Review

Recent progress in understanding Alzheimer's β-amyloid structures

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The formation of amyloid fibrils, protofibrils and oligomers from the β-amyloid (Aβ) peptide represents a hallmark of Alzheimer's disease. Aβ-peptide-derived assemblies might be crucial for disease onset, but determining their atomic structures has proven to be a major challenge. Progress over the past 5 years has yielded substantial new data obtained with improved methodologies including electron cryo-microscopy and NMR. It is now possible to resolve the global fibril topology and the cross-β sheet organization within protofilaments, and to identify residues that are crucial for stabilizing secondary structural elements and peptide conformations within specific assemblies. These data have significantly enhanced our understanding of the mechanism of Aβ aggregation and have illuminated the possible relevance of specific conformers for neurodegenerative pathologies.

Structural diversity of β-amyloid aggregates

The β-amyloid (Aβ) peptide occurs naturally inside the human brain as a proteolytic fragment of the amyloid precursor protein [1–3]. The peptide possesses an amphiphilic structure with a hydrophilic N- and a hydrophobic C terminus. The C-terminal end of the peptide is variable, and produces peptides with lengths ranging at least from 37 to 42 residues [1,4]. The two most intensively studied Aβ alloforms are Aβ(1–40) and Aβ(1–42) [3], which consist of 40 and 42 residues, respectively. More than 10 single-site sequence variants of this peptide have been described, most of which relate to familial forms of Alzheimer's disease (AD; termed FAD) [1]. Naturally occurring Aβ peptides can be chemically modified, for example oxidized side chains, truncated main chains, and pyroglutamate-modified N termini have been described [1,5]. Aβ amyloid fibrils form the core of dense amyloid plaques within the brain parenchyma, one of the hallmarks of AD, or they accumulate at the walls of cerebral blood vessels, associated with cerebral amyloid angiopathy (CAA) [1]. Amyloid fibrils are the end products of a complex fibrillation pathway, and their formation is preceded by numerous on- or off-pathway intermediates. These intermediate structures can include Aβ dimers, oligomers, amyloid-derived diffusible ligands, globulomers, paranuclei, and protofibrils (not to be confused with protofilaments, see below) [1,2,6,7]. However, the definition of these states strongly depends on the context of their preparation in different laboratories, and they often cannot be isolated or easily purified [6]. Therefore, reliable structural information on Aβ amyloid fibrils and other Aβ-derived aggregated states is difficult to obtain, although such data would be crucial for understanding their biological properties and for the design of structure-specific ligands or inhibitors.

In this review, we summarize the progress made over the past 5 years towards understanding the structures of Aβ peptide aggregates. The different structural levels of Aβ amyloid fibrils are presented, including quaternary structure, protofilament organization and packing of β-sheets. Moreover, we discuss several topics of particular interest, including the structural polymorphism of amyloid fibrils, oligomers, and the comparison of Aβ(1–40) and Aβ(1–42) fibrils. Key methods of structural investigation are introduced to help the reader assess the published, and sometimes seemingly conflicting, structural models, which have been proposed to explain the new experimental data. The available structural data also shed light on the various conformational states adopted by Aβ, and their possible role in human disease.

Cross-β structure of Aβ amyloid fibrils

Amyloid fibrils can be defined as fibrillar polypeptide aggregates with a cross-β structure [8]. Cross-β structures represent intermolecular polypeptide assemblies, in which the β-sheet plane and the backbone hydrogen bonds that connect the β-strands are oriented parallel to the main fibril axis. It follows that the β-strands run perpendicular ('cross') to this direction. The presence of a common cross-β structure in all amyloid fibrils was initially shown by X-ray diffraction measurements [9]. More recently, crystallographic studies of peptide microcrystals have revealed so-called steric zippers [10,11]. Steric zippers consist of a pair of two cross-β sheets with interdigitating side chains (Figure 1a). They can be formed by several different short peptide chains (usually 4–7 amino acids), such as from Aβ residues 37–42 (Figure 1a) or 35–40 [11], and it has been suggested that steric zippers constitute, in the context of full-length polypeptide chains, the structural spine of amyloid fibrils. Indeed, the cross-β structure of Aβ fibrils has been shown to be formed by discrete sequence segments at the peptide center or C-terminus [20–24]. Most studies have described 2–4 β-strands, usually involving residues

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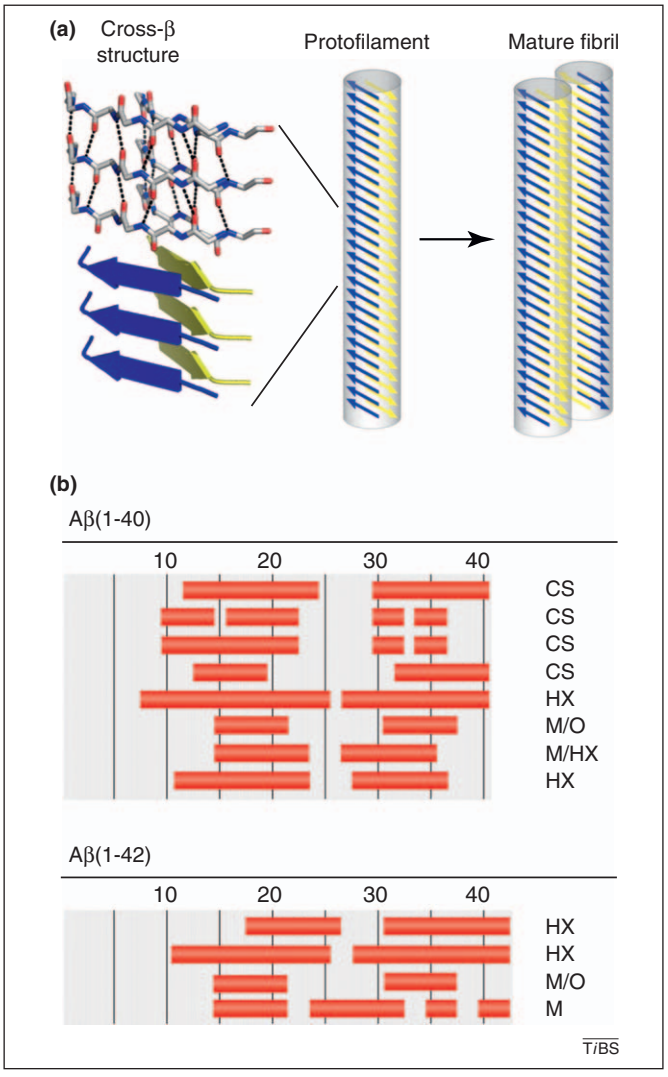

Figure 1. Structural hierarchy and β-sheet structure of Aβ fibrils. Mature amyloid fibrils encompass one or several protofilaments. Their cores are formed by peptides that adopt a cross-β structure. **(a)** Structural hierarchy of amyloid fibrils, exemplifying the cross-β structure by a steric zipper structure from Aβ residues 37–42 (PDB-code: 2ONV) [11]. Only the backbone is drawn; the upper three strands are shown in stick representation, highlighting the hydrogen bonds; the bottom three strands are displayed as a ribbon diagram. The cross-β structure forms the structural spine of an amyloid protofilament; the filamentous substructure of mature Aβ fibrils. **(b)** Possible β-sheet forming residues of Aβ(1–40) and Aβ(1–42), as suggested by chemical shift data (CS) [21,29,34], protection from hydrogen exchange (HX) [20,22,41,42], mutational perturbation without (M) [39] and with hydrogen exchange (M/HX) [22], mutagenic changes combined with structural order analysis (M/O) [23].

16–20 and 31–36 (Figure 1b). Differences exist concerning the exact residues that were reported to form the β-sheet segments, either reflecting real structural differences or experimental ambiguities (e.g. gaps within the structural assignment).

The amyloid fibrils formed from the full-length Aβ peptide display the typical characteristics of amyloid fibrils, including a high affinity to amyloid-specific dyes (Congo red and/or Thioflavin T) and fibril-specific conformation-sensitive antibodies [12,13]. Based on the shape of the amide I regions, their infrared spectroscopic properties correspond to other amyloid fibrils [14–16], and additionally suggest a parallel orientation of the β-sheets in Aβ(1–40) and Aβ(1–42) amyloid fibrils [12,17,18]. The assembly of full-length Aβ fibrils differs from the antiparallel β-sheet characteristics seen in the fibrils formed, for instance, from the Aβ(11–25) peptide fragment, Aβ-derived variant peptides carrying the 'Iowa' Asp23Arg substitution [19] and certain Aβ-derived steric zippers [11]. These examples illustrate the β-sheet variability of the fibrils arising from Aβ and its derived peptide fragments or variants.

General topology and polymorphism of mature amyloid fibrils

Transmission electron microscopy (TEM) and atomic force microscopy have shown that mature amyloid fibrils can have a length of >1 μm, whereas the lateral width of previously analyzed fibrils rarely exceeds 25 nm [15,20,25–27]. Mature Aβ amyloid fibrils comprise one or several protofilaments (not to be confused with protofibrils) [4,7]. Analogous to other filamentous structures, such as microtubules, amyloid protofilaments represent the substructures of mature fibrils (Figure 1a). Mature Aβ fibrils are often, but not always, twisted (Figure 2a) and result in regular crossovers that are visible by TEM (Figure 2a). All analyzed superhelical Aβ(1–40) and Aβ(1–42) fibrils have a left-handed twist [14,15,26,27], and they usually possess a polar structure [14,15,27]. Polarity refers in this case to the directionality of the fibril structure, similar to the polarity of actin filaments or microtubules. Most of the 3D reconstructions of Aβ amyloid fibrils are limited to resolutions of 0.8–3 nm, at which they exhibit twofold fibril symmetry [15,25,27,28]; that is, the fibril cross-section superimposes with itself after 180° rotation. However, a minority of the obtained reconstructions do not comply with twofold

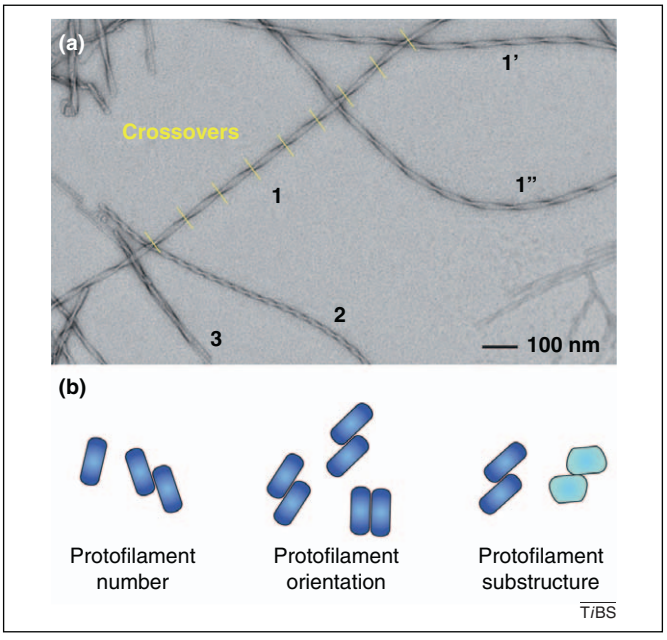

Figure 2. Polymorphism and structural deformations of Aβ fibrils. Typical Aβ fibril samples are affected by heterogeneity, which arises both from the intra-sample polymorphism of different fibril structures and from their deformations from ideal helical symmetry (bending and twist variability). **(a)** Negative stain TEM image of Aβ(1–40) fibrils illustrating the fibril variability. Fibrils 1, 2 and 3 show fibril polymorphism. Isomorphous fibrils 1, 1′ and 1″ differ in bending. The twist variability (crossover distance) of fibril 1 is highlighted in yellow. **(b)** Schematic representations of fibril cross-sections to illustrate three types of fibril polymorphism. Differences depend on the number, orientation or substructure of the underlying protofilaments.

symmetry [27], and non-twofold symmetrical fibrils have also been assumed by some published models [29].

An important structural feature emerging from these and many other studies is the immense structural polymorphism of amyloid fibrils. Structural polymorphism is defined here as the variability in peptide conformation or intrafibrillar arrangement of different fibrils. Specific fibril morphologies can be identified, for instance, by TEM, by their characteristic widths or crossover distances (Figure 2a). 3D reconstructions of polymorphic amyloid fibrils have revealed three distinct types of polymorphisms; that is, fibrils that differ in: (i) the number of protofilaments (Figure 2b); (ii) relative protofilament orientation (Figure 2b); and (iii) internal protofilament substructure (Figure 2b) [30]. The latter polymorphism type can be further subdivided, as indicated by a recent analysis of different steric zippers, depending on differences in the participating sequence segments and packing modes; thus, they are termed segmental, registration, combinatorial and packing polymorphisms [31].

Polymorphism is potentially relevant for human disease, because it might underlie the natural variability of some amyloid diseases, such as amyloid light chain (AL) amyloidosis, or the protein-encoded inheritance phenomena of prion strains [31]. It also constitutes an important biophysical difference between amyloid fibril formation and native protein folding reactions. The latter are usually characterized by a unique correspondence between amino acid sequence and folded state, whereas amyloid fibril formation can lead, for the same polypeptide sequence, to many well-defined end states.

The term polymorphism frequently refers to an inter-sample variance that can arise from different incubation or solution conditions. For example, different fibril morphologies occur in the presence of salt, Zn^{2+} ions, or in conjunction with the use of different buffer systems [27,32,33]. In addition, it has been reported that agitation or quiescent incubation conditions can produce different fibril morphologies that are visible by TEM and encompass different peptide folds, as judged by NMR [34]. These studies were self-consistent to the extent that the experiments were carried out with comparable peptide aliquots and pretreatments, whereas cross-study comparisons are often problematic. Besides differences in the incubation conditions, variability is also caused by different peptide batches or sample pretreatments. Numerous attempts have been made to reduce these problems by application of 'disaggregation' protocols [35,36], but the comparability of peptide samples remains a major problem in the analysis of fibril structures.

In addition to inter-sample polymorphism, the detailed examination of Aβ fibril samples with single particle techniques has revealed substantial intra-sample polymorphism [27,33]. For example, a systematic analysis of Aβ(1–40) fibrils formed in 50 mM sodium borate, pH 9.0, has revealed variations in the fibril width from 13 to 29 nm; by contrast, most fibrils present well-resolved crossover distances from 100 to 200 nm [33]. A dramatically different spectrum of morphologies has been obtained when fibrils were grown under this buffer condition, but in the presence of 0.5 M sodium or potassium chloride. The average width of fibrils was significantly decreased, with many being thinner than 13 nm or having no discernible crossover distances [33]. However, all these samples presented intra-sample polymorphism, which indicated that changing the incubation conditions does not necessarily cause a switch between single fibril morphologies. Instead, it alters the distribution of morphologies and favors one polymorphic ensemble over another. Thus, intra-sample polymorphism constitutes a major obstacle to high-resolution structural techniques that cannot separate the signal coming from different morphologies.

Structural deformations report on the nanoscale flexibility properties of amyloid fibrils

Besides polymorphism, structural deformation is one further cause for the heterogeneity of amyloid samples. These deformations manifest themselves in different degrees of bending or twisting, as demonstrated by the variable crossover distances within the same fibril (Figure 2a). Although these deformations create a further potential problem to structural analysis, they can be used to infer nanoscale mechanical properties of amyloid fibrils. For an Aβ(1–40) fibril morphology with an average crossover distance of 140 nm and width of 19 nm, the values for the Young's modulus and shear modulus were found to be similar to those of most other protein filaments [37]. The considerable bending rigidity of amyloid fibrils is relevant when considering the pathogenic activity of rigid structures that can occur and impair normal function in naturally contractile or elastic tissues. For example, CAA is known to be associated with micro-hemorrhages caused by the deposition of Aβ fibrils within cerebral vessel walls [1].

Structural methods for studying amyloid fibrils

To date, atomic structures of full-length Aβ fibrils have not been determined. Suitable crystals of full-length Aβ fibrils for X-ray crystallography have not been obtained, and the large fibril size precludes conventional solution-state NMR techniques. The absence of atomic structures has led to the proposal of several structural models. Before entering a more detailed discussion of the available structural data on Aβ fibrils, it is important to clarify the difference between 'structure' and 'model' (Boxes 1 and 2).

Models can be partially based on structural constraints from biophysical measurements. Their critical assessment requires at least some knowledge about the structural constraints that can be derived from the employed biophysical techniques. Direct information about the secondary structure of fibrils and aggregates can be obtained by X-ray fiber diffraction, circular dichroism (CD) and infrared spectroscopy [4,14,16,38]. In addition, the two spectroscopy methods can be used to quantify the secondary structural content of a sample. Combined with isotope editing, infrared spectroscopy can even provide direct, residue-specific structural information [38].

Residue-specific information can also be obtained by site-specific mutagenesis coupled with kinetic or thermodynamic measurements [39,40], hydrogen exchange in conjunction with NMR or mass spectrometry [20,22,40–42], or electron paramagnetic resonance spectroscopy [23]. However, the structural data that result from these

Box 1. Use of the term 'structure'

The spatial coordinates of atoms and specification of their covalent and non-covalent interactions are collectively referred to as the structure of a protein or peptide, if they are primarily determined based on experimental data obtained, for example, by X-ray crystallography, NMR or electron microscopy. The quality of the experimental data must allow the accurate placement of the major structural elements, such as amino acids and their side chains. In X-ray crystallography and electron microscopy, this accuracy is directly related to the spatial resolution of the experiment and usually needs to be better than 0.4 nm to allow reliable placement of amino acids. NMR experiments provide distance information and other data that constrain the arrangement of atoms, which gives rise to a number of possible atomic coordinates. The variability of these coordinates is expressed as a root mean square deviation (RMSD) and values <0.15 nm are usually sufficient for reliable amino acid placements. However, a structure can also be represented through a density map at lower resolution. For example, a cryo-EM map at 1 nm resolution can also be referred to as a structure (although it is often called reconstruction). If a representation that is based more or less equally on experimental and other information, the distinction between structure and model becomes more difficult. However, if it is called a structure, spatial resolution information or RMSD values should always be provided in the initial publication.

techniques are more indirect, because the methods are, in contrast to CD or infrared spectroscopy, not themselves able to distinguish β-sheet structure from other stable and ordered conformations. Although changes measured with these methods are usually interpreted with β-sheet formation, the more immediate readouts are the speed of aggregation, the aggregate stability [39,40], protection from hydrogen exchange [20,22,40–42], and the hydrophobicity and structural order at discrete sites after cysteine replacement and cysteine side chain modification [23].

Solid-state NMR and electron cryo-microscopy (cryo-EM) have the potential to determine the structure of Aβ amyloid fibrils at atomic or near-atomic resolution. Solid-state NMR determines structural constraints such as chemical shift values, bond angles or specific interatomic distances. It allows the direct identification of the residues of Aβ participating in the β-sheet structure of fibrils (Figure 1b). Such measurements have revealed, for example, similar structural characteristics of *in vitro* grown amyloid fibrils and fibrils seeded with brain extracts [43]. The second technique, cryo-EM, directly visualizes the fibrils and allows the calculation of their 3D density (reconstruction). The observation of individual fibrils enables selection of specific fibril morphologies. Therefore, only data from a chosen morphology will be averaged in a 3D reconstruction. This possibility allows cryo-EM to avoid many of the problems of intra-sample polymorphism. Given a sufficient amount of data and accurate alignment, near-atomic resolution (0.3–0.4 nm) can be obtained [44,45]. In the case of Aβ fibrils, this technique is able to visualize the 3D structure of several fibril morphologies at up to 0.8 nm resolution, which provides information about the global fibril architecture, protofilament substructure, and location of the cross-β structure within the symmetrical fibril helix [14,15,25,27].

Box 2. Use of the term 'model'

If the arrangement of atoms is primarily based on general reasoning, homology between molecules, general side chain geometry or chemical bond lengths obtained from a database, it is commonly called a model. A model can still be partially based on experimental data to guide model building. For example, the strong demand for detailed descriptors of amyloid structures often leads to atomic models in the literature that are based on some experimental data, but that encompass many details that have not been established experimentally (hence they are models). When comparing models, it is important to consider not only the structural detail shown in the model, but also the primary experimental data leading to it and the degree of extrapolation the model represents. An atomic model that includes the coordinates of side chains is, therefore, not necessarily more informative than a simpler model that includes only a backbone trace or, indeed, a model that outlines only the folding motif of a peptide. Models can also consist of more simplistic representations and symbols, for example cylinders and arrows to symbolize α-helical and β-sheet secondary structural elements, or a simple line to indicate the path of the peptide backbone.

Protofilament structure of mature Aβ fibrils

The protofilament substructure of an Aβ fibril has been identified by cryo-EM [15,25]. The observed protofilaments show cross-sectional dimensions of 4 × 11 nm and a cross-sectional subdivision into a single central region of quasi twofold symmetry (4 × 5 nm) and two peripheral regions (Figure 3a–e). The central region is formed by two paired elongated density cores, corresponding to two cross-β sheets. Mass-per-length (MPL) measurements suggest that each protofilament contains ~2.5 peptides per cross-sectional layer. This protofilament structure has been observed in two fibril reconstructions: one obtained with the Aβ(1–40) peptide [25], and the other with Aβ(1–42) [15]. The analyzed Aβ(1–40) fibril contains two such protofilaments, whereas there was only one within the Aβ(1–42) fibril. The single-protofilament Aβ(1–42) fibril presents two equally shaped peripheral regions that are fully solvent-exposed and structurally disordered (Figure 3d). By contrast, the two-protofilament Aβ(1–40) fibril contains an arch-shaped peripheral region at the protofilament–protofilament interface, whereas the other peripheral region is solvent-exposed and structurally disordered (Figure 3a–c). This protofilament architecture is apparently conserved in different Aβ amyloid fibrils, and several other Aβ(1–40) fibril reconstructions are consistent with this protofilament substructure [27]. Nevertheless, some evidence points to additional types of Aβ protofilament substructures, as suggested by cryo-EM reconstructions [27,28] (Figure 3f) or MPL measurements of certain fibril morphologies [26,29,34].

Possible folds and intermolecular arrangement of fibrillar Aβ peptide

Several models of the Aβ fold in amyloid fibrils have been proposed; sometimes combining structural constraints from different experimental techniques or from samples that are formed under different conditions. However, different conditions can produce fibrils with different peptide conformations (see above); thus, caution should be exercised when comparing the respective models. Most fibril models assume a U-shaped peptide fold (Figure 4a,b). This fold has been termed a β-arc (or β-arch) [46]. U-shaped peptide models are derived from molecular dynamics simu-

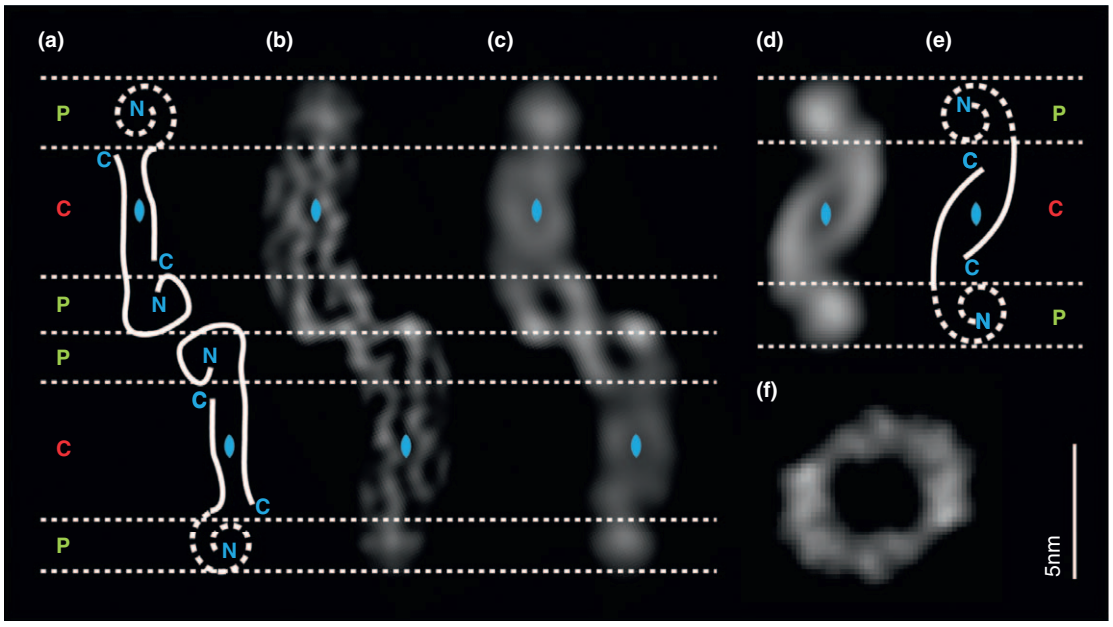

Figure 3. Cryo-EM cross-sections of different Aβ fibril morphologies. Cryo-EM reconstructions reveal significant similarities in the protofilament structure of two Aβ(1–40) and Aβ(1–42) fibril morphologies, which suggests homologous peptide assemblies. **(a–c)** Aβ(1–40) fibril cross-section filtered at 0.8 nm (b) and 1.5 nm (c) [25]. Structural interpretation of the cross-section (a). **(d)** Cross-section of the Aβ(1–42) fibril structure from pH 7.4 at 1.5 nm resolution [15]. **(e)** Structural interpretation of the cross-section. Structures in (b–d) present a similar subdivision into one central region (C, red), which possesses approximate twofold symmetry (blue symbol), and two peripheral regions (P, green). **(f)** Aβ(1–42) fibril at pH ~2 [28].

lations, partially implementing structural constraints from solid-state NMR, or other biophysical techniques. Occasionally, it has been suggested that these models fit cryo-EM reconstructions [14,28]. However, reconstruction of an Aβ(1–40) fibril at higher resolution (0.8 nm), in which the cross β-sheet structure is directly resolved, does not comply with the previous U-shaped peptide models [25,37,47]. The 0.8 nm reconstruction presents cross-sectional dimensions that are significantly larger than those predicted by the U-shaped models, and therefore, must encompass the peptide in a different structural arrangement (Figure 4c–e).

Based on MPL measurements as well as the shape, symmetry and subdivision of the protofilament cross-section of the 0.8 nm reconstruction, it has been proposed that one protofilament cross-β repeat comprises two oppositely directed Aβ molecules (Figures 3a–e,4e). These peptides are part of two separate, paired cross-β sheets. Therefore, the basic peptide arrangement resembles that of a steric zipper structure (Figure 1a). The two peptides are structurally equivalent in the single-protofilament fibril (Figure 3d,e), whereas the cross-sectional density in the two-protofilament fibril implies that the two peptides differ in conformation (Figure 3a–c). In addition, the peptide that forms the protofilament–protofilament interface is arch-shaped (with dimensions different from the published β-arch), whereas the other peptide contains significant structural disorder [47]. The possibility that a specific Aβ(1–40) fibril morphology might encompass two different peptide folds has also been raised by solid-state NMR [29]. Nevertheless, the current cryo-EM structures do not exclude the possibility that other Aβ fibril morphologies exist that possess a U-shaped peptide architecture and a protofilament substructure different from the one described here. All current models are consistent in assuming that a significant fraction of the peptides in the fibril have solvent-exposed N termini. This conjecture is consistent with findings that the peptide N terminus can be flexible and constitutes an important epitope for AD immunotherapy [1–3].

Structural comparison of Aβ(1–40) and Aβ(1–42) fibrils

The Aβ(1–42) peptide is generally believed to be more pathogenic than the Aβ(1–40) peptide [1–3]. When expressed in *Drosophila melanogaster*, the Aβ(1–42) peptide is highly toxic and reduces the life-span of the affected animals, whereas Aβ(1–40) transgenic flies do not present a discernible phenotype [48]. The significant chemical similarities of the two peptides (the first 40 residues are identical) suggest that their conformational properties are also largely similar. Yet, inevitable differences must exist, associated with the additional two C-terminal residues of Aβ(1–42). The clearest biophysical difference is the higher aggregation propensity of Aβ(1–42) [49]. Moreover, Aβ(1–40) can affect, in mixtures, the aggregation mechanism of Aβ(1–42), thereby preventing the formation of mature Aβ(1–42) fibrils [50] by stabilizing intermediate conformations [51].

The available cryo-EM fibril reconstructions from the two peptides show marked differences in protofilament packing. Published Aβ(1–42) fibrils possess either a single-protofilament arrangement or a two-protofilament assembly with a hollow core [15,28]. All of the more than 10 published Aβ(1–40) fibril reconstructions are differently structured [14,15,25,27,37]. In addition, there is evidence that fibrils formed by the two peptides differ, at least

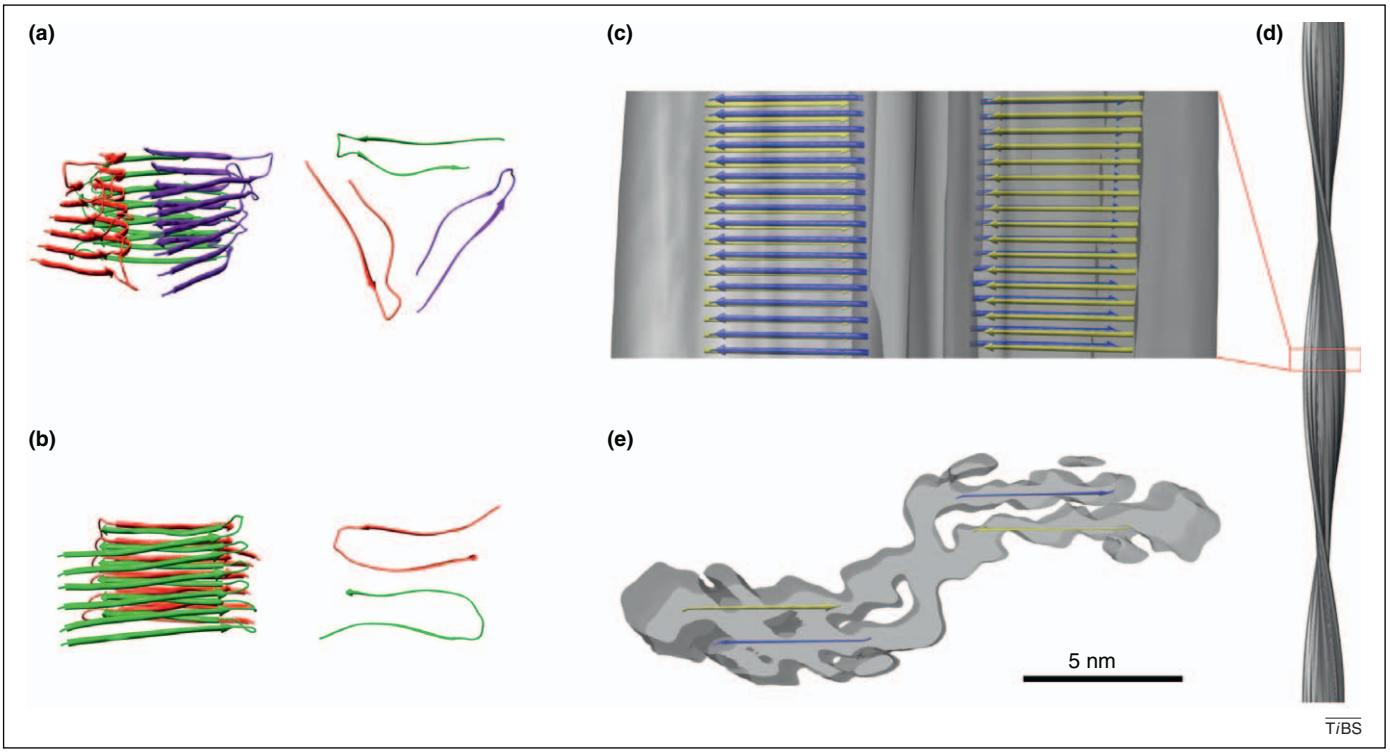

Figure 4. Structural models of Aβ fibrils. Previous structural models deviate significantly for the most highly resolved Aβ fibril structures, which were obtained by cryo-EM. Hence, the latter encompass a different peptide assembly. **(a,b)** Structural models assuming a U-shaped peptide fold; side views and top views shown; only residues 9–40 modeled. (a) Three Aβ(1–40) molecules per cross-sectional layer [29]. (b) Two Aβ(1–40) molecules per cross-sectional layer [24]. **(c,d)** Cryo-EM structure of an Aβ(1–40) fibril (0.8 nm). (c,d) side views, **(e)** cross-section. Images in (c,e) are superimposed with a β-sheet model, which is derived from these cryo-EM data, and highlights the peptides forming the cross-β regions in yellow or blue (the lines are not meant to imply continuous β-strands over their entire length; these regions might instead contain several shorter strands). Images in (a–c) and (e) are displayed with the same scale.

slightly, in the exact residues that form the secondary structural elements Fig. 1b). By contrast, it has also been shown that the protofilaments of Aβ(1–42) and Aβ(1–40) fibrils are highly similar. For example, they can produce identical MPL values, cross-sectional areas and shapes, and their protofilament cross-sections show a similar division into one central and two peripheral regions [15]. These data suggest similar peptide folds in the two fibrils, which is consistent with the fact that the β-sheet structure is usually assigned to similar sequence segments of Aβ(1–40) or Aβ(1–42) peptides (Figure 1b). In addition, infrared and NMR data indicate that both fibrils contain a parallel β-sheet structure [14,15,20,24], and there is long-standing evidence that Aβ(1–42) fibrils are able to seed fibril formation from Aβ(1–40) peptides [49]. Taken together, these data demonstrate many similarities, but also significant differences between specific samples prepared by the two peptides. Additional analyses will be required to establish whether these differences represent sample-specific variations or systematic differences between the two Aβ alloforms.

Structure of Aβ(1–40) and Aβ(1–42) oligomers

In vitro Aβ fibrillation reactions involve a range of different on- or off-pathway intermediates, and it is thought that similar structures, such as oligomers or protofibrils, can become stabilized during disease [2,6,7,52]. In fact, the onset of AD is thought to depend on the action of Aβ fibrillation intermediates [1–3], but there is uncertainty about the exact mechanism, and different possible pathways to disease have been proposed. For example, it has been suggested that annular Aβ aggregates are toxic and kill the affected cells by perforating their membranes [7,53]. Other mechanisms involve Aβ-dependent excitotoxicity reactions, which require dendritic tau protein [54], or mitochondrial dysfunctions [18]. The available data suggest that, although the Aβ peptide and its derived amyloid fibrils are typically located extracellularly, the formation of these deposits and their pathogenic activity arises from intracellular Aβ structures [55,56].

Although solving the atomic structures of Aβ fibrils is already difficult, addressing the structures of their various precursors is even more challenging. Such aggregates can exhibit variable molecular weights and overall shapes, including spherical oligomers, curvilinear protofibrils and annular pores. Despite evidence for random coil-like conformations in some assemblies [57], many analyzed Aβ(1–40) and Aβ(1–42) oligomers display appreciable β-sheet content [12,17,58,59]. Chemical shift measurements by solid state NMR and Fourier transform infrared spectroscopy (FTIR) analyses have consistently demonstrated that similar residues are involved in the formation of the β-sheet structure of Aβ(1–40) oligomers and fibrils, and that the two states display similar FTIR spectral characteristics [12,17,18,59]. Yet, FTIR also indicates that the β-sheet structure of some Aβ(1–40) and Aβ(1–42) oligomers is considerably antiparallel, whereas full-length Aβ fibrils possess parallel β-sheet characteristics [12,17,18]. Moreover, NMR experiments suggest that the β-sheet packing distance and assembly could be different in Aβ(1–42)

oligomers and Aβ(1–42) fibrils [58]. Other important structural differences between Aβ oligomers and fibrils are their higher diffusibility, hydrophobicity and ability to interact with membranes [6], which could explain the oligomer-dependent pathogenicity mechanisms described above.

Concluding remarks

Recent improvements in techniques, in particular cryo-EM and NMR, have enabled the localization of the cross-β structure within mature Aβ fibrils, and have identified some of the residues that stabilize fibrils and oligomers. The structures underlying the different aggregation states of the Aβ peptide are important for a mechanistic understanding of related diseases. For example, Aβ fibrils seem to underlie the pathogenicity in CAA, whereas oligomers or other premature Aβ aggregates are probably more relevant for AD [1–3]. To understand any one disease, it might be necessary to study entire populations of oligomers or fibrils that capture the natural polymorphic states of Aβ peptide. Continued technical developments raise hopes that more comprehensive structural information will become available in the next 5–10 years that could potentially form the basis for further studies and developments, such as in disease treatment, as well as in understanding the aggregation pathways that lead to the characteristic polymorphisms seen in all amyloid fibrils.

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