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The on-fibrillation-pathway membrane content leakage and off-fibrillation-pathway lipid mixing induced by 40-residue β-amyloid peptides in biologically relevant model liposomes☆

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ABSTRACT

Disruption of the synaptic plasma membrane (SPM) induced by the aggregation of β-amyloid (Aβ) peptides has been considered as a potential mechanism for the neurotoxicity of Aβ in Alzheimer's disease (AD). However, the molecular basis of such membrane disruption process remains unclear, mainly because of the severe systematic heterogeneity problem that prevents the high-resolution studies. Our previous studies using a two-component phosphatidylcholine (PC)/phosphatidylglycerol (PG) model liposome showed the presence of Aβ-induced membrane disruptions that were either on the pathway or off the pathway of fibril formation. The present study focuses on a more biologically relevant model membrane with compositions that mimic the outer leaflet of SPMs. The main findings are: (1) the two competing membrane disruption effects discovered in PC/PG liposomes and their general peptide-to-lipid-molar-ratio dependence persist in the more complicated membrane models; (2) the SPM-mimic membrane promotes the formation of certain "on-fibrillation-pathway" intermediates with higher α-helical structural population, which lead to more rapid and significant of membrane content leakage; (3) although the "on-fibrillation-pathway" intermediate structures show dependence on membrane compositions, there seems to be a common final fibril structure grown from different liposomes, suggesting that there may be a predominant fibril structure for 40-residue Aβ (i.e. $A\beta_{40}$) peptides in biologically-relevant membranes. This article is part of a Special Issue entitled: Protein Aggregation and Misfolding at the Cell Membrane Interface edited by Ayyalusamy Ramamoorthy.

1. Introduction

The main hypothesis for the pathology of Alzheimer's disease (AD), known as the amyloid cascade hypothesis (ACH), has been challenged over the past few years because of a number of current failures in the anti-amyloid drug developments [1–4]. Several types of drugs, including the active and/or passive antibodies for the β-amyloid (Aβ) aggregates and inhibitors for β/γ-secretases and co-factors, have shown only mild effects on the progression of the disease [2]. However, it is worth noting that some of these drugs, such as Bapineuzumab and Semagacestat, did reduce the production of Aβ or clear the existing Aβ plaques in early-phase clinic tests [3,5]. Therefore, it is prompt to address the question about the correlation between the aggregation of Aβ and the downstream consequences such as the disruption of neuronal cells, i.e. the molecular basis of the neurotoxicity of Aβ peptides.

Cellular membrane disruption induced by the aggregation of Aβ has been considered widely as a main neurotoxicity mechanism [6–9]. Particularly, the disruption of synaptic plasma membranes (SPMs) is thought to be highly biologically relevant, because the synaptic loss correlates strongly to the levels of cognitive decline and dementia in AD [10]. However, it remains a major challenge to understand the mechanistic details, and particularly the molecular basis, of the Aβ-induced membrane disruption because the model systems that are typically utilized for such mechanistic studies possess severe heterogeneity [11,12]. Aggregation of Aβ peptides may lead to the formation of multiple intermediates, which can be either on or off the pathway of fibrillation, and all intermediates may interact with membranes and result in multiple disruption pathways. The original ACH mainly focused on the fibrillation pathway, which may be insufficient to explain the neuronal toxicity. It has been recognized recently that the spherical Aβ oligomers, which are generally considered to be off the fibrillation pathway, might possess higher levels of neurotoxicity [13–18]. Therefore, such systematic heterogeneity can be biologically significant in the neurotoxicity mechanisms of Aβ. On the other hand, the heterogeneity

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also hinders the application of high resolution techniques such as the solid-state nuclear magnetic resonance (ssNMR) spectroscopy, which potentially serves as a highly suitable technique to probe the detailed molecular basis of Aβ aggregation and membrane interactions [19–21]. Therefore, it is necessary to reduce the heterogeneity so that individual membrane disruption effects can be studied in separated, well-controlled model systems.

There have been a large number of studies on the Aβ-induced membrane disruption mechanisms using model liposomes over the past decades [12,22]. Overall, these studies may be categorized by their approaches to generate model systems. The first category involves the addition of Aβ peptides into pre-formed liposomes, usually the large unilamellar vesicles (LUVs). In these studies, the peptides were usually treated before usage to remove any pre-existed large oligomers. Therefore, the initial states of Aβ peptides in these works could be considered as monomer or low-order oligomers. Fibrillation was typically reported in such systems when the initial Aβ concentrations were equal or higher than ~10 μM [23–28]. One recent single-molecular imaging study utilized sub-μM Aβ concentration and reported formation of small oligomers on membrane surfaces with restricted mobility, which might represent the nucleation step of Aβ in membrane-related environments [29]. A variety of membrane disruption effects have been observed in these model systems, including the leakage of liposome content [23,30], changes in membrane curvatures [31], lipid uptake [32] and vesicle fusion [33,34]. There may be a mixture of several different membrane disruption effects in a particular system.

The second category of model systems are usually generated with pre-formed large Aβ oligomers in liposomes, where the initial peptide concentrations were higher than the previous model systems (i.e. ~100 μM or higher). These oligomers typically showed spherical morphologies on transmission electron microscopy (TEM) and were considered to be off the pathway of fibril formation [14,35]. A major membrane disruption mechanism that has been proposed in such model systems was the formation of cation-selected ion channels, which was supported by previous atomic force microscopy (AFM), the Black Lipids Membrane (BLM) assays and computational modeling [36–39]. It has been considered that the formation of ion channel might serve as a common mechanism in different types of amyloid diseases. In addition to the ion channel hypothesis, it has also been reported that large Aβ oligomers had the ability to fragmentize the lipid bilayers through detergent-like mechanisms, where the oligomeric cores were surrounded and stabilized by lipids [30,32]. Recently, it was suggested that the two processes might occur in steps, where the ion channels formed initially, and membrane fragmentation was induced at later stages. Changes in certain membrane compositions such as the gangliosides populations may trigger the membrane fragmentation process [32].

We have recently showed the reduction of systematic heterogeneity using simple model liposomes with only the zwitterionic lipid 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphocholine (POPC) and the negatively charged 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphoglycerol (POPG) and 40-residue Aβ ($A\beta_{40}$) peptides [30]. Our results suggested three distinct membrane disruption effects, namely the leakage of liposome contents, the vesicle fusion and the membrane fragmentation, became predominant in individual model systems with well-controlled conditions. Our results indicated that the final structures of the $A\beta_{40}$ aggregates in these three model systems were homogeneous and distinguishable to the resolution of ssNMR measurements, which suggested that the systematic heterogeneity of $A\beta_{40}$-induced membrane disruption in POPC/POPG model liposomes could be reduced and the molecular basis (i.e. $A\beta_{40}$-membrane interactions) in individual model systems could be studied in high-resolution details. Within these three distinct membrane disruption pathways, the first two involved addition of the $A\beta_{40}$ peptides externally to liposomes, and the $A\beta_{40}$-to-lipid molar ratio played key roles in shifting the predominance of liposome content leakage and lipid mixing [34].

This work describes an expansion of such systematic studies of the competition between fibrillation-induced membrane content leakage and non-fibrillation-induced lipid mixing in model liposomes with more biologically relevant membrane compositions. We prepared model liposomes with lipid compositions that mimic the outer leaflet of synaptic plasma membranes [40,41], where the major membrane interactions may occur when the peptides were released from the neuronal cells after enzymatic cleavage. Different events upon the external addition of $A\beta_{40}$, such as the binding between peptides and membranes, the early-stage aggregation and membrane interactions, and the final-state $A\beta_{40}$ structures, are investigated with systematic changes in the $A\beta_{40}$-to-lipid molar ratios and membrane compositions. Our results illustrate the distinct major membrane disruption effects that may occur both on and off the pathway of fibrillation with the externally added $A\beta_{40}$ peptides. The biologically relevant membrane compositions, such as cholesterol, sphingomyelin and gangliosides, seem to have more significant influences on the initial binding and early-stage peptide conformation/membrane interactions rather than the final fibril structures.

2. Experimental section

2.1. Peptide synthesis and purification

All $A\beta_{40}$ peptides, including the isotope-labeled and unlabeled sequences, were synthesized manually using routine Fmoc solid-phase peptide synthesis protocols. The crude products were cleaved from Val-pre-loaded Wang resin using a mixture of trifluoroacetic acid/phenol/H_2O/1,2-ethanedithiol/thioanisole with volume ratio 9:0.5:1:0.5:0.25, purified using reversed-phase high-performance liquid chromatography (HPLC) with C18 reversed-phase columns, lyophilized and stored at −20 °C until usage. For all experiments described below, the peptides were freshly-dissolved in dimethyl sulfoxide (DMSO) and quantified using a nanodrop ultraviolet-visible (UV-VIS) spectrometer before the addition to pre-formed liposomes.

2.2. Liposome preparation

Three model liposomes were studied in this work, and their compositions were: (1) 1,2-dimyristoyl-*sn*-glycerol-3-phosphocholine(DMPC)/1,2-dimyristoyl-*sn*-glycerol-3-phospho-L-serine (DMPS)/cholesterol with 1.5:0.3:0.5 molar ratio; (2) DMPC/DMPS/sphingomyelin/cholesterol with 1.5:0.3:1.0:0.5 molar ratio; and (3) DMPC/DMPS/sphingomyelin/cholesterol/ganglioside GM1 with 1.5:0.3:1.0:0.5:0.15 molar ratio. For the fluorescence lipid mixing assay, liposomes were prepared with additional 0.5 mol% 1,2-dipalmitoyl-*sn*-glycerol-3-phosphoethnolamine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-DPPE) and 1 mol% 1,2-dipalmitoyl-*sn*-glycerol-3-phosphoethnolamine-*N*-(lissamine rhodamine B sulfonyl) (Rh-DPPE) relative to the total lipids. All liposomes were prepared by mixing the lipid/cholesterol components in chloroform, followed by the formation of dried lipid film under N_2 flow and high-vacuum desiccator, 10 cycles of freeze-thaw in 10 mM phosphate buffer (pH 7.4 with 0.01% NaN_3) using liquid N_2 and 50 °C water-bath and 30 cycles of extrusion with 200 nm pore-size membranes.

2.3. Analytical HPLC quantification of the binding of $A\beta_{40}$ to membranes

A total 1.0 mL liposome solution was mixed with 20 μL $A\beta_{40}$ stock solution in DMSO. The initial concentration of $A\beta_{40}$ was kept at 10 μM for all HPLC quantifications and the $A\beta_{40}$-to-lipid molar ratios varied from 1:30, 1:60, 1:90 to 1:120 for individual samples. For one set of measurements, the mixture was briefly vortexed for 5 min and the liposomes were pelleted down using ultracentrifugation (432,000 Xg for 30 min at 4 °C). For the other set of measurements, the mixture was vortexed and incubated quiescently at 37 °C for 4 h before ultracentrifugation. In both sets, supernatants were analyzed by HPLC with a reversed-phase C18 analytical-scale column and the linear H_2O-