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著者(和文)	TEHRANIMohammad
Author(English)	Mohammad Tehrani
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Structural insights into Arctic mutant (E22G) Aβ40 fibrils using solid-state NMR and cryoEM

Introduction

Misfolded aggregates of amyloid-β (Aβ) peptide are a defining hallmark of Alzheimer's disease (AD).[1] While the 40-residue Aβ peptide (Aβ40) is the most prevalent form in the brain, Aβ fibrils in amyloid plaques predominantly consist of the more aggregation-prone and cytotoxic 42-residue variant (Aβ42).[2] The cytotoxicity of Aβ aggregates may be intricately linked to their structural properties, as different fibril structures have been associated with varying clinical and pathological features of AD. Hence, elucidating the structural characteristics of amyloid fibrils is critical for understanding the disease's molecular mechanisms and for developing therapeutic or preventive strategies.[3,4]

Certain pathogenic mutations in the Aβ region of the amyloid precursor protein (APP) are linked to familial Alzheimer's disease (FAD). These mutations often accelerate Aβ aggregation, making the peptide more prone to fibrillogenesis, and in some cases, promote the formation of toxic oligomers.[5] Several FAD-associated APP mutations cluster within residues 21–23 of the Aβ sequence, a hydrophobic region critical for fibril formation. Notable examples include the Flemish (A21G), Osaka (E22Δ), and Arctic (E22G) variants.[6] The Arctic (E22G) mutation promotes early-onset FAD by enhancing the assembly of Aβ into oligomers and fibrils.[7] This mutation is unique in that it is the only known FAD-related mutation associated with clinical features typical of sporadic AD, unlike other mutations at position 22, such as the Italian (E22K) and Dutch (E22Q)

variants, which primarily induce cerebral amyloid angiopathy (CAA). E22G carriers exhibit classical AD pathology, including senile plaques, neurofibrillary tangles, progressive cognitive decline, and in some cases, cerebrovascular events.[8] Interestingly, the amyloid plaques in Arctic FAD patients resemble cotton-wool plaques, characterized by a core rich in Aβ40 and surrounded by a ring of both Aβ42 and Aβ40 fibrils, distinguishing them from the Aβ42-enriched plaques commonly observed in sporadic AD.[9,10]

The structural investigation of amyloid fibrils harboring FAD-related mutations has garnered significant interest, as these studies may shed light on how specific mutations drive the molecular onset of AD. Notably, fibril structures from Arctic mutant Aβ (E22G) are of particular interest, as they may provide insights into the mechanisms of sporadic AD. Previous studies using electron microscopy and solid-state NMR (SSNMR) have revealed at least five distinct fibrillar morphologies of E22G Aβ40, [11,12] with structural plasticity allowing the peptide to adopt both U-shaped and S-shaped β-sheet motifs[13]. More recent cryoEM studies have shown that E22G Aβ fibrils in human brains exhibit a tetrameric structure, with two pairs of protofilaments adopting U- and S-shaped β-sheet motifs within V12 – V40 and E11 – G37 residues respectively.[14]

Results and discussion

In this study, we developed a methodology to prepare a monomorphic E22G Aβ40 fibril sample amenable to high-resolution structural analysis. Unlike the densely bundled fibrils observed in wild-type (WT) Aβ40 and Aβ42 fibrils, the E22G Aβ40 fibrils formed in our preparations exhibited a less compact, twisted morphology with a uniform cross-over distance of 35 – 45 nm. It has been reported that E22G Aβ40 has high propensity to form multiple forms of

fibrils (i.e., polymorphs), which have long hindered structural analysis of resultant fibrils. The fibrils prepared by our optimized method has a highly homogeneous twisted morphology with a uniform short cross-over distance of 35 – 45 nm. This morphological homogeneity facilitated structural elucidation using SSNMR and cryoEM, which revealed an atomic-resolution structure of the E22G Aβ40 fibril. The structure comprised two Aβ molecules forming inner layers with slight distortions in C_2 symmetry, each adopting a unique W-shaped parallel β-sheet motif. While cryoEM data suggested the presence of additional β-sheet layers, these were not confirmed by SSNMR. Compared to previous structural studies on WT Aβ40 and Aβ42 fibrils, which exhibited U- and S-shaped motifs,[15,16] the E22G Aβ40 fibril presented here exhibits an extended conformation similar to the I-shaped Aβ40 fibril polymorph[17]. This less compact fibrillar structure is consistent with the Aβ40-rich cores observed in the cotton-wool plaques from Arctic FAD patients.[18] Furthermore, the low Thioflavin-T (ThT) fluorescence associated with these fibrils supports their structural similarity to the Aβ40-enriched plaques seen in Arctic mutation carriers,[19] suggesting that the distinct structural features of the E22G Aβ40 fibril may underpin the unique pathological characteristics of Arctic FAD.

We also investigated the misfolding kinetics of E22G and WT Aβ40/Aβ42 monomers, as well as the cross-seeding potential of E22G Aβ40 fibrils. Our findings reveal that E22G Aβ40 fibrils self-assemble more rapidly than WT Aβ40 or Aβ42 at physiologically relevant concentrations (Aβ40 : β42 ~9:1). Moreover, E22G Aβ40 fibrils were able to cross-seed WT Aβ40 and Aβ42 fibril formation, suggesting that this mutation may facilitate pathological aggregation in a mixed Aβ environment.

Conclusion

Our results offer new insights into the structural properties of E22G Aβ40 fibrils, highlighting their unique W-shaped β-sheet motif and less compact morphology. These findings suggest that the specific structure of the E22G Aβ40 fibrils contributes to the distinct clinical and pathological features observed in Arctic FAD. Furthermore, the ability of E22G Aβ40 fibrils to cross-seed WT Aβ fibrils provides a mechanistic explanation for their potential role in accelerating amyloid aggregation in the brain.

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