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Recent atomic models of amyloid fibril structure

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Despite the difficulties associated with determining atomic-level structures for materials that are fibrous, structural biologists are making headway in understanding the architecture of amyloid-like fibrils. It has long been recognized that these fibrils contain a cross-β spine, with β-strands perpendicular to the fibril axis. Recently, atomic structures have been determined for some of these cross-β spines, revealing a pair of β-sheets mated closely together by intermeshing sidechains in what has been termed a steric zipper. To explain the conversion of proteins from soluble to fibrous forms, several types of models have been proposed: refolding, natively disordered and gain of interaction. The gain-of-interaction models may additionally be subdivided into direct stacking, cross-β spine, three-dimensional domain swapping and three-dimensional domain swapping with a cross-β spine.

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Introduction

In 1935, the pioneering biophysicist Astbury [1] placed poached, stretched egg white in the X-ray beam and observed a diffraction pattern with perpendicular reflections at ~4.7 Å along the meridional (stretched or fibril) direction and ~10 Å along the equatorial direction. The pattern suggested that the protein chains of the egg white pack in an extended or β-conformation, with the chains perpendicular to the long (stretched) axis. Later, this cross-β X-ray diffraction pattern was observed for the elongated unbranched fibrils of amyloid deposits in diseased tissues [2]. More recently, the cross-β X-ray pattern was observed for numerous fibrils formed by removing non-pathological proteins from native conditions [3–6]. Today, pathologists term those extracellular fibrils that are associated with disease as 'amyloid' and fibrils of normal proteins formed by changing solution conditions as 'amyloid-like' [7]. X-ray, electron microscopy (EM) and biochemical studies [2,8,9] have shown that amyloid and amyloid-like fibrils share common properties, including an elongated unbranched morphology, a substructure composed of multiple protofilaments and protofilament cores containing β-sheets with strands perpendicular to the long fibril axis. In this review, we focus on models of the arrangement of protein chains in the protofilament proposed from 1999 to 2005.

At present, no single model accounts adequately for all properties of all fibrils, but some models explain many properties of a range of different fibrils. The models discussed below are useful in coming to terms with fundamental questions about amyloid fibrils, such as do fibril-forming proteins exist in two distinctly different states (native and fibrillar); what is the nature of the conversion from native state to fibril; what is the structure of the common cross-β spine; is there an amino acid sequence signature for the formation of the cross-β spine; and what is the structural basis of the self-complementation of proteins?

Models of amyloid-like fibrils

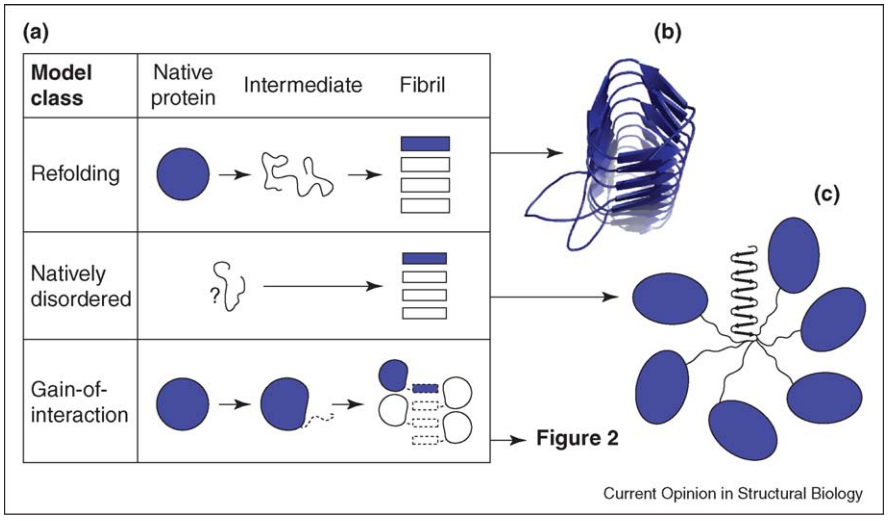
A variety of atomic-level models have been proposed for amyloid fibrils [10,11], with representatives shown in Figures 1 and 2 for each model class.

Refolding models

Refolding models depict each fibril-forming protein as existing in either its native state or a distinctly different fibril state. In the conversion from native to fibril, the protein must unfold and then refold. Because the fibrillar state has many common properties, such as morphology and diffraction pattern, that are independent of the fibril-forming protein, some investigators [4] suggest that the fibrillar state is defined by backbone hydrogen bonds, which are common to all proteins. In this class of model, the specific sequence of amino acid sidechains is unimportant, although the composition can affect the rate of fibrillization and the stability of the fibrillar state [6]. Specific refolding models have been proposed for insulin [12], an SH3 domain [13] and prion protein [14].

In terms of the fundamental questions raised above, refolding models depict the conversion from native to amyloid state as being one of unfolding one structure and forming a second. This second structure is enriched in β-sheet and depends on backbone hydrogen bonding for its stability rather than sidechain interactions. In some such models, for example, that of prion protein [14], refolding is proposed to span only part of the molecule.

Figure 1



Models of amyloid structure. **(a)** Cartoon depicting the three general types of models for the conversion of proteins from their native state to the amyloid-like state. In refolding models, the protein unfolds and then refolds into a different structure, which is stabilized largely by backbone hydrogen bonds. In natively disordered models, the cross-β spine forms from protein segments that are poorly structured in the native state. In gain-of-interaction models, a change in the conformation of the protein frees a segment for interaction with segments from other molecules. An extensive portion of the native structure is maintained in the fibril. **(b)** Ribbon diagram showing an example of a left-handed parallel β-helix, taken from the structure of UDP *N*-acetylglucosamine *O*-acyltransferase from *Escherichia coli* (PDB code 1LXA). This helix was used in modeling the structure of the refolded portion of prion protein in fibrils [14]. **(c)** Cartoon depicting the parallel superpleated β-structure proposed for Ure2p fibrils [19]. The view looks down the long fibril axis. Each arrow represents a view down a single β-sheet. The blue ovals represent the natively folded C-terminal domains, showing how stacked domains could pack around the serpentine core.

Parallel β-helices (Figure 1b) have been frequently suggested as the structure in these models [14,15], although this specific structure would not seem to be a requirement of the concept of a refolding model. For example, the pair of sheets with a dry steric zipper (discussed below) could serve as the spine. The question of protein self-complementation is not usually addressed for refolding models; they emphasize the structure of single refolded molecules and seem to assume that these stack on one another, mating open edges of β-sheets [16].

A specific example of a refolding model is that proposed for insulin by Jiménez *et al.* [12], based on a cryo-EM reconstruction of insulin fibrils. The protofilaments were found to have a cross-section of approximately 30×40 Å. Jiménez *et al.* [12] fit into this density insulin molecules transformed from their native, largely α-helical, structure into four β-strands constrained by two interchain disulfide bonds. These refolded monomers stack in parallel to form extended β-sheets, with a left-handed twist of $\sim 2^{\circ}$ between strands, mimicking the observed twist of the fibril. The refolded insulin molecule is compatible in dimensions with the reconstructed fibril, but the resolution of the experiment is too coarse to rule out other types of models.

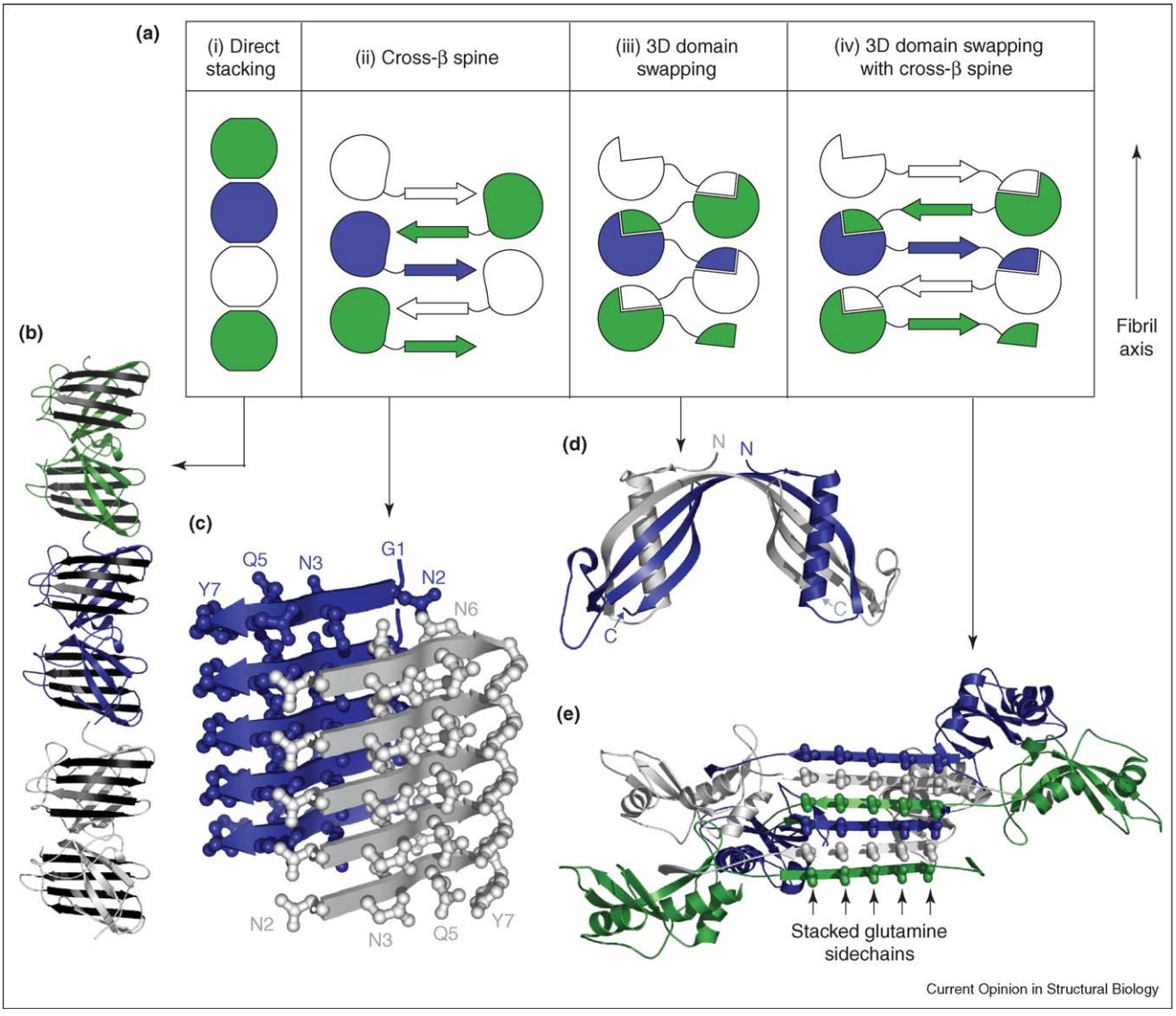
Natively disordered models

Certain proteins, or segments of proteins, are poorly ordered in their native states [17]. Among these natively disordered proteins are several that form amyloid-like fibrils. In the process of forming a fibril, all or part of the previously unstructured polypeptide becomes structured to form the cross-β spine. Models of this cross-β spine have been proposed recently for the natively disordered C-terminal segment of HET-s from fungus [18••], N-terminal segments of the yeast prions Ure2p [19•,20•] and Sup35p [21••,22•], the expanded polyglutamine segment of huntingtin protein [23,24,25•] and the β-amyloid polypeptide (Aβ) [26–29,30••].

Figure 1c depicts a model of the cross-β fibrils of Ure2p [19•]. The previously disordered N-terminal segment of the protein is suggested to form a serpentine arrangement of β-strands, each contributing to a separate β-sheet. The monomers stack parallel and in register to form a serpentine core of β-sheets, termed a 'parallel superpleated β-structure' [19•]. This model is supported by other recent studies of Ure2p, which suggest that the Ure2p fibrillar core is composed of parallel, in-register stacks of the N-terminal domain [20•,31].

The 39–43 residue Aβ peptide, when cleaved from the β-amyloid precursor protein, has a poorly defined monomeric structure [32], but quickly forms amyloid fibrils. Recent studies using solid-state NMR [26,33–36], quenched hydrogen-deuterium exchange NMR [30••,37], and other biochemical and biophysical techniques [27–29,30••,38]

Figure 2



Gain-of-interaction models. **(a)** Cartoon depicting the four subtypes of gain-of-interaction models. In direct stacking models (panel i), the gained interaction is achieved via simple stacking of subunits. Alternatively, in the cross-β spine models (panel ii), a segment of the protein separates from the core domain to stack into a cross-β spine, with the core domain decorating the edges of the spine. In the somewhat more elaborate model shown in panel iv, the molecules at the edges of the spine domain swap with identical molecules. This permits a wider range of stable geometries around the cross-β spine. In the remaining subtype (panel iii), proteins first domain swap and then stack into the fibril. **(b)** Ribbon diagram showing a crystalline filament of human superoxide dismutase mutant S134N (PDB code 1OZU [39]). Three dimers stack in an example of a direct stacking model. The β-strands highlighted in black are arranged roughly perpendicular to the fibril axis. **(c)** Ribbon diagram showing the pair of sheets of the GNNQQNY cross-β spine, with backbones represented by arrows and sidechains by ball-and-stick structures (PDB code 1YJP [21••]). The asparagine and glutamine sidechains facing into the space between the two sheets (N2, Q4, N6) pack to form a steric zipper. **(d)** Ribbon diagram showing the crystal structure of a 3D domain-swapped dimer of human cystatin C (PDB code 1G96 [46]). The monomers are colored blue and light gray, to highlight the swapped domains. N and C termini are indicated. **(e)** Ribbon diagram showing one sheet of the 3D domain-swapped cross-β spine model of fibrillar polyglutamine mutants of RNase A [45••]. The view shows one face of the proposed steric zipper, with aligned stacks of glutamine sidechains (shown as sticks) forming hydrogen bonds along the length of the fibril.

have helped to define the structures of the fibrils. There is general agreement that Aβ(1–40) and Aβ(1–42) peptides stack parallel and in register to form a set of β-sheets, with the N-terminal ~10 amino acids being poorly structured. Less clear are the boundaries of the core β-strand and turn regions, as the various studies seem to give conflicting results. The solid-state NMR studies [26,33–36] confer the most constraints on the structure and suggest that a bend in the chain (residues 25–29) brings two β-strands (residues 12–24 and 30–40) into proximity.

Gain-of-interaction models

The third general class of model is termed gain of interaction [39]. In these models, a conformational change in a limited region of the native protein exposes a previously inaccessible surface. This newly exposed surface binds to a surface of another molecule, building up a fibril. In gain-of-interaction models, most of the structure of the native protein is retained in the fibril (Figure 2). Only the interaction surface and its links to the core domain are changed.

Gain-of-interaction models can, in turn, be divided into four classes. The first class comprises direct stacking models. In these models, the newly exposed surface attracts a complementary surface of an identical molecule and the molecules stack on top of each other to form a fibril (Figure 2a, panel i). Molecules of mutant superoxide dismutase are found to stack this way in several different crystal forms [39] (Figure 2b), but it is not known if these stacks satisfy the other characteristics of amyloid-like fibrils, such as a cross-β diffraction pattern or unusual stability. Direct stacking models have also been proposed for transthyretin [40,41].

In terms of the fundamental questions raised above, the transition from native protein to fibril involves the exposure of a sequence segment that binds to a complementary segment of other superoxide dismutase molecules. There is no evidence that this sequence segment occurs frequently, so the self-complementation exhibited by superoxide dismutase would not be expected to be general for all proteins. Similarly, there seems to be no reason to expect that direct stacking of molecules would invariably lead to a cross-β diffraction pattern. In the case of the stacks of superoxide dismutase molecules, certain β-strands happen to be perpendicular to the direction of the stack (Figure 2b). Thus, this model of fibril formation appears to be at least partly compatible with the cross-β diffraction pattern (for the 4.7 Å reflection). But there seems to be no reason to expect a general tendency of fibril-forming proteins to stack with their β-strands perpendicular to the fibril axis. Thus, the generality of the direct stacking model for all amyloid-like fibrils seems unlikely.

Cross-β spine models are a second class of gain-of-interaction model. In these models, the short segment of the protein chain that becomes exposed has a tendency to stack into a β-sheet. The fibril grows with the stacking of the short segments of many identical molecules into β-sheets. The segment may be located at the end of a folded domain or between two folded domains, and in either case the domains are proposed to retain their native structure in the fibril.

Therefore, for the cross-β spine models, the structure of the short segment in the cross-β spine becomes the focus of structural studies. We have determined high-resolution atomic structures of a couple of short peptides in a cross-β spine [21••], revealing the details of the atomic interactions. The peptide GNNQQNY, taken from the sequence of the yeast (*Saccharomyces cerevisiae*) prion Sup35p, forms amyloid-like fibrils [21••,42,43]. The 1.8 Å resolution structure of the cross-β spine formed by this peptide, shown in Figure 2c, is based on X-ray diffraction from microcrystals closely related to the fibrils [21••]. The structure reveals that identical peptides in an extended conformation stack parallel and in register to form β-sheets. Two identical β-sheets face each other, with their sidechains intermeshing in a zipper-like, tightly packed, highly complementary interface, termed a steric zipper. The region between the sheets excludes all water, making a dry strip that runs the length of the pair of sheets. Similar structures were determined for the cross-β spines formed by a shorter peptide, NNQQNY [21••], and proposed for a glutamine-rich peptide [25•].

In cases in which the stacking-prone segment of protein occurs at a protein terminus, it is easy to envisage the rest of the protein retaining its native conformation and dangling off the end of the β-strand to which it is covalently bound (Figure 2a, panel ii). Such a model has been proposed for the fibril form of β_2-microglobulin [44]. But what if the spine-forming segment lies towards the middle of the polypeptide chain? In this case, protein molecules could retain native-like structures by domain swapping around the cross-β spine. That is, each natively folded protein would contain two domains, each from a different polypeptide chain. This situation is depicted in panel iv of Figure 2a. There is evidence that such a cross-β spine with a domain swap is present in a designed amyloid-like fibril of ribonuclease A (RNase A) [45••] (Figure 2e).

In terms of the fundamental questions posed above, the conversion from native to amyloid state in cross-β spine models consists of exposing the stacking-prone segment of the protein and forming the cross-β spine by the stacking of many such segments from identical molecules. The main protein domains retain their native structures. These folded domains could dangle at the sides of the growing spine or they could swap with complementary domains (Figure 2a, panels ii and iv). Self-complementation of proteins is achieved by the tight complementarity of sidechains in the steric zipper and, if domain swapping occurs, then also by domain swapping. Formation of the fibril depends directly on the amino acid sequence of the cross-β spine. The sidechains of the spine peptide must be able to form a steric zipper with other molecules of the same type.

Still another type of gain-of-interaction model involves domain swapping without a cross-β spine. This type of model has been proposed for fibrils of cystatin C [46,47]

and β_2-microglobulin [48]. Domain-swapped dimers (Figure 2d) or oligomers could stack on each other to form a fibril or there could be a run-away domain swap, as shown in panel iii of Figure 2a. In a run-away domain swap, each monomer swaps a domain into the next monomer along the fibril. Either of these arrangements would produce a cross-β diffraction pattern only if native β-strands of the molecules happen to sit roughly perpendicular to the fibril axis. Thus, although an amyloid fibril could form through a domain-swapping gain of interaction, it is unlikely that this is a general mechanism of formation for all amyloid-like fibrils. Strand swapping [49], a subclass of domain swapping, can also lead to fibril formation.

Conclusions

Recent progress in understanding amyloid structure includes the first atomic-level structures of the cross-β spine and the development of a range of models, using a wide variety of structural tools. These models are helpful in defining questions for the next stages of research. Important questions to answer include: what is the range of structures that form the cross-β spines of amyloid fibrils; to what extent do the remaining segments of the fibril-forming proteins retain their native structure, or refold into the spine or some other structure; what is the basis of the self-complementarity of proteins that form fibrils; and to what extent is there an amino acid sequence signal for the formation of amyloid fibrils?

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