Unzipping the mysteries of amyloid fiber formation

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Interest in the phenomenon of protein aggregation is as old as protein research. For example, changes in activity and covalent structure accompanying “coagulum” formation by insulin were important to the elucidation of the molecular origins of hormone action (1). In recent memory, the phenomenon of aggregation served only as an annoyance to the research biochemist and a serious economic consideration in the production and distribution of protein pharmaceuticals. Now, however, one class of aggregate has drawn considerable interest from a range of disciplines. These aggregates are readily identified ultrastructurally by the presence of nonamorphous, filamentous structures, termed amyloid fibers.

Function and Pathology
Two diverse groups share an interest in these systems. The first are those interested in the biological and biomedical relevance. Amyloid fibers are involved in a range of human conditions, e.g., Alzheimer’s disease and type II diabetes (2, 3). The conversion of a normally soluble protein into amyloid can give rise to a gain in toxic function or a loss of function, or can result in occlusion of normal cellular or organ function. Furthermore, there is growing evidence that amyloid can be used productively by organisms (4). For example, in Escherichia coli, the deposition of the protein curli as amyloid serves as a substrate for colony and biofilm formation (5). In humans, it has recently been suggested that similar conversions in conformation of cytoplasmic polyadenylation element-binding proteins are central to the maintenance of long-term memory (6). The second group are those interested in the development of novel materials (7, 8). Amyloid fibers template their own assembly, giving rise to reproducible structures on the nanometer scale. The precursors are readily synthesized or are biologically expressed, allowing for a range of derivitization. The reaction conditions for assembly can be environmentally friendly and yet yield fibers that are physically and chemically robust. For example, the fibrillogenic NM domain of the yeast prion Sup35 has recently been derivitized to create conductive gold wires with diameters of ~100 nm (9).

Effective insight into biological problems and control in material sciences require a much deeper understanding of the physical chemistry of amyloid formation. In this issue of PNAS, Kammerer et al. (10) rigorously characterize an important tool for making these insights. This tool is a 17-residue de novo-designed peptide, ccβ, in which considerations relevant to amyloid formation were overlaid onto a coiled-coil protein design (11). The coiled-coil protein motif is both a well used motif in biological systems (12) [e.g., in regulation of influenza membrane fusion (13, 14)], and a basic unit of tertiary structure for de novo design. The basic structure can be visualized as a seven-residue or heptad repeat, (abcdefg)n, of amino acids with α-helical propensity. The intrinsic pitch of an α-helix is 3.6 residues per turn. By spacing small aliphatic side chains, notably leucine, at positions a and d, a hydrophobic stripe occurs on average every 3.5 residues. In solution, hydrophobic association gives rise to a supercoiling of the helices into dimer, trimer, or tetrameric assemblies with many of the properties of a folded protein. This association includes hydrogen–deuterium exchange protection, cooperative unfolding transitions, and well defined 1H NMR spectra.

Structural Properties
To understand the interest and possibilities, it is important to understand the physical properties of amyloid systems. All amyloid fibers are composed of β-strands. This is true regardless of the structural origins of the precursor. For example, islet amyloid precursor polyglobulin (Aβ), in which amyloid after exposure to divalent Cu(II).

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ity and intrinsic helicity. Not surprisingly, this variant’s thermodynamic properties are comparable to its parent. Remarkably, oxidation of the methionines to methoxide, ccβ-MetO, produced a construct wholly incapable of generating fibers. One principle of mutational analysis is to introduce the minimum perturbation required to generate a measurable effect. In the case of ccβ-MetO/ccβ-MetO, a change of polarity with minimal change in size and weight illustrates the importance of specific side-chain–side-chain interactions in establishing and stabilizing the amyloid fiber.

**Kinetics of Assembly**

A protein placed under amyloidogenic conditions will initially remain soluble. This quiescent period or lag phase is followed by cooperative assembly into the aggregated state. Like crystallization, a hallmark of this process is the capacity of fiber formation to be bypassed by providing exogenous seed from a previously conducted reaction. From a physical chemist’s point of view, it is seeding that blurs the distinction between infectious and degenerative forms of amyloid disease. For example, it may be the case that spontaneous conversion of the cellular conformation of prion protein, PrPc, to a pathological form, PrPSc, perhaps by ingestion of infected bovine tissue, will endogenous PrPc convert. This example highlights a central question in amyloid formation.

**Peptide Model Systems**

The benefit of studying fibrillogenesis in peptides is the ease of implementing design, incorporation of nonamino acids, and incorporation of site-specific labels for spectroscopy. Polypeptides have an intrinsic ability to form amyloids, the sequence preferences of which are readily explored by this approach. For example, systematic analysis of the amyloidogenicity of 6-mer sequences has enabled the description of motifs suitable for genome-wide investigations (27). Whereas intrinsic propensities of polypeptides are readily investigated by this approach, caution must be exercised in studies of the peptides of parent amyloidogenic proteins. A consequence of the assertion that any protein can form an amyloid (28) is that any subpeptide of any protein can form an amyloid, provided suitable conditions are found. For example, the putative core of IAPP, IAPP19–29, was suggested and then identified as amyloidogenic by using conditions of 10 mg/ml in 10% acetic acid (29). By contrast, full-length IAPP readily forms fibers at 10 μg/ml. The origins of this disparity are structural with long-range aromatic/amine contacts central to the establishment of prefibrilligenic structure (30). Inhibition of parent precursor fiber formation by a subpeptide is therefore more suggestive of the subpeptide structural role in amyloid formation. For ccβ, long-range interactions are accessible to measurement by using isotopic labeling and solid-state NMR. This permitted careful distinction between intra- and intermolecular contacts between disparate residues, specifically Ala-7 and Leu-14. Clearly, the coiled-coil design permits a breadth of analysis available only to synthetic methods. Furthermore, despite its small size, the ccβ sequence yields a system in which the relationship between refolding transitions, oligomerization events, and fibrillogenesis can be systematically compared with those found in biomedical systems.

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