Direct Evidence for Self-Propagation of Different Amyloid-β Fibril Conformations

Thomas Spirig Oxana Ovchinnikova Toni Vagt Rudi Glockshuber
Department of Biology, Institute of Molecular Biology and Biophysics, ETH Zurich, Zurich, Switzerland

Key Words
Alzheimer’s disease · Thioflavin T · Electron microscopy · Aggregation · Prion

Abstract
Background: Amyloid fibrils formed by amyloid-β (Aβ) peptides are associated with Alzheimer’s disease and can occur in a range of distinct morphologies that are not uniquely determined by the Aβ sequence. Whether distinct conformations of Aβ fibrils can be stably propagated over multiple cycles of seeding and fibril growth has not been established experimentally. Objective: The ability of the 40-residue peptide Aβ1–40 to assemble into fibrils with the conformation of the mutant Aβ1–40 peptide containing the ‘Osaka’ mutation E22Δ was investigated. Methods: Fibril formation of highly pure, recombinant Aβ1–40 in the presence of distinct, pre-formed seeds in vitro was recorded with thioflavin T fluorescence, and distinct fibrillar structures were identified and distinguished by fluorescence spectroscopy and electron microscopy. Results: We propagated the specific quaternary structure of Aβ1–40 E22Δ fibrils with wild-type Aβ1–40 over up to seven cycles of seeding and fibril elongation. As a result of a 107-fold dilution of the initially present Aβ1–40 E22Δ seeds, the vast majority of fibrils formed after the seventh propagation cycle with Aβ1–40 did not contain a single molecule of Aβ1–40 E22Δ, but still retained the conformation of the initial Aβ1–40 E22Δ seeds. Increased critical concentrations of Aβ1–40 fibrils formed in the presence of Aβ1–40 E22Δ nuclei suggest that these fibrils are less stable than homologously seeded Aβ1–40 fibrils, consistent with a kinetically controlled mechanism of fibril formation. Conclusion: The propagation of a distinct Aβ fibril conformation over multiple cycles of seeded fibril growth demonstrates the basic ability of the Aβ peptide to form amyloid strains that in turn may cause phenotypes in Alzheimer’s disease.

Introduction

The formation and deposition of abnormally aggregated proteins has been implicated in various neurodegenerative disorders, including Alzheimer’s disease (AD), Parkinson’s disease, Huntington disease and spongiform encephalopathies [1]. The highly stable protein aggregates known as amyloid fibrils are characterized by the presence of an ordered β-sheet structure and a unique fibrous appearance [2]. There is a continuous debate regarding the destructive versus protective effect of such protein aggregates. The abnormal accumulation of fibrillar protein aggregates, however, appears to be a common feature of many progressive neurodegenerative diseases [3, 4]. Moreover, the ability to form amyloid fibrils is not limited to a specific set of proteins involved in disease, but appears to be a common property of any polypeptide under appropriate conditions [5–7].

Amyloid-β (Aβ) peptides are the main component of neuritic plaques present in individuals suffering from AD, the most prevalent neurodegenerative disease in el-
Aβ is formed by endoproteolytic cleavage of the β-amyloid precursor protein. Alternative cleavage of this protein results in various Aβ isoforms, of which Aβ1–40 represents the major isoform in the human brain [9]. While increasing age is considered to be the greatest AD risk factor, a small number of AD cases have a familial background. Familial AD can be associated with mutations in the Aβ sequence such as the deletion of glutamate 22 (‘Osaka’ mutation) identified in Japanese patients [10]. Aβ1–40 E22Δ forms amyloid fibrils with a unique quaternary structure distinct from wild-type (WT) fibrils, shows an increased tendency to form fibrillar bundles and is more toxic than WT Aβ1–40, possibly explaining the early onset of AD symptoms associated with this mutation [11, 12].

The molecular pathway of Aβ fibril formation is still not completely understood, in particular because the isolation and characterization of intermediates has proven to be very challenging. It is, however, widely accepted that fibrillization follows a nucleation-dependent polymerization mechanism, which requires the formation of an ordered fibrillar nucleus (seed) followed by fibril elongation through the incorporation of additional Aβ monomers [13–15]. The formation of nuclei with seeding activity represents the rate-limiting step in Aβ fibrillization and typically results in a lag phase in fibril growth, which can be eliminated by the addition of preformed seeds [16]. Solid-state nuclear magnetic resonance combined with mutational analysis has recently provided detailed insight into the three-dimensional structures of a multitude of polymorphs of Aβ fibrils, which usually form parallel β-sheet structures along the fibril axis [17, 18]. Aβ fibrils formed by the same peptide can occur in a range of structurally different morphologies. Depending on the experimental conditions, Aβ1–40 can adopt a variety of distinct fibril structures in vitro [19–22]. Notably, the conformational variability of protein aggregates has also been implicated in prion diseases. The existence of prion strains with distinct phenotypes can be explained by several different, self-propagating conformations of prion protein fibrils [23]. Whether conformational strains resulting in distinct disease phenotypes also exist in AD and Aβ amyloids behave like an infectious agent remains a matter of debate [24–28]. Studies with transgenic mice demonstrated that in vivo seeding of Aβ can induce amyloid formation and generate distinct phenotypes depending on the injected amyloid agent [29–32]. Recently, distinct fibril conformations of tissue-derived Aβ1–40 from patients with different clinical histories suggested that variations in fibril structures might correlate with variations in AD development [33]. In vitro seeding experiments indicated that Aβ fibrils can adopt distinct conformational properties from their seeds [11, 34–36]. However, repeated self-propagation of distinct Aβ fibrils retaining their specific quaternary structure, which is the basis for possible strain phenomena in AD, has not been reported so far for Aβ peptides. Here, we show that specific conformations of Aβ1–40 fibrils can be propagated over multiple cycles of seeding and fibril elongation. The data provide a molecular basis for the existence of strains in AD and contribute to a mechanistic understanding of Aβ fibril formation.

Materials and Methods

Preparation of Recombinant Aβ1–40
The plasmids for the expression of Aβ1–40 WT and Aβ1–40 E22Δ as fusions to the peptide sequence (NANP)15 with an N-terminal (His)6 tag have been described previously [12, 37]. The fusion proteins were expressed in Escherichia coli BL21 (DE3) cells (New England BioLabs) under T7 promoter control in Terrific Broth medium containing 0.1 mg/ml ampicillin. Protein expression was induced with 1 mM IPTG for 4 h at 37°C. The Aβ fusions were purified and cleaved with tobacco etch virus protease, and the resulting Aβ peptides were purified by reverse-phase HPLC as described [12]. The purity and identity of the Aβ preparations were verified by analytical reverse-phase HPLC on a Zorbax SB300 C8 column (Agilent) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry, respectively (data not shown). Pure peptides were aliquoted in Protein LoBind Eppendorf tubes (Vadaux-Eppendorf), lyophilized and stored at –80°C.

Fibril Growth
Solutions of Aβ1–40 WT and Aβ1–40 E22Δ monomers were prepared by dissolving the lyophilized Aβ peptides in 10 mM NaOH followed by ultracentrifugation (135,500 g, 1 h, 4°C) to remove any residual aggregates. The soluble Aβ monomers in the supernatant were carefully removed and their concentration determined via the specific absorbance of Aβ at 280 nm (1,730 M–1 cm–1). Aβ monomer stock solutions were generated by dilution of the peptides to a concentration of 100 μM in 10 mM NaOH. The Aβ fibrils were formed at 25°C with shaking in a volume of 150 μl in a black 96-well plate with a clear bottom (Greiner Bio-One) sealed with a VIEWseal foil (Greiner Bio-One) to prevent evaporation in a microtiter plate reader (BioTek Instruments). The fibrilization reactions were initiated by dilution of the Aβ stock solution to a final concentration of 10 μM Aβ peptide in aggregation buffer (10 mM H3PO4-NaOH pH 7.4, 100 mM NaCl, 35 μM thioflavin T (ThT)). Fibril formation was followed by monitoring ThT fluorescence using wavelengths of 440 ± 15 and 485 ± 10 nm for excitation and emission, respectively. All fibrillation curves represent the average of three replicate wells.

Seeding Experiments
To generate seeds for subsequent fibrilization reactions, fibrils were collected after completion of the assembly reaction and sonicated for 12 min at 4°C in a water bath-based sonicator (Bandelin...
Electronic). The kinetics of ThT fluorescence change for Aβ1–40 WT were followed in the presence of seeds from sonicated Aβ1–40 WT or Aβ1–40 E22Δ fibrils. All seeding experiments were performed with 10% of freshly prepared seeds and 90% of soluble Aβ monomer, resulting in a total Aβ concentration of 10 μM. The reactions were otherwise performed under the conditions described above for spontaneous fibrilization reactions.

**ThT Fluorescence Spectra**

The fluorescence spectra of aliquots of the Aβ1–40 aggregation reactions were measured after maximum ThT fluorescence had been reached. The spectra were recorded on a QuantaMaster fluorescence spectrometer (QM-7/2003; Photon Technology International) in the range of 450–550 nm (excitation at 440 nm) and corrected for the buffer.

**Electron Microscopy**

Samples for transmission electron microscopy were removed from the aggregation reactions after the maximum ThT fluorescence had been reached. The fibril suspensions were applied to carbon-coated copper grids (Quantifoil Micro Tools) for 1 min and stained with 2% (w/v) uranyl acetate for 30 s. Electron micrographs were recorded at 100 MeV on an FEI Morgagni 268 transmission electron microscope.

**Critical Concentrations**

Aliquots were removed from fibrillization reactions 12 h after a constant plateau in ThT fluorescence had been reached and subjected to ultracentrifugation (135,500 g; 20 min, 25 °C) in order to precipitate formed fibrils. The concentration of the soluble Aβ monomers in the supernatants was then determined using an Aβ1–40-specific quantitative enzyme-linked immunosorbent assay (ELISA). Appropriate dilutions of the supernatants were incubated in high-binding polystyrene 96-well microplates (Greiner Bio-One) for 2 h at room temperature to allow binding of Aβ monomers to the wells. After blocking with 200 μl of 2% bovine serum albumin in PBS overnight at 4 °C, rabbit anti-β-amyloid 1–40 antibody (0.1 μg/ml; Merck Millipore) in 100 μl of blocking buffer was added and incubated for 2 h at room temperature, followed by the addition of 100 μl of horseradish peroxidase-conjugated anti-rabbit IgG antibody (0.27 μg/ml; Merck Millipore) in blocking buffer as a secondary antibody. After incubation for 2 h at room temperature, the antibody-bound Aβ was visualized by the addition of 100 μl of detection solution (0.1 mg/ml 3,3′,5,5′-tetramethylbenzidine, 0.01% (v/v) H2O2, 0.1 M acetic acid-NaOH pH 6.0). The reaction was stopped with 2 M H2SO4 (100 μl) and the absorbance at 450 nm was determined using a microtiter plate reader (BioTek Instruments). After each step the wells were washed 3 times with 250 μl PBS + 0.05% (v/v) Tween 20. As a reference for quantification, standard curves for Aβ1–40 WT and Aβ1–40 E22Δ monomers were established (online suppl. fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000363623). Samples with known concentrations (0.5–10 nM) of soluble Aβ1–40 WT or Aβ1–40 E22Δ were generated from Aβ stock solutions (100 μM) by serial dilutions in 0.2 M Na2CO3/NaHCO3 pH 9.5. The absorbance at 450 nm was then plotted against the Aβ concentration, and the standard curves were fitted empirically with a 4-parameter sigmoidal model.

The apparent free energies of fibril elongation/dissociation (ΔGppp) were calculated according to the equation ΔGppp = −RT ln (1/Cs), as the critical concentration (Cs) is the inverse of the amyloid fibril growth equilibrium constant under the assumption that the molar concentration of growing ends does not change as monomers associate to and dissociate from the ends of the fibrils in a dynamic equilibrium [16, 38, 39].

**Results**

Aβ1–40 Can Be Efficiently Seeded to Form Distinct Fibril Structures

Fibril formation of Aβ1–40 and the mutant peptide Aβ1–40 E22Δ (pH 7.4, 25 °C) and initial Aβ monomer concentrations of 10 μM was followed in a microtiter plate reader monitoring the fluorescence increase of the amyloid-specific dye ThT. As recently demonstrated for aggregation reactions under similar conditions, Aβ1–40 E22Δ fibrils adopted a quaternary structure distinct from that of Aβ1–40 WT fibrils (fig. 1a) [12]. Characteristic properties of these Aβ1–40 E22Δ fibrils include about a 5-fold higher specific ThT fluorescence intensity and a shift in the ThT fluorescence emission maximum from 485 to 474 nm (fig. 2), which allowed us to follow the propagation of this specific fibril conformation in cross-seeded fibrilization reactions in quantitative terms. Fibrils of Aβ1–40 WT and Aβ1–40 E22Δ were sonicated to produce smaller fragments, which were used to seed subsequent fibril formation. We analyzed aggregation kinetics of Aβ1–40 WT in the presence of 10% of preformed Aβ1–40 WT seeds (homologous seeding) and in the presence of 10% of Aβ1–40 E22Δ seeds (heterologous seeding or cross-seeding). As expected, a lag phase in the aggregation kinetics of Aβ1–40 WT was no longer observable when seeded with Aβ1–40 WT fibrils (fig. 1b). Aggregation of Aβ1–40 WT was also accelerated when cross-seeded with Aβ1–40 E22Δ fibrils, indicating coaggregation of Aβ1–40 WT with preformed fibrils of Aβ1–40 E22Δ (fig. 1b). Importantly, the ThT fluorescence intensity reached the high values characteristic for Aβ1–40 E22Δ fibrils, suggesting that the new fibrils formed from Aβ1–40 WT adopted the conformation of the E22Δ nuclei. The final ThT fluorescence value for the cross-seeded reaction was slightly higher compared to the unseeded Aβ1–40 E22Δ fibrilization reaction, possibly due to larger amounts of well-structured fibrils and a smaller percentage of amorphous Aβ1–40 aggregates. In contrast to Aβ1–40 WT cross-seeded with Aβ1–40 E22Δ seeds, Aβ1–40 E22Δ monomers were not efficiently seeded by Aβ1–40 WT nuclei, which was manifested by the presence of a lag phase and final ThT fluorescence levels specific for Aβ1–40 E22Δ fibrils (data not shown).
Self-Propagation of Aβ₁₋₄₀ Fibril Conformations Can Be Followed over Multiple Seeding Cycles

To our knowledge, repeated seeding over more than two cycles with the retention of a specific fibril conformation has not been demonstrated experimentally for Aβ fibril propagation in vitro. To test whether Aβ can stably propagate a specific quaternary structure in a prion-like fashion, we followed the kinetics of multiple seeding cycles in which newly formed fibrils of Aβ₁₋₄₀ WT, initially seeded with fibrillar Aβ₁₋₄₀ E₂₂Δ nuclei, were used as seeds in successive aggregation reactions with fresh preparations of monomeric Aβ₁₋₄₀ WT. We observed that the increased ThT fluorescence levels of the cross-seeded fibrils were preserved and transmitted during seven seeding cycles (fig. 1c). Moreover, all fibrils formed during these multiple seeding cycles also displayed ThT fluorescence emission spectra almost identical to those of pure Aβ₁₋₄₀ E₂₂Δ fibrils, with a characteristic shift of the emission maximum from 485 to 474 nm compared to Aβ₁₋₄₀ WT (fig. 2). These results indicate that seven generations of fibrils of Aβ₁₋₄₀ WT initially seeded with Aβ₁₋₄₀ E₂₂Δ nuclei adopt a quaternary structure similar to the Aβ₁₋₄₀ E₂₂Δ seeds. To confirm that specific conformational properties of Aβ₁₋₄₀ E₂₂Δ seeds are propagated with Aβ₁₋₄₀ WT, we examined the morphologies of formed fibrils by electron microscopy (fig. 3).
**Fig. 2.** ThT fluorescence emission spectra of Aβ<sub>1-40</sub> WT fibrils and fibrils of Aβ<sub>1-40</sub> WT initially seeded with Aβ<sub>1-40</sub> E22Δ fibrils after each seeding/propagation reaction (the seeding cycle numbers S1–S7 are indicated). Fluorescence emission spectra of fibril-bound ThT for Aβ<sub>1-40</sub> WT, Aβ<sub>1-40</sub> E22Δ and fibrils from the seeding experiments in figure 1 are shown. The spectra were normalized to the respective fluorescence maximum to illustrate differences in spectral shapes. The fluorescence maxima of Aβ<sub>1-40</sub> WT fibrils (485 nm), Aβ<sub>1-40</sub> WT fibrils formed with Aβ<sub>1-40</sub> WT seeds (485 nm), Aβ<sub>1-40</sub> E22Δ fibrils (474 nm) and Aβ<sub>1-40</sub> WT initially seeded with Aβ<sub>1-40</sub> E22Δ after up to 7 seeding/propagation cycles (474 nm) are indicated.

**Fig. 3.** Negatively stained electron micrographs of seeded and unseeded Aβ<sub>1-40</sub> fibrils. Representative transmission electron microscopy images of fibrils formed from Aβ<sub>1-40</sub> WT (a), Aβ<sub>1-40</sub> WT in the presence of Aβ<sub>1-40</sub> WT seeds (b), Aβ<sub>1-40</sub> E22Δ (c), Aβ<sub>1-40</sub> E22Δ seeds after sonication (e), and Aβ<sub>1-40</sub> WT in the presence of Aβ<sub>1-40</sub> E22Δ seeds after 1 (f), 4 (g) and 7 (h) seeding cycles are shown (the seeding cycle numbers S1, S4 and S7 are indicated). The scale bars correspond to 100 nm.
Aβ_{1–40} WT incubated in the absence of seeds as well as in the presence of Aβ_{1–40} WT seeds mainly formed straight individual fibrils that laterally associated to discrete small bundles, while Aβ_{1–40} E22Δ fibrils displayed a high tendency to develop networks of fibrillar bundles, in agreement with earlier studies [12]. Aβ_{1–40} WT fibrils formed during multiple seeding/growth experiments that had initially been cross-seeded with Aβ_{1–40} E22Δ displayed morphologies very similar to the mutant form even after seven seeding cycles. Although less curved than Aβ_{1–40} E22Δ fibrils, these cross-seeded fibrils aggregated to extended networks of fibrillar bundles, which are clearly distinct from Aβ_{1–40} WT fibrils. Fibrils formed during seeding cycles 1 through 7 were indistinguishable based on their fluorescence properties (fig. 3) and appearance in electron micrographs (data not shown).

After serial dilutions of the initial Aβ_{1–40} E22Δ seeds as a result of repetitive seeding and growth in the presence of Aβ_{1–40} WT, only 1 molecule of Aβ_{1–40} E22Δ was present in the reaction mixture per 10 million molecules of Aβ_{1–40} WT after cycle 7. Thus, less than 1 in 1,000 fibrils still contains a single molecule of Aβ_{1–40} E22Δ after seven cycles of seeding, assuming that Aβ_{1–40} fibrils with an average length of 1 μm with mass-per-length values of 18–21 kDa/nm are formed (corresponding to an average of 4,000–5,000 Aβ_{1–40} monomers per fibril) [20, 35, 40]. Therefore, although the vast majority of fibrils after seven seeding cycles are exclusively made up of Aβ_{1–40} WT fibrils, these fibrils still retain the conformation of the initial Aβ_{1–40} E22Δ seeds (fig. 4). The results confirm a significant structural plasticity of Aβ fibrils, highlighting the ability of Aβ fibrils to retain a specific quaternary structure under defined conditions of fibril propagation and demonstrate that Aβ fulfills the molecular requirements for forming prion-like amyloid strains in AD patients.

**Cross-Seeded Fibrils Are Thermodynamically Disfavored but Kinetically Stable**

As proposed by Wetzel and coworkers [41], a comparison of fibril elongation thermodynamics, rather than the kinetics of fibril formation, was used to assess the effects of cross-seeding on Aβ fibril formation. An Aβ_{1–40}-specific ELISA was used to determine the concentrations of soluble monomers after the completion of fibrillization. This so-called critical concentration C_r is formally equivalent to the dissociation constant of the monomer binding to the growing end of the fibril and can be used to compare thermodynamic stabilities of different fibrils [16, 38, 39]. The data in table 1 show that the homologously seeded Aβ_{1–40} WT and Aβ_{1–40} E22Δ fibrils display similar residual monomer concentrations in the range of 60 ± 13 and 72 ± 18 nM, respectively. Aβ_{1–40} WT fibrils formed in the presence of Aβ_{1–40} E22Δ seeds displayed a significantly higher C_r value of 145 ± 25 nM. The observed increase in C_r corresponds to an apparent destabilization of the formed fibrils relative to Aβ_{1–40} WT fibrils of 2.2 kJ/mol through the imposed Aβ_{1–40} E22Δ fibril structure. Although thermodynamically disfavored, the cross-seeded Aβ_{1–40} WT fibrils were kinetically stable and did not spontane-

---

**Table 1.** C_r values and apparent free energies of fibril elongation (ΔG_{app})

| Aβ fibrils                          | C_r, nM  | ΔG_{app}, kJ/mol |
|-------------------------------------|----------|------------------|
| Aβ_{1–40} WT + Aβ_{1–40} WT seeds   | 60±13    | −41.2±0.5        |
| Aβ_{1–40} E22Δ + Aβ_{1–40} E22Δ seeds | 72±18    | −40.8±0.6        |
| Aβ_{1–40} WT + Aβ_{1–40} E22Δ seeds | 145±25   | −39.0±0.3        |

C_r: Data are presented as means ± standard deviations of 6 independent experiments performed in duplicate.

**Fig. 4.** A model of fibril formation of Aβ_{1–40} WT in the presence of Aβ_{1–40} E22Δ seeds. Spontaneously aggregating Aβ_{1–40} WT (blue) and Aβ_{1–40} E22Δ (red) peptides form fibrils with distinct conformations. Monomeric Aβ_{1–40} WT incubated with seeds of sonicated Aβ_{1–40} E22Δ fibrils adopts the conformation of Aβ_{1–40} E22Δ fibrils and propagates it over several seeding cycles in a prion-like fashion.
ously convert to the thermodynamically preferred Aβ$_{1–40}$ WT conformation after 24 h of incubation, as evidenced by a constant, high ThT fluorescence signal of the cross-seeded fibrils (data not shown). De novo formation of nuclei with the more stable WT conformation may be suppressed by the low concentrations of free Aβ$_{1–40}$ monomers [42], and high activation energy barriers may prevent the direct interconversion between different fibril polymorphs due to the highly cooperative character of these reactions [22, 43]. Under physiological conditions, Aβ$_{1–40}$ WT fibrils cross-seeded with Aβ$_{1–40}$ E22Δ nuclei may thus only convert back to the more stable WT conformation after an incubation period of several weeks or even much longer.

**Discussion**

Structural polymorphism and the ability of a specific fibrillar quaternary structure to propagate is not limited to yeast and mammalian prions. Previous studies with Aβ$_{1–40}$, insulin and lysozyme showed that distinct amyloid conformations can be propagated in vitro through seeding and imply that prion-like strain behavior may be an inherent feature of proteinaceous amyloids [11, 34, 35, 44–46]. However, the latter experiments were limited to one or two seeding cycles. The existence of polymorphic strains in AD with varying biological activities or even potentially infectious properties would require multiple, continuous seeding and elongation cycles, which have not been reported for Aβ. Using pure, recombinant Aβ peptides, we analyzed cross-seeding of Aβ$_{1–40}$ by Aβ$_{1–40}$ E22Δ seeds. Pronounced differences in their ThT fluorescence properties and distinct fibril morphologies demonstrated that Aβ$_{1–40}$ WT can adopt the conformation of Aβ$_{1–40}$ E22Δ fibrils and retain it over up to seven cycles of seeded polymerization. Our data thus provide direct evidence for the self-propagation of different Aβ conformations and demonstrate that Aβ possesses the basic property of forming amyloid strains in AD, analogous to prion strains.

The β-sheet is the dominant, common structural feature of amyloid fibrils. However, structural models of Aβ fibrils reported so far differ in the extent of β-sheet structure, and the exact positions of the β-strands in the Aβ sequence vary in different structural models described for Aβ$_{1–40}$ fibrils [35, 39, 47, 48]. The efficient seeding of Aβ$_{1–40}$ WT by Aβ$_{1–40}$ E22Δ nuclei observed in our study suggests that position 22 is located in a turn region. An amino acid deletion within a β-strand would flip all side chains of the following amino acid residues within this strand by 180° from the outside to the interior of the fibril core and vice versa. The resulting conformational changes would be likely to prevent Aβ$_{1–40}$ E22Δ to seed Aβ$_{1–40}$ WT peptides, as successful seeding relies on conformational complementarity between the seed fibril and the monomeric peptide associating with the growing fibril end. Our observations are consistent with simulation studies on the ensemble of monomeric Aβ$_{1–40}$, which indicate that the side chains of E22 and D23 remain exposed to the solvent [49].

In contrast to Aβ$_{1–40}$ WT seeded with Aβ$_{1–40}$ E22Δ nuclei, Aβ$_{1–40}$ E22Δ monomers were not efficiently cross-seeded by Aβ$_{1–40}$ WT seeds. As proposed by Wetzel and coworkers [34] for Aβ and islet amyloid polypeptide, a lack of reciprocity in cross-seeding might arise from different degrees of complementarity between the aggregation product and the seed. The missing residue in Aβ$_{1–40}$ E22Δ might significantly decrease the conformational plasticity of this region and explain the failure to adopt the conformation of Aβ$_{1–40}$ WT fibrils. Alternatively, increased hydrophobicity due to the absence of the glutamate in Aβ$_{1–40}$ E22Δ might result in a higher amyloidogenic potential of this peptide. Using simulated free-energy profiles, Caflisch and coworkers [50] recently showed that fibril morphogenesis can switch from kinetic to thermodynamic control with increasing amyloidogenic potential, a scenario in which the Aβ$_{1–40}$ E22Δ monomers would not be allowed to adopt an alternative, less stable conformation during cross-seeding.

Using a highly sensitive, quantitative ELISA assay, we determined critical monomer concentrations of 60 ± 13 and 70 ± 18 nM for homologously seeded Aβ$_{1–40}$ WT and Aβ$_{1–40}$ E22Δ fibrils, respectively. These values are in agreement with C$_{r}$ values recently determined by atomic force microscopy by Tycko and coworkers [42], but considerably lower than most previously reported C$_{r}$ values for Aβ$_{1–40}$ WT or Aβ$_{1–40}$ E22Δ, which range from 0.2 to 8 μM [11, 36, 38, 39, 51–54]. The low C$_{r}$ values observed in our study might be a consequence of the high purity of the recombinant Aβ peptides used, which are likely to have yielded more homogeneous and more stable fibrils compared to fibrils formed by synthetic peptides. Our determined C$_{r}$ value for Aβ$_{1–40}$ may thus be a good estimate of the minimum Aβ$_{1–40}$ monomer concentration required for spontaneous fibril formation in vivo. Aβ$_{1–40}$ monomer levels in human brain extracellular fluids were indeed reported to be in the low nanomolar range [55–57].
Acknowledgments

We would like to thank Hiang Dreher-Teo for the purification of tobacco etch virus protease. We acknowledge Peter Tittmann (Electron Microscopy Center, ETH Zurich) for technical support and Serge Chesnov (Functional Genomics Center Zurich) for the acquisition of mass spectra. This work was funded by the ETH Zurich and the Swiss National Science Foundation within the framework of the program 'NCCR Neural Plasticity and Repair'.

References

1. Shastri BS: Neurodegenerative disorders of protein aggregation. Neurochem Int 2003;43:1–7.
2. Carrell RW, Lomas DA: Conformational disease. Lancet 1997;350:134–138.
3. Bacciantini M, Giannoni E, Chiti F, Baroni F, Formigli L, Zurdo J, Taddei N, Ramponi G, Dobson CM, Stefani M: Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 2002;416:507–511.
4. Caughey B, Lansbury PT: Prototifbrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. Annu Rev Neurosci 2003;26:267–298.
5. Guijarro JI, Sunde M, Jones JA, Campbell ID, Shastry BS: Neurodegenerative disorders of cerebral beta-amyloidogenesis is governed by agent and host. Science 2006;313:1781–1784.
6. Cloe AL, Orgel JP, Sachleben JR, Tycko R: Solid-state NMR studies of amyloid fibril structure. Annu Rev Phys Chem 2011;62:279–299.
7. Hardj J, Selkoe DJ: The amyloid hypothesis of Alzheimer’s disease: progress and problems on the road to therapeutics. Science 2002;297:353–356.
8. Finder VH, Gloshuber R: Amyloid-beta aggregation. Neurodegener Dis 2007;4:13–27.
9. Tomiyama T, Nagata T, Shimada H, Teraoka Finder VH, Glockshuber R: Amyloid-beta aggregation: could prion-like templated misfolding play a role? Brain Pathol 2013;23:333–341.
10. Lomakin A, Chung DS, Benedek GB, Kirschner DA, Teplow DB: On the nucleation and growth of amyloid beta-protein fibrils: detection of nuclei and quantitation of rate constants. Proc Natl Acad Sci USA 1996;93:1125–1129.
11. Lomakin A, Teplow DB, Kirschner DA, Benedek GB: Kinetic theory of fibrillogenesis of amyloid beta-protein. Proc Natl Acad Sci USA 1994;91:7942–7947.
12. Harper JD, Lansbury PT Jr: Models of amyloid seeding in Alzheimer’s disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. Annu Rev Biochem 1997;66:385–407.
13. Tycko R: Progress towards a molecular-level structural understanding of amyloid fibrils. Curr Opin Struct Biol 2004;14:96–103.
14. Tycko R: Solid-state NMR studies of amyloid fibril structure. Annu Rev Phys Chem 2003:352–382.
15. Tycko R: Progress towards a molecular-level structural understanding of amyloid fibrils. Proc Natl Acad Sci USA 2010;107:3487–3492.
16. Haider J, Selkoe DJ: Amyloid beta (Aβ) prions. Proc Natl Acad Sci USA 2002;109:11025–11030.
17. Petkova AT, Leapman RD, Guo Z, Yau WM, Westermark P: Amyloid fibril secondary structure using scanning proton magnetic resonance. EMBO Rep 2013;14:1017–1022.
18. Lu JX, Qiang W, Yau WM, Schwieters CD, Meredith SC, Tycko R: Molecular structure of beta-amyloid fibrils in Alzheimer’s disease brain tissue. Cell 2013;154:1257–1268.
19. O’Nuallain B, Williams AD, Westermark P, Wetzl R: Seedling specificity in amyloid growth induced by heterologous fibrils. J Biol Chem 2004;279:17490–17499.
20. Petková AT, Leopan RD, Guo Z, Yau WM, Mattson MP, Tycko R: Self-propagating, molecular-level polymorphism in Alzheimer’s beta-amyloid fibrils. Science 2005;307:262–265.
21. Kodosi R, Williams AD, Cheunuru S, Wetzl R: Aβ1–40 forms five distinct amyloid structures whose beta-sheet contents and fibril stabilities are correlated. J Mol Biol 2010:401:503–517.
22. Finder VH, Vodopivec I, Nitsch RM, Gloshuber R: The recombinant amyloid-beta peptide Aβ1–40 aggregates faster and is more neurotoxic than synthetic Aβ1–40. J Mol Biol 2010;396:9–18.
23. O’Nuallain B, Shivaprasad S, Khetarpal I, Wetzl R: Thermodynamics of Aβ1–40 amyloid fibril elongation. Biochemistry 2005;44:12709–12718.
24. Williams AD, Portelius E, Khetarpal I, Guo JT, Cook KD, Xu Y, Wetzl R: Mapping Aβ amyloid fibril secondary structure using scanning proton mutagenesis. J Mol Biol 2004;335:833–842.
25. Spirid/Ovichnikova/Vagt/Glockshuber

DOI: 10.1159/000363623

Neurodegener Dis 2014;14:151–159
158
Self-Propagation of Aβ Fibril Conformations

DOI: 10.1159/000363623

40 Goldsbury CS, Wirtz S, Muller SA, Sunderji S, Wicki P, Aebersold R, Frey P: Studies on the in vitro assembly of Aβ₁–₄₀: implications for the search for Aβ fibril formation inhibitors. J Struct Biol 2000;130:217–231.

41 Williams AD, Shivaprasad S, Wetzel R: Alanine scanning mutagenesis of Aβ₁–₄₀ amyloid fibril stability. J Mol Biol 2006;357:1283–1294.

42 Qiang W, Kelley K, Tycko R: Polymorph-specific kinetics and thermodynamics of beta-amyloid fibril growth. J Am Chem Soc 2013;135:6860–6871.

43 Dzwolak W, Grudzielanek S, Smirnovas V, Ravindra R, Nicollini C, Jansen R, Loksztejn A, Porowski S, Winter R: Ethanol-perturbed amyloidogenic self-assembly of insulin: looking for origins of amyloid strains. Biochemistry 2005;44:8948–8958.

44 Paravastu AK, Qahwash I, Leapman RD, Meredith SC, Tycko R: Seeded growth of beta-amyloid fibrils from Alzheimer’s brain-derived fibrils produces a distinct fibril structure. Proc Natl Acad Sci USA 2009;106:7443–7448.

45 Dzwolak W, Smirnovas V, Jansen R, Winter R: Insulin forms amyloid in a strain-dependent manner: An FT-IR spectroscopic study. Protein Sci 2004;13:1927–1932.

46 Morozova-Roche LA, Zurdo J, Spencer A, Noppe W, Receveur V, Archer DB, Joniau M, Dobson CM: Amyloid fibril formation and seeding with wild-type human lysozyme and its disease-related mutational variants. J Struct Biol 2000;130:339–351.

47 Olofsson A, Lindhagen-Persson M, Sauer-Eriksson AE, Ohman A: Amide solvent protection analysis demonstrates that amyloid-β₁–₄₀ and amyloid-β₁–₄₂ form different fibrillar structures under identical conditions. Biochem J 2007;404:63–70.

48 Török M, Milton S, Kayed R, Wu P, McIntire T, Glabe CG, Langen R: Structural and dynamic features of Alzheimer’s Aβ peptide in amyloid fibrils studied by site-directed spin labeling. J Biol Chem 2002;277:40810–40815.

49 Vitalis A, Caflisch A: Micelle-like architecture of the monomer ensemble of Alzheimer’s amyloid-beta peptide in aqueous solution and its implications for Aβ aggregation. J Mol Biol 2010;403:148–165.

50 Pellarin R, Schuetz P, Guarnera E, Caflisch A: Amyloid fibril polymorphism is under kinetic control. J Am Chem Soc 2010;132:14960–14970.

51 Harper JD, Wong SS, Lieber CM, Lansbury PT Jr: Assembly of Aβ amyloid protofibrils: an in vitro model for a possible early event in Alzheimer’s disease. Biochemistry 1999;38:8972–8980.

52 Doran TM, Anderson EA, Latchney SE, Opanashuk LA, Nilsson BL: Turn nucleation perturbs amyloid beta self-assembly and cytotoxicity. J Mol Biol 2012;421:315–328.

53 Inayathullah M, Teplow DB: Structural dynamics of the ΔE22 (Osaka) familial Alzheimer’s disease-linked amyloid-β protein. Amyloid 2011;18:98–107.

54 Hasegawa K, Oku K, Yamada M, Naiki H: Kinetic modeling and determination of reaction constants of Alzheimer’s beta-amyloid fibril extension and dissociation using surface plasmon resonance. Biochemistry 2002;41:13489–13498.

55 Kanai M, Matsubara E, Iosek K, Urakami K, Nakashima K, Arai H, Sasaki H, Abe K, Iwatsubo T, Kosaka T, Watanabe M, Tomidokoro Y, Shizuka M, Mizushima K, Nakamura T, Igeta Y, Ikeda Y, Amari M, Kawarabayashi T, Ishiguro K, Harigaya Y, Wakabayashi K, Okamoto K, Hirai S, Shoji M: Longitudinal study of cerebrospinal fluid levels of tau, Aβ₁–₄₀, and Aβ₁–₂₄(43) in Alzheimer’s disease: a study in Japan. Ann Neurol 1998;44:17–26.

56 Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, Sinha S, Schlossmacher M, Whaley J, Swindlehurst C, et al: Isolation and quantification of soluble Alzheimer’s beta-peptide from biological fluids. Nature 1992;359:325–327.

57 Schoonenboom NS, Mulder C, Van Kamp GJ, Mehta SP, Scheltens P, Blankenstein MA, Mehta PD: Amyloid beta 38, 40, and 42 species in cerebrospinal fluid: more of the same? Ann Neurol 2005;58:139–142.