BBA - Biomembranes xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

BBA - Biomembranes

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

Fluorescence imaging of the interaction of amyloid beta 40 peptides with live cells and model membrane

Elaheh Jamasbi[a], Mohammed Akhter Hossain[a,c], Marsha Tan[b], Frances Separovic[a,*], Giuseppe D. Ciccotosto[b,*]

[a] School of Chemistry, Bio21 Institute, The University of Melbourne, VIC 3010, Australia
[b] Department of Pathology, The University of Melbourne, VIC 3010, Australia
[c] The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, VIC 3010, Australia

A B S T R A C T

Amyloid beta peptides (Aβ) found in plaques in the brain have been widely recognised as a hallmark of Alzheimer's disease although the underlying mechanism is still unknown. Aβ40 and Aβ40(A2T) peptides were synthesized and their effects on neuronal cells are reported together with the effect of tetramer forms of the peptides. ThT assay revealed that mutation affected the lag time and aggregation and the presence of lipid vesicles changed the fibril formation profile for both peptides. The A2T mutation appeared to reduce cytotoxicity and lessen binding of Aβ40 peptides to neuronal cells. Fluorescence microscopy of the interaction between Aβ40 peptides and giant unilamellar vesicles revealed that both peptides led to formation of smaller vesicles although the tetramer of Aβ(A2T) appeared to promote vesicle aggregation.

This article is part of a Special Issue entitled: Protein Aggregation and Misfolding at the Cell Membrane Interface edited by Ayyalusamy Ramamoorthy.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in aging population and is pathologically characterised by the accumulation of excess amyloid beta (Aβ) peptide which will aggregate to eventually form plaques that will deposit in the brain of sufferers, who exhibit a gradual decline in cognitive function [1,2]. Aβ is a 38–43 amino acid peptide that is derived from the amyloid precursor peptide (APP) through the sequential cleavage by the β- and γ-secretase proteases [3]. While Aβ40 and Aβ42 peptides are the major identifiable cleavage products, Aβ40 peptide is more highly expressed in neurons and cerebrospinal fluid and Aβ42 is by far the most abundant species in brain plaques and the synthetic or purified peptide is more neurotoxic, aggregates more readily, and has a higher affinity for lipid membrane binding [1,4–7]. In over 90% of AD patients, the cause of this disease is relatively unknown while in early-onset familial AD (FAD), the disease is caused by the inheritance of genetic risk factors (reviewed by [8]). The common feature of these FAD cases is that the genetic mutation typically results in an increased production of Aβ peptides in the brain, which acquire toxic properties and cause the AD pathogenesis.

In a single non-demented patient who displayed no AD like pathology, a mutation at alanine-637 to threonine (T) in the APP gene was identified [9] and the same coding mutation was identified in a cohort of Icelanders who were found to be protected against AD and age-related cognitive decline [10]. In contrast, an Italian patient was identified with a mutation at the same APP693 site but different amino acid substitution of alanine (A) to valine (V) resulted in early onset AD and developed severe cognitive deficits at a young age [11]. The A-673 residue lies within the β-secretase recognition sequence and is part of the amyloid-β (Aβ) peptide cleavage product (position 2 of Aβ) and, therefore, the A673T substitution makes APP a less favourable substrate for cleavage by BACE1 [10,12]. The identity of the AβA2T mutant form is thought to be the only known example of an Aβ variant that demonstrates protection against AD pathogenesis and cognitive decline.

The results describing the biophysical and toxic properties of synthetic Aβ peptide with the A2T mutation are somewhat controversial. The major reasons for this are the differences in experimental conditions that have been used to investigate the properties of the mutant peptides. Nevertheless, what remains unclear is how the mutant Aβ40(A2T) peptide interacts with a lipid membrane considering that neurons, which are the primary target site for the Aβ peptide interaction to exert its pathological effect, are primarily composed of lipid membrane material [4,13–15]. Therefore, understanding the physical nature of the Aβ/membrane interaction is important for deciphering

* Corresponding authors.
E-mail addresses: fs@unimelb.edu.au (F. Separovic), j.ciccotosto@unimelb.edu.au (G.D. Ciccotosto).

https://doi.org/10.1016/j.bbamem.2018.01.024
Received 14 December 2017; Received in revised form 23 January 2018; Accepted 27 January 2018

the protective properties of the A2T mutant Aβ40 peptide. Wild type and A2T mutant type of Aβ40 peptides were synthesized chemically and we compared the effects on lipid model membranes and binding to cultured cortical neurons. Here we report the effect of the mutant Aβ40(A2T) peptide which when prepared as a monomeric peptide species was not toxic, displayed diminished cell binding, and had little effect disrupting lipid model membranes. However, when we prepared oligomers from the mutant Aβ40(A2T) peptide by photo-induced cross-linking of unmodified proteins (PICUP) [4,16], it caused significant disruption of the lipid model membrane and cultured cells.

2. Methods

2.1. Materials

All chemicals and solvents were obtained from Sigma-Aldrich (Sydney, Australia) unless otherwise stated.

2.2. Peptide synthesis

Both native Aβ40 and mutant Aβ40(A2T) were chemically synthesized using previously reported in-house procedures for peptide synthesis [17,18], including some modifications and additional optimisation steps required to ensure the highest level of purity and yields. For the mutant Aβ40(A2T) peptide, the alanine (A) residue was substituted with the threonine (T) residue. Fmoc protected amino acids were used for peptide synthesis (GL Biochem, China). Briefly, Aβ peptides were prepared using the CEM Liberty automated microwave peptide synthesizer set at 86 °C. The histidine residue in the Aβ40 peptide sequence was coupled without microwave at 50 °C. Peptides were cleaved using trifluoracetic acid (TFA), anisol, triisopropylsilane, Milli-Q water in a v/v ratio of 94:3:2:1.

Our previous study with melittin [17] and Aβ [18] revealed that adding the fluorophore at the N-terminus affects peptide activity while adding it at position 26 in the case of the latter led to similar aggregation profiles to that of the native peptide [17]. Therefore, to prepare fluorescently labelled Aβ peptides, the serine (S) at position 26 in the peptide sequence was substituted with a cysteine residue (C). These Aβ40(S26C) and Aβ40(A2T/S26C) peptides were synthesized and labelled with AlexaFluor 430 as NHS ester forms (Invitrogen, Sydney, Australia) using thiol-maleimide conjugation chemistry as previously described [18]. The Mal-AlexaFluor was used to react with the thiol group of the cysteine residue. The final Aβ-AlexaFluor labelled peptides (termed $_{F430}$Aβ40(S26C) and $_{F430}$Aβ40(A2T/S26C), respectively; Fig. 1) were purified using RP-HPLC on Phenomenex C4 column (particle size 5 µm, 4.6 × 150 mm), with gradient of 10–90% of 0.1% TFA in ACN and 0.1% TFA in H_2O for 30 min.

2.3. Peptide preparation

Lyophilised peptides were prepared by dissolving in a sequence of buffers in a ratio of 2:7:1 v/v. Firstly, 20 mM NaOH was added to peptide and vortexed and sonicated in an ice chilled water bath for 15 min. Then Milli-Q water and 10× PBS (2.7 mM KCl, 1.5 mM KH_2PO_4, 138 mM NaCl, 8 mM Na_2HPO_4, at pH 7.4) buffer were added to the solubilised peptide before centrifuging at 16,000*g* for 5 min (to remove amorphous aggregates) and the supernatant transferred to a clean tube and stored on ice until used (typically within 1 h of its preparation). The final Aβ40 peptide concentrations were determined by UV absorption spectroscopy using the molar extinction coefficient of 55,771 M^{-1} cm^{-1} at 214 nm.

2.4. Oligomerization and purification of Aβ40 peptides by PICUP reaction

Aβ oligomers were prepared by PICUP reaction based on our previously published methods [4,19,20]. Briefly, while in the dark room, 5 mM Tris bipyridyl ruthenium(II) complex and 100 mM ammonium persulfate were mixed with 150 µM Aβ40 monomer solution in a 1:1:18 v/v ratio. The reaction mix, with the tube uncapped and placed 15 cm from the light source, was exposed to a 200 W light globe source for 6 s resulting in crosslinking of the peptide. To stop the reaction, 50 mM of tris(2-carboxyethyl)phosphine (Thermo Scientific, Australia) in sodium dodecyl sulfate loading buffer containing 10% (v/v) of 2-β-mercaptoethanol was added to the mixture.

The cross-linked oligomeric peptides were separated using the NuPAGE 12% Bis-Tris gel (Invitrogen, Mt. Waverley, Australia). Oligomeric bands (monomer to tetramer) were individually excised and Aβ peptide eluted from the gel pieces using a model 422 electro-eluter (BioRad, Australia). The eluted peptide solution was placed in dialysis tubing with a 3.5 kDa molecular weight cut-off (Fisher Biotech, Wembley, WA Australia) and dialysed against acidic water (pH 4–5) for 2 cycles for 1 h buffer and 1 cycle of overnight exchange followed by 1 cycle of 2 h exchange in neutral water (pH 7). The dialysed Aβ peptide oligomers were lyophilized by freeze drying. Aβ oligomers were solubilised using the same procedures described for preparation of Aβ40 peptide.

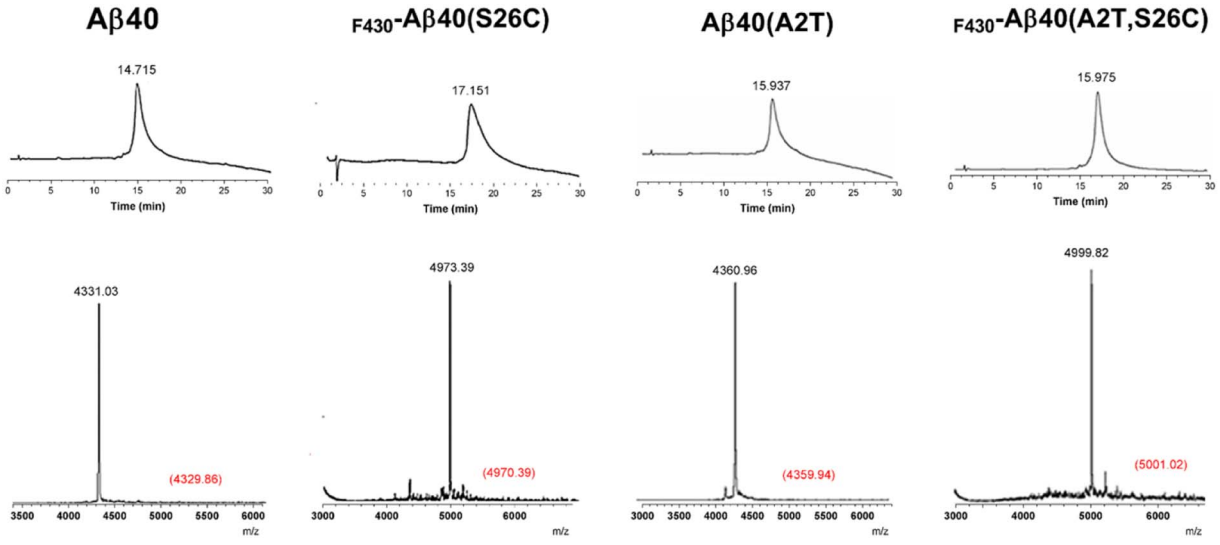

Fig. 1. Characterisation of purified synthesized Aβ40 peptides by chromatographic analysis. RP-HPLC (top panel) displaying peak elution times and MALDI TOF MS trace analysis (bottom panel) displaying calculated (black) and observed (red) mass values for each peptide synthesized.