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Failure of Aβ(1-40) amyloid fibrils under tensile loading

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ABSTRACT

Amyloid fibrils and plaques are detected in the brain tissue of patients affected by Alzheimer's disease, but have also been found as part of normal physiological processes such as bacterial adhesion. Due to their highly organized structures, amyloid proteins have also been used for the development of nanomaterials, for a variety of applications including biomaterials for tissue engineering, nanolectronics, or optical devices. Past research on amyloid fibrils resulted in advances in identifying their mechanical properties, revealing a remarkable stiffness. However, the failure mechanism under tensile loading has not been elucidated yet, despite its importance for the understanding of key mechanical properties of amyloid fibrils and plaques as well as the growth and aggregation of amyloids into long fibers and plaques. Here we report a molecular level analysis of failure of amyloids under uniaxial tensile loading. Our molecular modeling results demonstrate that amyloid fibrils are extremely stiff with a Young's modulus in the range of 18–30 GPa, in good agreement with previous experimental and computational findings. The most important contribution of our study is our finding that amyloid fibrils fail at relatively small strains of 2.5%–4%, and at stress levels in the range of 1.02 to 0.64 GPa, in good agreement with experimental findings. Notably, we find that the strength properties of amyloid fibrils are extremely length dependent, and that longer amyloid fibrils show drastically smaller failure strains and failure stresses. As a result, longer fibrils in excess of hundreds of nanometers to micrometers have a greatly enhanced propensity towards spontaneous fragmentation and failure. We use a combination of simulation results and simple theoretical models to define critical fibril lengths where distinct failure mechanisms dominate.

1. Introduction

Initially solely associated with severe disorders [1], amyloid protein materials are now recognized also as common protein structures with important biological functional roles [1–3] as bacterial coatings [1], protective materials in egg envelopes of several fish species and insects [4,5] and scaffold for catalytic reactions [6]. Amyloid protein materials often result from protein misfolding pathways that generate fibrillar aggregates with a common core structure consisting of an elongated stack of beta-strands stabilized by a dense network of hydrogen bonds [7]. This structural arrangement confers high stability and remarkable mechanical properties, which have been investigated both theoretically and experimentally [8–11]. In particular, amyloids feature a high elasticity, with Young's moduli ranging between 10 GPa and 20 GPa as well as a high bending stiffness [9,10]. According to earlier experimental and theoretical studies these mechanical properties are related to their molecular structure [10,12]. The exceptional mechanical properties of amyloids make them good candidates for a wide range of potential technological applications, and specifically as new bionanomaterials utilizing them as nanowires [13–16], gels [17–22], scaffolds and biotemplates [13,22–27], liquid crystals [28], adhesives [29] and biofilm materials [30]. These applications often imply the functionalization of the amyloid fibrils with the introduction of additional elements, including enzymes, metal ions, fluorophores, biotin or cytochromes. Amyloids have been also proposed for biological applications in cell adhesion [31] and as bioadhesives for tissue regeneration and engineering [32], on the basis that amyloid toxicity is associated with oligomeric species or pre-fibrillar intermediates rather than mature fibrils [33,34].

Measurements of the mechanical properties of amyloid fibrils and, in particular of their strength, are crucial to understand their potential and performance limits in applications, and the quest to elucidate the mechanics of amyloid protein materials has been

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going on for several years. Several experimental techniques, based on the mechanical manipulation of the individual nanostructures, have been used to measure elastic modulus and strength [8,35–41] and even provided a first experimental evaluation of the failure stress in the range of 0.2–1.0 GPa [8]. Recently, other experimental works based on sonication [42] and laser-induced shock wave propagation and destruction [43] have revealed a heightened tendency to breakage, and clarified some aspects of the kinetics of proliferation of amyloid fibrils. The observed high fragility of amyloid fibrils combined with the elongation mechanism that occurs only at the tails of each fibril, explain the explosive proliferation of the amyloid fibrils observed during laser irradiation due to the increased density of fibril terminals that accelerate growth [8,43]. The understanding of proliferation mechanisms is fundamental not only for material science applications, but also for biomedicine, which faces issues related to deposition and uncontrolled self-assembly of amyloids in the form of large plaques with dimensions on the order of micrometers. They result from a hierarchical organization that, from the atomistic level reaches the nanometer scale, where one or more fibrils arrange to form protofilaments [44–46] and the assembly of multiple protofilaments results in a variety of morphologies, including twisted rope-like structures, flat-tapes with nanometer-scale diameters [47,48], spherulitic structures [49] and, at a higher level of complexity, the characteristic amyloid plaques found in affected tissues.

The understanding of the failure mechanism under tensile stress and the transfer of the mentioned brittleness over larger size scales is crucial in several application areas, including amyloid aggregate growth, the development of biomaterials, and the understanding of changes of mechanical properties across multiple material scales. To date, however, no direct tensile loading test of amyloid fibrils has been conducted. The molecular structure of the amyloid fibrils and the presence of the stabilizing hydrogen bonds network makes such tests extremely challenging from an experimental perspective, in particular pertaining to the application of load at the level of individual fibrils. Further, a systematic study of the mechanical properties of amyloid fibrils over different length scales is currently missing. This is an important issue to shed light onto the mechanism of growth of amyloid fibrils, since, despite the importance of the self-propagating nature of amyloids, many questions related to how the fibers grow and form remain open. Some experimental works suggest that amyloid growth occurs by the addition of oligomeric intermediates at fiber ends [50], while other results demonstrate that amyloids grow efficiently by the addition of monomers to fiber ends [51].

In this paper, we report a series of molecular dynamics simulations investigating the mechanical failure response of amyloid fibrils with lengths up to $\approx$190 Å due to direct tensile loading. The insight derived here has important implications for the development of models of larger-scale amyloid plaques where thousands of fibrils supposedly approach the micrometer scale [52] and interact determining the observed stiffness of amyloid plaques [30,53].

2. Materials and methods

2.1. Amyloid fibril geometry setup

We investigate Aβ(1-40) amyloid fibrils associated with Alzheimer's disease as a model system. The basic structure of this amyloid fibril has been determined through solid state Nuclear Magnetic Resonance (ssNMR) investigations [54]. Each fibril is characterized by a beta-cross structure, deriving from the repetition of layers stacking on top of each other and stabilized by a dense hydrogen bond network. Each layer comprises two, sequence-wise identical, U-turns (sequence: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV) [54,55], and is twisted by an average angle θ with respect to the nearest neighbor layers. According to the ssNMR data, the first eight residues show structural disorder [54] and the corresponding coordinates are not yet available. Therefore only the last 32 amino acids are taken into account to build the fibrils, starting from the glycine (G) at position 9. Each U-turn contains 32 alpha carbons and we will consider a number $N_c = 64$ of alpha carbons per layer. According to the adopted Cartesian representation, the fibril grows along the z axis, while the twist rotation occurs in the x-y-plane. Fibrils of $n = 20$, 30 and 40 layers are built following the method described in references [56] and [11]. Thereby the coordinates of one layer are copied and translated along the fibril axis imposing the typical beta-sheet interlayer distance (4.8 Å) and any interlayer twist rotation [56]. The length (L_n) of fibrils composed of n layers ($n = 20$, 30, 40) is evaluated as the distance between the centroids of the alpha carbons composing the top and bottom layers. The values of L_n result to be $L_{20} = 91.87$ Å, $L_{30} = 144.33$ Å and $L_{40} = 189.75$ Å for 20, 30 and 40 layers, respectively. The simulation of larger fibrils is computationally prohibitive due to the size of these larger molecular fibrils. The subsequent molecular relaxation process will drive the fibril towards the optimized interlayer distances and twist angles [56] (see next sections for details on the molecular dynamics simulation approach used in our study).

2.2. Mechanical characterization

The relaxed configuration of each amyloid fibril is extracted from the corresponding relaxation trajectory and forms the reference structure to investigate the mechanical response to applied strain. A relaxed fibril with length L_{20} is represented in the snapshot of Fig. 1A corresponding to 0% applied strain as an example. Deformed systems with applied strain are generated by homogenously rescaling the coordinates of the relaxed fibrils along the growth axis. In each case, the imposed strain (denoted by ε) ranges between 1% and a maximum value in excess of the strain where failure is observed, which determines a variation of the overall fibril length from L_n to $L_n^* = L_n(1+\varepsilon)$.

Molecular dynamics simulations are performed to relax the deformed fibrils under displacement boundary conditions. To apply displacement boundary conditions (to realize prescribed strain), the positions of the alpha carbons composing the top and the bottom layers are fixed in the axis direction, but are allowed to move freely in the plane orthogonal to the fibril axis (Fig. 1B). This allows to account for winding/unwinding rotational motions that are expected as a consequence of the molecular rearrangements at each applied load (following earlier results reported in [10]). A quantitative analysis of fibrils, carefully relaxed at each level of applied strain, is performed by averaging relevant quantities (twist, number of hydrogen bonds, energy, etc.) over the last 1 ns of relaxation, using the CHARMM molecular dynamics program [57]. The energy values reported here (e.g. in Fig. 2) are averaged over the last 1 ns relaxation and normalized by the starting volume (V) of the fibril. The volume V is evaluated as $L_n A_c$, being $A_c = 1414.32$ Å^2 (the cross-section area) estimated as described in earlier work [10]. The variations of the number of hydrogen bonds is calculated using the software VMD [58] by imposing a cutoff distance of 4 Å and a cutoff angle of 40°. The interlayer twist angle is evaluated by considering the position of the alpha carbons of the residue serine 26 along the sequence given above [56].

The average local displacement is a useful variable to visualize the distribution of the strain along each fibril and to gain information on the occurrence of the failure. In a fibril composed of n layers, for each layer (j) we define a local interlayer distance

$$d_j^0 = \frac{1}{N_c}\sum_{i=1}^{N_c}\left|r_{ij}^0 - r_{i(j+1)}^0\right| \tag{1}$$

where r_{ij}^0 and $r_{i(j+1)}^0$ identify the positions of the ith alpha carbon in the layer j and j+1, respectively, and N_c is the number of alpha-carbon atoms involved. Similarly, for each time step (k) extracted from the relaxation trajectory of the deformed fibrils, we define the local distance as

$$d_{jk} = \frac{1}{N_c}\sum_{i=1}^{N_c}\left|r_{ijk} - r_{i(j+1)k}\right| \tag{2}$$

where r_{ijk} and $r_{i(j+1)k}$ identify the positions of the ith alpha carbon in the layer j and j+1of the deformed fibrils at time step k, respectively. The local strain per layer and per time step, ε_{jk} is

$$\varepsilon_{jk} = \frac{d_{jk} - d_j^0}{d_j^0} \tag{3}$$

The maps shown in Fig. 4 report the values of the local strain $<\varepsilon_j>$ averaged over the last 1 ns relaxation, evaluated for each applied strain.

2.3. Molecular dynamics simulation approach

Molecular interactions are modeled on the basis of the all-atom CHARMM19 polar force field combined with an effective Gaussian model for the water solvent (EEF1) [59], where the implicit solvent model is chosen because of the size of the investigated systems. Its applicability is supported by earlier studies that compare the utilization of the explicit and implicit models [60] and validate the results against the experimental data [10,61]. The non-deformed fibrils are first minimized to relax the system to a favorable starting configuration, and then equilibrated at