Analytical Biochemistry 481 (2015) 18–26

Contents lists available at ScienceDirect

Analytical Biochemistry

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

Uptake of raft components into amyloid β-peptide aggregates and membrane damage

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ARTICLE INFO

Article history:
Received 11 December 2014
Received in revised form 13 March 2015
Accepted 12 April 2015
Available online 20 April 2015

Keywords:
Amyloid β-peptide
Amyloid aggregation
Model membrane
Membrane heterogeneity
Lipid raft

ABSTRACT

Amyloid aggregation and deposition of amyloid β-peptide (Aβ) are pathologic characteristics of Alzheimer's disease (AD). Recent reports have shown that the association of Aβ with membranes containing ganglioside GM1 (GM1) plays a pivotal role in amyloid deposition and the pathogenesis of AD. However, the molecular interactions responsible for membrane damage associated with Aβ deposition are not fully understood. In this study, we microscopically observed amyloid aggregation of Aβ in the presence of lipid vesicles and on a substrate-supported planar membrane containing raft components and GM1. The experimental system enabled us to observe lipid-associated aggregation of Aβ, uptake of the raft components into Aβ aggregates, and relevant membrane damage. The results indicate that uptake of raft components from the membrane into Aβ deposits induces macroscopic heterogeneity of the membrane structure.

Alzheimer's disease (AD)[1] is the most common form of dementia among aged individuals. The formation of extracellular senile plaques in the cerebral cortex is one of the pathological hallmarks of AD [1–3]. The plaques are insoluble deposits primarily comprising fibrillar aggregates of amyloid β-peptide (Aβ), the predominant forms of which comprise 40 or 42 amino acid residues [4,5]. Aβ is generated from a type I transmembrane protein, termed amyloid precursor protein (APP), through proteolytic cleavage by β- and γ-secretases [6]. The conversion of normally soluble Aβ to fibrillar aggregates rich in β-sheet structures is considered to be a key step in the pathological process of AD [1–3]. Oligomeric species and/or fibrillar intermediates being formed during the aggregation process have been implicated as the primary neurotoxic species associated with AD [7,8]. The 42-residue form [Aβ(1–42)], which has 2 additional hydrophobic residues at the C terminus, is known to be more fibrillogenic and more prone to form toxic assemblies than the 40-residue form [Aβ(1–40)], suggesting that Aβ(1–42) plays a critical role at the earliest stage of plaque formation [4,9].

Although numerous studies have identified several factors that may contribute to the pathology of AD, there is increasing evidence that lipid membranes act as a site for the nucleation/accumulation of fibrillar aggregates of Aβ and become a possible target for the toxic assemblies that accumulate during the aggregation process [10–13]. Biological membranes are highly dynamic and complex assemblies; the fluidity, structural asymmetry, and heterogeneity of membranes are critical to their biological functions [14,15]. Lipid rafts are membrane microdomains rich in cholesterol (Chol) and sphingomyelin (SM) and have been proposed to play pivotal roles in various cellular processes such as cell signaling, cell adhesion, protein sorting, and protein trafficking [16,17].

The mechanism by which Aβ causes neuronal death has not been fully elucidated. However, recent in vitro studies have shown that membrane dysfunction and disruption can be caused by the ability of amyloid-forming Aβ to form ion channel-like pores and to induce membrane permeabilization through detergent-like action [18–20], and Aβ–membrane interaction is significantly affected by membrane components such as Chol and gangliosides [10–13,20,21]. In particular, the interaction of Aβ with ganglioside GM1 (GM1), which is abundant in neuronal membranes, has been demonstrated to be a crucial factor in mediating Aβ aggregation and related membrane damage [22–24].

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[1] *Abbreviations used:* AD, Alzheimer's disease; Aβ, amyloid β-peptide; APP, amyloid precursor protein; Chol, cholesterol; SM, sphingomyelin; GM1, ganglioside GM1; SPB, substrate-supported planar bilayer; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; TR-PE, Texas Red 1,2-dihexadecanoyl-*sn*-glycero-phosphoethanolamine; CTB-488, cholera toxin subunit B conjugated with Alexa Fluor 488; ThT, thioflavin T; DMSO, dimethyl sulfoxide; L_o domain, liquid ordered domain; L_d domain, liquid disordered domain; hIAPP, human islet amyloid polypeptide.

http://dx.doi.org/10.1016/j.ab.2015.04.014
0003-2697/

Yanagisawa and coworkers identified a specific form of Aβ associated with GM1 in the brains of patients with AD and suggested that the GM1-associated form of Aβ may act as a seed for its accumulation [25]. Subsequent studies have shown that GM1 clusters formed in lipid rafts promote the accumulation and fibrillar aggregation of Aβ, which in turn perturbs the organization and functions of membranes [10,22,23]. These results have implications for the role of lipid rafts containing GM1 in Aβ-induced neurotoxicity [26]. In this context, it is important to develop new experimental approaches to characterize the association of Aβ that assembles into amyloid deposits with lipid membranes containing raft components and GM1.

Substrate-supported planar bilayers (SPBs) have been widely used as simple model systems for cellular membranes [27]. SPBs are usually prepared using two methods [28,29]: (i) sequential transfer of two lipid monolayers from an air–water interface to a solid substrate surface (Langmuir–Blodgett method) and (ii) spontaneous spreading of lipid vesicles on a solid substrate (vesicle fusion method). The latter method has become popular owing to its relative ease of preparation [30]. Here, we observed the lipid-associated aggregation of Aβ(1–42) in the presence of lipid vesicles containing SM, Chol, and GM1 and on an SPB formed using the vesicle fusion method. Similarly, we observed amyloid aggregates of a shorter peptide comprising 25 to 35 residues of Aβ [i.e., Aβ(25–35)]. Aggregation of this peptide has been studied in comparison with that of full-length Aβ because this fragment is physiologically present in elderly people and retains much of the fibrillogenic and neurotoxic abilities of Aβ(1–42) [31,32]. We focused on the uptake of GM1 into two types of Aβ aggregates and the related membrane damage.

Materials and methods

Materials

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), SM (egg, chicken), Chol (ovine wool), and GM1 (brain, ovine–ammonium salt) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Texas Red 1,2-dihexadecanoyl-*sn*-glycero-phosphoethanolamine (TR-PE) and cholera toxin subunit B conjugated with Alexa Fluor 488 (CTB-488) were purchased from Molecular Probes (Eugene, OR, USA). Aβ(1–42) and Aβ(25–35) were purchased from Peptide Institute (Osaka, Japan); their purities were greater than 95% according to their elution profiles during high-performance liquid chromatography. Thioflavin T (ThT) was obtained from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of reagent grade. The buffer solution (20 mM phosphate [pH 7.0] with 100 mM sodium chloride) was passed through a filter (pore size 0.22 μm) and used throughout the experiments. Pure (Milli-Q) water was used for the preparation of all solutions.

Preparation of lipid vesicles

DOPC, SM, and Chol were dissolved in chloroform, and GM1 was dissolved in methanol. Two types of vesicle suspension were prepared by mixing these lipid solutions: DOPC/TR-PE and DOPC/SM/Chol/GM1/TR-PE. The ratio of main lipids (DOPC/SM/Chol, 0.5:0.25:0.25) was given by their molar ratio (mol mol^{-1}). GM1 was added at 2.0 mol% of the total amount of main lipids. A TR-PE fluorescence marker in chloroform was added at 1.0 mol% of the amount of main lipids. The mixed lipids were dried under a stream of nitrogen, and the dried films were placed in a vacuum desiccator for 4 h to remove any residual solvents. The films were hydrated in buffer overnight to yield a total lipid concentration of 5 mM. The lipid suspensions were dispersed by five freeze–thaw cycles and stored in the dark at 4 °C. Just prior to use, the suspensions were extruded 21 times through a polycarbonate filter (pore size 50 nm) using a mini-extruder (Avanti Polar Lipids) and diluted with degassed buffer to a concentration of 1 mM.

Preparation of Aβ(1–42) and Aβ(25–35)

Aβ(1–42) and Aβ(25–35) were dissolved in dimethyl sulfoxide (DMSO) at 4.0 mg ml^{-1} and diluted with pure water to a concentration of 0.40 mg ml^{-1}. The Aβ(1–42) solution was further diluted with degassed pure water to concentrations of 0.5 to 10 μM (45 μg ml^{-1}) and used as soluble forms. The aggregated forms (10 μM) of Aβ(1–42) were prepared by mixing the peptide solution at 0.40 mg ml^{-1} with degassed buffer and incubating the mixture at 37 °C for 6 or 24 h. The aggregated forms of Aβ(25–35) were prepared similarly; the peptide solution at 0.40 mg ml^{-1} was diluted with degassed buffer to a concentration of 42 μM (45 μg ml^{-1}) and incubated at 37 °C for 6 or 24 h. The final Aβ solution (45 μg ml^{-1}) contained 1.2% (v/v) DMSO. The aggregates of Aβ(1–42) and Aβ(25–35) formed by incubation for 24 h were fragmented into smaller sizes in an ultrasonic bath (the frequency was 42 kHz, and the power output was 40 W) for 10 min (see online supplementary material).

Substrate cleaning

Observations of Aβ aggregation were conducted using a glass-bottom dish with a diameter of 35 mm (product code D111300, Matsunami Glass, Osaka, Japan) in which a microscopy coverslip (22 × 22 mm) was bonded to the bottom with a hole of 14 mm diameter of a round plastic container (mark 1 in Fig. 1A). The space (~150 μl) surrounded by the coverslip (mark 2) and dish material was used as a well (mark 3) for the deposition of solutions onto the coverslip (i.e., substrate). The dishes (substrates) were cleaned using a commercial detergent solution (0.5% Hellmanex/water, Hellma, Mühlheim, Germany) for 20 min under sonication, rinsed with pure water, and dried. Solutions were deposited gently at the center of the substrate using a micropipette. The well was covered with a coverslip (18 × 18 mm, Matsunami) (mark 4) during incubation.

SPB formation

A vesicle suspension (150 μl, 1 mM) was deposited onto the substrate (glass-bottom dish) to form a bilayer by the vesicle fusion method. The dish was incubated for 3 h at room temperature after covering the well with a coverslip. The substrate surface was extensively rinsed with pure water to remove extra lipids after removing the coverslip. Water covering the substrate was replaced with buffer using a micropipette.

Microscope observation

An inverted confocal laser-scanning microscope (LSM 700, Carl Zeiss, Germany) equipped with a 63× oil immersion (NA 1.4) objective (Carl Zeiss) was used to make the observations. The fluorescence images of TR-PE (red), CTB-488 (green), and amyloid-sensitive dye ThT (blue) were acquired sequentially by altering the excitation wavelength using the 505-, 488-, and 405-nm lines of a diode laser and emission bandpass filters of 555–640, 500–555, and 405–500 nm, respectively. In the dish, the final concentrations of CTB-488 and ThT in the buffer were 0.16 μg ml^{-1} and 5 μM, respectively. For observations of the surfaces of substrate and SPBs, image acquisition, which was controlled by software (ZEN 2009, Carl Zeiss), was reset to encompass the distributions of fluorescence intensities for each sample.