Biochemical and Biophysical Research Communications 557 (2021) 122–126

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Lipid membranes induce structural conversion from amyloid oligomers to fibrils

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ARTICLE INFO

Article history:
Received 6 March 2021
Accepted 31 March 2021

Keywords:
Alzheimer's disease
Protein aggregation
Liposome
Amyloid fibrils
Aβ oligomers

ABSTRACT

Formation of amyloid oligomers and fibrils underlies the pathogenesis of a number of neurodegenerative diseases such as Alzheimer's. One mechanism of action by which Aβ aggregates cause neuronal toxicity is through interactions with cellular membranes. Aβ aggregates have been shown to disrupt membrane integrity via pore formation, membrane thinning, or lipid extraction. At the same time, lipid membranes also affect the rate of Aβ aggregation and remodel pre-formed Aβ fibrils. Here we show that Aβ42 globulomers, a type of well-characterized and stable Aβ oligomers, convert to amyloid fibrils in the presence of DOPC liposomes. Electron paramagnetic resonance studies show that the fibrils converted from Aβ42 globulomers adopt the same structure as fibrils formed directly from monomers. Our results suggest that the interactions between Aβ oligomers and cellular membranes are dynamic. By converting Aβ oligomers to fibrils, the lipid membrane can reduce the membrane-disrupting activities caused by these oligomers. Modulation of Aβ-membrane interactions as a therapeutic strategy should take into account the dynamic nature of these interactions.

1. Introduction

Protein aggregation and formation of amyloid oligomers and fibrils play a central role in the pathogenesis of a number of neurodegenerative diseases including Alzheimer's and Parkinson's diseases [1,2]. Aβ aggregation is a supersaturation-driven process [3] and leads to the formation of both soluble oligomers and insoluble amyloid fibrils [4]. Soluble oligomers have been considered to be the main toxic species leading to a pathological cascade, although the exact mechanism by which Aβ aggregates cause toxicity is not totally clear [5]. Interactions between Aβ aggregates and cellular membranes have long been a topic of intense research [6]. A large body of experimental evidence suggests that Aβ oligomers increase membrane permeability, leading to loss of membrane ion gradients and cellular membrane depolarization [7,8]. Aβ oligomers may form pores or ion channels in the membrane [9–11]. Alternatively, Aβ oligomers may disrupt membrane integrity via membrane thinning and carpeting [12,13]. Aβ aggregates may also have a detergent-like effect of lipid extraction and membrane dissolution [14,15]. On the other hand, lipid membranes have been shown to affect Aβ aggregation. Contradictory results have been obtained regarding the effect of liposomes on the rate of Aβ aggregation. Some studies showed that liposomes inhibit Aβ aggregation [16,17], while other studies showed aggregation-promoting effect of liposomes [18]. The different effects of liposomes may be partly due to the composition of lipids [19,20]. One consensus finding is that lipid membranes reduce the overall amount of Aβ aggregates [17,18]. Lipid membranes have also been shown to remodel pre-formed Aβ fibrils, leading to formation of protofibrils [21] or thinner filaments [20].

One aspect of Aβ-lipid interaction that has not been well studied is how lipid membranes affect the structure of Aβ oligomers, which have been widely regarded as the toxic species leading to Alzheimer's pathology [22]. In this work, we investigated lipid membrane-induced structural changes of Aβ42 globulomers, which are formed by the 42-residue isoform of Aβ protein, Aβ42, in the presence of low concentrations of SDS [23]. Globulomer-specific antibodies recognize endogenous Aβ oligomers in the brain slice of Alzheimer's patients, suggesting that globulomer-like structures are pathologically relevant [23,24]. Aβ42 globulomers adopt antiparallel β-sheet structures based on studies using electron paramagnetic resonance (EPR) [25,26] and NMR [27,28]. Previous studies have shown that Aβ42 globulomers are extremely stable, devoid of fibril formation even after incubation at room

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https://doi.org/10.1016/j.bbrc.2021.03.174
0006-291X/

temperature for 7 days [29]. Furthermore, Aβ42 globulomers did not form fibrils even in the presence of pre-formed fibril seeds [29]. Upon incubation with large unilamellar vesicles of DOPC at 4 °C, we found that Aβ42 globulomers formed amyloid fibrils. EPR studies confirm that the globulomer-converted fibrils adopt parallel in-register β-sheet structures, just like fibrils formed directly from Aβ42 monomers.

2. Material and methods

Preparation of Aβ42 and spin labeling. The Aβ42 construct is a fusion protein of GroES-ubiquitin-Aβ42 [30], which allows the purification of full-length Aβ42 without any extra residues upon digestion with a deubiquitylating enzyme, Usp2-cc [31]. A cysteine mutant of Aβ42, L17C, was constructed using site-directed mutagenesis. Protein purification of Aβ42 has been described previously [32]. For spin labeling, we added TCEP to Aβ42 L17C at 10 mM concentration and incubated it at room temperature for 20 min to break any disulfide bonds. Then TCEP was removed using buffer exchange with the labeling buffer (20 mM MOPS, 7 M guanidine hydrochloride, pH 6.8). Spin labeling was performed at room temperature for 1 h by adding MTSSL, 1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl methanethiosulfonate (Adipogen) at 10-fold molar excess. The sample was then buffer exchanged to 30 mM ammonium acetate (pH 10), lyophilized, and stored at −80 °C. Labeling efficiency was evaluated with mass spectrometry. Only samples with labeling efficiency of >95% were used in the subsequent experiments. The spin label side chain is named R1 and the spin-labeled Aβ42 mutant is thus named L17R1.

Preparation of Aβ42 globulomers. Lyophilized powder of wild-type Aβ42 L17R1 was first dissolved in 100% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at 1 mM, bath-sonicated for 5 min, and then incubated at room temperature for 30 min. HFIP was then evaporated overnight in a fume hood. HFIP-treated samples were dissolved in dimethyl sulfoxide (DMSO) at 5 mM and bath-sonicated for 5 min. The Aβ concentration was determined using a fluorescamine method [33]. PBS buffer (20 mM phosphate, 140 mM NaCl, pH 7.4) and 10% SDS were used to dilute the Aβ sample to 400 μM containing 0.2% SDS (final concentration). The sample was incubated for 6 h at 37 °C, and then diluted with 3 vol of deionized water to a final Aβ concentration of 100 μM and incubated for another 18 h at 37 °C. The sample was centrifuged at 14,000 g for 20 min to remove insoluble aggregates, and the supernatant was concentrated using an ultrafiltration filter with 30-kD molecular mass cut-off. The 30-kD retentate is the globulomer sample.

Incubation of Aβ42 globulomers with DOPC liposomes. To prepare large unilamellar vesicles, we added 20 mM HEPES (pH 7.4) to dried 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) lipid film (Avanti Polar Lipids) to 2 mM concentration. The solution was incubated at room temperature for 1 h with vortexing every 10 min. The lipid suspension was then subject to 5 freeze-thaw cycles using a dry ice isopropanol bath and a 37 °C water bath. Then we passed the lipid suspension through a mini-extruder (Avanti Polar Lipids) with 0.1 μm membrane 21 times. Equal volumes of DOPC liposome and Aβ42 L17R1 globulomers were mixed to achieve a lipid to protein ratio of 20:1. The globulomer-lipid mixture was incubated at 4 °C. Samples were taken out at 24 and 72 h for transmission electron microscopy studies. EPR studies were performed at 72 h.

Fibril formation from Aβ42 L17R1 monomers. HFIP-treated Aβ42 L17R1 was first dissolved in a buffer containing 20 mM CAPS, 7 M guanidine hydrochloride, pH 11, to 1 mM concentration. Then the sample was diluted 20-fold with PBS buffer and incubated at 37 °C without agitation. When the thioflavin T fluorescence reached plateau, an aliquot of the fibril sample was taken for electron microscopy studies and the rest of the fibrils was collected for EPR measurements.

Transmission electron microscopy. Glow-discharged copper grids covered with 400-mesh Formvar/carbon film (Ted Pella) were used to prepare globulomer and fibril samples for electron microscopy. The samples were negatively stained using 2% uranyl acetate and examined under a JEOL JEM-1200EX transmission electron microscope at 80 kV.

EPR spectroscopy. Aβ42 L17R1 globulomers after incubation with or without DOPC liposomes at 4 °C for 72 h were loaded into glass capillaries (VitroCom) sealed at one end. A Bruker EMX EPR spectrometer equipped with an SR4102ST cavity was used for X-band continuous-wave EPR measurements at room temperature. A microwave power of 20 mW and a modulation frequency of 100 kHz were used. Modulation amplitude was optimized for each individual sample. Scan width was 200 G. The EPR spectra of freshly prepared Aβ42 L17R1 globulomers and fibrils prepared directly from monomers were reproduced using data from Gu et al. [25] and Wang et al. [34], respectively. All EPR spectra were normalized to the same number of spins.

3. Results and discussion

We investigated the effect of lipid vesicles on Aβ42 globulomers using a spin-labeled Aβ42 variant, L17R1 (R1 represents the spin label). The freshly prepared globulomer sample consists of mostly globular structures under electron microscope (Fig. 1A). These globulomers have a size range of approximately 5–15 nm, similarly as in a previous study of wild-type Aβ42 globulomers [25]. Elongated structures can also be spotted in the freshly prepared globulomers, although they are rare (Fig. 1A, arrowheads). The width of these elongated structures is the same as the diameter of the globulomers, suggesting that the elongated structures are formed by oligomers joining together. Upon incubation at 4 °C for 3 d, elongated and networked structures became the dominant feature in the electron micrograph (Fig. 1B). These networked structures do not have a smooth appearance of fibrils or protofibrils.

To study the effect of lipid membranes on the structural changes of Aβ42 globulomers, we prepared large unilamellar vesicles using a zwitterionic lipid, DOPC. After Aβ42 L17R1 globulomers were incubated with DOPC liposomes at 4 °C for 1 d, short fibrils formed out of clusters of lipid vesicles (Fig. 1C). Some fibrils appeared to wrap around the lipid vesicles (Fig. 1C and D, arrowheads). At 1 day of incubation with DOPC liposomes, all the fibrils in the electron micrographs are associated with liposomes (Fig. 1C and D). After 3-d incubation at 4 °C with DOPC vesicles, large amount of long fibrils are dominant (Fig. 1E and F). Under the electron microscope, the fibrils outnumber liposomes, in contrast to the 1-d incubation when the liposomes outnumber the fibrils. The fibrils converted from globulomers are similar in morphology to Aβ42 fibrils formed directly from monomers (Fig. 1G).

We then performed EPR studies to gain insights into the molecular structure of Aβ42 L17R1 globulomers as a result of membrane interactions. Previously, we have used EPR to study the structures of both amyloid oligomers and fibrils [25,26,32,34]. In a parallel in-register β-sheet structure found in the core of amyloid fibrils, stacked spin label side chains have strong spin-spin exchange interactions, leading to a characteristic single-line EPR spectrum (Fig. 2A) [35,36]. The EPR spectrum of the freshly prepared globulomers consists of three resonance lines (Fig. 2B), typical of a nitroxide spin label such as R1 in this work. We previously measured the spin-spin distance at 14 residue positions, including L17, in Aβ42 globulomers and the overall results are consistent with antiparallel β-sheet structure [25]. Upon 3-day incubation at 4 °C in the absence of DOPC liposomes, the EPR

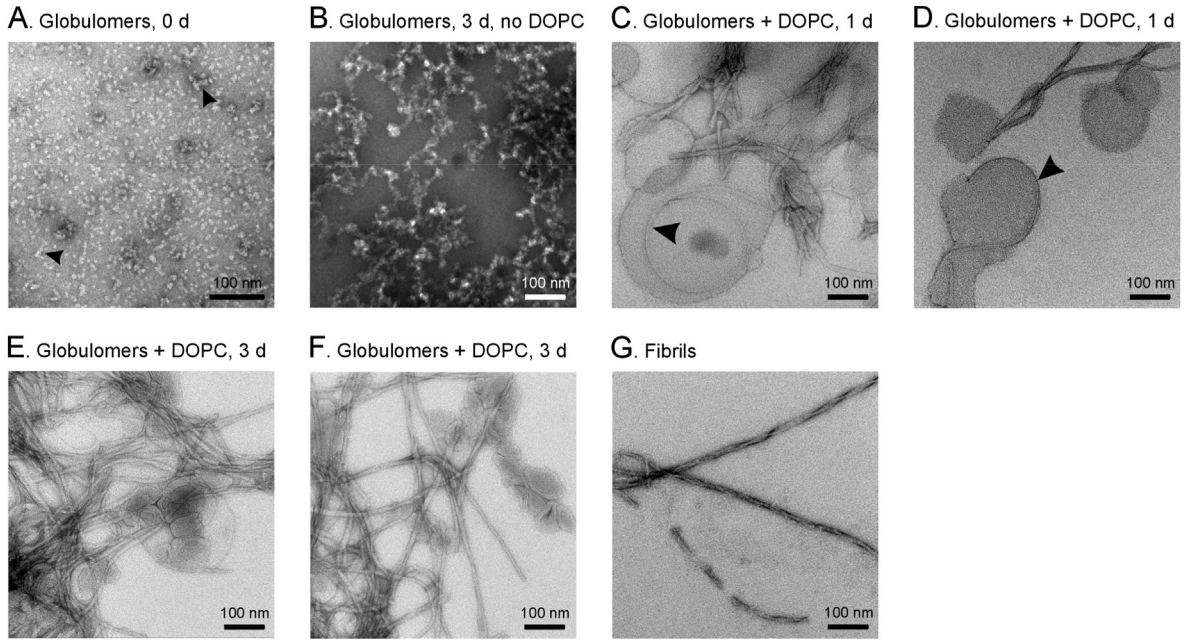

Fig. 1. Transmission electron micrographs of Aβ42 globulomers and fibrils. (*A*) Freshly prepared Aβ42 L17R1 globulomers. Arrowheads point to some elongated structures. (*B*) Aβ42 L17R1 globulomers without DOPC large unilamellar vesicles incubated at 4 °C for 3 d. (*C, D*) Aβ42 L17R1 globulomers with DOPC vesicles incubated at 4 °C for 1 d. Arrowheads point to fibrils wrapping around the surface of the lipid vesicles. (*E, F*) Aβ42 L17R1 globulomers with DOPC vesicles incubated at 4 °C for 3 d. (G) Aβ42 L17R1 fibrils prepared directly from monomers.

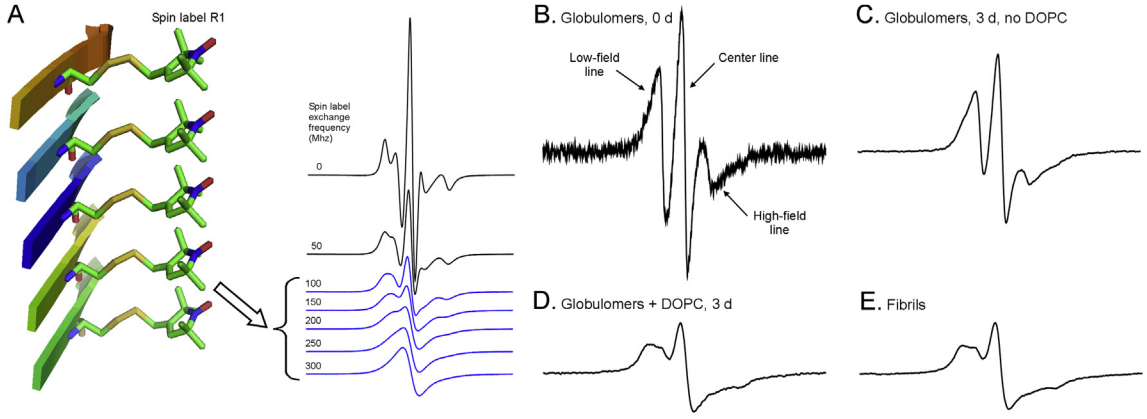

Fig. 2. EPR spectra of Aβ42 globulomers and fibrils. (*A*) Spin labels in a parallel in-register β-sheet structure show characteristic single-line EPR spectra. A stick model of the spin label R1 is shown on the left. Simulated EPR spectra with different strength of spin-spin interactions are shown on the right. Spin labels in the core of amyloid fibrils typically show spin exchange frequency of 100 MHz or higher. (*B*) EPR spectrum of freshly prepared Aβ42 L17R1 globulomers. (*C*) EPR spectrum of Aβ42 L17R1 globulomers incubated at 4 °C for 3 d without DOPC large unilamellar vesicles. (*D*) EPR spectrum of Aβ42 L17R1 globulomers incubated at 4 °C for 3 d with DOPC large unilamellar vesicles. (*E*) EPR spectrum of Aβ42 L17R1 fibrils prepared directly from monomers. EPR scan width is 200 G. The EPR spectra in panels *B*–*E* are normalized to the same number of spins.

spectral lineshape remained largely unchanged (Fig. 2C), suggesting that the structure of Aβ42 globulomers remained the same even though the globulomers formed networked structures (Fig. 1B). In the presence of DOPC liposomes, the EPR spectrum of Aβ42 globulomers changed dramatically, showing a single-line feature with the low-field and high-field resonance lines collapsing into the center-field line (Fig. 2D). The EPR spectrum of globulomers after incubation with DOPC liposomes is almost identical to the EPR spectrum of amyloid fibrils prepared directly from Aβ42 monomers (Fig. 2E). The single-line EPR spectrum is a fingerprint feature of parallel in-register β-sheet structures in the core of amyloid fibrils, which we have previously studied extensively for both Aβ40 and Aβ42 fibrils [34,35,37,38]. Therefore, the EPR data show that DOPC liposomes induced the structural change from Aβ42 globulomers to amyloid fibrils, which adopt parallel in-register β-sheet structures similar to the fibrils formed directly from monomers.

Our studies suggest that the interactions between Aβ aggregates and lipid membranes are dynamic. Aβ oligomers are capable of changing the properties of the lipid membranes such as pore formation [9–11] and membrane thinning [12,13]. In return, the membranes can also change the structure of Aβ aggregates. Martins et al. [21] showed that lipid membranes converted pre-formed fibrils to protofibrils. Chaparro Sosa et al. [20] showed that lipid membranes reduced both the number and the width of pre-formed Aβ42 fibrils. Mrdenovic et al. [39] studied the interactions between lipid membranes and Aβ42 oligomers and found that the small oligomers disrupted membranes of small unilamellar vesicles through membrane insertion and lipid extraction, but large

oligomers formed fibrils in the presence of lipid membranes without membrane insertion. The Aβ42 oligomers in the study of Mrdenovic et al. [39] were prepared using a protocol for Aβ-derived diffusible ligands (ADDLs). The ADDL protocol involves first solubilization of Aβ42 in DMSO, followed by dilution with F12 medium [40] or a buffered salt solution such as PBS [41] and incubation at 4 °C. Mrdenovic et al. [39] found that Aβ42 formed mostly small oligomers with an average diameter of ~6 nm at 24-h incubation time and large oligomers of ~10 nm in diameter at 48-h incubation. The finding [39] that small and large Aβ42 oligomers have different modes of interaction with lipid membrane echoes other studies that show different biological activities from small and large oligomers [42]. We previously found that small and large Aβ42 oligomers aggregate at different rate and may form fibrils of different structures, but the small and large oligomers co-exist in the same sample [43]. Globulomers are another type of Aβ42 oligomers prepared by diluting Aβ42 from DMSO to PBS containing small amount of SDS, followed by incubation at 37 °C for 24 h [23]. Gellermann et al. [29] showed that globulomers do not convert to fibrils even with fibril seeds or incubation at room temperature for 7 d. In this work, we show that lipid membranes induced structural conversion of Aβ42 globulomers to form amyloid fibrils, suggesting that the membrane surface serves as a catalyst to help the globulomers to overcome a high energy barrier of fibril nucleation. The mutual effect of oligomers and membranes on each other suggests that, while oligomers affect membrane integrity, membranes also induce structural changes of the oligomers, and consequently change the effect of oligomers on the affected membranes. This should be taken into account in understanding the mechanism of Aβ-lipid interactions and designing therapeutics to modulate these interactions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank So Hui Won and Tiffany Y. Lin for the preparation of Aβ proteins. This work was supported by the National Institutes of Health (Grant number R01AG050687).

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