CellPress

Neuron
Review

Amyloid Polymorphism: Structural Basis and Neurobiological Relevance

Robert Tycko[1,*]
[1]Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0520, USA
*Correspondence: robertty@mail.nih.gov
http://dx.doi.org/10.1016/j.neuron.2015.03.017

Our understanding of the molecular structures of amyloid fibrils that are associated with neurodegenerative diseases, of mechanisms by which disease-associated peptides and proteins aggregate into fibrils, and of structural properties of aggregation intermediates has advanced considerably in recent years. Detailed molecular structural models for certain fibrils and aggregation intermediates are now available. It is now well established that amyloid fibrils are generally polymorphic at the molecular level, with a given peptide or protein being capable of forming a variety of distinct, self-propagating fibril structures. Recent results from structural studies and from studies involving cell cultures, transgenic animals, and human tissue provide initial evidence that molecular structural variations in amyloid fibrils and related aggregates may correlate with or even produce variations in disease development. This article reviews our current knowledge of the structural and mechanistic aspects of amyloid formation, as well as current evidence for the biological relevance of structural variations.

Aberrant aggregation of certain peptides and proteins causes many neurodegenerative diseases, including Alzheimer disease (AD), Parkinson disease (PD), transmissible spongiform encephalopathies (TSEs), Huntington disease, frontotemporal dementia, and amyotrophic lateral sclerosis. The association of protein aggregation with neurodegeneration motivates efforts in many laboratories to elucidate the detailed molecular aspects of protein aggregation, including mechanisms and pathways of aggregation and molecular structures of the aggregated states of the relevant peptides and proteins.

The thermodynamic endpoints of protein aggregation (i.e., the most stable self-assembled states under typical conditions) are often filamentous assemblies called amyloid fibrils. Figure 1 shows examples of amyloid fibrils prepared in vitro. Amyloid fibrils are inherently noncrystalline, insoluble materials, making it difficult to determine their internal molecular structures by traditional methods for high-resolution structure determination, especially X-ray crystallography and multidimensional nuclear magnetic resonance (NMR) spectroscopy. Over the past 10–15 years, progress has been made on the amyloid structure problem through the application of less traditional methods. One of the most powerful methods for structural studies of amyloid fibrils is solid state NMR (ssNMR), which means a set of NMR techniques that are designed specifically for applications to molecular assemblies that are not soluble and not necessarily crystalline (Tycko, 2011). Other methods that have contributed to recent progress include electron paramagnetic resonance (EPR) (Margittai and Langen, 2008), electron microscopy (Goldsbury et al., 2005; Serpell and Smith, 2000), cryo-electron microscopy (cryo-EM) (Jiménez et al., 1999; Meinhardt et al., 2009), and hydrogen/deuterium exchange measurements (Kheterpal et al., 2006; Lührs et al., 2005; Olofsson et al., 2007; Toyama et al., 2007). In addition, X-ray crystallographic studies of peptides in amyloid-like crystals have been quite valuable (Nelson et al., 2005; Sawaya et al., 2007). Detailed structural information at the molecular level is essential for a full understanding of the amyloid formation process, for understanding of biological effects arising from structural variations, and for the rational development of compounds that can inhibit amyloid formation (Estrada and Soto, 2007) or bind specifically to amyloid fibrils for diagnostic imaging (Fleisher et al., 2011; Klunk et al., 2004).

A fundamentally important property of amyloid fibrils is their ability to amplify and propagate their own structures by recruitment of additional protein molecules from their surroundings. Recent studies in a number of laboratories highlight the biomedical significance of amyloid self-propagation in neurodegenerative diseases (Eisele et al., 2010; Frost et al., 2009a; Iba et al., 2013; Kfoury et al., 2012; Langer et al., 2011; Luk et al., 2012a, 2012b; Meyer-Luehmann et al., 2006; Sanders et al., 2014; Stöhr et al., 2012, 2014; Volpicelli-Daley et al., 2011; Watts et al., 2014). Amyloid self-propagation is a likely underlying mechanism for the infectious nature of prion protein (PrP) particles in TSEs (Collinge and Clarke, 2007; Prusiner, 2013). Thus, the self-propagation of amyloid fibrils formed by proteins other than PrP is now often called ''prion-like'' behavior.

If one accepts the definition that prions are disease-causing, infectious, proteinaceous particles, then in vitro self-propagation should not be called ''prion-like.'' Other issues regarding this term, such as the distinction between propagation within a single organism and transmission between organisms and the distinction between naturally occurring and artificially induced transmission, have been discussed by others (Ashe and Aguzzi, 2013; Hardy and Revesz, 2012).

Polymorphism is another important property of amyloid fibrils (Tycko, 2014). As shown in Figure 1 for the amyloid-β (Aβ) peptide associated with AD and the α-synuclein (α-syn) protein associated with PD, fibrils formed by a single peptide or protein can be polymorphic, i.e., can exhibit multiple distinct

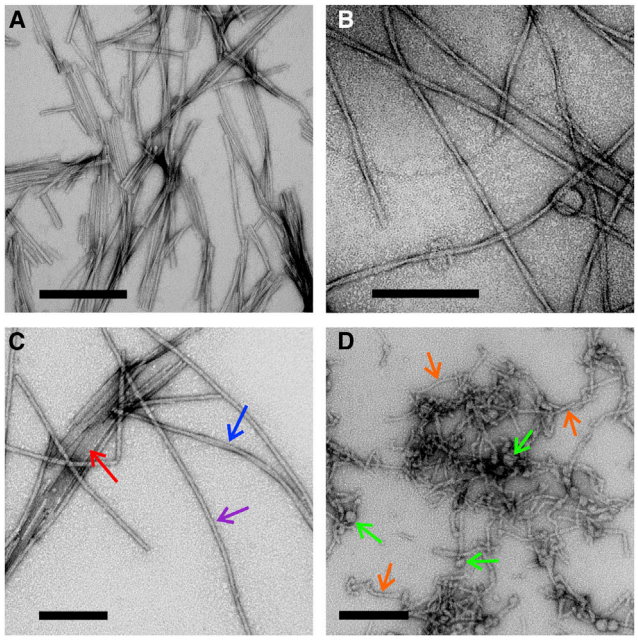

Figure 1. Polymorphism of Amyloid Fibrils and Aggregation Intermediates, as Seen in Transmission Electron Microscope Images with Negative Staining

(A) Synthetic Aβ40 fibrils with "striated ribbon" morphologies.
(B) Synthetic Aβ40 fibrils with "twisted" morphologies.
(C) Recombinant α-synuclein fibrils with striated ribbon (red arrow), twisted (blue arrow), and rod-like (purple arrow) morphologies.
(D) Synthetic Aβ40 aggregation intermediates with protofibrillar (orange arrows) and nonfibrillar (green arrow) morphologies, prepared by quiescent incubation of a 100 μM peptide solution at 24°C and pH7.4 for 36 hr. Scale bars represent 200 nm.

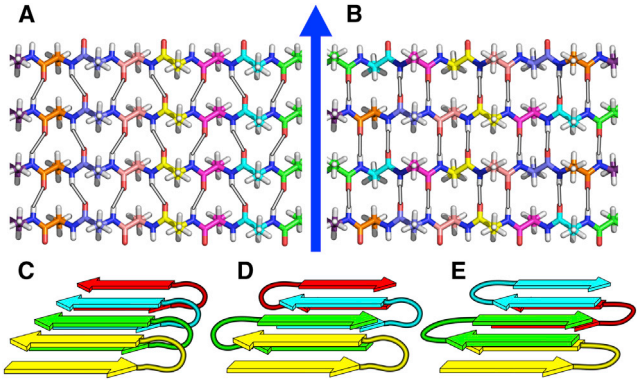

Figure 2. Varieties of Cross-β Structures in Amyloid Fibrils

(A) An "in-register" parallel cross-β structure, in which β strand segments from adjacent protein or peptide molecules align in parallel and with no shift of their amino acid sequences (represented by the varying colors of carbon atoms) relative to one another.
(B) An antiparallel cross-β structure. Silver bars indicated hydrogen bonds between backbone carbonyl and backbone amide groups. The fibril growth direction is indicated by the blue arrow.
(C–E) Schematic representations of cross-β structures that could be formed by a peptide that contains two separate β strand segments, separated by a loop or turn segment. Colors indicate successive copies of the same peptide molecule. From left to right, the structures are a double-layered, in-register parallel cross-β unit, a double-layered antiparallel cross-β unit, and a double-layered antiparallel β-hairpin structure.

appearances in transmission electron microscopy (TEM) images. Although in principle amyloid polymorphs could merely be different bundled arrangements of the same basic amyloid "protofilament" structure, in fact ssNMR measurements clearly show that amyloid polymorphs contain distinct molecular structures, and that each molecular structure can propagate itself (Bousset et al., 2013; Frederick et al., 2014; Gath et al., 2014; Paravastu et al., 2008; Petkova et al., 2005). Self-propagating, molecular-level polymorphism of amyloid fibrils is a likely underlying mechanism for the occurrence of distinct prion strains in TSEs (Collinge and Clarke, 2007; Safar et al., 1998; Toyama and Weissman, 2011; Wickner et al., 2010). As discussed below, recent studies suggest that neurodegenerative diseases such as AD, PD, and tauopathies (involving aggregation of the tau protein) may exhibit variations in clinical characteristics and neuropathology that are attributable to amyloid polymorphism, in analogy to prion strains.

This article reviews recent work on amyloid formation, molecular structure, and polymorphism that relates to the issues described above. Several excellent reviews of relevant experiments in cell cultures and animal models have appeared recently (Ashe and Aguzzi, 2013; Guo and Lee, 2014; Hardy and Revesz, 2012; Jucker and Walker, 2013; Walker et al., 2013). Therefore, this article focuses primarily on physical and structural properties of amyloid fibrils and related aggregates that could be the basis for the biological phenomena. Although this article emphasizes work on Aβ, α-syn, and tau, similar ideas apply to other proteins whose aggregation is associated with neurodegeneration. It should also be noted that amyloid formation has been shown to be a biologically functional, presumably evolved, property of a variety of proteins (Pham et al., 2014).

Principles of Amyloid Formation from In Vitro Studies

Amyloid fibrils are typically 5–15 nm in width, unbranched, straight over length scales approaching 1 micron, and often many microns long. Measurements on bundles of aligned fibrils by X-ray fiber diffraction first revealed that they contain "cross-β" structures (Eanes and Glenner, 1968), which are ribbon-like β sheets in which β strand segments run approximately perpendicular to the fibril growth direction and hydrogen bonds between β strands are approximately parallel to the growth direction (Sunde et al., 1997). Figure 2 shows atomic models of ideal cross-β structures, which can involve either parallel or antiparallel alignments of adjacent β strands. Figure 2 also shows examples of double-layered cross-β structures, as may exist in fibrils formed by peptides or proteins with two separate β strand segments. As discussed below, a single amyloid fibril can contain several cross-β subunits, with two or more β sheet layers within each subunit. The spacing between β strands in a β sheet is always 0.46–0.48 nm. Therefore, a one micron length of amyloid fibril typically contains thousands of protein molecules, with the exact number depending on the number of cross-β subunits, the number of β strand segments contributed to each cross-β subunit by one molecule, and the number of β sheet layers within each subunit.

In vitro, amyloid fibrils are readily prepared from purified synthetic peptides or recombinant proteins by incubation in simple