Synthetic Amyloid Beta Peptides Aid Alzheimer Investigation

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Aggregates of the amyloid beta (Aβ) peptide are a hallmark of the Alzheimer's disease brain. Rather than presenting a single well-defined moiety, the Aβ peptide pool is complex and composed from peptides truncated at the N-terminus and varying in length at the C-terminus. A range of synthetic amyloid beta peptides with C-terminal variation were produced to systematically investigate the role of peptide composition on aggregation. Thioflavin T binding, far-UV circular dichroism and transmission electron microscopy were used to investigate the properties of the formed aggregates.

Introduction

Alzheimer's disease is a progressive neurodegenerative disease and one of the first symptoms includes the loss of cognitive function which generally presents itself around the age of 65. Accumulations of Aβ, a product of the processing of the amyloid precursor protein (APP), present the typical pathogenic feature in the Alzheimer's disease brain (1,2). Over the years the Aβ peptide pool has been identified as highly complex and consists, amongst others, of peptides containing 37, 38, 40, 42 and 43 amino acids (3,4), mainly as a result of an ill-defined cleavage site of the γ-secretase enzyme for APP. The Aβ peptide pool in patients with Alzheimer's disease tend to over represent longer Aβ peptides (42 amino acids and up) compared to healthy subjects. While it has already been reported that Aβ1-42 aggregates more rapidly than Aβ1-40 (5), until recently the properties of other Aβ peptides in the brain were unknown. In this work, the aggregation properties of C-terminal variations of Aβ are compared using Thioflavin T binding, circular dichroism, and transmission electron microscopy (6).

Materials & Methods

Peptides were provided as HFIP films by JPT Peptide Technologies (Berlin, Germany). They were synthesized using an ABI 433A Peptide Synthesizer and purified by preparative high performance liquid chromatography. Purity and identity of the peptides were evaluated using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Peptides were dissolved using a sequential procedure involving 1,1,1,3,3,3-hexafluoro-2-propanol and dimethyl sulfoxide followed by incubation in 1 mM EDTA and 50 mM Tris pH 7.5 (7). Aggregation of Aβ was monitored using Thioflavin T binding, circular dichroism and transmission electron microscopy (TEM) upon incubation for 15 h, 2 weeks and 2 weeks, respectively.

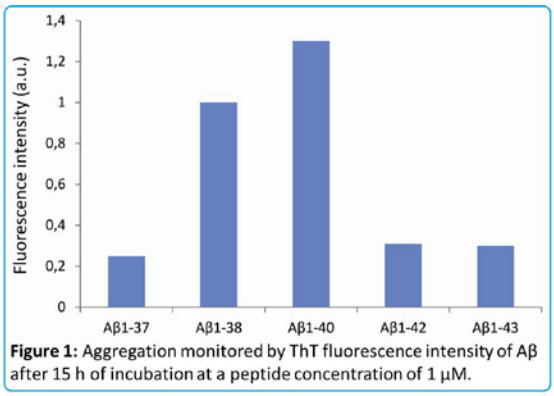

Figure 1: Aggregation monitored by ThT fluorescence intensity of Aβ after 15 h of incubation at a peptide concentration of 1 µM.

Results

The affinity of formed aggregates to bind Thioflavin T, a dye reporting on aggregate formation, was monitored upon incubation of Aβ1-37, Aβ1-40, Aβ1-42 and Aβ1-43 for 15 hours (**Figure 1**). The formation of Thioflavin T-positive aggregates does not correlate in a linear manner to Aβ peptide length. Short Aβ1-37 and long Aβ1-42 and Aβ1-43 have similar low Thioflavin T fluorescence intensity after 15 h incubation while Aβ1-38 and Aβ1-40 exert five- to six-fold higher fluorescence intensity under similar conditions. A morphological description of the formed aggregates was obtained by using transmission electron microscopy (**Figure 2**).

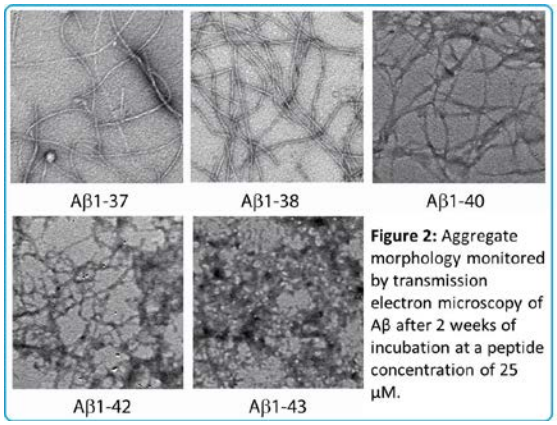

Figure 2: Aggregate morphology monitored by transmission electron microscopy of Aβ after 2 weeks of incubation at a peptide concentration of 25 µM.

All peptides have a strong propensity to self-assemble, but the morphology of the resulting aggregates varies with peptide length. Shorter peptides Aβ1-37 and Aβ1-38 form extended negatively stained and semi-flexible fibrils without obvious branching. Upon extending the peptide length towards the C-terminus, the fibrils formed gradually transform into densely stained networks in which individual strands can no longer be distinguished. Even though Aβ1-37 and Aβ1-42 show similar Thioflavin T binding properties upon aggregate formation, fibril morphologies vary strongly. To establish the conformation in terms of secondary structure of the aggregates formed, far-UV circular dichroism was employed (**Figure 3**). Data show a gradual conversion from random coil (negative values around 198 nm) to β-sheet enriched (negative peak around 210 nm) aggregates with C-terminal extension.

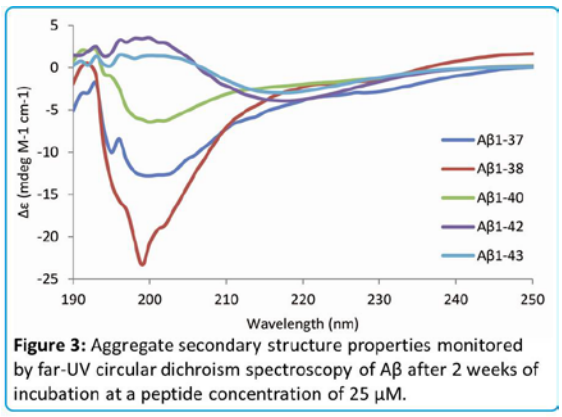

Figure 3: Aggregate secondary structure properties monitored by far-UV circular dichroism spectroscopy of Aβ after 2 weeks of incubation at a peptide concentration of 25 µM.

Discussion & Conclusions

Synthetic Aβ peptides were tested for their effect of C-terminal variation on aggregation propensity, morphology and secondary structure using Thioflavin T fluorescence, transmission electron microscopy, and far-UV circular dichroism, respectively. Aβ peptides investigated in this study contained 37, 38, 40, 42 and 43 amino acids which are peptides also naturally found in the brain. Strong differences were observed in the propensity of the peptides to self-assemble and form Thioflavin-T positive aggregates largely resulting from varying morphology. Even though extensive β-sheet enriched aggregation was observed for Aβ1-42 and Aβ1-43 using transmission electron microscopy and circular dichroism spectroscopy, as consistent with literature, these aggregates only exert limited Thioflavin T binding (8,9). It is hypothesized that the dense networks observed for these two peptides disable direct access of the Thioflavin T dye, thereby inhibiting binding and fluorescence intensity. The Aβ peptide pool in the brain is mainly composed from Aβ1-40 and Aβ1-42 which properties have been extensively studied. The presence of Aβ1-37, Aβ1-38 and Aβ1-43 in the brain has been recently recognized and, moreover, these peptides are now anticipated to play a role in modulating progress of Alzheimer's disease (10).

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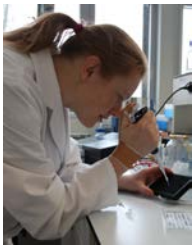

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