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Feature article

Amyloids: From molecular structure to mechanical properties

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ABSTRACT

Many proteins of diverse sequence, structure and function self-assemble into morphologically similar fibrillar aggregates known as amyloids. Amyloids are remarkable polymers in several respects. First of all, amyloids can be formed from proteins with very different amino acid sequences; the common denominator is that the individual proteins constituting the amyloid fold predominantly into a β-sheet structure. Secondly, the formation of the fibril occurs through non-covalent interactions between primarily the β-sheets, causing the monomers to stack into fibrils. The fibrils are remarkably robust, considering that the monomers are bound non-covalently. Finally, a common characteristic of fibrils is their unbranched, straight, fiber-like structure arising from the intertwining of the multiple β-sheet filaments. These remarkably ordered and stable nanofibrils can be useful as building blocks for protein-based functional materials, but they are also implicated in severe neurodegenerative diseases. The overall aim of this article is to highlight recent efforts aimed at obtaining insights into amyloid proteins on different length scales. Starting from molecular information on amyloids, single fibril properties and mechanical properties of networks of fibrils are described. Specifically, we focus on the self-assembly of amyloid protein fibrils composed of peptides and denatured model proteins, as well as the influence of inhibitors of fibril formation. Additionally, we will demonstrate how the application of recently developed vibrational spectroscopic techniques has emerged as a powerful approach to gain spatially resolved information on the structure–function relation of amyloids. While spectroscopy provides information on local molecular conformations and protein secondary structure, information on the single fibril level has been developed by diverse microscopic techniques. The approaches to reveal basic mechanical properties of single fibrils like bending rigidity, shear modulus, ultimate tensile strength and fracture behavior are illustrated. Lastly, mechanics of networks of amyloid fibrils, typically forming viscoelastic gels are outlined, with a focus on (micro-) rheological properties. The resulting fundamental insights are essential for the rational design of novel edible and biodegradable protein-based polymers, but also to devise therapeutic strategies to combat amyloid assembly and accumulation during pathogenic disorders.

1. Introduction

Amyloids constitute a fascinating class of biopolymers that consist of non-covalently bound aggregates of misfolded polypeptides, resulting in insoluble fibrous protein aggregates sharing specific structural traits. Amyloids are set apart from other biopolymers such as DNA and polysaccharides, in that the respective monomers of amyloids are comparatively large polypeptides which polymerize to form a so-called cross-β structure. Moreover, the bonds between the monomers are non-covalent. As a result of misfolding, the individual polypeptides constituting an amyloid fibril fold predominantly into a β-sheet structure. The strong, but non-covalent interaction between the β-sheets gives rise to stacking of the peptides into protofibrils, which can subsequently assemble into large fibrils with a diameter of several nm's and a length up to many microns. Many different biological and artificial peptides can form amyloid structures under the right conditions. *In vivo*, amyloids are often associated with neurodegenerative diseases like Alzheimer, diabetes and Parkinson's disease. However both biogenic and artificial amyloids are also widely applied, for instance, in structuring foodstuffs.

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Amyloid fibrils can be regarded as biopolymers very similar to silk [1], exhibiting common key features: they originate primarily from unstructured precursor proteins and the definition of the materials occurs through its specific structural and mechanical properties rather than through its detailed chemical composition. The cross-β sheet core structure of amyloid fibrils is very rigid and confers superior mechanical properties. The fibrils can exhibit a Young's modulus similar to that of silk [2] and an ultimate strength similar to steel [2,3]. As such, amyloid fibrils constitute promising building blocks for bio-inspired materials. The fibrils form spontaneously from a wide range of natural proteins. The structural and mechanical properties are relatively insensitive to the protein amino acid sequence [4] and to a wide range of chemical and biochemical modifications [5]. The unique structure of amyloid fibrils renders them relatively robust, even under extreme conditions such as high and low temperature, the presence of proteases, detergents and denaturants, and physical forces [6]. The recent increase in research activity in the area of amyloid-based materials [7] is therefore not surprising. Amyloid fibrils have been used as templates for metallic nanowires that could be used for molecular electronics [6], have been proven to be efficient drug delivery vehicles [8] and are useful as scaffolds for tissue engineering [9]. In the context of human diseases (see Section 2), their robustness against chemicals generally impedes effective medical treatment of amyloid-based diseases.

Despite the broad interest in this relatively novel class of materials, a detailed understanding of the relation between the molecular properties of amyloids and their material characteristics like stiffness and mechanical strength has remained challenging. For instance, the high β-sheet content as the main secondary structure characteristic of amyloids is related to their structural stability [3]. But amyloids contain additional secondary structures and exhibit a distinct polymorphism [10–12], seeming to weaken the exclusive role of β-sheets as the determinant of amyloid stiffness and mechanical strength. In particular, the role of the amino acid side chains remains to be elucidated in that context. The lag in our basic understanding of the relation between amyloid structure and mechanical properties may be traced back to the limited number of techniques that can provide information on the molecular interactions underlying the assembly and material properties of protein fibrils. The material properties of amyloids are based on delicately interconnected effects occurring at a variety of length scales, as illustrated in Fig. 1. Our article will highlight recent applications of various techniques aimed at elucidating the molecular structures and mechanical properties of amyloid-fibrils and derived materials.

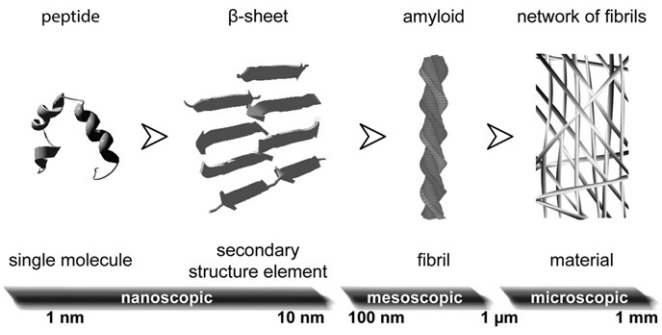

Fig. 1. Hierarchical length scales relevant in amyloid research, starting from the level of single molecules (*left side*: hIAPP, PDB 2L86) in the nanoscopic regime. Single or several peptides and proteins build up secondary structure elements, which are dominated in amyloids by β-sheets (PDB 2KIB). Mature amyloid fibrils have mesoscopic dimensions and consist typically of two or more twisted strands, forming thin and long fibrils. Networks of fibrils constitute micro- and macroscopic materials. Vibrational spectroscopies focus on insights at the molecule and secondary structure element level, atomic force and electron microscopy (AFM and EM) at the level of single fibrils and (micro-) rheology on networks of amyloids.

Specifically, this feature article describes recent investigations into the structural and mechanical properties of amyloids at different length scales. Nanoscopic level insights are obtained with recently developed vibrational spectroscopic techniques. The vibrations of molecular groups within amyloid structures, and in particular the amide I mode (with predominant C=O stretch character), are sensitive reporters of the local environment of those groups, as well as the protein structure. Hence, vibrational spectroscopies are very useful for quantifying secondary structure and intermolecular interactions in amyloid systems. Moreover, surface-specific vibrational spectroscopies like vibrational sum frequency generation (vSFG) can provide information about amyloid formation at (lipid-) interfaces, which are known to catalyze the polymerization of amyloid precursors. The application of tip-enhanced Raman spectroscopy (TERS) offers the possibility to spatially resolve structural characteristics of amyloids in the nm regime. Two-dimensional infrared spectroscopy (2D-IR) reveals amyloid interactions down to the level of single amino acids, by selective isotopic substitution.

At the mesoscopic level, we review results from biophysical assays using transmission electron-, atomic force and fluorescence microscopy to measure single fibril mechanical properties. Among others, several different approaches to determine values for bending rigidity, shear modulus, ultimate tensile strength and extensibility are presented. Rheology measurements have been applied at the microscopic scale to access the mechanical properties of networks of fibrils, which constitute promising materials like viscoelastic gels.

2. Background

Many biological materials rely on fibrous networks of proteins for their mechanical strength. Some well-known examples include tissues such as skin, blood clots, and spider webs. Proteins are normally folded in a specific geometry dictated by their primary structure (amino acid sequence). Fibrils are then formed by supramolecular assembly of protein building blocks, which are often globular (as in the case of actin and microtubules) or rod-like (as in the case of collagen and fibrin blood clots).

There is also an alternate path of fibril formation: misfolded or partially unfolded proteins tend to form amyloid fibrils. This pathway was originally discovered in the context of several neurodegenerative diseases (notably Alzheimer's, Parkinson's, Huntington's, and Prion disease) and late onset diabetes [13,14]. These 'conformational diseases' (or amyloidoses) are characterized by the deposition of insoluble plaques of aggregated amyloid fibrils, which can lead to cell death in specific organs. Surprisingly, several organisms use amyloids to build natural, protective materials, such as protective coatings of bacteria and spores and the silkmoth eggshell [15]. It is increasingly apparent that the amyloid state is an inherent characteristic of polypeptide molecules under denaturing conditions, independent of the native structure or primary sequence. In the context of food, this has been long known, for instance in the cases of heat-denatured gelation of β-lactoglobulin from milk and lysozyme from egg white [4].

Amyloid fibrils formed from structurally unrelated peptides and proteins share a surprisingly similar structure. The change in secondary structure of the native protein to a β-sheet rich secondary structure represents one of the main determinants of amyloid formation. Amyloid fibrils show a characteristic X-ray diffraction pattern, caused by a so-called cross-β core structure. The amyloid core structure is composed of a stack of β-strands perpendicular to