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Structural and nanomechanical comparison of epitaxially and solution-grown amyloid β25–35 fibrils

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ABSTRACT

Aβ25–35, the fibril-forming, biologically active toxic fragment of the full-length amyloid β-peptide also forms fibrils on mica by an epitaxial assembly mechanism. Here we investigated, by using atomic force microscopy, nanomechanical manipulation and FTIR spectroscopy, whether the epitaxially grown fibrils display structural and mechanical features similar to the ones evolving under equilibrium conditions in bulk solution. Unlike epitaxially grown fibrils, solution-grown fibrils displayed a heterogeneous morphology and an apparently helical structure. While fibril assembly in solution occurred on a time scale of hours, it appeared within a few minutes on mica surface fibrils. Both types of fibrils showed a similar plateau-like nanomechanical response characterized by the appearance of force staircases. The IR spectra of both fibril types contained an intense peak between 1620 and 1640 cm^{-1}, indicating that β-sheets dominate their structure. A shift in the amide I band towards greater wave numbers in epitaxially assembled fibrils suggests that their structure is less compact than that of solution-grown fibrils. Thus, equilibrium conditions are required for a full structural compaction. Epitaxial Aβ25–35 fibril assembly, while significantly accelerated, may trap the fibrils in less compact configurations. Considering that under *in vivo* conditions the assembly of amyloid fibrils is influenced by the presence of extracellular matrix components, the ultimate fibril structure is likely to be influenced by the features of underlying matrix elements.

1. Introduction

Amyloid fibrils are nanoscale proteinaceous filaments that become deposited, in the form of plaques, in the extracellular space of different tissues in various degenerative disorders [1–3]. The main constituent of amyloid plaques in the brains of patients with Alzheimer's disease are amyloid beta (Aβ) fibrils composed of 39- to 43-residue-long Aβ peptides, which are proteolytic by-products of the transmembrane amyloid precursor protein (APP) [4]. The undecapeptide Aβ25–35 is a naturally occurring proteolytic product of the full-length Aβ [5,6]. It has been proposed that Aβ25–35 represents the biologically active region of Aβ because it is the shortest fragment that exhibits β-sheet-containing aggregated structures and retains the toxicity of the full-length peptide [7]. The peptide, which has a net charge of +1, contains four polar residues at its N-terminus and seven predominantly hydrophobic residues at its C-terminus [8]. The basic features of the Aβ25–35 fibril are similar to those formed from other Aβ peptides. Accordingly, β-strands in an orientation perpendicular to the fibril axis connect to each other via hydrogen bonds and line up to form β-sheet ribbons. The fibril contains several β-sheets that associate *via* amino acid side-chain packing to form the final protofilament structure [9].

Aβ25–35 peptides incubated *in vitro* for an extended period of time (hours to days) form mature amyloid fibrils which are often used as an amyloid model. We have recently shown that the growth of Aβ25–35 amyloid fibrils can be greatly facilitated by an epitaxial mechanism on mica surface. Under these conditions, the peptides form oriented fibrillar network on mica surface within a few minutes [10–12]. Although it has been hypothesized that the epitaxially grown fibrils are identical to the ones evolving under equilibrium conditions in solution, a detailed structural comparison has not yet been carried out. Addressing the structure of epitaxially grown fibrils is compromised by the fact that only a fibrillar monolayer is available for investigation. In the present work we used atomic force microscopy, nanomechanics and FTIR spectroscopy in total internal reflection mode for the structural comparison of Aβ25–35 fibrils grown epitaxially or in bulk solution. We find that although both fibril types are dominated by β-sheet structural elements

Abbreviations: AFM, atomic force microscopy; PBS, phosphate-buffered saline; FTIR, Fourier transform infrared

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that display similar nanomechanical properties, the fibrils grown in solution have more compact and polymorphic structure.

2. Materials and methods

2.1. Sample preparation

Aβ25–35 ($^{+}$H3N-GSNKGAIIGLM-COO^{-}) was produced by solid-state synthesis as published earlier [13]. For the study of epitaxially grown fibrils, the peptides were dissolved in dimethyl sulfoxide (DMSO) and transferred to Na-phosphate-buffered saline (Na-PBS) buffer (10 mM Na-phosphate, pH 7.4, 140 mM NaCl, 0.02% NaN_3) at a final concentration of 0.5–1 mg/ml. Insoluble aggregates ("seeds") were removed by centrifugation at 250.000 g and 4 °C for 2 h (Beckman Coulter OptimaTM MAX Ultracentrifuge). The supernatant was diluted to appropriate concentrations prior to further use. According to AFM analysis, the amount of remaining amorphous aggregates was <0.1%. In case of fibrils grown in solution, 0.5–1 mg peptide was dissolved in 10 μl DMSO solution and further diluted with Na-PBS buffer to a final concentration of 0.5–1 mg/ml. The Aβ25–35 fibrils were grown in solution at room temperature for several (typically 2–10) days. The sample was then diluted prior to further investigations. In the case of FTIR experiments, 1 mg/ml Aβ25–35 fibril suspension was concentrated to 25 mg/ml by first vacuum drying in a SpeedVac instrument followed by dissolution of the pellet in D_2O. Two microliters of 25 mg/ml Aβ25–35 samples was used for each measurement. Peptide concentration was measured with the quantitative bicinchoninic acid assay [14].

2.2. Atomic force microscopy

AFM was carried out by steps described in our previous publications [10–12,15–17]. Typically, 100 μl samples were applied to a freshly cleaved mica surface. We used high-grade mica sheets (V2 grade, #52-6, Ted Pella, Inc., Redding, CA). For the study of epitaxially grown fibrils, the seedless sample was incubated for 10 min on the mica surface. In case of fibrils grown in solution, 100 μl of the several-day-old fibrils was pipetted onto freshly cleaved mica surface and then incubated for 30 min. After washing the surface with buffer to remove the unbound fibrils, we scanned the surface with AFM. The samples were imaged with AFM in buffer or in air. Non-contact mode AFM images were acquired with an Asylum Research MFP3D instrument (Santa Barbara, CA) using silicon-nitride cantilevers (Olympus BioLever, resonance frequency in buffer ~ 9 kHz; Olympus AC160 cantilever, resonance frequency in air ~ 330 kHz;). The 512 × 512-pixel images were collected at a typical line-scanning frequency of 0.6–1.5 Hz and with a set point of 0.5–0.8 V.

2.3. Force measurements

Force spectroscopy on Aβ25–35 fibrils was carried out by established protocols [11,12,15–17]. Briefly, a 100 μl sample of Aβ25–35 (8 μM and 950 μM for epitaxially and solution-grown fibrils, respectively) was pipetted on freshly cleaved mica and incubated for 10 min at room temperature. Unbound fibrils were removed by washing gently with buffer (Na-PBS). Surface-bound fibrils were mechanically manipulated by first pressing the cantilever (Olympus BioLever, lever A) tip against the surface, then pulling the cantilever away with a constant, pre-adjusted rate. Typical stretch rate was 500 nm/s. Experiments were carried out under aqueous buffer conditions (Na-PBS buffer, pH 7.4). Stiffness was determined for each cantilever by using the thermal method [18].

2.4. FTIR spectroscopy

The Fourier transform infrared (FTIR) spectra of amyloid fibrils growing in solution were investigated in a diamond anvil cell (Diacell,

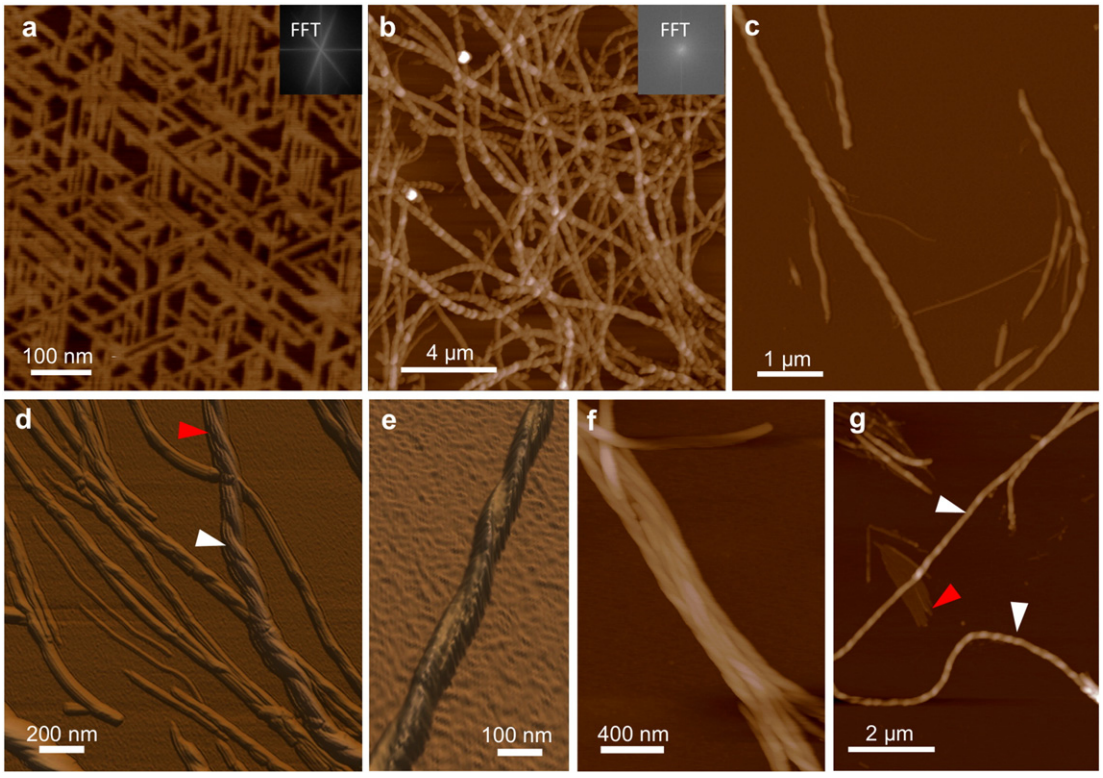

Fig. 1. AFM images showing the morphological appearance of Aβ25–35 fibrils. (a) Epitaxially grown, oriented Aβ25–35 fibril network on mica surface. (b–g) Mature Aβ25–35 fibrils assembled in solution and adsorbed subsequently onto mica. Fibrils display structural polymorphism and different levels of organizational hierarchy: (b) beaded appearance, (c) left-handed helix, (d) fibrils with apparent twist (white arrowhead) and striations (red arrowhead), (e) two fibrils twisted around each other, (f) bundle of twisted fibrils and (g) fibrils with left-handed twist (white arrowheads) and ones showing sheet-like appearance (red arrowhead). Insets, 2D-FFT of the respective AFM image.