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Aggregation and fibril morphology of the Arctic mutation of Alzheimer's Aβ peptide by CD, TEM, STEM and *in situ* AFM

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ABSTRACT

Morphology of aggregation intermediates, polymorphism of amyloid fibrils and aggregation kinetics of the "Arctic" mutant of the Alzheimer's amyloid β-peptide, $A\beta_{(1-40)}$(E22G), in a physiologically relevant Tris buffer (pH 7.4) were thoroughly explored in comparison with the human wild type Alzheimer's amyloid peptide, wt-$A\beta_{(1-40)}$, using both *in situ* atomic force and electron microscopy, circular dichroism and thioflavin T fluorescence assays. For arc-$A\beta_{(1-40)}$ at the end of the 'lag'-period of fibrillization an abrupt appearance of ~3 nm size 'spherical aggregates' with a homogeneous morphology, was identified. Then, the aggregation proceeds with a rapid growth of amyloid fibrils with a variety of morphologies, while the spherical aggregates eventually disappeared during *in situ* measurements. Arc-$A\beta_{(1-40)}$ was also shown to form fibrils at much lower concentrations than wt-$A\beta_{(1-40)}$: $\leqslant$2.5 μM and 12.5 μM, respectively. Moreover, at the same concentration, 50 μM, the aggregation process proceeds more rapidly for arc-$A\beta_{(1-40)}$: the first amyloid fibrils were observed after *c.a.* 72 h from the onset of incubation as compared to approximately 7 days for wt-$A\beta_{(1-40)}$. Amyloid fibrils of arc-$A\beta_{(1-40)}$ exhibit a large variety of polymorphs, at least five, both coiled and non-coiled distinct fibril structures were recognized by AFM, while at least four types of arc-$A\beta_{(1-40)}$ fibrils were identified by TEM and STEM and their mass-per-length statistics were collected suggesting supramolecular structures with two, four and six β-sheet laminae. Our results suggest a pathway of fibrillogenesis for full-length Alzheimer's peptides with small and structurally ordered transient spherical aggregates as on-pathway immediate precursors of amyloid fibrils.

1. Introduction

Alzheimer's disease (AD) is the most common age-related dementia whose hallmark is the abundance of amyloid plaques in the brain of AD patients. The principal constituent of these plaques are fibrils mainly composed of amyloid β-peptides most commonly as 40 or 42 amino acid long $A\beta_{(1-40)}$ and $A\beta_{(1-42)}$ variants. It is generally accepted that aggregation of Aβ-monomers or oligomers is involved in AD pathogenesis and there is increasing evidence that specific fibrillar species and, in particular, prefibrillar intermediates (oligomers) play a central role in the neurodegeneration (Selkoe, 1995; Lansbury, 1999; Dahlgren et al., 2002; Lashuel et al., 2002; Walsh et al., 2002; Petkova et al., 2005; Lal et al., 2007; Chimon et al., 2007; Inoue, 2008; Zheng et al., 2008; Small, 2009; Ono et al., 2009; Ahmed et al., 2010; Jang et al., 2010b; Sandberg et al., 2010), but as yet the precise mechanism is unknown. The structures of Aβ-monomers, dimers and oligomers have been visualized with high-resolution scanning tunneling microscopy at sufficient resolution to suggest the folding of the polypeptide chain (Losic et al., 2006). Nevertheless, different fibril morphologies can display distinguished molecular structures and neurotoxicity (Petkova et al., 2005, 2002; Paravastu et al., 2008; Tycko et al., 2009; Miller et al., 2010). Supramolecular models for $A\beta_{(1-40)}$ and $A\beta_{(1-42)}$ have been obtained using structural constraints from STEM mass-per-length measurements, solid-state nuclear magnetic resonance spectroscopy (NMR) (Petkova et al., 2002, 2005; Paravastu et al., 2008), as well as cryo-electron microscopy (Sachse et al., 2008; Schmidt et al., 2009). Although some features of the fibril

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