Biochemical and Biophysical Research Communications 439 (2013) 321–326

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Fibrillar seeds alleviate amyloid-β cytotoxicity by omitting formation of higher-molecular-weight oligomers

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ARTICLE INFO

Article history:
Received 27 August 2013
Available online 6 September 2013

Keywords:
Alzheimer's disease
β-amyloid
Aggregation
Oligomer
Fibril
Monomer

ABSTRACT

Amyloid-β (Aβ) peptides can exist in distinct forms including monomers, oligomers and fibrils, consisting of increased numbers of monomeric units. Among these, Aβ oligomers are implicated as the primary toxic species as pointed by multiple lines of evidence. It has been suggested that toxicity could be rendered by the soluble higher-molecular-weight (high-n) Aβ oligomers. Yet, the most culpable form in the pathogenesis of Alzheimer's disease (AD) remains elusive. Moreover, the potential interaction among the insoluble fibrils that have been excluded from the responsible aggregates in AD development, Aβ monomers and high-n oligomers is undetermined. Here, we report that insoluble Aβ fibrillar seeds can interact with Aβ monomers at the stoichiometry of 1:2 (namely, each Aβ molecule of seed can bind to two Aβ monomers at a time) facilitating the fibrillization by omitting the otherwise mandatory formation of the toxic high-n oligomers during the fibril maturation. As a result, the addition of exogenous Aβ fibrillar seeds is seen to rescue neuronal cells from Aβ cytotoxicity presumably exerted by high-n oligomers, suggesting an unexpected protective role of Aβ fibrillar seeds.

1. Introduction

Amyloid-β (Aβ) peptide, which is believed to play a causative role in the pathogenesis of Alzheimer's disease (AD), can exist in multiple forms including monomers, oligomers or protofibrils, fibrils, and amyloid plaques that contain densely packed Aβ fibrils and other molecules [1]. Amyloid fibrils or plaques are considered to be a pathological hallmark of AD. It has been suggested that the two extremes of Aβ forms, monomers and insoluble fibrils are unlikely the candidates for synaptic plasticity and memory impairment in AD [2]. As such, non-fibrillar Aβ aggregates are more critical in the pathogenesis of AD [3–6].

The identification of the toxic oligomeric variants responsible for long term potential (LTP) impairment and synaptic dysfunction *in vivo* has been a focus of current research [6–9]. On one hand, Soluble low-molecular-weight (low-n) oligomers including Aβ dimers [5,8,10,11], trimers [12], tetramers [13], 12-mers [6,13] are individually proven to be neurotoxic and to be able to impair synaptic functions. On the other hand, high-molecular-weight (high-n) oligomers which are spherical Aβ assemblies [14–16], have also been isolated from AD brain tissues and suggested to be the main toxic Aβ oligomeric species. It seems plausible that both soluble low-n oligomers and high-n oligomers could be responsible for the pathogenesis of AD.

However, whether the low-n oligomer species are the main responsible neurotoxic Aβ forms is much disputed. In most of the relevant literatures, Aβ oligomers were analyzed by SDS–PAGE [7]. As the essential material in this method, the detergent SDS, is known to induce the artificial oligomerization of Aβ [17] – a inherent caveat to prevent the precise determination of Aβ stoichiometry. Take the Aβ dimer as an example, this low-n oligomer was investigated as the minimal sized toxic species *in vivo*[5,8]. Due to the intrinsic thermodynamic trend to assemble into high-n metastable oligomers, whether the synaptotoxicity observed was indeed directly caused by the dimers present in their purified fractions remains unproven [7]. Recently, the synaptic toxic Aβ dimers

Abbreviations: AD, Alzheimer's disease; Aβ, β-amyloid; ITC, isothermal titration calorimetry; SEC, size exclusion chromatography; PBS, phosphate buffered saline; ThT, Thioflavin T; MTT, 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazoliumbromide; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; DMSO, dimethyl sulfoxide; DMEM, Dulbecco's modified Eagle's medium; MEM, minimum essential media; FBS, fetal bovine serum; TEM, transmission electron microscope; FTIR, Fourier transform infrared spectroscopy.

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http://dx.doi.org/10.1016/j.bbrc.2013.08.088

were captured by cross-linking, recapitulating the transient nature of these low-n oligomers [11].

Since the metastable high-n assemblies could be the potential culprit for AD development [7,11], it is of great importance to re-evaluate the roles of monomers and fibrils because they might act as sources and reservoirs of the those soluble synaptotoxic and neurotoxic oligomers [2]. That mature Aβ fibrils can seed accelerated aggregation of both monomer and oligomer Aβ hints at the possible interactions between fibrils and other forms of Aβ [18–20]. Despite the great progress made in this field, the following mechanistic questions remain unanswered: Why can fibrillar seeds regulate the Aβ aggregation process and how do fibrillar seeds affect the levels of the different Aβ aggregated forms, especially toxic soluble high-n oligomer species? To our best knowledge, little literature is available on the detailed interaction – on a molecular level – between fibrils or amyloid plaques and other forms of Aβ [21–23]. In this study, we found that the interaction between the monomeric and fibrillar Aβ is a pivotal event for fibrillar seeds to modulate Aβ toxicity. Once monomer Aβ binds to fibrillar seed, its conversion to fibril is greatly accelerated, as evidenced by significantly reduced levels and shortened lifespan of the oligomeric forms, especially the high-n species. The thermodynamic favorable interaction of monomeric Aβ with fibrillar seeds results in less cytotoxicity of Aβ in neuroblastoma N2a cell line and rescues cells by omitting the formation of high-n oligomer. Since the high-n oligomer Aβ species, not other Aβ species, have been implicated as the primary pathological species in the pathogenesis of AD [2,7,11], our results imply that amyloid plaques may play a positive protective role by interacting with monomers, subsequently accelerating their fibrillation and thus preventing them from forming the neurotoxic oligomers.

2. Materials and methods

2.1. Preparation of monomer Aβ

Aβ42 was from R-peptide Company, USA. Monomeric forms of Aβ42 were prepared by dissolving to 5 mg/ml in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP, Acros, USA), incubated overnight at room temperature and stored at −20 °C in HFIP. The HFIP was then evaporated off by a speed vac before using. After that, dimethyl sulfoxide (DMSO) was added and the solutions (5 mg/ml) were sonicated for 15 min before using.

2.2. Preparation of fibrillar Aβ seeds

Aβ42 fibrils were prepared by incubating 50 μM monomer Aβ42 for 84 h. The fibrils were centrifuged at 10,000*g* at 4 °C for 30 min (Biofuge, Heraeus Instruments, Germany) and 100 μl of supernatant was injected into a size exclusion chromatography column (TSK-GEL G3000PW$_{XL}$, TOSOH corporation) to quantify the relative amount of soluble monomer Aβ and oligomer Aβ in PBS solution. By comparing with the amount of the fresh monomer Aβ that was initiated to form fibrils, the amount of formed fibrils was estimated from the area of relative peaks (Fig. S1). The pellet was reconstituted into PBS (1×, pH 7.4) with v/v 1% DMSO and the sample contained fibrillar Aβ42 at the estimated concentration of 75 μM. The solution was mechanically fragmented into smaller fibrillar structures by sonication on ice for 45 min. The resultant stock solution was used as fibrillar Aβ seeds in following experiments.

2.3. Isothermal titration calorimetry (ITC) analysis

All calorimetric experiments were performed on a fully computer-operated and thermostated VP-ITC calorimeter (Microcal Inc., USA) at 30 °C. Each titration to determine the enthalpy changes of the adsorption of monomer Aβ on the fibrillar seeds consisted of 14 successive injections of 20 μl seed solution (75 μM) into the reaction cell (1.4616 ml) containing 20 μM Aβ42 in the same buffer.

2.4. Quenched tyrosine fluorescence titration

20 μM Aβ42 or PBS blank solution with v/v 1% DMSO was placed in a four-sided quartz fluorescence cuvette with total volume of 700 μl. Fibrillar seed stock solution, 9.58 μl each time, was added into the cuvette. Such volume was designed to simulate the ITC condition. Fluorescence spectra were collected using a Hitachi FP-4500 fluorescence spectrophotometer (Japan). An excitation wavelength of 280 nm (slit width = 5 nm) was used and data were collected over 290–350 nm (slit width = 5 nm).

2.5. Fibrillation experiments

Aβ42 fibrillation was studied at 37 °C in the absence or presence of fibrillar seeds. The monomer Aβ42 concentration was 20 μM in PBS. Fibrillar seeds at different concentrations (1, 2, and 4 μM, respectively) were incubated with Aβ42. Each sample of 1.2 ml was shaken in a 1.5 ml Eppendorf tube at 200 rpm. To monitor the growth of fibrils, 20 μl aliquots from the tubes were taken at different time points and mixed with Thioflavin T (ThT, 10 μM) in a four-sided quartz fluorescence cuvette. The ThT fluorescence was measured at 489 nm with excitation at 440 nm in a Hitachi FP-4500 fluorescence spectrophotometer (Japan).

2.6. Analytical size exclusion chromatography (SEC)

Analytical SEC was utilized to quantify the relative amounts of soluble (monomer and oligomer) Aβ42 in solution at selected time points during Aβ aggregation process. An SEC column TSK-GEL G3000PW$_{XL}$ (TOSOH Corporation) was connected to a Waters 600 (Waters, USA). Aliquots (200 μl) of the samples were injected into the column. The running buffer was PBS (1×, pH 7.4) and the elution was monitored by UV 215 and 280 nm at a flow rate of 0.5 ml/min. Integration was operated on Origin 7.0 ®Software.

2.7. Cell viability experiments

N2a WT cells were cultured in the medium containing 42.5% (v/v) DMEM, 42.5% (v/v) Optical MEM, 5% (v/v) FBS, 100 units/ml penicillin, 0.1 mg/ml of streptomycinin a density of 5000 per well in 96 wells plate for first 24 h. After the medium was replaced by 50% (v/v) DMEM and 50% (v/v) Optical MEM, 20 μM Aβ with or without 1, 2 , and 4 μM of seeds was adding into wells for further incubation for 10 or 20 h. 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium-bromide (MTT) was added to each well for 3.5 h. MTT reduction was assessed by measuring the absorption at 570 nm by a BioTek Synergy 4 microplate reader.

3. Results

3.1. Fibrillar seeds interact with monomer Aβ at the ratio around 1:2

We first characterized the interaction between Aβ fibrillar seeds and monomers by measuring the changes of tyrosine intrinsic fluorescence. Tyrosine intrinsic fluorescence has been applied to assess interactions of Aβ with other molecules [24,25]. Tyrosine intrinsic fluorescence emission at 309 nm (excited at 280 nm) is usually quenched on the occasion of molecular interactions. A marked quenched tyrosine fluorescence signal at 309 nm was observed