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Structural and Toxic Properties of Protein Aggregates: Towards a Molecular Understanding of Alzheimer's Disease

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1. Introduction

Alzheimer's disease (AD) is an age-related irreversible brain disorder that progresses over 10 to 20 years. AD gradually destroys human memory and thinking skills, leads to severe loss of mental function, and eventually causes death (Querfurth and LaFerla, 2010). Normally, AD symptoms first appear after age 65. It is estimated that AD affected 36 million people globally in 2009, and the number will be more than doubled by 2050. Currently, there is no cure for AD, except few medications that can relieve AD symptoms (Brunden et al., 2009; Citron, 2004).

AD is accompanied by a significant shrunk of brain tissue, which is a result of brain neuron degeneration. In the brains of AD patients, neurons are found to have lost their synaptic connections with other neurons and are unable to survive (Wenk, 2003). The synaptic failure is indeed the earliest event associated with the cognitive impairment caused by the disease (Selkoe, 2002). In addition, the brains of AD patients are characterized by the presence of two types of hystopathological hallmark lesions: amyloid plaques and neurofibrillary tangles (NFTs), which are composed of aggregated proteinaceous material (Tiraboschi et al., 2004). Although both amyloid plaques and NFTs are also present in brains of healthy individuals, the amount of these aggregates in the brains of AD patients is significantly higher. Early studies correlated neuron degeneration in AD with the formation of these insoluble protein aggregates. However, recent studies suggest that soluble protein aggregates of the same protein composition are more toxic to neurons (Rahimi et al., 2008). It has also been suggested that the insoluble aggregates actually play a beneficial role in that they sequester the toxic soluble aggregates into less toxic or non-toxic insoluble aggregates (Greenwald and Riek, 2010).

At current stage, we are still trying to understand how different protein aggregates in the brain mediate neuron degeneration and lead to AD. Like any other protein activity, the neurotoxicity of protein aggregates must be associated with their specific molecular structures. Therefore, investigating the high-resolution structures of amyloid plaques, NFTs and soluble aggregates would greatly facilitate the development of diagnostic and therapeutic strategies. Here, in this chapter, we review current knowledge about the structure and toxicity of the aggregates involved in AD.

2. Structure and toxicity of different protein aggregates involved in AD

2.1 Amyloid-β peptides, tau protein and their relation to AD

Amyloid plaques are extracellular deposits mainly composed of full length and truncated fragments of amyloid-β (Aβ) peptides. Aβ peptides are produced upon cleavage of the amyloid precursor protein (APP) by β- and γ- secretases (Hooper, 2005). APP is an integral membrane protein expressed in many tissues and concentrated in the neuronal synapses (Turner et al., 2003). The γ-secretase protease cleaves APP at different positions, thus producing different Aβ fragments composed by 36 to 43 amino-acid-residues (Kang et al., 1987; Masters et al., 1985). Among those, the Aβ fragments having 40 ($A\beta_{40}$) or 42 ($A\beta_{42}$) amino-acid-residues are the most common (Hartmann et al., 1997). The amino-acid sequences of $A\beta_{40}$ and $A\beta_{42}$ are:

$A\beta_{40}$: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV
$A\beta_{42}$: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

In the amyloid plaques, Aβ peptides form highly-ordered insoluble fibrillar aggregates, termed amyloid fibrils (Ohnishi and Takano, 2004). Besides, in the brain of AD patients, Aβ peptides can also form different types of soluble aggregates, such as small spherical aggregates, large spherical aggregates and small curved fibrils that are different from mature amyloid fibrils found in amyloid plaques (Dahlgren et al., 2002). An important role for of Aβ peptides in AD pathogenesis is also suggested by the fact that many mutations related to early-onset familial forms of AD increase total Aβ peptides levels and/or the relative concentration of $A\beta_{40}$ and $A\beta_{42}$, which is an important factor for AD since the latter peptide is more hydrophobic and hince aggregation prone (Borchelt et al., 1996; Eckman et al., 1997).

Neurofibrillary tangles (NFTs) are intracellular deposits mainly composed of the tau protein. Normally, tau interacts with tubulins and facilitates their assembly into microtubules, which act as cell skeleton and are important for cell health (Hernandez and Avila, 2007). Tau sequence can be divided into two major domains: a projection domain and a C-terminal microtubules binding domain. The latter contains similar but not identical repeats of 31-32 residues responsible for tubulin binding (Kosik, 1990). Six isoforms of tau are present in the brain, with 352 to 441 amino-acid-residues (Goedert et al., 1992). They differ from each other in the number of tubulin binding domains. Tau can undergo multiple types of post-translational modification. Its most important modification in AD is phosphorylation. When tau is hyperphosphorylated, it looses binding affinity to the microtubules, and starts to self-assemble into paired helical filaments (PHFs), which in turn deposit into NFTs (Alonso et al., 2001). Tau deposition as NFTs is also a characteristics of a subset of frontotemporal dementia. The fact that mutations in the gene encoding for tau are related to familial forms of frontotemporal dementia indicate an important role for tau in neurodegeneration (Heutink, 2000).

In early reports, spreading of NFTs in the brain had been observed to correlate with the progression of AD, suggesting a central role for tau in AD, but later studies rather led to the dominant idea that the aggregation of Aβ peptides in the brain is the primary event in AD and occurs before tau aggregation (Hardy and Selkoe, 2002). The amyloid cascade hypothesis for AD proposes that the imbalance between the production and clearance of Aβ peptides leads to Aβ aggregation and plaques formation, initiating a complex cascade of

events, such as synaptic failure, tau hyperphosphorylation and inflammation, that finally lead to AD (Tanzi and Bertram, 2005). So far, the correlation between Aβ amyloid fibrils and AD onset remains weak (Schmitz et al., 2004), wheareas soluble Aβ aggregates are believed to play the most important role in AD (Rahimi et al., 2008).

Despite the primary role of Aβ aggregates in AD, Aβ and tau have been demonstrated to interact with each other and most likely they cause AD through synergic effects. Indeed, the presence of Aβ peptides in transgenic mice influences the aggregation of tau into NFTs and, on the other hand, tau protein mediates Aβ toxicity (Lewis et al., 2001; Rapoport et al., 2002). Recently, a novel hypothesis linking Aβ and tau has been proposed on the basis of the observation that tau can also have a dendritic localization under physiological conditions and that tau-mediated postsynaptic targeting of the tyrosine protein kinase FYN confers Aβ toxicity (Ittner et al., 2010).

2.2 Structure of $A\beta_{42}$ amyloid fibrils

$A\beta_{42}$ amyloid fibrils are the most abundant aggregates in amyloid plaques (Roher et al., 1993). Under electron microscope, they look like unbranched filaments with a diameter of ~ 10 nm, and their length can reach up to several micrometer (Antzutkin et al., 2002) (Figure 1A). The X-ray diffraction pattern of aligned $A\beta_{42}$ amyloid fibrils shows two characteristic bands: one sharp band at ~ 4.7 Å position, and another diffused orthogonal band at ~ 10 Å (Kirschner et al., 1986) (Figure 1B).

Two structural models of $A\beta_{42}$ amyloid fibrils are available. In the first model, the structural information was obtained by using solid-state nuclear magnetic resonance (ssNMR) and solution NMR combined with the hydrogen/deuterium exchange (H/D-exchange) method. The ssNMR techinque provides distance restraints within amyloid fibrils while H/D-exchange monitored with solution NMR allows the identification of the regions of the protein sequence involved in the core structure of amyloid fibrils (Hoshino et al., 2002; Wang et al., 2008; Wang et al., 2010). The obtained model shows that: (a) in amyloid fibrils, amino-acid-residues 1-10 of $A\beta_{42}$ are unstructured, residues 11-22 and 31-42 form two β-strands (β1 and β2), and residues 23-30 form a bend (Figure 1C) (Ahmed et al., 2010; Olofsson et al., 2006); (b) through backbone hydrogen bonds, β1- and β2-strands interact with β1- and β2-strands in other $A\beta_{42}$ molecules and form two parallel β-sheets. All β-strands are perpendicular to the fibril axis, and both β-sheets are parallel to the fibril axis. This structure is called the cross-β-sheet structure (Figure 1E) (Balbach et al., 2002; Kirschner et al., 1986; Tycko, 2004); (c) the two β-sheets are connected by the intramolecular side chain interaction between Phe19 and Leu34, the salt bridge between Asp23 and Lys28, and the intermolecular side chain interaction between Gln15 and Gly37 (Figure 1C & 1F) (Ahmed et al., 2010). The distance between two adjacent β-strands is ~ 4.7 Å, and the distance between two β-sheets is ~ 10 Å, corresponding to the two characteristic bands observed in X-ray diffraction of amyloid fibrils.

In the second model, $^{35Mox}A\beta_{42}$ was used to produce amyloid fibrils. $^{35Mox}A\beta_{42}$ contains a methionine sulfoxide at position 35. This structural model was obtained by using solution NMR combined with H/D-exchange method and pairwise mutagenesis experiments. The resulting structure shows that: (a) in the fibrils, amino-acid-residues 1-17 of $^{35Mox}A\beta_{42}$ are unstructured, residues 18-26 and 31-42 form two β-strands (β1 and β2), and residues 27-30 form a bend (Figure 1D) (Luhrs et al., 2005); (b) β1- and β2-strands in different

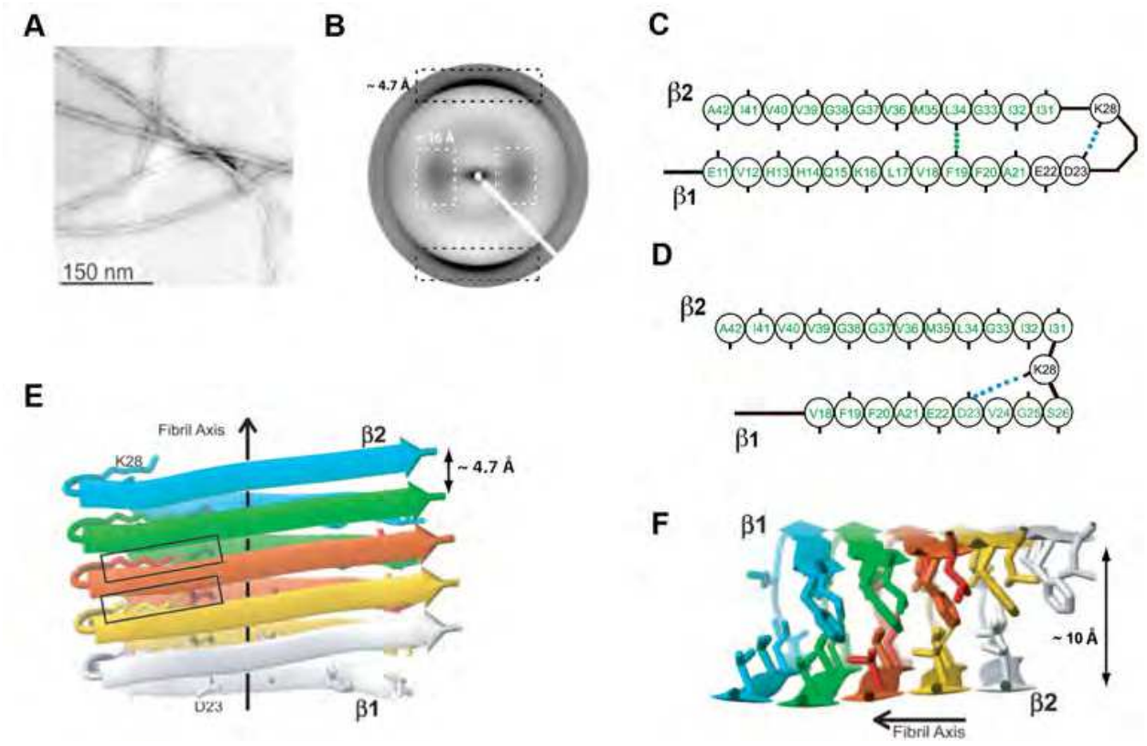

Fig. 1. Structural model of $A\beta_{42}$ amyloid fibrils. (A)Electron microscope image of $A\beta_{42}$ amyloid fibrils. (B) X-ray diffraction pattern of amyloid fibrils. (C) Illustration of $A\beta_{42}$ in amyloid fibrils. (D) Illustration of $^{Mox}A\beta_{42}$ in amyloid fibrils. (E) The cross-β-sheet structure of amyloid fibrils. (F) The side chain arrangement in amyloid fibrils. This figure is adapted from Fig 4 (Antzutkin et al., 2002), Fig 1 (Ahmed et al., 2010) and Fig 4 (Luhrs et al., 2005).

$^{35Mox}A\beta_{42}$ molecules interconnect and form the cross-β-sheet structure (Figure 1E); (c) the two β-sheets are connected by the intermolecular side chain interactions between Phe19 and Gly38, Ala21 and Val36, and the salt bridge between Asp23 and Lys28 (Figure 1D & 1F) (Luhrs et al., 2005). Compared to the structure of $A\beta_{42}$ amyloid fibrils, the structural difference of $^{35Mox}A\beta_{42}$ amyloid fibrils may reflect the effect of Met35 modification, or, it may reflect the a structural polymorphism of $A\beta_{42}$ amyloid fibrils.

2.3 Structure of $A\beta_{40}$ amyloid fibrils

$A\beta_{40}$ can form at least three types of amyloid fibrils with distinct morphology, which include striated-ribbon fibrils (Figure 2A), twisted-pair fibrils (Figure 2B) and brain-seeded fibrils (Figure 2C) (Paravastu et al., 2009; Petkova et al., 2005). The polymorphism of the fibrils is induced by the different conditions used to form them. In particular, incubation of $A\beta_{40}$ under agitation leads to the formation of straight-rod shaped protofilaments with a width of 6 nm, and these protofilaments associate laterally to form striated-ribbon fibrils. Quiescent growth rather leads to the formation of twisted-pair fibrils that are composed of single protofilaments (Petkova et al., 2005). Seeding $A\beta_{40}$ aggregation with purified fibrils from brain tissue of AD patients leads to the formation of curved and untwisted fibrils (Paravastu et al., 2009). All three $A\beta_{40}$ fibrils have different underlying molecular structures.

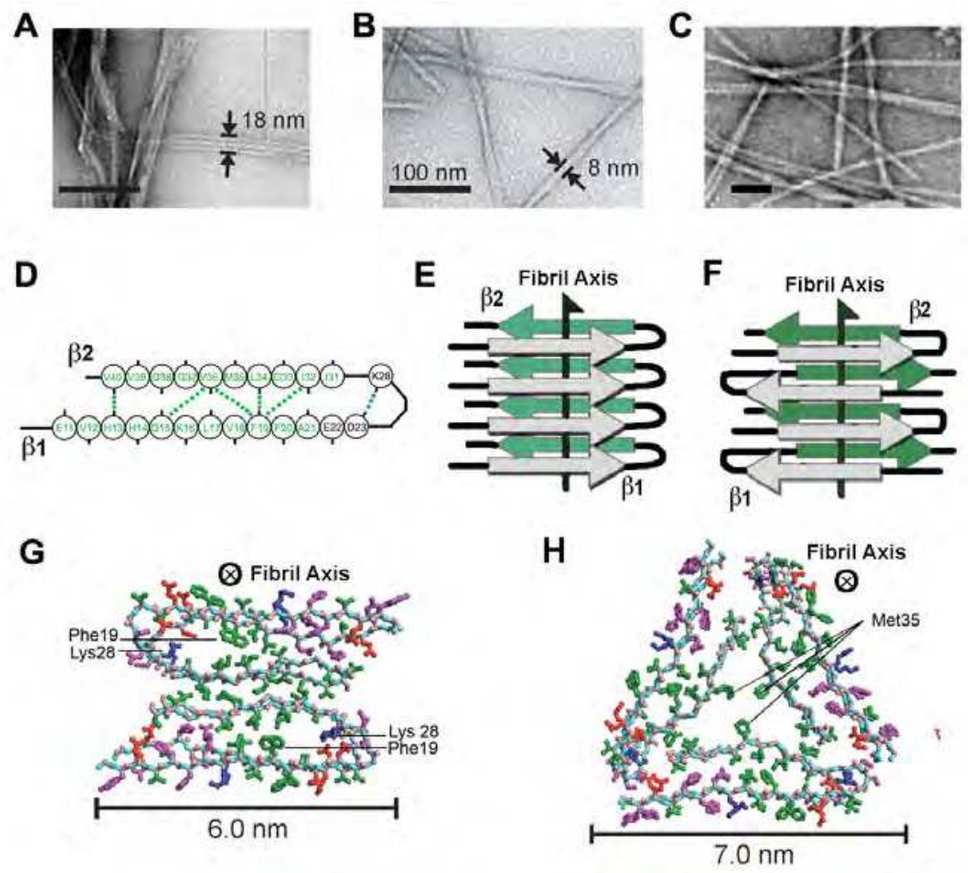

Fig. 2. Structural model of $A\beta_{40}$ amyloid fibrils. (A-C) Electron microscope images of striated-ribbon fibrils (A), twisted-pair fibrils (B) and brain-seeded (C) amyloid fibrils of $A\beta_{40}$. Scale bar = 100 nm. (D) Illustration of $A\beta_{40}$ in amyloid fibrils. (E) The parallel cross-β-sheet structure of $A\beta_{40}$ amyloid fibrils. (F) The antiparallel cross-β-sheet structure of $A\beta_{40}$–D23N amyloid fibrils. (G) One protofilament in the striated-ribbon fibrils is composed of two cross-β-sheet units. (H) One protofilament in the twisted-pair fibrils is composed of three cross-β-sheet units. This figure is adapted from Fig 4 (Paravastu et al., 2008), Fig 1 (Paravastu et al., 2009), and Fig 4 (Sawaya et al., 2007).

A structural model for striated-ribbon fibrils of $A\beta_{40}$ was obtained using solid-state NMR spectroscopy and electron microscopy. It shows that: (a) in amyloid fibrils, amino-acid-residues 1-9 of $A\beta_{40}$ are unstructured, residues 10-22 and 30-40 form two β-strands (β1 and β2), and residues 23-29 form a bend (Figure 2D) (Petkova et al., 2006); (b) through backbone hydrogen bonds, β1- and β2-strands interact with β1- and β2-strands in other $A\beta_{40}$ molecules and form two parallel β-sheets. All β-strands are perpendicular to the fibril axis, and both β-sheets are parallel to the fibril axis. This structure is considered as one cross-β-sheet unit (Figure 2E); (c) the two β-sheets are connected by side chain interactions, including the intermolecular interaction between Phe19 in one molecule and Leu34 in the neighboring molecule, and the intramolecular salt bridge between Asp23 and Lys28 (Petkova et al., 2006) (Figure 2D); (d) the individual protofilament in the striated-ribbon fibrils is composed of two cross-β-sheet units, which are two-fold

rotationally symmetric to each other along the fibril axis (Sciarretta et al., 2005; Tycko, 2010) (Figure 2G).

The structural model for twisted-pair fibrils of $A\beta_{40}$ contains similar cross-β-sheet unit as in striated-ribbon fibrils (Petkova et al., 2006) (Figure 2D & 2E). But the individual protofilament in the twisted-pair fibrils is composed of three cross-β-sheet units, which are three-fold rotationally symmetric to each other along the fibril axis (Sciarretta et al., 2005) (Figure 2H). This is the biggest difference from the protofilament composed of two cross-β-sheet units obtained for striated-ribbon fibrils. Other structural differences in twisted-pair fibrils include the absence of the salt bridge between Asp23 and Lys28, and different side chain interactions among cross-β-sheet units.

Brain-seeded fibrils have been produced to mimic amyloid fibrils of $A\beta_{40}$ formed *in vivo*. Due to experimental limit, currently it is not possible to measure the structure of fibrils directly from human tissue. As an alternative, fibrils extracted from the brain tissue of AD patients were used as seeds to produce large amount of isotopically labeled brain-seeded fibrils for structural study (Paravastu et al., 2009). These fibrils are thus likely to represent the amyloid fibrils formed *in vivo*. It has been shown that the ssNMR spectra of the brain-seeded fibrils are different from that of the striated-ribbon or twisted-pair fibrils, suggesting that brain-seeded fibrils have a different structure than previous identified structures of $A\beta_{40}$ fibrils (Tycko, 2010). However, a detailed structural model for brain-seeded fibrils is not yet available.

Disease-related mutants of $A\beta_{40}$ also form amyloid fibrils. The structure of the amyloid fibrils formed by the Iowa mutant ($A\beta_{40}$-D23N), associated with early-onset familial AD, is composed of a different cross-β-sheet unit, in which β1- and β2-strands of $A\beta_{40}$-D23N molecules are interconnected and form two antiparallel β-sheets (Tycko et al., 2009) (Figure 2F).

2.4 Structure of paired helical filaments of tau

As mentioned before, neurofibrillary tangles (NFTs) deposits are composed of paired helical filaments (PHFs) of tau protein (Wischik et al., 1988) (Figure 3A). Although the high-resolution structure of tau PHFs has not been solved yet, several structural models are available based on various experimental data. Fourier transform infrared (FT-IR) spectroscopy experiments show that tau PHFs are composed of a large amount of random coil structure together with some α-helical and β-sheet structure (Sadqi et al., 2002). It has also been found that the residues 265-338 in PHFs (PHF43) are protected against proteases digestion, and PHF43 alone can also form amyloid fibrils (von Bergen et al., 2000). Further investigation shows that the amino-acid-residues 306-311 (sequence: VQIVYK) (PHF6) within PHF43 are responsible for PHF43 amyloid fibrils formation. PHF6 alone forms amyloid fibrils that resemble tau PHFs (von Bergen et al., 2005; von Bergen et al., 2000). X-ray microcrystallography data show that PHF6 forms parallel β-sheets in amyloid fibrils, and the β-sheets are connected by side chains that form steric zippers (Sawaya et al., 2007) (Figure 3B & 3C).

One structural model of tau PHFs suggests that they contain one cross-β sheet composed of PHF6 while the rest of the sequence is structurally disordered or contains some α-helical structure (von Bergen et al., 2005) (Figure 3D). Another structural model suggests that PHFs may contain an additional cross-β sheet, which is composed of amino-acid-residues 272-289 (S2) (Figure 3E). This model is based on the finding that isolated S2 can also form amyloid fibrils under certain conditions (Margittai and Langen, 2006).

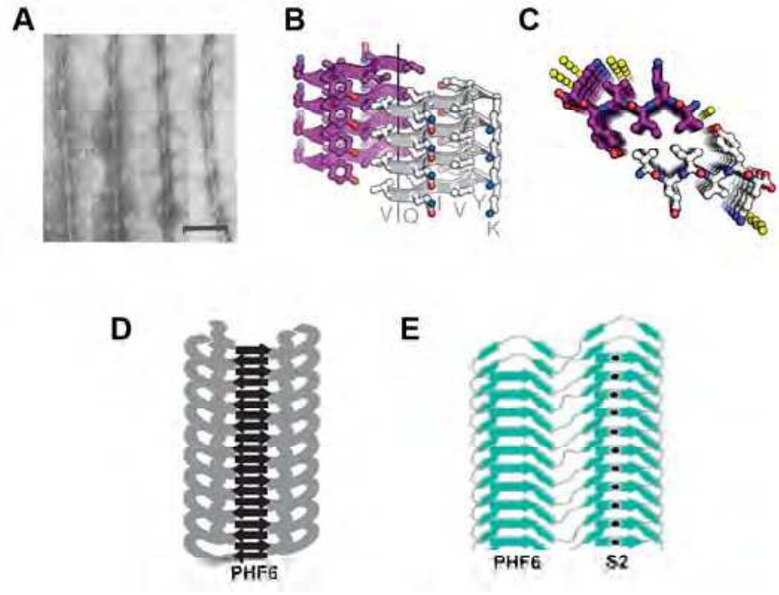

Fig. 3. Structural model of paired helical filaments (PHFs) of tau. (A) Electron microscope image of tau PHFs. Scale bar = 100 nm. (B-C) The side and top view of the cross-β-sheet structure of PHF6 amyloid fibrils. (D) The proposed structural model for tau PHFs, in which PHF6 form the cross-β-sheet structure, and the rest of tau PHFs is not/least structured. (E) Another proposed structural model for tau PHFs, in which PHF6 and S2 form two cross-β-sheet structures. This figure is adapted from Fig 1B (Wischik et al., 1988), Fig 2 & 3 (Sawaya et al., 2007), Fig 6 (von Bergen et al., 2005) and Fig 4A (Margittai and Langen, 2006).

2.5 Soluble Aβ aggregates: Mechanism of toxicity and structure

Increasing evidence suggest that the pathogenic agents in amyloid-related diseases are the transient, pre-fibrillar Aβ assemblies preceding the formation of mature fibrils, whereas amyloid fibrils rather represent the protective end state of the protein misfolding event (Sakono and Zako, 2010; Rahimi et al., 2008; Chiti and Dobson, 2006). This is particularly valid in the case of AD, where a weak correlation exists between cognitive impairment and amyloid plaques formation (Terry et al., 1991) and, in transgenic mouse lines used to model AD, synaptotoxicity is observed before and/or independently of amyloid plaque formation (Mucke et al., 2000). Instead, the levels of soluble Aβ oligomers in AD patients correlate much better with the onset of neurodegeneration (for a review on the oligomer-toxicity hypothesis see Klein et al., 2001). Remarkably, a mutated form of Aβ showing enhanced oligomerization but no fibrillation was identified in a Japanese pedigree of AD patients with little deposition of fibrillar amyloid (Tomiyama et al., 2008).

Despite their relevance to disease, a detailed structural and functional characterization of amyloid pre-fibrillar species is considerably hampered by their transient and heterogeneous nature. In addition, in the case of Aβ pre-fibrillar species, further complications arise due to the various methods used to produce the synthetic peptide/s used in the experiments and the fact that the $A\beta_{40}$ and $A\beta_{42}$ are more aggregation prone than other amyloidogenic peptides and protein and form ensembles of oligomeric species already upon dissolution of the lyophilized peptides in aqueous buffer (Teplow, 2006). Describing in detail all types of Aβ oligomers reported in the literature up to date would be beyond the scopes of this book chapter; we will

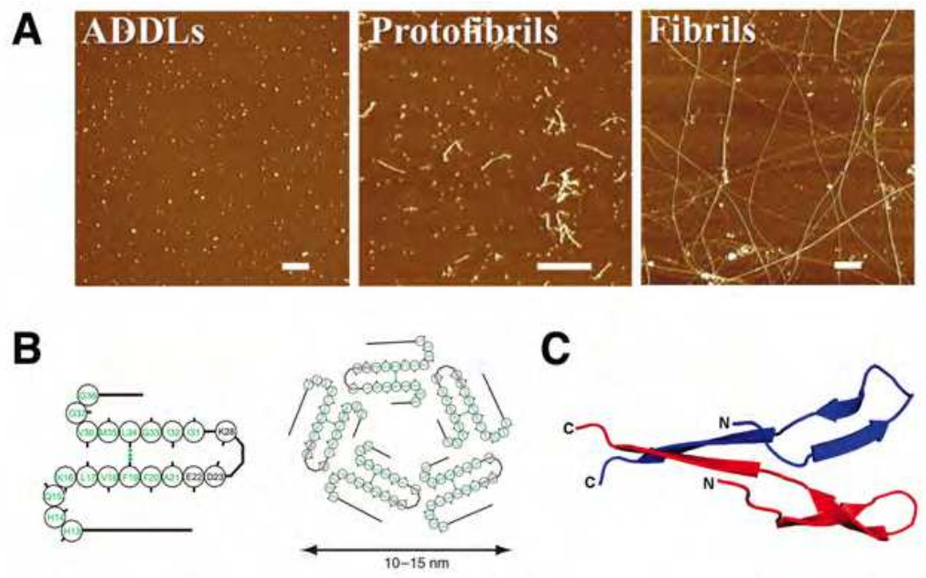

Fig. 4. Morphology and conformation of different aggregates formed by $A\beta_{42}$ under different incubation conditions. (A) Atomic force microscopy images of ADDLs (left), protofibrils (middle) and mature fibrils (right). (B) Schematic model of $A\beta_{42}$ monomer within a spherical pentameric aggregated from solid-state magic angle spinning data. Light blue squares connect Phe19 and Leu34. The areas showing little protection from hydrogen-deuterium exchange are coloured in green. (C) Model for the structure of $A\beta_{42}$ preglobulomers obtained from solution state NMR. According to this model, preglobulomers are formed by dimer units adopting mixed parallel and antiparallel structure. This figure is adapted from Fig 2 & 4 (Klein et al., 2001), and Fig 3 (Yu et al., 2009).

thus limit our description to a summary of the most consolidated and recent information on the species thought to play an important role in AD disease. Although all these species are generally referred to as "Aβ oligomers", the readers should keep in mind that they may differ in structure, pathway of formation and mechanism of toxicity as well.

2.5.1 Aβ pre-fibrillar aggregates formed in vitro

In studies performed *in vitro*, Aβ peptides have been found to form various types of aggregates having spherical morphology. Cross-linking experiments show that $A\beta_{40}$ and $A\beta_{42}$ preparations differ in their oligomers distributions, with the former peptide forming roughly equimolar mixtures of aggregates ranging from dimers to tetramers, whereas the latter preferentially forms pentamers/hexamers (termed paranuclei), which self-associate into dodecamers and octadecamers (Bitan et al., 2001; Bitan et al., 2003). These larger species appear not to be artifacts of the cross-linking technique since $A\beta_{42}$ stable dodecamers formed by stacked hexamer units have been detected also in the absence of any cross-linking (Bernstein et al., 2009). Here we will summarize present information on the toxic effect and the conformational properties of the most investigated oligomeric species.

Aβ-derived diffusible ligands (ADDLs) are slowly sedimenting aggregates observed only in preparations of synthetic $A\beta_{42}$ peptides; they appear as globular species with 3-8 nm height (Figure 4A) and they are believed to be composed of 3-24 monomers (Oda et al., 1995;

Lambert et al., 1998; Klein et al., 2002; Chromy et al., 2003). Their pathological relevance is supported by the detection of aggregates (in particular dodecamers) immunoreactive to anti-ADDLs antibodies in the brain and cerebrospinal fluid of AD patients (Gong et al., 2003; Georganopoulou et al., 2005). ADDLs are potent neurotoxins: they induce the death of principal neurons of the hippocampus and, in rat hippocampal brain slices, they inhibit long-term potentiation (LTP) but not long-term depression (LTD), two forms of synaptic plasticity implicated in learning and memory (Lambert et al., 1998; Wang et al., 2002). Binding of ADDLs to hippocampal neurons appears to occur at the postsynaptic termini of excitatory synapses and stimulates aberrant expression of the activity-regulated cytoskeletal-associated (Arc) protein (implicated in long-term memory formation), tau hyperphosphorylation, synapses deterioration and loss, and enhanced formation of reactive oxygen species (ROS) thought to play a major role in the pathogenesis of AD (Lacor et al., 2004; Guzowski 2002; Hsieh et al., 2006; De Felice et al., 2007a; De Felice et al., 2007b; Lacor et al., 2007; Shankar et al., 2007). Monoclonal antibodies targeting ADDLs can prevent their aberrant binding and synaptotoxicity (Lambert et al., 2007). *In vivo*, insulin signaling and the cellular prion protein (PrP^C) have been suggested to mediate the toxicity of ADDLs, even if in the latter case an effective involvement in AD is currently under debate (De Felice et al., 2009; Laurén et al., 2009; Gimbel et al., 2010; Calella et al., 2010; Caetano et al., 2011). Despite the large amount of data on the toxic effect of ADDLs, still little is known about their structure; mainly because they are heterogeneous mixtures of oligomers of different sizes. The structure of neurotoxic $A\beta_{42}$ pentamers has been recently investigated (Figure 4B). Hydrogen-deuterium exchange measurements indicate that the first nine residues are solvent exposed, as well as residues 13-15, 25-29 and 37-38 that presumably form solvent-accessible turns (Ahmed et al., 2010). Solid-state magic angle spinning measurements show the lack of parallel, in-register β-sheets, but the C-terminal regions of the constituting monomers are packed together and adopt a conformation similar to that observed in the mature fibrils (U-shaped hairpin structure), with Phe19 in contact with Leu34 (Ahmed et al., 2010).

Globular oligomers having a size of 4-5 nm, composed of twelve monomeric units and forming independently from fibril formation have been obtained from $A\beta_{42}$ samples incubated at high concentration (400 μM) in the presence of sodium dodecyl sulfate (SDS) or fatty acids; such species are known as Aβ globulomers (Barghorn et al., 2005; Gellermann et al., 2008; Yu et al., 2009). Similarly to ADDLs, globulomers selectively bind to neuronal cells, inhibit LTP in hippocampal brain slices and their toxicity can be rescued with a globulomer-specific antibody (Barghorn et al., 2005; Hillen et al., 2010). Moreover, antibodies against globulomers detect immunoreactive species in tissue sections from the brains of AD patients and human APP transgenic mice (Barghorn et al., 2005). In contrast to ADDLs preparations, globulomer samples are stable and homogeneous; thus can be used for structural studies. Protease digestion, cross-linking and antibody binding data suggest that residues 1-20 of $A\beta_{42}$ are accessible to the solvent while the rest of the sequence forms the core of the aggregates. Solution NMR data on smaller pre-globulomeric species (tetramers) indicate that they are composed of dimer repeating units (Figure 4C) with residues 18-23 and 28-40 forming β-structure in mixed parallel and antiparallel β-sheets (Yu et al., 2009). Finally, hydrogen-deuterium exchange measurements indicate that globulomers and pre-globulomers have similar secondary structures and that the former species result from association of pre-globulomeric units.

$A\beta_{40}$ and $A\beta_{42}$ can also form, off-pathway from fibril formation, large globular aggregates with little or no cross-β structure (they do not bind the amyloid-specific dye thioflavin T) termed amylospheroids (ASPDs) (Hoschi et al., 2003; Matsumura et al., 2011). ASPDs of 10-15 nm diameter are potent neurotoxins to primary cultures from rat septum regions; the most toxic species being 32-mers (Hoschi et al., 2003; Matsumura et al., 2011). Antibodies generated to recognize ASPDs stain AD brains and have been used to immunoisolate ASPDs directly from AD and dementia with Lewy bodies brains (Noguchi et al., 2009). Levels of ASPDs in AD patients correlate with the severity of disease and the immunoprecipitated species induce degeneration on neurons *in vitro* (Noguchi et al., 2009). Chimon et al. investigated the structure of globular $A\beta_{40}$ aggregates formed by at least 150 monomers that impair the viability of cultured PC-12 cells and have a diameter similar to that of ASPDs, but, in contrast to ASPDs, they are rich in β-sheet structure and are on-pathway intermediates of fibril formation (Chimon and Ishii, 2005; Chimon et al., 2007). Solid-state NMR spectra show that these assemblies have ordered parallel, in regisister, β-stucture, extremely similar to that of mature $A\beta_{40}$ fibrils, particularly in the hydrophobic core and C-terminal regions (Chimon and Ishii, 2005; Chimon et al., 2007).

Aβ protofibrils (Figure 4A) also belong to the wide range of possible Aβ pre-fibrillar aggregates. These are soluble aggregates formed by both $A\beta_{40}$ and $A\beta_{42}$ *via* association of smaller globular units, with an apparent mass higher than 100 kDa and appearing as curved fibril-like structures of 3-8 nm diameter and less than 200 nm length (Harper et al., 1997; Walsh et al., 1997; Walsh et al., 1999; Harper et al., 1999). PFs formed *in vitro* alter the viability of cultured rat primary cortical neurons and induce neuronal injury, impaired electrophysiological activities and neuronal loss (Walsh et al., 1999; Hartley et al., 1999). The fact that the early-onset AD-related Arctic mutation enhances PF formation suggests that these species might be relevant for the disease (Nilsberth et al., 2001). Moreover, 4-hydroxy-2-nonenal (HNE), a metabolite of oxidative stress found to exist at increased concentrations in AD patients and to co-localize with Aβ deposits, causes *in vitro* accumulation of $A\beta_{40}$ PFs and prevents their conversion into mature fibrils, thus leading to sustained neurotoxicity to cultured cells (Sayre et al., 1997; Siegel et al., 2007; Johansson et al., 2007). PFs appear transiently and are considered to be the direct precursors of long and rigid amyloid fibrils (Harper et al., 1997; Walsh et al., 1997). Indeed, they already possess a high content of β-sheet structure and the capability to bind the WO1 amyloid fibrils-specific antibody (Williams et al., 2005). Nonetheless, they are still metastable and can dissociate into low molecular weight oligomers (Walsh et al., 1999; Harper et al., 1999). $A\beta_{40}$ PFs possess a stable structural core (Kheterpal et al., 2003). Proline substitution and hydrogen-deuterium exchange experiments on $A\beta_{40}$ PFs stabilized by a small molecule show that the first N-terminal 14-19 residues and the C-terminal 3-5 residues are not involved in the formation of their structural core and that, in comparison to mature fibrils, PFs are less structured in the fragment spanning residues 20-34 (Williams et al., 2005; Kheterpal et al., 2006).

Protofibrillar aggregates formed by the E22G (Arctic mutation) mutant of $A\beta_{40}$ can also adopt annular structures (Lashuel et al., 2002). These species have a pore-like appearance with an outer diameter of 7-10 nm and an inner diameter of 1.5-2.0 nm, and appear to be composed of 40-60 peptide molecules (Lashuel et al., 2002). Structures similar to annular PFs have been observed upon incorporation of different Aβ peptides into artificial and natural membranes and have been hypothesized to serve as calcium channels that mediate Aβ-induced toxicity in AD (Arispe et al., 2007). However, the formation of Aβ pores is still

debated since spherical Aβ aggregates can increase membrane permeability and intracellular calcium levels without any evidence of discrete channel of pore formation (Kayed et al., 2004; Demuro et al., 2005). Antibodies specific for annular protofibrils, but not spherical oligomers and fibrils, also recognize heptameric alpha-hemolysin pores, suggesting that the antibody recognizes an epitope that is specific for a β barrel structural motif (Kayed et al., 2009).

2.5.2 Cell-derived pre-fibrillar aggregates

Oligomerization studies performed *in vitro* with synthetic peptides have various limitations: 1) the usually employed peptide concentrations are in the micromolar range, whereas Aβ concentration *in vivo* is rather in the nanomolar or even subnanomolar range (Seubert et al., 1992; Suzuki et al., 1994; Tabaton et al., 1994); 2) *in vitro*, peptides of specified lengths are used, whereas numerous Aβ species, with extensive amino and carboxyl terminal heterogeneity, are usually present *in vivo* (reviewed in Golde and Younkin, 1996); 3) aggregation is usually examined under non-physiological conditions, with the peptides solubilized in organic solvents and then diluted in water or aqueous buffers free of other proteins, macromolecules or small molecules. These limitations imply that pathologically relevant aggregates may differ substantially from those produced *in vitro*. Thus, considerable effort has been recently spent in isolating and characterizing Aβ oligomers formed *in vivo*. Detailed structural characterization of cell-derived oligomers is lacking due to their low concentration, but their ability to cause toxicity and cognitive impairment has been deeply investigated.

Small SDS-stable oligomers have been isolated at nanomolar concentration from the medium of caltured cells, human cerebrospinal fluid, APP transgenic mouse brain and human brain (reviewed in Selkoe 2008; Rahimi et al., 2008). Aβ dimers have been detected and proved to form intracellularly in primary human neurons and in both neuronal and non-neural cell lines (Walsh et al., 2000). Stable dimers and trimers of Aβ isolated from the neuritic plaques of AD patients and leptomeningeal vessels compromise the viability of cultured rat hippocampal neuron glia cells through a microglia-dependent mechanism and Aβ dimers from the cerebral cortex of AD patients can also impair synaptic plasticity and memory in rats (Roher et al., 1996; Shankar et al., 2008). Synaptic dysfunction is also caused by dimers from *ex vivo* human cerebrospinal fluid (Klyubin et al., 2008).

Oligomers (in particular trimers) secreted in the medium of Chinese Hamster Ovary cells over-expressing the β-amyloid precursor protein inhibit hippocampal LTP, decrease the density of dendritic spines, impair cognitive function in rats and inhibit the remodeling of synapses, a prerequisite for memory consolidation (Walsh et al., 2002; Cleary et al., 2005; Townsend et al., 2006; Shankar et al., 2007; Poling et al., 2008; Freir et al., 2010). Compounds that inhibit oligomers formation or antibodies that bind to the oligomers rescue their toxic effects (Walsh et al., 2005a; Walsh et al., 2005b).

A soluble, SDS-stable dodecamer termed Aβ*56 was found in the brain of middle-aged APP transgenic mice carrying a familial AD-linked double mutation (Lesné et al., 2006). Levels of Aβ*56 dodecamers in middle-aged APP transgenic mice correlate with cognitive deficits and administration of purified Aβ*56 to young rats disrupts their memory (Lesné et al., 2006). In a recent study, synthetic $A\beta_{42}$-derived oligomers, cell- and brain-derived low molecular weight oligomers, and Aβ*56 have been compared for their ability to produce deficits in learned behavior of rats, finding that dimers derived from the culture medium of APP transgenic cells are the most potent neurotoxins (Reed et al., 2009).

Oligomers of modified forms of N-terminally truncated Aβ peptides having pyroglutamate as first residue in the sequence (pE-Aβ) have also been detected in the cerebral cortex of AD patients (Piccini et al., 2005). pE-Aβ peptides are believed to play an important role in the pathogenesis of AD because they are highly abundant in the brains of AD patients and they are major constituents of the amyloid plaques (for a recent review, see Gunn et al., 2010). *In vitro* studies indicate that pE-Aβ peptides are more aggregation prone and neurotoxic than full length Aβ (Harigaya et al., 2000; Russo et al., 2002). When a novel mouse monoclonal antibody specifically targeting low molecular weights oligomers of pE-Aβ is used to passively immunize transgenic mice, plaque load and Aβ levels are reduced and behavioral deficits are normalized, suggesting that pE-Aβ oligomers are valuable targets for AD diagnosis and therapeutic intervention (Wirths et al., 2010).

3. Conclusion

In this chapter, we reviewed current knowledge of the structure and toxicity of different protein aggregates that are involved in Alzheimer's disease (AD). Currently, two structural models are available for $A\beta_{42}$ amyloid fibrils. Both models show a parallel cross-β-sheet structure. But in the first model, amino-acid-residues 11-22 and 31-42 adopt β-strand conformation ($A\beta_{42}$); wheareas in the second model, residues 18-26 and 31-42 adopt β-strand conformation ($^{35Mox}A\beta_{42}$). For $A\beta_{40}$ amyloid fibrils, structural polymorphism has been observed. The striated-ribbon fibrils, twisted-pair fibrils and brain-seeded fibrils of $A\beta_{40}$ all have different morphology and molecular structure. Tau PHFs also contain a cross-β-sheet structure, in which amino-acid-residues 306-311 and possibly 272-289 adopt the β-strand conformation. The rest of tau is either disordered or contains small amount of α-helical structure.

Recent studies suggest that the soluble Aβ aggregates (pre-fibrillar aggregates), instead of Aβ amyloid fibrils and tau PHFs, correlate more closelly with neuron degeneration in AD. Several types of Aβ pre-fibrillar aggregates have been described. Their common toxicity may be due to multiple reasons such as the heterogeneity of Aβ aggregates preparations, most likely containing different amounts of monomeric, oligomeric and fibrillar Aβ rather than single species, and the fact that all these species may share a "misfolded" nature, meaning that they most likely expose on their surfaces repetitive clusters and/or arrays of groups that enable their interaction with different targets. It has been recently suggested that it's the ongoing Aβ polymerization process involving the elongation and growth of pre-fibrillar aggregates such as protofibrils, rather than the formation of a specific toxic oligomeric species to be responsible for Aβ toxicity in AD (Jan et al., 2011). Similarly to what has been proposed for another amyloid forming peptide, the cooperative binding of Aβ peptides to cell membranes, and the growth and elongation of Aβ aggregates at the level of the membrane could result in changes in membrane curvature and weakened lipid packing, leading to membrane permeabilization and toxicity (Engel et al., 2008; Friedman et al., 2009). Further studies are therefore necessary to clearly elucidate the structural properties and mechanism of toxicity of Aβ aggregates in order to develop efficient therapeutic strategies for AD.

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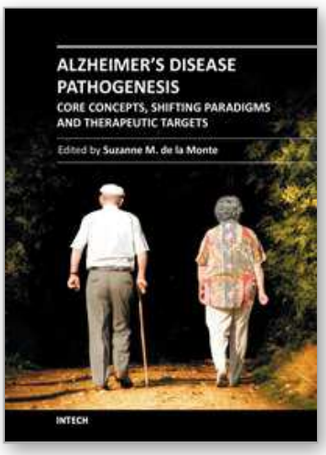

Alzheimer's Disease Pathogenesis-Core Concepts, Shifting Paradigms and Therapeutic Targets

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Alzheimer's Disease Pathogenesis: Core Concepts, Shifting Paradigms, and Therapeutic Targets, delivers the concepts embodied within its title. This exciting book presents the full array of theories about the causes of Alzheimer's, including fresh concepts that have gained ground among both professionals and the lay public. Acknowledged experts provide highly informative yet critical reviews of the factors that most likely contribute to Alzheimer's, including genetics, metabolic deficiencies, oxidative stress, and possibly environmental exposures. Evidence that Alzheimer's resembles a brain form of diabetes is discussed from different perspectives, ranging from disease mechanisms to therapeutics. This book is further energized by discussions of how neurotransmitter deficits, neuro-inflammation, and oxidative stress impair neuronal plasticity and contribute to Alzheimer's neurodegeneration. The diversity of topics presented in just the right depth will interest clinicians and researchers alike. This book inspires confidence that effective treatments could be developed based upon the expanding list of potential therapeutic targets.

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