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GM1 ganglioside-mediated accumulation of amyloid β-protein on cell membranes

Masaki Wakabayashi[a], Takuma Okada[b], Yasunori Kozutsumi[b,c], Katsumi Matsuzaki[a,*]

[a] Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan
[b] Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan
[c] Glyco-chain Expression Laboratory, Supra-biomolecular System Research, RIKEN Frontier Research System, Wako 351-0198, Japan

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Abstract

The conversion of soluble, nontoxic amyloid β-protein (Aβ) to aggregated, toxic Aβ is the key step in the development of Alzheimer's disease. Liposomal studies proposed that Aβ specifically recognizes a cholesterol-dependent cluster of monosialoganglioside GM1 and a conformationally altered form of Aβ promotes the aggregation of the protein. In this study, the accumulation of Aβ on living cells was investigated for the first time. The interaction of fluorescein-labeled Aβ (FL-Aβ) with rat pheochromocytoma PC12 cells was visualized using confocal laser microscopy. FL-Aβ was found to colocalize with GM1-rich domains on cell membranes and to accumulate in a concentration- and time-dependent manner, leading to cytotoxicity. Cholesterol depletion significantly reduced Aβ accumulation. These observations corroborate the GM1-mediated Aβ accumulation model.

Keywords: Amyloid β-protein; Ganglioside; Cholesterol; Alzheimer's disease; Lipid raft

The conversion of soluble, nontoxic amyloid β-protein (Aβ) to aggregated, toxic Aβ rich in β-sheet structures is considered to be the key step in the development of Alzheimer's disease (AD) [1–3]. Membranes play an important role in the aggregation of Aβ. Yanagisawa et al. [4,5] discovered monosialoganglioside GM1-bound Aβ (GM1-Aβ) in the brains of patients with AD and suggested that GM1-Aβ may act as a seed for the polymerization of Aβ. Indeed, in human neuroblastoma cells, approximately half of all detergent-insoluble Aβ was present in lipid rafts [6] rich in gangliosides, sphingomyelin (SM), and cholesterol [7]. Aβ was also found to be highly concentrated in lipid rafts of the brain in the Tg2576 transgenic mouse [8]. Furthermore, gangliosides including GM1 [9–11] as well as oxidized phospholipids [12] have been shown to accelerate the rate of amyloid fibril formation of soluble Aβ in vitro.

Based on spectroscopic studies using liposomes, we have proposed the following model for the interactions of Aβ with lipid rafts [11,13]. Aβ specifically recognizes a ganglioside cluster, the formation of which is facilitated by cholesterol. Aβ undergoes a conformational transition from an α-helix-rich structure to a β-sheet-rich one with increasing protein density on the membrane. The β-sheet-rich Aβ serves as a seed for protein aggregation. However, it is not yet clear whether Aβ accumulates on cell membranes in a ganglioside- and cholesterol-dependent manner.

In this study, the accumulation of Aβ on living cells was investigated for the first time. The interaction of fluorescein-labeled Aβ (FL-Aβ) with rat pheochromocytoma PC12 cells, which are often used to evaluate Aβ cytotoxicity [14–16], was visualized using confocal laser microscopy. FL-Aβ was found to colocalize with GM1 on cell membranes and accumulate in a concentration- and

* Corresponding author. Fax: +81 75 753 4578.
E-mail address: katsumim@pharm.kyoto-u.ac.jp (K. Matsuzaki).

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time-dependent manner, leading to cell death. Cholesterol depletion significantly reduced Aβ accumulation.

Materials and methods

Materials. Dulbecco's Modified Eagle's Medium (DMEM), horse serum, fetal bovine serum, penicillin, and streptomycin were purchased from Invitrogen (Carlsbad, CA). Human Aβ-(1–40) labeled with the fluorescein fluorophore at its N-terminus (FL-Aβ) was purchased from AnaSpec (San Jose, CA). Alexa Fluor 647-conjugated cholera toxin subunit B (CTX-B), propidium iodide (PI), and ethidium homodimer-1 (EthiD-1) were obtained from Molecular Probes (Eugene, OR). Methyl-β-cyclodextrin (MβCD) was purchased from Sigma (St. Louis, MO). Micro BCA protein assay kit was obtained from Pierce (Rockford, IL).

Aβ solution. FL-Aβ was always handled in light-protected, capped tubes under a nitrogen atmosphere to avoid photodegradation. The protein was dissolved in 0.02% ammonia on ice. Any large aggregates that may act as a seed for fibril formation were removed by ultracentrifugation in 500 μl polyallomer tubes at 100,000*g*, 4 °C for 3 h [17]. The peptide concentration of the supernatant was determined in triplicate using the Micro BCA protein assay.

Cell culture. Rat pheochromocytoma PC12 cells were cultured in DMEM containing 5% horse serum, 10% bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin at 37 °C with 5% CO_2. After being plated at densities ranging from 1 to 3 million cells on a poly-D-lysine-coated 35-mm glass-bottomed dish, cells were incubated for 24 h.

Aβ accumulation on cells. Cells were incubated with freshly prepared 10 or 25 μM FL-Aβ in medium at 37 °C with 5% CO_2, and then rinsed twice with Tris-buffered saline (TBS) (150 mM NaCl, 10 mM Tris, and 1 mM EDTA, pH 7.4). The labeling of cell surface GM1 was performed by incubating the cells with 10 μg/ml CTX-B in TBS for 20 min at room temperature. Fluorescently labeled cells were visualized using the 63× C-Apochromat objective of a Zeiss LSM 510 confocal laser scanning microscope.

Interaction of preincubated Aβ with cells. Twenty-five micromolar FL-Aβ was preincubated in medium for 48 h at 37 °C with 5% CO_2. The incubated peptide was directly applied to plated PC12 cells and further incubated for 30 min at 37 °C with 5% CO_2. GM1 labeling was performed as described above.

Dead cell staining. Plated PC12 cells were incubated with 25 μM FL-Aβ in medium for 48 h at 37 °C with 5% CO_2 and then rinsed twice with TBS. Dead cells were stained with 0.2 μM EthiD-1 in TBS for 30 min at room temperature. Apoptotically dead cells were stained as follows. Plated PC12 cells were incubated for 48 h in serum-free medium (DMEM) at 37 °C [18,19]. Dead cells were stained with 0.5 μM PI in TBS for 3 min and then with 10 μg/ml CTX-B in TBS for 20 min at room temperature.

Cholesterol depletion. Plated PC12 cells were incubated for 3 h in serum-free medium (DMEM) and then treated with 5 mM MβCD in serum-free medium at 37 °C for 8 min. Under these conditions, the cellular cholesterol level was reduced to about 40% of that in untreated cells. Treated cells maintained their viability and were partly protected from Aβ-induced cytotoxicity [14]. The MβCD-containing medium was replaced with fresh, FL-Aβ (25 μM)-containing medium. The MβCD-treated cells were incubated for 24 h. GM1 labeling was performed as described above.

Results

Time- and concentration-dependent Aβ binding

First, cells were incubated only with CTX-B as a control (Fig. 1A). CTX-B was mainly distributed on plasma

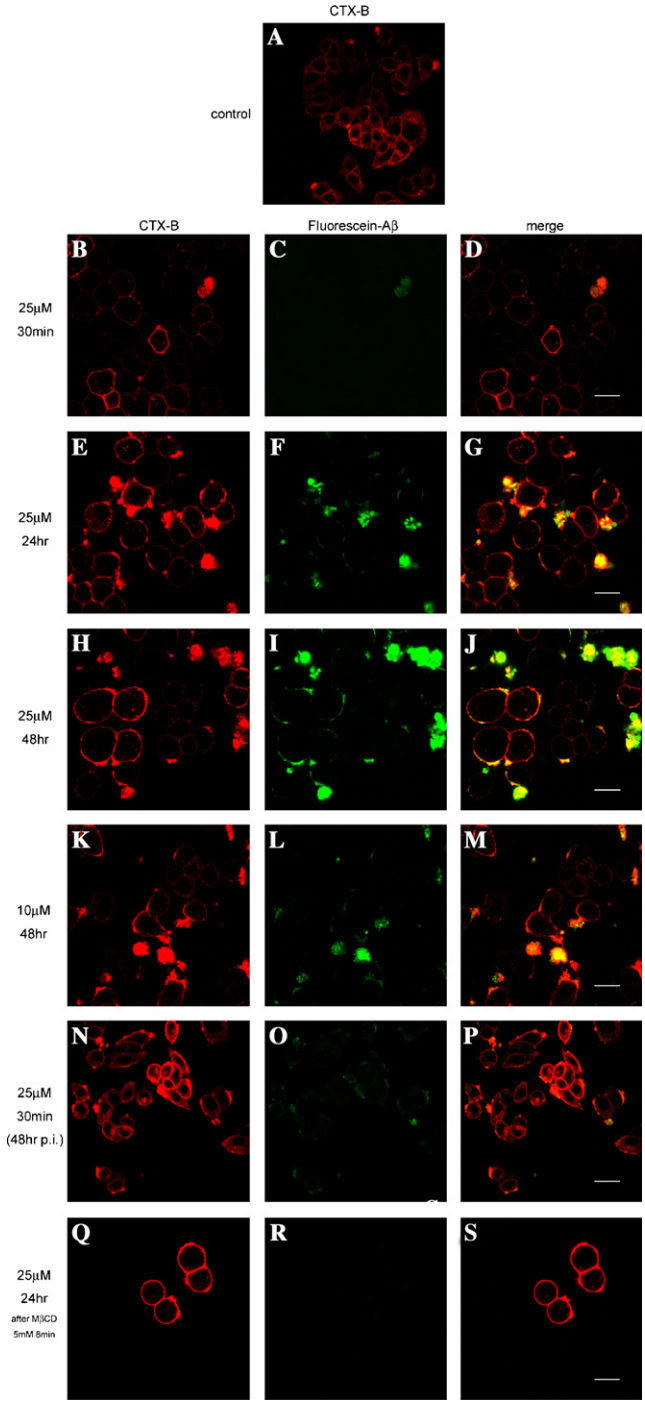

Fig. 1. Concentration- and time-dependent accumulation of FL-Aβ on PC12 cells. PC12 cells were incubated with FL-Aβ at 37 °C under various conditions, and the distribution of GM1 and Aβ was visualized by CTX-B staining (shown in red, the left columns) and fluorescein fluorescence (shown in green, the middle-column figures), respectively, under a confocal laser microscope without fixation. The figures in the right column represent the merged images. (A) Untreated PC12 cells as a control. FL-Aβ concentration: (B–J and Q–S), 25 μM; (K–M), 10 μM. Incubation time: (B–D), 30 min; (E–G and Q–S), 24 h; and (H–J and K–M), 48 h. (N–P), FL-Aβ (25 μM) was preincubated in medium for 48 h and was applied to PC12 cells for 30 min. (Q–S) Cells were pretreated with 5 mM MβCD for 8 min.