Explanation for the mechanical strength of amyloid fibrils

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Received 30 March 2006; accepted 2 June 2006; published online 6 July 2006

The presence of “proteinaceous β-sheet rich fibrillar structures” and amyloidogenic material, has been alluded to extensively in the literature, in association with natural materials exhibiting superior mechanical strength per unit volume. Here we provide a clear experimental demonstration and explanation for why individual amyloid quaternary structures themselves have beneficial mechanical characteristics.

KEY WORDS: biotribology, nanotribology, adhesion, AFM

1. Introduction

Amyloid fibrils are quaternary protein structures formed from the non-specific folding (or misfolding), and subsequent aggregation, of proteins into intermolecular β-sheets of infinite propagation. These supramolecular crossed β-sheet structures are considered to be the fundamental motif of amyloid fibril architecture [1]. Whilst many aspects of the actual self-assembly process, and the internal structure of individual fibrils, remain unknown, evidence suggests that at least some of these aspects are amino-acid specific [1]. Amyloid fibrils are commonly associated with neurodegenerative diseases, although there is evidence that the amyloid structure is a generic form into which any polypeptide can fold, particularly in vitro under slightly denaturing conditions [2,3]. Recently there has been an interest in the mechanical properties of amyloid [4], which in part, has been triggered by the observed amyloidogenic nature of spider silk [5]. This amyloidogenic nature takes the form of a β-sheet enriching structural conversion of the major spider-silk protein, spidron, inside the silk gland of the spider. Similar conformational changes have been observed for the silkworm protein, fibroin, from random coil/helix to β-sheet [6]. This, together with the suspected nucleation-dependent aggregation mechanism during silk production [7], have lead to the suggestion that such conformational changes during the spinning process are as much (or more) important as (than) the protein sequence and native structure [6]. To date, direct measurements of the nanoscale surface mechanical properties of spider silk have not been able to provide additional insight, into the validity of this suggestion, or into the potential mechanical role of amyloid within the silk [8].

The amyloidogenic nature of natural adhesives and cements has also been described. In particular, Kamino et al. [9] observed a number of similarities between a barnacle underwater adhesive protein, and proteins involved in the formation of amyloid plaque. This, together with the insoluble behaviour of the adhesive protein, led them to speculate that the molecular mechanisms for forming an insoluble proteinaceous multimer may be similar between amyloid plaque and barnacle cement [9]. Recently, unusually well ordered, repetitive nanomechanical responses have been observed in the natural adhesive of terrestrial algae, with additional histochemical evidence suggesting that the mechanical responses can be attributed to the presence of amyloid within the material [10].

To date, the reason why quaternary amyloid structures may contribute to the exceptional mechanical strength of the materials described above, particularly under tension, has not been fully explored or explained.

Here we use an ultra-low noise atomic force microscope (AFM) [11] to investigate the tensile response of model amyloid fibrils formed in vitro (also known as amyloid-like fibrils [12]). In recent years, significant effort has been made to determine and understand the complex molecular structure of amyloid fibrils by investigating model amyloid fibrils such as these, formed from short peptide segments [13].

2. Experimental Section

2.1. Materials

Amyloid fibrils were formed from 11 amino acid long, peptide segments of transthyretin, TTR105–115 (amino acid sequence Tyr-Thr-Ile-Ala-Ala-Leu-Leu-Ser-Pro-Tyr-Ser) purchased from CS Bio Co., CA, U.S.A. The fibrils were formed following the method of Jaroniec et al. [14]. The TTR fibrils (10 mg/mL) were diluted 1:5 in MilliQ water. A 40 μL sample was then deposited

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directly onto freshly cleaved mica, incubated for 60 s, rinsed three times in MilliQ water, and dried in a stream of N₂ gas. The deposited amyloid fibrils were first imaged and characterised at high resolution in air in order to confirm the distribution and density of fibrils on the mica surface, and to explore the structural morphology of the fibrils, figure 1.

2.2. AFM Measurements

All measurements were performed with a custom designed, thermal noise limited AFM, capable of routine true atomic resolution imaging on mica when operating in frequency-modulation detection mode [11]. The AFM is interfaced to a commercially available AFM controller (Asylum Research: MFP-3D Bipolar Controller).

High resolution imaging of fibrils was performed in air using intermittent-contact mode. For stability when imaging at high resolution, stiff silicon cantilevers (NCH: Nanosensors) with a spring constant of approx. 40 N/m and resonance frequency of approx. 300 kHz in air, were used.

The tensile response of the fibrils was measured in static pull off mode in MilliQ water to remove meniscus forces. In this case, compliant silicon cantilevers were used to optimise force sensitivity (BSI: Nanosensors), with spring constants in the range 0.05–0.07 N/m. The exact spring constant for each lever was calibrated using the method of Sader et al. [15]. For the pulling measurements, fibrils were located and imaged in intermittent-contact mode at the second resonance frequency of the cantilever (typically in the range 34–38 kHz in water). Imaging resolution was found to degrade relative to imaging in air with stiffer levers, but was adequate for positioning the tip at specific locations along the length of the fibrils. Pulling measurements were made in static mode with tip velocities in the range of 20–30 nm/s.

3. Results and Discussion

Isolated fibrils were first imaged, and subsequently pulled (with non-specific binding to the tip) at different points along their length, in order to investigate their mechanical response. Various mechanical responses were observed with random force peaks and plateaus predominating. Irregularly spaced force peaks are typical of complex multi-molecular interactions. Figure 2 shows an example curve where a clear plateau was observed. Such plateaus often extended for tens of nanometres, which is far greater than either the width or the length of the individual peptide fragments. This response was attributed to the AFM tip attaching close to the end of an intermolecular β-sheet, as shown schematically to the right of figure 3. The sheet picked up by the tip is peeled from the sheet below at constant average force, resulting in a force plateau. This is because, with this pulling geometry, the bonds laterally connecting one β-sheet to the underlying sheet are loaded in series. This mechanism was proposed and observed recently by Kellermayer et al. [16].

However, if the tip attaches to an intermolecular β-sheet somewhere along its length, then many of the bonds laterally connecting that sheet to the underlying sheet are loaded in parallel. This strongly pins the sheet to the bulk of the fibril and thus the applied tensile force can result in the successive unravelling of the peptide molecules from the bulk of the fibril, as shown schematically to
the left of figure 3, and experimentally in figure 4. The sawtooth mechanical response observed in the force versus extension curve of figure 4 relates to the sequential removal of the $\beta$-strands from within the intermolecular $\beta$-sheet. The magnitude of the force at the point the bonds rupture is approximately 20 pN and will relate to both the number and type of bonds between the $\beta$-strands.

Despite our sawtooth mechanical response being associated with pulling on the bulk of the fibril it was observed less frequently than the plateau type response associated with pulling close to the end of the fibril. We found from imaging the fibrils after the measurement that this was associated with the high probability of damaging the intermolecular $\beta$-sheets with the tip during the initial approach and indentation. This results in two ‘ends’ being created in the middle of the fibril, as seen in figure 5. We found that for these fibrils and AFM tips, compressive forces had to be kept to a minimum of around 100 pN or less to avoid damaging the fibrils. The cross-section along the length of the fibril in figure 5 shows a trough of approximately 60 nm in length and 1.3 nm in depth running along the fibril from the point of indentation. Intersheet distances are typically of the order of 1 nm [3], so it would seem likely that a single $\beta$-sheet has been peeled from the body of the fibril and has broken after peeling back 60 nm. The data implies that the peeling mechanism pulls $\beta$-sheets from within the main fibril structure rather than removing loosely
bound intermolecular \( \beta \)-sheets, which are not fully formed, and are lying at the surface of the fibril.

Previously Smith et al. [17] have explained how molecular level mechanical characteristics observed in adhesives, fibers and composites should contribute to their observed macroscopic mechanical strength. Specifically, they described how particular mechanical benefit is derived from a modular elongation mechanism [17]. This is regardless of the specific molecular composition and is due to the extra energy required to break the sacrificial bonds (typically within folded modular units or loops) before exposing the backbone of the material to a high enough force to induce rupture.

From the results shown in figure 4, this mechanism is clearly possible when a tensile load is applied at some point along the fibril. In a natural material, such as an adhesive, there should be a greater probability of tensile loading along the length of the fibril than at the ends, which are likely to be buried within the tangle of fibrils and other material in the adhesive matrix. It is also less likely that any compressive loading will damage the structure of the fibrils in natural materials for a number of reasons. Firstly the loading is unlikely to involve an object as sharp as an AFM tip. Secondly the fibrils are formed from whole proteins and are thus expected to be more robust (this could be further enhanced, by cross-linking between the proteins within the \( \beta \)-sheets). Finally the fibrils would commonly be in a soft biological matrix rather than lying directly on a rigid substrate.

We see qualitative similarities, between the mechanical responses of amyloids formed from peptide fragments and those formed from proteins in certain natural adhesives, measured previously [10]. Exact quantitative agreement is not observed, either in terms of the magnitude of the measured forces or the distance between the force peaks. This is probably because both parameters are likely to be highly dependent on the number of amino acids in the constituent proteins or polypeptide fragments. Each protein molecule or polypeptide fragment will form a \( \beta \)-strand or strands depending on their length. These molecules then self-assemble, to form intermolecular \( \beta \)-sheets, as shown schematically in figure 4. The peak force should increase if there are a greater number of amino acids in the constituent molecules because there are likely to be a higher number of “sacrificial bonds” and greater “hidden length”. There is also a greater opportunity for cross-linking between whole proteins than there is for short peptides. Cross-linking will significantly enhance the continuity of the \( \beta \)-sheet and has already been shown to facilitate amyloid formation [18].

In terms of the mechanical properties of natural materials, the interaction of the fibrils with any adjacent biomaterial, polysaccharide for example, is also important. In other words, on the mesoscopic scale the fibrils would play a mechanical role by simply distributing any load, as in conventional fiber composite materials. At the nanoscale, any tensile force, regardless of the applied direction, should elicit the same beneficial mechanical characteristics of “hidden length” and “sacrificial bonds” from the fibril, due to the highly repetitive structure and high degree of axial symmetry. The fact that the fibrils can play a beneficial mechanical role both on the meso- and nano-scale explains why the quaternary structure is mechanically advantageous and may be the reason why the structure has been evolutionarily conserved in certain organisms. The fantastic ability of amyloid fibrils to self-assemble could also be mechanically advantageous as it implies that the fibrils may be able to rapidly self-heal under appropriate conditions.

4. Conclusion

We have provided a clear explanation for the apparent connection between the amyloid structure and natural materials of superior mechanical strength, based on the beneficial features of “hidden length” and
“sacrificial bonds” present within the amyloid crossed-
β-sheet structure when under a tensile axial load. Given
the generic nature of this amyloid crossed-β-sheet
structure [2,3], it is feasible that this mechanism is the
unidentified cause of superior mechanical strength in a
broad range of natural materials.

Evidence suggests that the amyloid structure can
form from any polypeptide, under appropriate condi-
tions in vitro [3], making it a prime target for biomim-
icry. However, it is clear from our results that it will be
necessary to explore methods of forming amyloid fibrils
from cross-linked whole proteins, as opposed to short
peptide segments, in order to reap the full benefit of
these mechanical characteristics.

Acknowledgments

This work was funded by Science Foundation Ireland
(01/PI.2/C033).

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