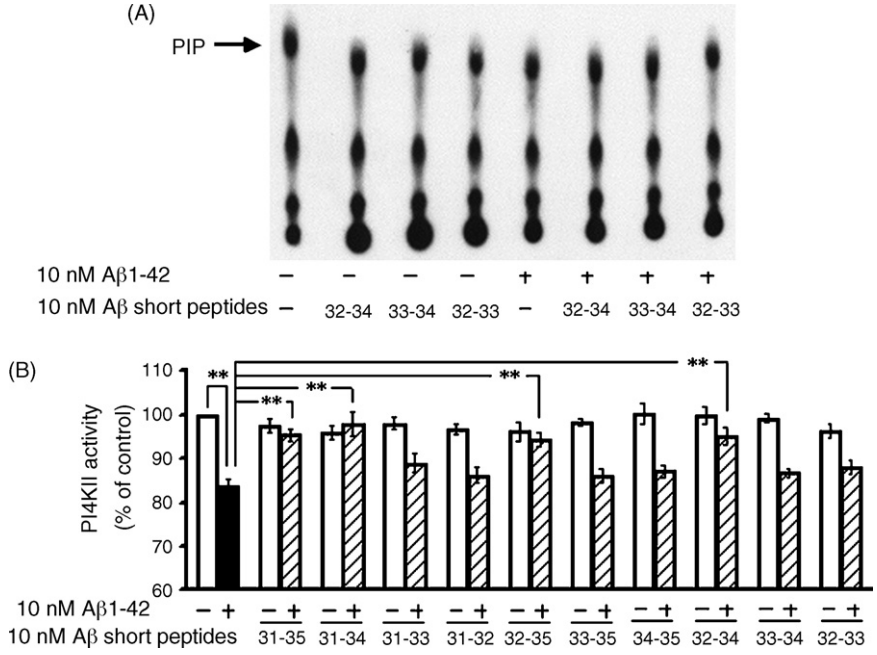

Fig. 1. Effects of Aβ short peptide derived from $A\beta_{31–35}$ on the $A\beta_{1–42}$-induced inhibition of recombinant human PI4KIIα activity. PI4KIIα was prepared freshly and was pre-incubated with or without $A\beta_{1–42}$ in the presence or absence of different short peptides for 30 min at room temperature. The kinase activity assay was initiated by the addition of [γ-^{32}P] ATP and $MgCl_2$ at 37 °C for 10 min, then terminated by the addition of four volumes of chloroform/methanol/HCl (20:40:1, v/v). (A) Typical spots of phosphatidylinositol monophosphate (PIP) as products of PI4KII activities. (B) Summary of the effects of nine Aβ short peptides as well as $A\beta_{31–35}$, (**)$p \leq 0.01$, $n = 4–5$. Each bar represents the mean ± S.E.M.