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Author(s)	Vestergaard, Mun'delanji C.; Morita, Masumune; Hamada, Tsutomu; Takagi, Masahiro
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Description	

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Alzheimer's amyloid beta:lipid membrane interactions, detected in real-time

Mun'delanji C. Vestergaard, Masumune Morita, Tsutomu Hamada, and Masahiro Takagi

School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi City, Ishilkawa, 923-1292, Japan.

Abstract:

There are strong implications that Amyloid beta (Aβ) peptide causes neurotoxicity in Alzheimer's disease (AD) through (i) pore formation, (ii) the disruption of ionic channels that could affect calcium homeostasis, and (iii) receptor binding. The actual mechanism(s) remains unclear. In this study, we utilised cell-sized model membranes to observe the stability of lipid vesicles, in real-time, in the presence of Aβ-peptides. Using fluorescence-labelled Aβ-peptides, we imaged the localisation of pre-fibrillar Aβ species on the membrane surface, whereas mature fibrils hardly co-localised with the membrane surface. Further, we investigated the interaction of different oligomeric species of the Aβ peptide with lipid vesicles, observing membrane stability in terms of fluctuations and morphological changes. We observed a few different membrane morphological changes, with oligomeric species inducing a higher level of membrane instability. Interestingly, gramicidin A, a pore-forming peptide, did not induce any membrane transformations. We propose that this membrane transformation may be a different toxicity mechanism, from the proposed pore-formation hypothesis. This 'toxicity' mechanism may aid in real-time observation of these morphological understanding the mechanisms behind Aβ-induced neuro-toxicity in Alzheimer's.

1. INTRODUCTION

Alzheimer's disease (AD) is an age-related cognitive impairment that affects more than 18 million people world-wide [1]. It is mainly characterised by formation of senile (amyloid) plaques and neurofibrillary tangles in the brain of AD individuals, involving amyloid beta (Aβ) peptides and tau protein, respectively [2-4]. The mechanisms by which the two exert neurotoxicity are not well understood. The current work investigates possible mechanisms for Aβ's-mediated membrane malfunction.

Amyloid β-peptides are natively unfolded protein but aggregates into β-sheet structure of ordered fibrils [5-7]. Fibril formation proceeds from nucleation, via oligomers to mature fibrils. A detailed account of the pathways for Aβ oligomerisation and assembly can be found in a review by Murphy [8]. Soluble oligomers are a common intermediate in the pathway for fibril formation, and some have held these intermediates as the most toxic species of amyloid related to neurodegenerative disease [9,10]. Others hold the view that fibrils are the principle triggers of neuronal cell toxicity or disorder [6,11-13]. The possible roles in Aβ-induced neuronal cell toxicity have been reported as due to (i) pore formation, a (ii) disruption of ionic channels that could affect calcium homeostasis [6,14,15], and (iii) lipids peroxidation via membrane associated free radical formation [16-18]. Still, the mechanism of Aβ-induced neuronal cell toxicity remains unclear.

Current research on Aβ's role in AD is wide and vast. Some research groups have studied the interactions between Aβ and lipids [19]. The effect of lipid and cholesterol compositions on oligomerisation and reverse ('backward') oligomerisation has been reported [20,21]. Small and large lipid vesicles (< μm) have also been exploited to study the influence of lipid bilayer on Aβ-peptide assembly [22]. As is perhaps apparent, all these studies have concentrated on the effect of biomolecules on Aβ oligomerisation and assembly. From the results, an inference has been on how different Aβ molecular species cause neurotoxicity. The deductions have been made mainly using histochemical dyes [21], and not direct real-time observation of what may be taking place to the biological or lipid vesicles used. The studies on the interaction between Aβ-peptides and lipid vesicles have concentrated in the how the composition of the membranes affect the peptides. In particular, how levels of cholesterol and different lipid compositions in a given model membrane, affect oligomerisation and assembly of the peptides [23,24].

We are studying, in real-time, membrane responses to the presence of different Aβ-peptides species [25].

2. PREPARATION OF CELL-SIZED LIPID VESICLES.

Giant liposomes were prepared by gentle hydration method [26]. Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC, Avanti Polar Lipids) lipid was dissolved in chloroform / methanol (1:1 v/v) mixture. DOPC solution was transferred into a glass tube and gently dried using stream of nitrogen gas, to produce a thin lipid film. The thin film was subsequently dried under vacuum overnight, and was hydrated with ultrapure Milli Q (18 Ω) for 3 hours at 37 ℃. The final lipid concentration was 0.5 mM.

3. IMAGING OF AMYLOID BETA SPECIES USING ATOMIC FORCE MICROSCOPY

Amyloid β (trifluoroacetate salt, Peptide Institute Inc., Japan) was incubated at 37 °C in 20 mM Tris buffer, pH 7.4 at 80 μM concentration for various incubation periods. Immediately after incubation, Aβ samples were diluted with Milli Q to a final concentration of 8 μM, and immobilised on clean mica disk (Furuuchi Chemical Co., Japan). AFM images were obtained using an AFM unit (SPA400-SPI3800, Seiko Instruments Inc., Chiba, Japan) equipped with a calibrated 20 μm xy-scan and 10 μm z-scan range PZT-scanner and a silicon nitride tip (SI-DF40P, spring constant = 42 N/m, frequency resonance = 300 kHz,

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Seiko Instruments Inc.) was used for AFM imaging of samples. All AFM images were obtained in air in a dynamic force mode (DFM mode) at optimal force. All AFM operations were done in an automated moisture control box with 30-40% humidity at room temperature (RT). Typical AFM images of Aβ showed different Aβ species, from small oligomeric to mature fibrils with increase in incubation period (Fig. 1).

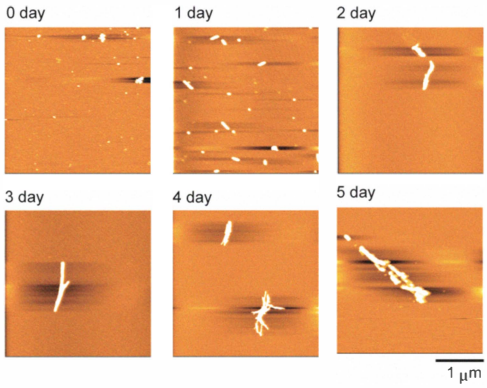


Figure 1. Typical atomic force microscopy images of Aβ-40 (10 μM) after incubation shows small oligomeric (Day 0), oligomeric (Day 1), proto-fibrillar (Days 2 and 3), and mature fibrillar (Days 4 and 5) species (C). Aβ-40 was incubated at 80 μM in 20 mM Tris buffer, pH 7.4, at 37 °C for 0 , 1, 2 , 3, 4 , and 5 days).

4. AMYLOID BETA: LIPID MEMBRANE INTERACTIONS

After incubation, Aβ was introduced to lipid vesicles at a final concentration of 2 μM in 0.5mM Tris buffer, by dilution in Milli Q water. Tris buffer of > 0.5 mM ionic strength interfered with membrane stability.

4.1 Membrane fluctuation

The interaction between Aβ-peptide and the DOPC cell-sized vesicles were observed in real-time for a total period of 20 min. Four μL of this vesicle solution was placed in a silicon well (100nm) on a glass slide, and covered with a cover slip. Examination was carried out at RT using a phase-contrast microscope (Olympus BX-51, Japan). The silicon well and the cover slip ensured that evaporation of the solution did not occur over the duration of the experiment

Some of the lipid vesicles were destabilized by the peptide and this was captured as vesicle fluctuation. Figure 2 shows typical membrane fluctuation 0 and 10 min after addition of Aβ.

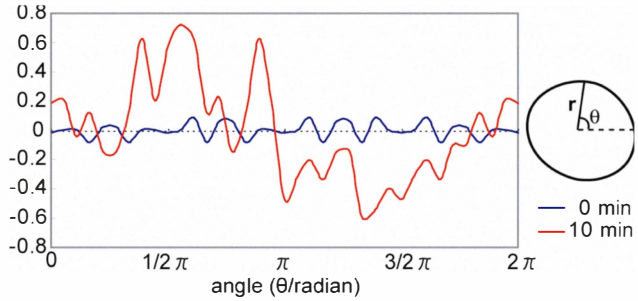
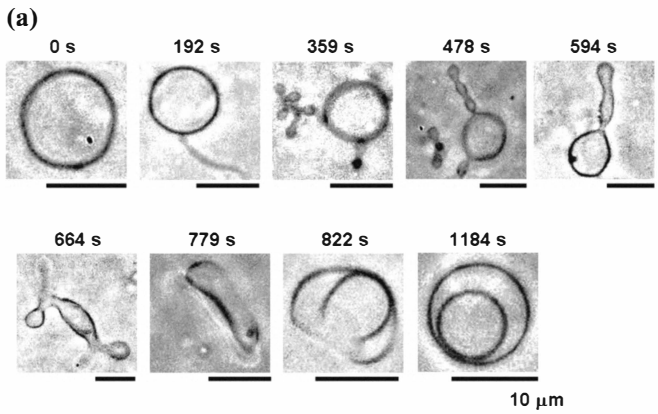


Figure 2. Degree of membrane fluctuation 0 and 10 min after introduction of Aβ-40 to lipid vesicle. Plotted the value of (r-<r>) in each θ (θ=2π/n, n=0,1…100).

4.2 Membrane morphological changes

Some of the 'fluctuating' vesicles underwent membrane shape changes. Figure 3 (a) is an example of a transformation pathways, captured in real-time. Other pathways are discussed by Morita and colleagues [25].



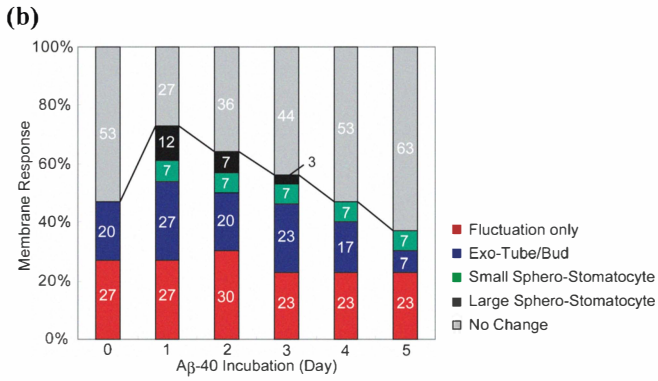


Figure 3. Effect of Aβ-peptide on membrane stability. (a) An example of a Lipid vesicle transformation pathway in response to Aβ-40 (2 μM) added after 0-5 days incubation in 80 μM in 20 mM Tris buffer, pH 7.4 at 37 °C. (b) Response of DOPC vesicles upon addition of Aβ-40 incubated for the indicated time periods. (*n=30*) .

Figure 3 clearly reveals that the frequency of membrane instability (fluctuation and morphological changes), was the highest when Aβ was introduced to the membrane surface after 1 day of incubation. We refer to this species are oligomeric Aβ. Fig. 3B provides detailed information on the types of membrane instability induced by the different Aβ species. Oligomeric (Day 1) and proto-fibrillar Aβ (Day 2 and 3) species exhibited the most diverse range in membrane responses, with the oligomeric species giving a the highest tendency to induce large sphero-stomatocytes. Large sphero-stomatocytes were formed only in response to addition of Aβ incubated for 1, 2 and 3 days. Our results (in terms of tendency to perturb the membrane and cause diverse morphological changes) are in line with reports that oligomeric (/ proto-fibrillar / intermediate) Aβ species have been widely reported as the most toxic species [9,10].

4.3 Localisation of amyloid beta species on membrane

HiLyte Fluor™ 488-labelled Aβ (Anaspec) and Aβ were mixed 1:2 (mol ratio). Aβ was added to lipid vesicles to a final concentration of 2 μM. Four μL of this solution was imaged using a confocal microscope (Olympus FV-1000, Japan). Pre-fibrillar Aβ localised on the membrane surface, and interacted with the vesicle (Fig. 3(a)). On the other hand, only a few mature fibrils were observed in close proximity to the membrane surface (Fig. 3(b)).

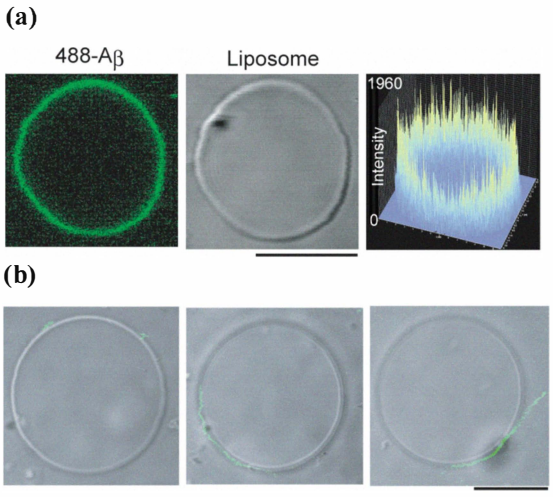


Figure 4. Localisation of Aβ s2 μM) species. (a) Pre-fibrillar Aβ localized on a DOPC membrane: fluorescence-labeled Aβ on DOPC vesicle (left); phase contrast image of DOPC vesicle (centre); and fluorescence intensity of Aβ (right) 10 min of interaction with the vesicle. (b) Image of mature fibriillar Aβ-peptide captured 0, 10 and 20 min after introducing the peptide to a DOPC lipid vesicle.

5. GRAMICIDIN A: LIPID MEMBRANE INTERACTIONS

Gramicidin A (MP Biochemicals Inc.) 1 mg/ml stock solution in dimethylsulfoxicide (DMSO) was diluted in Milli Q water. The vesicle containing 0.1 M sucrose solutions were suspended in isomolar glucose solution, because we wanted them to sink to the bottom of the experimental chamber to obtain an optimal image with phase contrast microscopy. The final concentration of lipid was 50 mM, and the Gramicidin A was 50 mg/ml. Real-time observation was carried out for a total of 20 min. Some of the vesicles fluctuated during observation. However, no lipid vesicles changed their morphology.

6. DISCUSSION

Engel et al. studied membrane damage by human islet amyloid polypeptide (IAPP) through fibril growth at the membrane, and not necessarily a particular IAPP species (fibrillar or pre-fibrillar) [27]. Previously, they reported that membrane barrier was compromised due to uptake of lipids from the lipid bilayer during amyloid fibril formation [28]. Our work shows that different Aβ species induced membrane fluctuation, and morphological changes to different extents (Fig. 3(b)). We show that oligomeric species has the most effect. Engel et al., also showed a similar change in membrane shape that we captured, which later regained the original shape [27]. The interaction of rigid fibrils with a soft DOPC membrane may change the spontaneous curvature of the membrane to initiate membrane fluctuation. In addition, we captured changes in the vesicle interior. The formation of an inner bud could influence the spatial localisation of membrane proteins such as receptors. Some of the vesicles had an increase an increase in surface area [25]. This increase could be due to incorporation of oligomeric Aβ into the membrane bilayer. The affinity of Aβ and Amylin (IAPP) to lipids is reported [27-30]. Using raft-exhibiting lipid vesicles, Aβ-peptide (Aβ-40) has been reported to localise within the non-raft domains [31]. Oligomeric Aβ species, due to natural affinity to lipids, recruited free lipids and small vesicles (micelles) into the mother vesicle. The uptake of lipids by amyloids from vesicles has been reported [29]. Since DOPC is a zwitterionic lipid, its interactions with Aβ were hydrophobic-driven. Arispe et al., first reported pore-formation of Aβ peptide as one of the main mechanisms for Aβ-induced toxicity [32]. Since then, there have been several reports on how the peptide breaches the membrane barrier [28,29,33]. In our study with a pore-forming peptide, Gramicidin A, we did not observe an

membrane transformations. Previously, we had proposed that the detected increase in vesicular surface could disturb the packing of lipid molecules in the bilayer. As a result, the packing would become sub-optimal, creating small pores [25]. Lack of membrane transformation observed using a pore-forming peptide has shifted our position towards membrane morphological change as a possible toxicity mechanism in its own right.

7. CONCLUSIONS

This work provided a real-time demonstration that Aβ-peptides may cause membrane damage by destabilizing the cell membrane. Using cell-sized lipid vesicles, we found that the presence of Aβ assembled species induced membrane fluctuations, which sometimes caused membrane transformation. We have made initial attempts to relate these differences with the degree of Aβ assembly. Using a pore-forming peptide, we have inequitably shown that the membrane transformation observed may not necessarily be due to pore-formation since Gramicidin A did not cause any membrane changes.

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