

Current Topics

Plasticity of Amyloid Fibrils[†]

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ABSTRACT: In experiments designed to characterize the basis of amyloid fibril stability through mutational analysis of the A β (1–40) molecule, fibrils exhibit consistent, significant structural malleability. In these results, and in other properties, amyloid fibrils appear to more resemble plastic materials generated from synthetic polymers than globular proteins. Thus, like synthetic polymers and plastics, amyloid fibrils exhibit both polymorphism, the ability of one polypeptide to form aggregates of different morphologies, and isomorphism, the ability of different polypeptides to grow into a fibrillar amyloid morphology. This view links amyloid with the prehistorical and 20th century use of proteins as starting materials to make films, fibers, and plastics, and with the classic protein fiber stretching experiments of the Astbury group. Viewing amyloids from the point of view of the polymer chemist may shed new light on a number of issues, such as the role of protofibrils in the mechanism of amyloid formation, the biological potency of fibrils, and the prospects for discovering inhibitors of amyloid fibril formation.

Amyloid fibrils and amyloid-related protein aggregates are associated with a variety of human diseases of the brain and the periphery (1, 2). The past decade has seen significant progress in our understanding of this alternatively folded protein structural motif. At the same time, much remains to be learned about the details of amyloid structure, the basis of fibrillogenesis, and the nature of protein aggregate cytotoxicity.

Experimental work over the past 10–15 years has revealed the amyloid folding motif to be a nearly ubiquitous alternative folded state that can be accessed by many polypeptides. For globular proteins, the route to amyloid often depends on the initial weakening of the native state; in addition, for

all proteins and peptides, amyloid formation also depends on packing constraints within the fibril (3). Reviewed here are mutagenesis experiments designed to focus on an understanding the nature of the packing constraints within the amyloid motif.

The results show that amyloid fibrils, like globular proteins, achieve stability through a mixture of hydrophobic and electrostatic forces. At the same time, the response of amyloid to mutagenesis differs in a number of ways from the typical response of globular proteins and suggests instead a significant resemblance to synthetic polymers and plastics. A relationship to synthetic polymers provides a comfortable context for a number important features of amyloid fibrils, including (a) the ability of so many naturally occurring proteins to form amyloid, (b) the observation of multiple conformational states of amyloid fibrils from the same polypeptide sequence, (c) the possible role of protofibrillar intermediates on the amyloid assembly pathway, (d) the biological potency of amyloid prions, and (e) the mixed success of attempts to identify inhibitors of the amyloid formation process.

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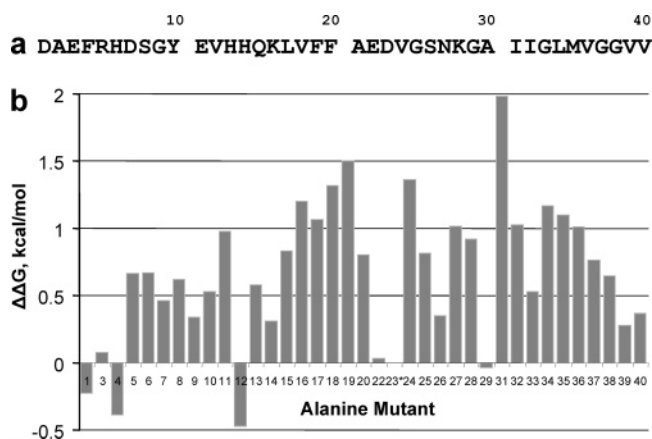


FIGURE 1: Elongation thermodynamics of the A β (1–40) amyloid fibril in response to alanine replacements. (a) Amino acid sequence of A β (1–40). (b) $\Delta\Delta G_{\text{Ala-WT}}$ of the elongation equilibrium for Ala mutants of A β (1–40). A positive value indicated destabilization of the mutant compared with the wild type. The x-axis shows the numbered residue replaced with Ala. The asterisk (*) at position 23 indicates that this mutant peptide could not be dissolved, and hence, fibrils could not be grown under defined conditions. Adapted from ref 5 (5) (Reprinted with permission from ref 5. Copyright 2006 Elsevier (Amsterdam).), with the inclusion of additional data (Williams, A. D., and Wetzel, R., previously unpublished data) obtained using published methods (61).

NATURE OF PACKING INTERACTIONS WITHIN THE AMYLOID FIBRIL

Much of the data and analysis discussed here comes from studies on A β ¹(1–40) amyloid fibrils, for which a characteristic equilibrium position for fibril elongation has been observed and exploited in the thermodynamic analysis of mutational effects. Thus, A β (1–40) fibril elongation tends toward a reproducible equilibrium position characterized by the concentration of monomeric peptide remaining in solution at the end of the fibril formation reaction (4–6). This critical concentration, C_r , is equivalent to the reciprocal of the fibril association equilibrium constant, K_a , which in turn can be used to calculate the ΔG for fibril elongation (6). Although the thermodynamics of elongation is technically not identical to the stability of the fibril, it would seem to be a good approximation of fibril stability because fibrils are built up by a series of elongation reactions in which, in each cycle, the incoming monomer explores the micro-environment at the growth end of the fibril.

Alanine Mutagenesis and the Fibril Elongation Equilibrium. Figure 1 shows the effects on the free energy of elongation in response to Ala substitution at different sequence positions in A β (1–40) (5). The results show that for many Ala replacements, especially in the C-terminal 2/3 of the molecule that is involved in the formation of the H-bonded amyloid core (Figure 2a) (7, 8), the elongation equilibrium is destabilizing by ≥ 1 kcal/mol. To check that this degree of destabilization is what might be expected, we compared our data on the A β (1–40) parallel, in-register amyloid fibril (Figure 2a) (7, 9), to the stabilities of G(β 1) variants in which residues at adjacent positions in the parallel β -sheet were mutated in pairwise fashion (10) (Figure 2b).

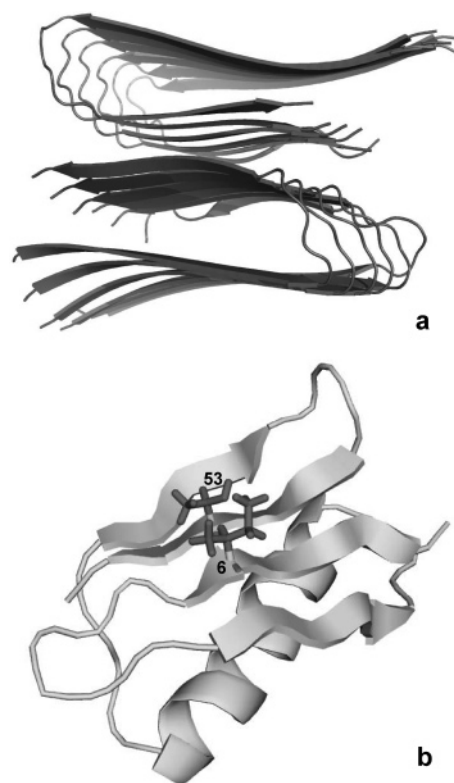


FIGURE 2: (a) Model of a section of the A β (1–40) protofibril based on solid-state NMR studies, based on pdb coordinates provided by Robert Tycko (7). (b) G(β 1) protein indicating residues 6 and 53 at adjacent positions in parallel β -sheet, from the pdb coordinates 2GB1. Figures rendered in PyMol (DeLano Scientific, LLC).

Table 1: Alanine Destabilization of Amyloid Fibrils and Globular Proteins^a

	$\Delta\Delta G_{(\text{Ala} - \text{residue})}$ (kcal/mol)						
	G(β 1) ^b	A β (1–40) amyloid fibrils					
mutation	6 + 53	18	19	20	31	32	36
Val \rightarrow Ala	1.25	1.3					1.0
Phe \rightarrow Ala	1.5		1.5	0.8			
Ile \rightarrow Ala	1.65				2.0	1.0	

^a The free energy effects of Ala mutations at different sequence positions on the protein folding equilibria of the G(β 1) domain and A β (1–40) fibrils are listed. Each column contains data for an X \rightarrow Ala mutation at a particular position (A β fibrils) or positions (G(β 1)).

^b G(β 1) data are from ref 10 (10). A β (1–40) data are from ref 5 (5). The values shown are on a per-residue basis so that the G(β 1) data for the effect of a double replacement (10) is divided by two.

The $\Delta\Delta G$ values for the two studies (Table 1) agree remarkably well, especially considering the differences between the two types of experiment and the expected contributions to the data of unique environments within the protein structures. Thus, although the globular protein data was obtained by the extrapolation of denaturation curves (10), whereas the fibril data was obtained from an equilibrium populated in native conditions (6), it would appear that side chain interactions make very similar contributions to β -sheet stability in fibrils and in globular proteins. This suggests that the analysis of elongation equilibria described here can provide useful data about amyloid fibril structure.

Lack of Additivity in Multiple Mutants. With some exceptions, mutations at distal positions in globular proteins tend to act independently of each other and, therefore, produce additive effects on protein stability and other

¹ Abbreviations: A β , amyloid beta protein; C_r , critical concentration; G(β 1), *Streptomyces griseus* immunoglobulin binding protein, β 1 domain.

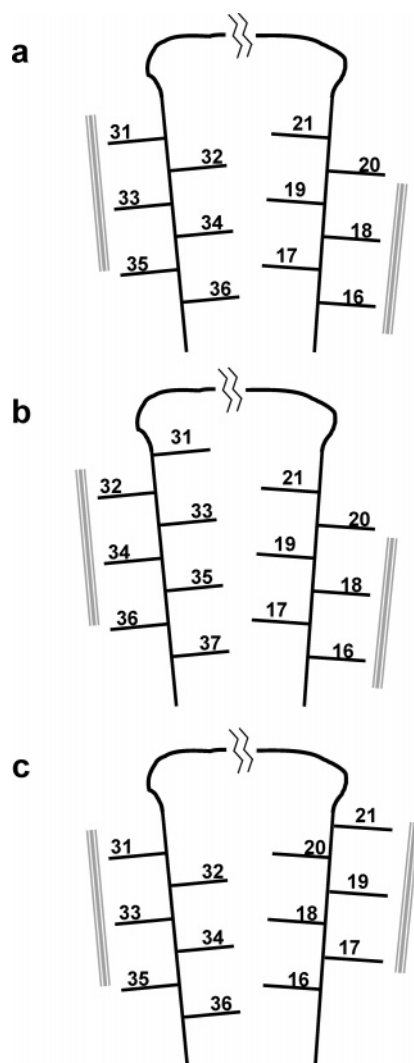


FIGURE 3: Schematic renderings of different models for the orientation of side chains within the A β hairpin in amyloid fibrils. The numbered lines represent the side chains of amino acids involved in β -sheet within the fibril. The gray bars represent apparent intermolecular packing residues. Part a is reproduced from ref 13 (13).

properties (11, 12). On the basis of structural models for A β (1–40) fibrils derived from solid-state NMR (7, 9), hydrogen–deuterium exchange (8), and cysteine mutational (13) studies, we conducted preliminary additivity experiments with Ala mutants of A β . We found that when Ala replaces both residues 17 and 34, which are well established to both be on the interior of the A β loop and in contact within the amyloid core (7, 13) (Figure 3), the cumulative destabilization of the two mutations is clearly non-additive (Figure 4a). Interestingly, when positions 17 and 27 are both replaced with Ala, the cumulative destabilization is also non-additive (Figure 3a). The lack of additivity between residues 17 and 27 is somewhat surprising, given their remote locations in the structural models (Figures 2a and 3a). Thus, the structural consequences of these mutations appear to be propagated at a significant distance through the protein structure, a result not usually observed in the analysis of globular proteins.

Atypical Responses of the A β Fibril to Proline Mutations: β -Sheet Migration within Fibrils. We also studied proline mutants to obtain information about the locations of

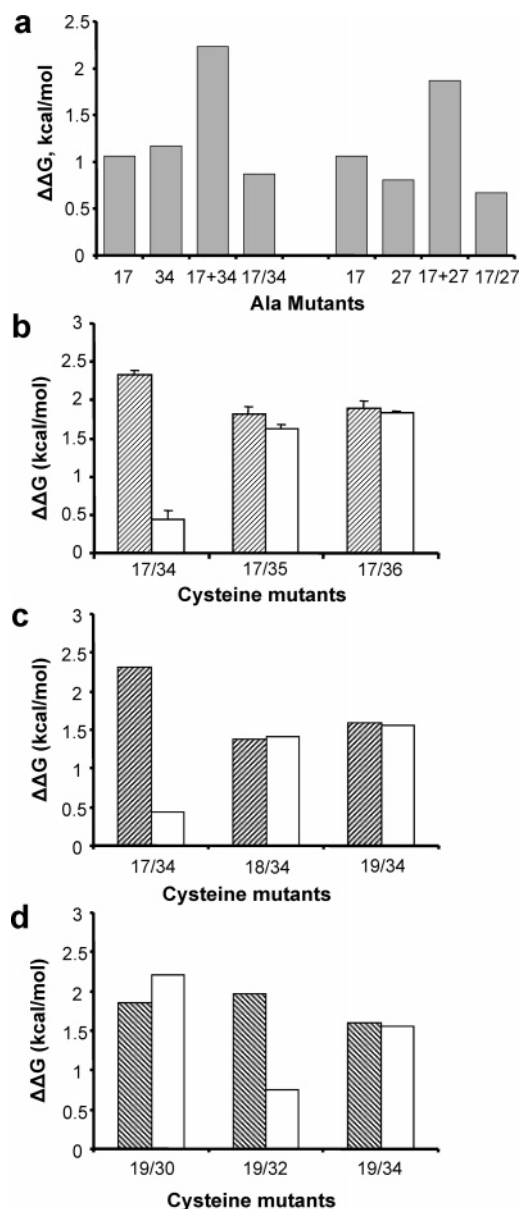


FIGURE 4: Elongation thermodynamics of multiple mutants of A β (1–40). (a) Additivity experiments of Ala mutants (see text). (b–d) Disulfide cross-linking experiments comparing the elongation equilibria of reduced (cross-hatched bars) and cross-linked, oxidized (open bars) double Cys mutants of A β (1–40) at the positions indicated. Part a reprinted with permission from ref 5. [Copyright 2006 Elsevier (Amsterdam)] and part b from ref 13. Parts c and d are based on previously unpublished data (Shivaprasad, S., and Wetzel, R.) obtained using published methods (62). The bars labeled $x + y$ are mathematical sums of x and y data; the bars labeled x/y show experimental data on x,y double mutants.

β -extended chain structure in the A β fibril (4, 14). The results (Figure 5a) indicated two or three contiguous segments of sequence that are highly sensitive to proline replacement, whereas the *N*- and *C*-termini as well as short elements between predicted β -extended chain segments are relatively insensitive to replacement (4). One surprising feature of the results, however, is that not a single mutation was found that completely destabilizes fibril structure. This is in sharp contrast to globular proteins like the G(β 1) domain (Figure 2b), in which single Pro replacements at β -sheet residues are so destabilizing that no significant fraction of folded protein is populated, leading to an estimated destabilization

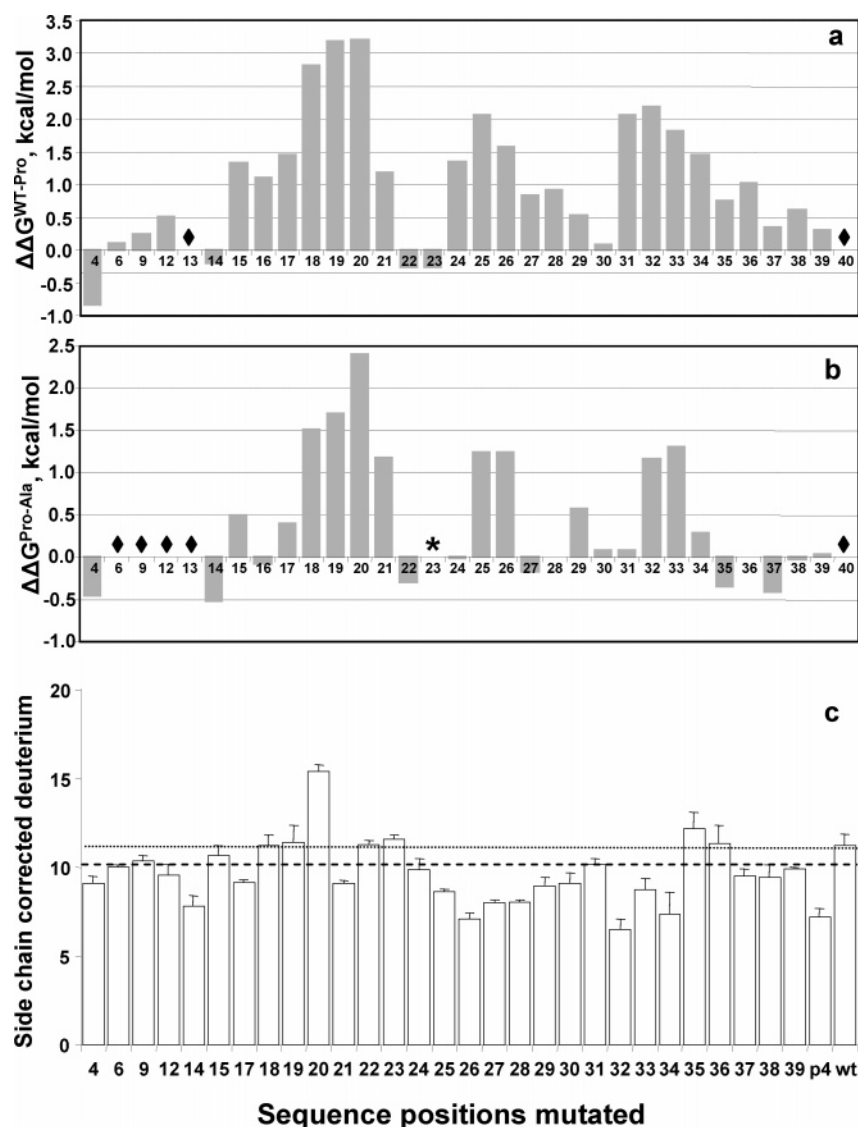


FIGURE 5: Proline mutagenesis of Aβ(1–40) amyloid structure and stability. (a) Plot of $\Delta\Delta G_{Pro-WT}$ with respect to the sequence position of proline mutation. (b) Plot of $\Delta\Delta G_{Pro-Ala}$ with respect to sequence position. (♦) Positions not analyzed; (*) not measurable because of solubility problems. (a) and (b) are reprinted with permission from ref 5. Copyright 2006 Elsevier (Amsterdam). (c) Hydrogen exchange protection of amyloid fibrils from Pro mutants of Aβ(1–40). Higher deuterium exchange (y-axis) indicates less protection and, therefore, fewer backbone amide hydrogens in β-sheet in the fibrils. (Reprinted with permission from ref 4. Copyright 2006 Elsevier (Amsterdam)).

of ≥ 4 kcal/mol (15, 16). Figure 5a shows that the highest destabilization found for any Pro mutation in the Aβ fibril is 3 kcal/mol, and many other Pro mutations at residues suspected to be in β-sheet in the fibril (7, 8) are destabilized even less. What is the source of this qualitative difference between an amyloid fibril and a globular protein?

One source of the missing folding energy appears to be the additional H-bonds that were surprisingly found to appear in proline-destabilized Aβ fibrils (4). When the number of backbone amide protons protected from hydrogen–deuterium exchange within the Aβ amyloid structure was assessed for wild type and Pro mutant fibrils, it was found that, in many cases, fibrils that are destabilized by a Pro mutation actually contain a larger number of protected amide protons (and, hence, most likely, more H-bonded β-sheet structure) than the WT fibrils (Figure 5c). This is especially surprising because it is expected that the effect of the Pro replacement will be a loss of H-bonded structure at the site of mutation. The site of the additional H-bonds must be one or more of

the regions of Aβ(1–40) that are not already involved in the H-bonded β-sheet in the wild type fibril, such as the N-terminal segment (7, 8, 17, 18). Because each added H-bond contributes stabilization energy, the added folding energy from these additional H-bonds appears to compensate for some of the destabilization caused by the Pro mutation. Many mutations in globular proteins affect the equilibrium by which the native structure is formed but alter the structure of the native folded state either modestly (19, 20) or not at all. In contrast, some mutations in the Aβ amyloid fibril appear to produce extensive remodeling of the β-sheet network of the fibril, which has the effect of allowing a fibril to form in spite of a severe mutation.

To further investigate how proline replacements affect elongation thermodynamics, the difference between the Pro–WT $\Delta\Delta G$ and the Ala–WT $\Delta\Delta G$ was determined and plotted. These values (Figure 5b), which are effectively the Pro–Ala $\Delta\Delta G$ for each sequence position, should report specifically on the ability of the backbone configuration at

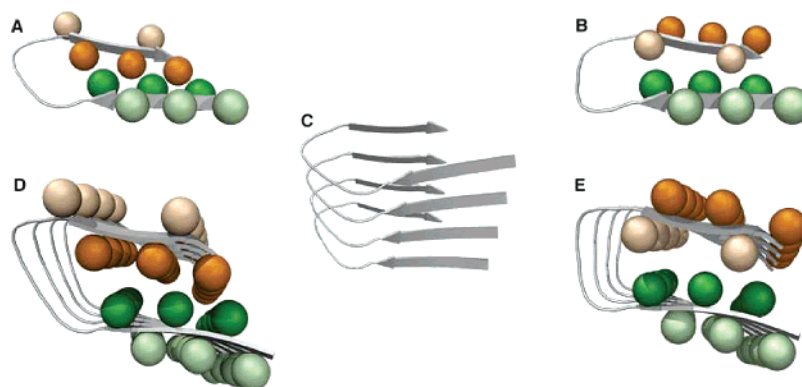


FIGURE 6: Schematic of the packing of interior residues in A β (1–40) fibrils from two different orientations of side chains within the A β hairpin. Parts A and D represent wild type A β (part a of Figure 3), and parts B and E represent an alternative conformation (part b of Figure 3) in which the C-terminal extended chain (residues 31–36) is rotated 180°. Part C shows the basic parallel, in-register structure of the A β fibril. Green shaded residues indicate residues 16–21, and tan/orange residues indicate residues 31–36. There is no side chain sphere for residue 33, which is Gly. The Figure was constructed by Sarina Bromberg (sarina@hughes.net).

a particular site to accept a Pro replacement (5). That is, the component of destabilization due to the loss of a hydrophobic side chain when a residue is replaced by Pro is largely factored out of the data in Figure 5b. The results are similar to those from the Pro scanning experiment (Figure 5a) but with some significant differences. In particular, some residues that are very likely to be in β -sheet in the fibrils according to structural probes (4, 7, 18), such as residues 31, 34, 35, and 36, exhibit little or no destabilization due to the main chain disruption effects of Pro (5). This apparent unresponsiveness to Pro is probably explained by compensatory gains from stabilizing interactions, such as additional H-bonds, as discussed above.

Resiliency in Fibril Structures from Disulfide Cross-linked Monomers. Disulfide engineering has been used to provide important side chain packing information within the A β (1–40) fibril (13). In one set of experiments, three double Cys mutants were examined: L17C/L34C, L17C/M35C, and L17C/V36C. Fibrils were grown from each under reducing conditions and then exposed to molecular oxygen. Only one of the three, the 17/34 double, efficiently produced a disulfide linkage within the monomer [revealed by HPLC after fibril dissolution (13)]. These results were interpreted to indicate that (a) the two β -extended chain segments 15–21 and 31–37 containing these residues are oriented within the fibril so that residues 15, 17, 19, and 21 from one segment and 32, 34, and 36 from the other segment are directed into the center of the sheet–sheet sandwich and that (b) within that packed sandwich, residues 17 and 34 are in contact (Figure 3a). [These results are consistent with independent solid-state NMR results (7).] This conclusion was also reached by comparing the elongation thermodynamics of the three double Cys mutants. In this case, oxidized, cross-linked monomers or, in separate experiments, reduced monomers, were allowed to form fibrils and their amyloid elongation equilibrium positions assessed. The results are shown in Figure 4b as a series of bar graphs. Confirming the results of the fibril cross-linking experiment described above, the 17/34 cross-linked monomer is the only one of the three to produce a fibril that is significantly more stable than the fibril from the reduced version of the same peptide. Furthermore, only the oxidized 17/34 fibrils exhibited stability very similar to that of wild type A β (1–40) fibrils.

The dramatic surprise in these experimental results was that the other two fibrils from oxidized double Cys mutants form readily, and are only modestly destabilized compared to wild type or reduced double Cys A β mutants. Yet, constrained by the covalent disulfide bond, both of these fibrils must be substantially different in structure compared to that of the 17/34 cross-linked fibrils or wild type fibrils. Cross-linked 17/36 fibrils must consist of the same faces of the two β -extended chains facing each other within the packed filament (as shown in Figure 3a) but with the register across the β -sandwich shifted so that residues 17 and 36 are now in contact. In a more extreme case, in cross-linked 17/35 fibrils, the 31–37 β -extended chain element must be engaged in fibril structure in a radically different way so that the face containing residues 31, 33, 35, and 37 will now be facing the interior of the filament, with the side chains of residues 17 and 35 in contact (as shown in Figure 3b). In spite of this potentially catastrophic structural change, the flipping of an extended chain element of the β -sheet by 180°, the fibrils so produced are only destabilized compared to wild type fibrils by about 2 kcal/mol. Although it is possible that the 17–35 disulfide produces a more local deformation in the β -sheet, rather than a rigid body, segmental 180° rotation, there appears to be no way that a rearrangement that accommodates the 17–35 disulfide can be subtle or innocuous. Figure 6 schematically shows how segmental rotation of the 31–37 extended chain by 180° dramatically changes the packing arrangements within the β -sheet of a single amyloid filament. In particular, this rotation introduces a potential void in sheet–sheet packing because of the altered placement of Gly 33.

Other disulfide experiments (Shivaprasad, S., and Wetzel, R., unpublished results) tell very similar stories. Figure 4c shows a comparison of the elongation $\Delta\Delta G$ s of the oxidized and reduced forms of the three double Cys mutants 17/34, 18/34, and 19/34. Of particular interest is the $\Delta\Delta G$ of the oxidized, cross-linked 18/34 mutant, which is only modestly destabilized compared to wild type A β (1–40). For this peptide to form fibrils, it would appear that the 16–21 β -extended strand of the peptide would have to undergo a 180° rotation (Figure 3c). Figure 4d shows a comparison of the elongation $\Delta\Delta G$ s of the oxidized and reduced forms of the three double Cys mutants 19/30, 19/32, and 19/34.

Consistent with the model shown in Figure 3a, this experiment shows that only the 19/32 double mutant, when oxidized, produces an amyloid fibril with stability minimally distorted from that of the wild type. Figure 4d also shows that the fibrils formed from oxidized, cross-linked 19/30 and 19/34 mutants are only modestly destabilized, despite the fact that the details of the hydrophobic packing across the loop must be significantly altered from that shown in Figure 3a.

The 17/34 packing interaction implicated in these Cys experiments is consistent with ss-NMR data on A β (1–40) fibrils (7). Interestingly, the 17–34 interaction is not part of a structural model for A β (1–42) on the basis of hydrogen exchange and other data (21). However, this is likely due to a real difference between A β (1–40) and A β (1–42) fibril structure and reflects the diversity of packing arrangements within the A β peptide capable of generating a stable amyloid fibril structure. Similarly, amyloid fibrils of the 10–40 fragment of A β appear to possess an alternative packing arrangement within the peptide as it folds into the amyloid motif (22). The resemblance between these polymorphic forms of A β fibrils and the fibril structures generated by the disulfide experiments described above is clear.

The packed, cross- β core of an amyloid fibril can be thought of as a 3D array in which each dimension consists of a type of stabilizing interaction: (1) covalent bonds (the β -extended peptide chain), (2) electrostatic bonds (the H-bonding direction of the β -sheet), and (3) packing interactions (hydrophobic and other interactions of the side chains). In the experiments described here, interactions in all three dimensions are dramatically altered without destroying the folding stability of the fibril. These experiments reveal a remarkable malleability or plasticity of the A β (1–40) fibril structure (4, 5, 13), in which radical changes in individual contacts can occur without a prohibitively large cost to stability. The resiliency of the amyloid fibril appears to be significantly greater than that of globular proteins. Because the folding of globular proteins tends to be highly cooperative, inputs of energy sufficient to disrupt the native state almost always lead to the formation of an essentially completely unfolded molecule, rather than the generation of an alternative structure. In contrast, when fibrils are exposed to mutations that in the globular protein world would be considered catastrophic, the amyloid tends to bend (that is, structurally adjust) rather than break (that is, unfold).

STRUCTURAL PLASTICITY OF AMYLOID FIBRILS

The word plasticity is sometimes applied to cases in which globular proteins, in response to point mutations, exhibit local structural polymorphism, lack of additivity, and so forth (20, 23, 24). The above review of mutational studies of A β (1–40) fibrils also uses the word plasticity in this sense to express the apparently very forgiving nature of amyloid structure, which appears to have the ability to extensively revise noncovalent packing interactions in response to potentially highly destabilizing mutations. Clearly, such examples of structural ambiguity or degeneracy are not formally equivalent to the plastic deformations of semicrystalline synthetic polymers that are associated with plastic materials (25). At the same time, plastic deformation implies

the ability of the material to exist in multiple stable or metastable states that are related to each other by changes in local packing and that are capable of interconversion without dissociation. Likewise, the ability of amyloid fibrils to readily engage alternative packing arrangements in response to mutation, as reviewed in the first half of this article, would seem to reflect a similar structural degeneracy, on a scale that is typically seen in semicrystalline polymers but almost never observed in globular proteins. The remainder of this article explores the relationship between amyloid and synthetic polymers and plastics.

Amyloid and Synthetic Polymers: Shared Features, Shared Vocabulary. A central conundrum of the amyloid phenomenon is the ability of many different polypeptide sequences to form stable, fibrillar, cross- β structures (26). Understanding how it can be that many different polypeptide sequences, each highly evolved to fold into a particular structure, can also fold into a cross- β amyloid motif has been one of the challenges in the amyloid field. Interestingly, synthetic polymer assemblies often exhibit a similar isomorphism. For example, “copolymers can ... show isomorphism if different monomeric units can replace each other without change of lattice structure. Isomorphism of chains is also possible if the two corresponding homopolymers have analogous crystal modifications, similar lattice constraints, and the same helix types.” (27). Might it be that the amazing ability of so many protein sequences to form fibrils is simply due to the resemblance of polypeptides to synthetic polymers?

With rare exceptions, globular proteins normally exhibit only one stably folded state. In contrast, polymorphism, the ability of a single polypeptide sequence to assemble into two or more structurally distinct and self-propagating amyloid fibrils, has been observed for several proteins, including the sup35 yeast prion (28), A β (1–40) (29), and a peptide fragment of β 2-microglobulin (30). The structural polymorphism of amyloid is biologically important because it is likely the molecular basis of both strain phenomena (28) and species barriers (31) in prion biology. Amyloid polymorphism has recently been reviewed from the point of view of polymer chemistry (32).

Structural polymorphism is commonly observed in plastic materials like the condensed states of synthetic polymers. In fact, a description of this phenomenon, from a basic text on plastics, reads remarkably like a summary of the recent discovery of multiple conformations of amyloid fibrils. “Most polymers show polymorphism: the same constitution and configuration leads to various energetically different crystal modifications. Polymorphism may be caused by different microconformations of polymer chains .. or by different packing of chains with identical chain conformations” (27). Synthetic polymers processed by different crystallization conditions are able to form fibrils, amorphous aggregates, stacks of platelets, and single lamellae (27), all of which are structures previously reported as alternative aggregated states during amyloid fibril formation (33–35). In a particularly striking case, the “Maltese cross” formations sometimes observed under a polarizing microscope in Congo red stained amyloid tissue (36) are also seen in synthetic polymers (27). In both settings, the molecular explanation for the Maltese cross is the formation of spherulites, spherical aggregates emanating from a central growth point that appear to form under conditions of limited nucleation. Given the differences

in chemical structure between proteins and synthetic polymers such as polypropylene, the resemblance of the spherulites formed from polypeptides and polypropylene is startling, suggestive of a deeper physical and structural resemblance of amyloid and synthetic polymers (37).

In fact, models for the internal structure of semicrystalline plastics and those that show how individual polymer chains fold in order to make up those structures (25, 27) bear a remarkable resemblance to models of amyloid fibrils derived from structural studies. Thus, although the structures and stabilities of amyloid fibrils appear to be dictated by the configurational constraints and microscopic interaction energies that also define globular protein structure, the morphological and energetic features, including structural polymorphism, that set them apart from globular proteins are more akin to the world of synthetic polymers and plastics (25, 27).

Amyloid as a Synthetic Polymer. The resemblance of amyloid fibrils to synthetic polymers and plastics should not be surprising. The first plastic materials known to have been used by man were in cave paintings such as those found at Altamira, in paints derived from egg white and blood proteins. Cow horn (keratin) was perhaps the first thermoplastic; it was molded into thin sheets to make lantern windows. One of the more well-known early commercial synthetic plastics was Galalith, produced by the treatment of casein with formaldehyde (27). Over 60 years ago, there was considerable interest in the commercial exploitation of the plastic and textile properties of artificial protein fibers, films, adhesives, and plastics (38, 39). Interestingly, Astbury's work on protein fibers can be viewed as plastic deformations induced by applying force to protein fibers. Starting in the 1920s, Astbury and colleagues performed electron diffraction experiments on wool and other protein fibers (40). The designation of α and β to the two main types of protein secondary structure derive from Astbury's definition of these motifs (long before their underlying structural meaning was clear) in the diffraction patterns of stretched and unstretched wool fibers. The term cross- β grew out of similar experiments in which poached eggs produced a cross- β pattern, whereas the stretching of this cross- β form produced fibers with diffraction patterns analogous to those of stretched wool (β -keratin) (39).

Synthetic polymers fall into two broad classes according to their microscopic structures and the properties defined by those structures. Amorphous polymers exhibit little or no long-range order and exist as a glass. In contrast, semicrystalline polymers exhibit a degree of 3D order that is normally thought of as reflecting a mix of highly regular crystallites and irregular elements. The degree of order can vary, even for a single polymer, and appears to do so as a result of vagaries of the crystallization process. Nucleation can be homogeneous, brought about by alignment within or between polymer molecules; alignment, in turn, can be induced by external forces such as shear forces. The requirement for normally rare homogeneous nucleation events can be abrogated by heterogeneous nucleation, in which exogenous factors, such as an added compound or the container wall, provide an amenable surface for initiating aggregation (27). Interestingly, both shear forces (stirring/agitation) (29) and the chemical nature of vessel walls (41) are well known to enhance amyloid growth rates.

In contrast to the simple, two-state crystallization of globular proteins, the crystallization of polymers can involve a number of intermediate states, each with different levels of crystallinity. The transition from a melt to the solid state sometimes only constitutes a primary stage crystallization. This is followed by an after-crystallization characterized by improved crystallinity, thicker lamellae, and transition to a denser state. Polymorphic forms of polymers can arise as different end states caused by differences in nucleation, cooling rates, and so forth or can be stages along the crystallization path toward maximized stability and improved crystallinity, through a kind of aging phenomenon (27).

Amyloidogenic proteins exhibit, in their condensed states, two kinds of polymorphism (32). Most of these peptides form spherical oligomers and protofibrils in the early stages of amyloid assembly; as alternative condensed states of these peptides, which in some cases are structurally related to the final amyloid fibrils (8), these states are themselves polymorphic variations. In addition, the final amyloid fibrils also often exist in different polymorphic forms (25, 27); these may be kinetically trapped alternative states or may be sequential intermediates toward a thermodynamically most stable form. In any case, it is likely that they differ from each other in their degrees of crystallinity, analogous to semicrystalline polymers (32).

What is it about amyloid fibril structure that produces properties more like those of synthetic polymers than those of globular proteins? Part of the answer likely lies in the malleability of both the H-bonding networks and the hydrophobic packing relationships in the amyloid β -sheets, as discussed above for A β fibrils. However, H-bonding networks cannot explain the structural plasticity of most synthetic polymeric materials because the interactions within or between the individual polymeric molecules (such as polypropylene and polyethylene) in many cases do not involve extended H-bonded networks. Instead, the most obvious general similarity between amyloids and synthetic plastics is that both involve condensed states in which individual polymer molecules are associated with each other through noncovalent interactions. That is, although the stability of both individual polymeric molecules and plastics (as well as globular proteins) depends on contributions from both intermolecular structural contacts and interactions with the solvent, the structural stability and properties of plastics (as well as amyloids) also depend on the interactions between the individual subunits (25). In amyloid, this means not only the H-bonds and hydrophobic packing between the layers of the basic cross- β filament (Figure 6) but also the less well-characterized interactions between the protofilaments. It seems clear that the more such intersubunit interactions contribute to overall fibril stability, the less necessary it is that the intramolecular packing contacts within the aggregate be completely optimized. This makes possible the existence of stable structures with only intermediate degrees of crystallinity in the core packing region. This analysis suggests that it will be very important to understanding the structural and energetic aspects of the interactions between the individual protofilaments that make up the mature amyloid fibril.

IMPLICATIONS OF AMYLOID PLASTICITY

Amyloid Assembly Mechanisms. Due especially to their potential role in human disease (42), there is currently intense interest in the structure of oligomeric pre-amyloid assemblies and their role in the amyloid assembly mechanism (32). Although it may turn out that early intermediates like spherical oligomers and protofibrils are off-pathway, in some cases, they may be on-pathway such that fibril nucleation and/or growth may involve concerted structural transformations within the aggregate as a key step to the formation of a final amyloid structure (43, 44). Such a mechanism would be supported, for example, by compositional (45) and structural (8) relationships between protofibrils and fibrils. Although such large scale structural rearrangements are not within the realm of experience of the protein chemist, they are commonplace among polymer chemists. Transformations can occur by two mechanisms. The first, plastic deformation, requires that the transformation be induced under physical stress, and generally implies that, on relief of stress, the transformation is reversed (25). The second, multi-stage crystallization, implies a conformational search for accessible positions of increased order and stability in a stepwise transition to higher crystallinities.

Fine Structure and Specificity Coexistent with Polymorphism. At first glance, the plastic nature of the amyloid fibril may seem at odds with two previously described properties of amyloid fibrils: self-propagation of polymorphic forms and well-defined packing interactions. However, although the plasticity of the amyloid folding motif leads to the existence of multiple possible conformers or polymorphs, any one of these polymorphic forms, once produced, exhibits a finite structure of sufficient integrity that it can propagate itself by seeded growth. This is perhaps not so difficult to visualize when the degree of order in the aggregate is high (46). However, even amyloid fibrils with intermediate levels of crystallinity appear to be able to self-replicate with high fidelity (29).

Implications for Inhibition of Protein Aggregation. Significant efforts have been made to discover small molecule inhibitors of amyloid growth, both in industry and academia, for example, by screening or design (47, 48). Very often, however, compounds that are discovered to influence the course of an aggregation reaction are required at high concentrations stoichiometric with the amyloidogenic peptide and/or are found to alter the nature of the aggregation product rather than completely inhibit aggregate formation (32, 49–51).

A small molecule capable of binding to the growth face of an amyloid fibril might be considered to be the equivalent of a highly destabilizing mutation within the amyloidogenic peptide such as the Pro replacements described above (Figure 5). If this is a reasonable analogy, then the expectation would be that as in the case of the Pro replacements, an aggregate might still be expected to form but with some alteration of its final structure. Furthermore, compounds that actually stimulate aggregation are well known in polymer chemistry, where small molecules are sometimes used as heterogeneous nucleating agents (25, 27, 52).

Given the above considerations, it may be a more realistic goal to search for small molecules that can change the course of an amyloid growth reaction so that toxic aggregates are

disfavored at the expense of nontoxic aggregate formation (53). This places an emphasis, however, on the detailed analysis of the reaction products formed in the presence of candidate inhibitor compounds (51).

Amyloid Materials Science. As discussed above, thinking of amyloid as a condensed polymer provides insights into the response of fibrils to mutations and to mechanisms of amyloid assembly. In addition, some properties of amyloid as a material appear to play a role in amyloid biology. It is also possible that amyloid will some day be engineered to serve materials science applications. Thus, the strength of a yeast prion phenotype has been shown to be related to the ease with which these fibrils fracture during amyloid elongation (28). Because growth rates depend on the elongation rate constant as well as the concentrations of both monomer and fibril growth ends, multiplication of the latter influences the ability of the prion to propagate in vitro. The strength and mechanical stiffness of insulin amyloid fibrils, assessed by atomic force microscopy, suggest that amyloid may have useful material properties (54). Other investigations of the utility of amyloid and amyloid-like aggregates as materials include surface properties for supporting cell culture (55), use as nanowires (56), and the ability to form liquid crystal phases (57). In contrast to traditional synthetic polymers, polypeptides offer a number of potential advantages as the basis of plastic materials, including uniform lengths, specificity and complexity of sequence, and generally good kinetic solubility, allowing slow crystallization.

Implications for Amyloid Structure. Some amyloid fibrils from short peptides appear to be very highly ordered (46), and microcrystals of short amyloidogenic peptides have supported high-resolution crystal structure determinations that have potentially provided general insights into the amyloid motif (58, 59). In polymer chemistry, however, longer chain lengths normally lead to lower degrees of crystallinity in the condensed state due to the constraints of chain folding (25). Furthermore, as discussed above, one way that polymorphic forms of polymers differ is in their degrees of crystallinity. It may thus be unrealistic to expect that all amyloids will exhibit the same high degrees of order that are found in structures formed by short peptides. Furthermore, although it is generally accepted in protein crystallography that multiple crystal forms may exhibit differences in quality and unit cell dimensions while not reflecting major differences in the structure itself, this may not be true for amyloids, for which, like for synthetic polymers, polymorphic forms are defined, at least in part, by their degrees of crystallinity. Improving the crystallinity of a particular amyloid polymorph may thus concomitantly alter the nature and structure of the polymorph.

CONCLUSIONS

Although the recent concentration of protein-related fields on amyloid fibrils and similar aggregates creates an impression of novelty and freshness, the observation and exploitation of the ability of soluble proteins to undergo a phase change well precedes the scientific enterprise itself, in such prehistoric exercises as the boiling of eggs and the curdling of milk, and also stands at the origins of protein science, in the characterization of the aggregation response of proteins to heat and denaturants (60). In the same way, the results

summarized here, while perhaps providing some new insights into the fundamental differences between the structural basis of globular proteins and amyloid fibrils, have precedents in the work of Astbury and others on transformations within protein fibers. The idea of the relationship of amyloid fibrils to plastics and synthetic polymers also has precedents in the historical (and pre-historical) use of proteins as precursors for synthetic plastics.

The plastic nature of amyloid appears to involve the malleability of both side chain interactions and H-bonded secondary structure and at its most basic level can be attributed to an apparently significant stability contribution from the noncovalent, multimeric nature of the amyloid fibril, a feature these fibrils share with more traditional plastic materials. At the same time, an amyloid of a particular protein appears to be an unusual plastic in some respects, such as in the homogeneous chain lengths of the monomers, the complexity and homogeneity of the side chain sequences, and the amphipathic nature of many polypeptides. Such unique features, combined with our ever-increasing abilities to dissect amyloid structure and the molecular basis of amyloid stability, may provide a level of control over the final product unprecedented in the synthetic plastics field and encourage practical uses of amyloid-like fibrils in material science and nanotechnology. In addition, the original impulse for the current focus on amyloid, its connection to human disease, can only be better served by an improved understanding of the biophysical basis of fibril structure, stability, and transformations.

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