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Pathophysiology of Amyloid Fibril Formation

George H. Sack Jr.

Abstract

All amyloid comprises fibrillar polymers of tightly associated protein monomers. Central to the fibril structure is a highly ordered β-pleated sheet domain although this interacting region may only be a relatively short stretch of each constituent polypeptide chain. Fibril formation begins as a nucleation event based either on the constituent monomer protein or its proteolytic fragment(s). The resulting fibrils are generally chemically inert and very stable.

Keywords: amyloid, fibril, β-sheet, polymer, nucleation

1. Introduction

The term "amyloid" is intrinsically a misnomer. It was derived in the nineteenth century to describe what was then thought to be amorphous material composed of carbohydrate (*Gk* "amyl," starch) in both plants and pathologic specimens from humans and animals. In human pathology these deposits were identified in the kidney, liver, heart, brain, and elsewhere and were often associated with organ dysfunction. In 1942, Hass [1] showed that the material was largely protein, not carbohydrate. An important observation in 1959 [2] used electron microscopy to show that the material was not amorphous but, rather, consisted of long fibrils. **Figure 1** shows an example of amyloid fibrils.

Subsequent studies have been directed to identifying the constituent proteins and clarifying the process(es) of fibril formation. Treatment for affected individuals depends upon addressing the underlying chemistry and biology. We will review these in turn.
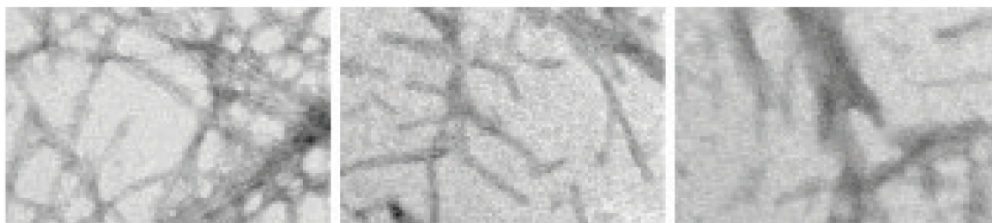

Figure 1.
Electron microscopic images of amyloid fibrils derived from synthetic sequences of Alzheimer β(1-42) polypeptides each containing different amino acid substitutions and yet forming characteristic fibrils, emphasizing the generalizability of amyloid fibril formation [3]. Copyright, 2005. National Academy of Sciences, used by permission.

2. Protein constituent(s) of amyloid fibrils

Many types of disorders are associated with amyloid deposits. Different fibril proteins underlie different pathologic contexts. **Table 1** presents examples of some types of amyloid diseases, the proteins from which their fibril proteins are derived, and their clinical associations. As will be noted below, it has become important to identify the precise molecular constituent(s) of the fibrils in order to establish the correct diagnosis and plan appropriate treatment.

In humans, the most frequently encountered pathologic amyloid fibrils are derived from fragments of immunoglobulin proteins. Some (but not all) immuno-globulin light chains (both κ and λ) have stretches of amino acids that can form the basic unit of amyloid fibrils. These are usually found in association with clonal pro-liferations of plasma cells which range from "monoclonal gammopathy of unknown significance" (i.e., "MGUS") to disorders such as myeloma. The fibrils themselves can cause distortion of organ microanatomy leading to cell dysfunction, disrupted cell-cell communication and, ultimately, organ failure.

Another well-studied type of amyloid fibril is derived from fragments of a small (104 amino acid) serum protein called "serum amyloid A (SAA)." Deposits of this type of amyloid are characteristic of chronic inflammatory or infectious disorders. It is likely that many of the examples of "amyloid" disease described in the pre-antibiotic era were derived from SAA (examples include tuberculosis and osteomyelitis).

Many genetic variants of transthyretin (TTR), another small, 127 amino acid serum protein, are associated with familial forms of amyloid disease. Affected indi-viduals show progressive neurologic and/or cardiac dysfunction with concomitant amyloid deposition [6]. In addition, some individuals develop TTR amyloid disease (particularly involving the heart) in the absence of an underlying mutation in the protein.

Amyloid fibrils and plaques are characteristic of the neurodegeneration of Alzheimer disease. In this case, the parent protein is a large membrane-spanning protein referred to as β-amyloid. Only a small fragment of the primary protein is found in amyloid fibrils [7]. Specific endoproteases release this fragment from the parent molecule. As **Figure 1** shows, characteristic amyloid fibrils can be formed from Aβ(1-42) polypeptides with individual amino acid variation(s).

Parkinson disease is another disorder of the central nervous system associ-ated with cellular depositions. In this case, α-synuclein accumulates in cells of the basal ganglia associated with loss of function. In neurons, relatively disorganized oligomers of α-synuclein appear first, and there is gradual compaction with β-sheet domains becoming prominent later [8, 9].

Considerable interest has arisen regarding prions. These are alternative conformations of proteins that can self-associate and self-propagate. First deter-mined to be causative agents of "spongiform encephalopathy" in humans (e.g., Creutzfeldt-Jakob disease), prions also have become recognized as respon-sible for transmissible neuropathies in animals (e.g., "mad cow" disease). Studies of Sup35 prions in yeast have been particularly informative, and multiple strains can be isolated [10].

Another interesting category of disorders associated with amyloid fibril forma-tion includes polypeptide hormones. Many of these are originally stored in relatively concentrated form within membrane-enclosed secretory granules. **Table 1** lists several associated types. In normal physiology dissociation of these organized structures must occur in order to release individual hormones [11]. In certain situa-tions, however, these same hormones (or their precursors) can persist and become detected as fibrils.

Designation	Precursor protein	Clinical example(s)
AL	κ, λ Light chains	Myeloma, MGUS
AA	Serum amyloid A	Secondary
ATTR	Transthyretin	Nerve/heart
Aβ	β-Protein precursor	Alzheimer
APrP	Prion protein	Encephalopathy
Aβ$_2$M	β$_2$-Microglobulin	Dialysis related
AIAPP	Amylin	Type II diabetes
ACal	(Pro)calcitonin	Medullary thyroid carcinoma
AANF	Atrial natriuretic factor	Atrial amyloid
AαSyn	α-Synuclein	Parkinson disease

Table 1.
Examples of pathologic human amyloid fibril proteins [4, 5].

3. Fibril structure

All amyloid fibrils share a basic unit comprising a relatively short and (usually) contiguous stretch of amino acids whose three-dimensional contours can be accommodated in a β-pleated sheet conformation (both parallel and antiparallel assemblies are recognized in fibrils). Hydrogen bonds between the amide groups polarize each other. Van der Waals forces also develop between the β-sheets. Water molecules are displaced from between the faces of the sheets. These important features emphasize that protein precursors of amyloid fibrils are not in their native state—the constituent monomer is in an altered conformation, and this state may only be transient prior to nucleation. Thus, there is an array of polypeptide species that may underlie fibril formation; the most (transiently) stable and/or abundant species is most likely to be captured in a stable β-sheet.

As shown by Riek and Eisenberg [12], amyloids derived from different protein constituents may have very different β-sheet domains. In addition, these regions may be rather small in comparison with the length of the parent protein(s). This implies that only a fragment of the parent (i.e., longer) protein may be found in the ultimate amyloid fibril, and this, in turn, implies that cleavage of the parent protein is often part of the process of fibril formation. **Figure 2** presents example details of the particularly well-studied Alzheimer Aβ(1-42) fibril.

The β-pleated sheet topology of the basic interacting region permits individual regions to stack upon one another in a highly ordered manner—thus extending into the long axis of the fibril (see **Figure 2**). The result is stabilization not only between β-sheet domains but also between stacked subunits. Fibrils often show a twist along their long axis.

Once the requisite region for forming β-sheets becomes available, fibril formation begins as a nucleation event. Usually small numbers of monomers interact, but once the primordial oligomeric fibrillary unit is assembled, further extension can occur by aligning new monomers with the growing fibril surface. The kinetics of this process are consistent with this scheme, there generally being a lag in assembling the oligomer followed by more rapid extension as subunits are added.

Most amyloid fibrils share relatively common dimensions although their lengths often vary. Because the fibrils are so tightly associated within their common nuclei of β-sheet domains, they are themselves rather resistant to dissociation (substantial free energy of formation) as well as to attack by proteases (regions susceptible to
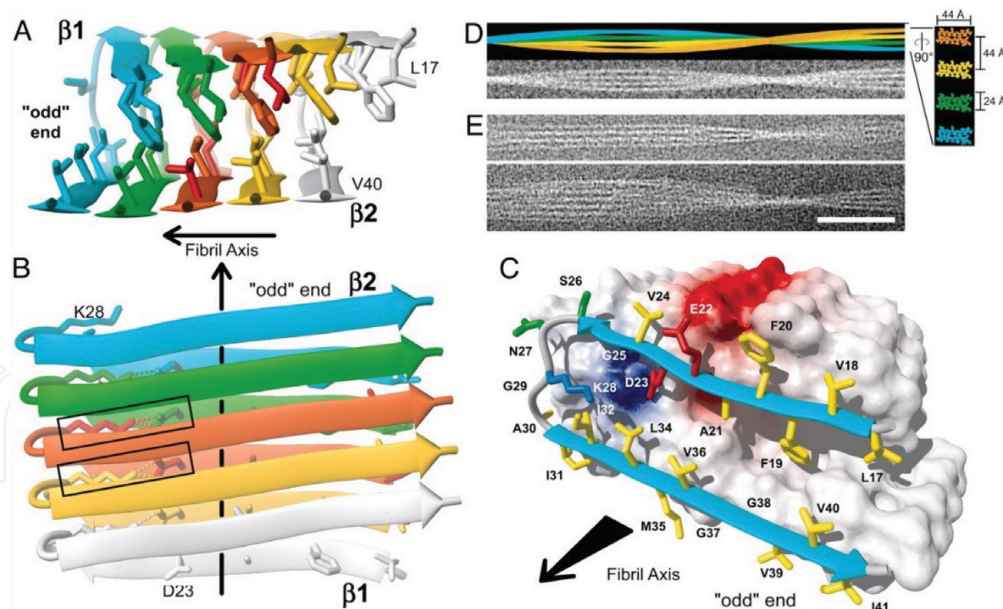
Figure 2.
Details of Alzheimer Aβ(1-42) fibril emphasizing amino acids 17-42. (A) Ribbon diagram of the core. (B) Salt bridges are indicated by the rectangles. (C) View of the "odd" end of the growing fibril showing the van der Waals surface as well as the amino acid side chains. The fibril axis is shown, corresponding to that in A and B. (D) Simulation of a fibril with four filaments (the adjacent figure shows the filament ends and the dimensions). (E) Cryoelectron micrographs of single fibrils [3]. Copyright 2005, National Academy of science, used by permission.

proteolysis may not be accessible). These molecular features help explain both the chronicity and the progressive nature of amyloid disorders.

Amyloid fibrils characteristically bind planar dye molecules. Upon binding to fibrils, these dyes can show birefringence when viewed using polarized light microscopy, providing a widely used method for identifying amyloid deposits in biologic tissues. The dyes Congo Red and Thioflavin T are used most frequently.

Another feature of amyloid fibrils is that they are usually found associated with other molecules. These include glycosaminoglycans (which may be responsible for calcium binding) as well as the pentraxin protein—"serum amyloid P" (SAP) [13]. These common features permit some imaging techniques to identify amyloid fibrils radiographically. For example, it is often possible to scan for SAP in order to localize amyloid deposits of various types [14].

Reference to **Table 1** emphasizes the variety of proteins that can form the β-pleated sheet structural units of amyloid fibrils. The proteins generally do not share common sequences or large-scale features because the region(s) essential for nucleating fibrils is(are) small (recall **Figure 2**). The protein constituent(s) of fibrils can frequently be determined using specific antibodies. The choice of antibodies to use is generally aided by the location of the deposit(s) and the clinical and/or pathologic context.

4. Fibril formation

As noted above, the fundamental event in amyloid formation is highly specific interaction between adjacent (usually) small protein units to form antiparallel (and, in some cases, parallel) β-sheets. The native state of the parent protein is generally thermodynamically more stable than the amyloid state. However, it is important to note that the stability of the amyloid state depends on protein concentration while that of the native precursor is largely independent of protein concentration. There thus arises a concentration above which the stability of the amyloid state can

become the same as or even transiently greater than that of the native state. Not surprisingly, the complex topology of long proteins makes them unlikely to nucleate and extend into stable fibrils. This generally limits formation of amyloid fibrils to proteins <150 aa [5].

Once the requisite region for forming the β-sheet becomes accessible, fibril formation begins as a stochastic nucleation event. Usually small numbers of monomers interact, but once the primordial oligomeric fibrillary unit is assembled, further extension can occur by aligning new monomers with the growing fibril surface. The kinetics of this process are consistent with this scheme, there generally being a lag in assembling the oligomer followed by more rapid extension as subunits are added.

Because different types of amyloid fibrils have different protein constituents and may be found in different locations, it is not always possible to identify common features. An important distinction is between fibrils that have soluble, generally blood-based, precursors and those derived from specific intracellular or organ-limited proteins. Among the former are immunoglobulins (usually κ or λ light chains) and serum amyloid A. The latter include hormone precursors such as procalcitonin, prolactin, as well as amylin and the β-protein precursor. Prions generally begin intracellularly.

As noted earlier, the protein precursors of amyloid fibrils are usually complex with multiple domains, and the critical, nucleating region is often small. Thus, many must undergo proteolysis in order to eliminate domains that are topologically incompatible with fibril dimensions and structure. In the best-studied examples, proteolysis occurs within lysosomes or related organelles although not all sites (or responsible proteases) have been determined.

Figure 3 presents a scheme for AA fibril formation from circulating SAA. SAA is usually found in association with high-density lipoprotein in the blood, and it must dissociate and enter the cell, often using clathrin-mediated endocytosis. Within the cell (in lysosomes or related structures), the acidic pH leads to unfolding of the precursor as reactive groups are titrated (steps A–C). This likely exposes region(s) with increased susceptibility to proteolysis. The considerable free energy of anti-parallel (or, for some other fibril precursors, parallel) β-pleated sheet formation and the relatively high local concentrations of appropriate domains leads to nucleation and at least some oligomer formation (steps C, D).

Intracellular oligomeric fibril nucleation ultimately causes organelle disruption followed by loss of cellular integrity (step E). The possibility of exosome participation at this stage has not been excluded. The result is a local mixture of nascent and growing amyloid fibrils and cell debris. Having oligomeric "seeds" already formed intracellularly, fibril elongation can then be extended. The result becomes a mixed,
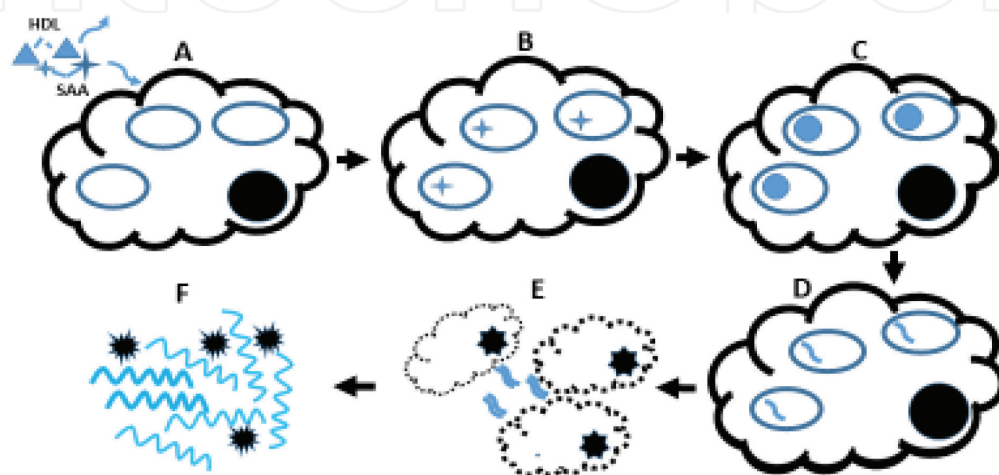

Figure 3.
Scheme for multiple steps in AA fibril formation from circulating SAA (from [16] see text for details).
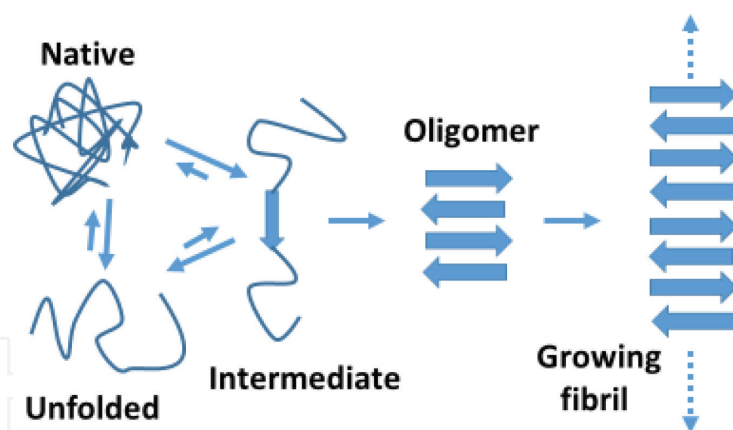
Figure 4.
Scheme for nucleation of TTR amyloid oligomers.

largely acellular, region where amyloid fibrils become the predominant structured species (step F) [15, 16].

A contrasting situation can occur in situations where the fibril precursor is not only soluble but also intrinsically capable of β structure formation without cleavage. **Figure 4** shows events for transthyretin (TTR). TTR is a 127 amino acid protein circulating in the blood as a stable tetramer that binds thyroid hormone and retin A (hence, its name). The TTR monomer itself contains prominent β-sheet domains. If the tetramer dissociates, the free monomers can misfold into various forms. Among these, some can associate as oligomers which then can be extended into fibrils. TTR amyloid fibrils are particularly prone to cause dysfunction in nerves and the heart. Interestingly, over 100 amino acid substitutions (i.e., mutations) have been identified in TTR [17]. Mutations differentially affect tetramer stability—some increase it, while others reduce it. Among the latter are several that are associated with inherited amyloid diseases, and the Val30Met and Val122Ile mutations have been particularly well-studied (affecting nerves and/or the heart). Kinetic and other evidence implicates the oligomer form(s) as directly involved in organ dysfunction. Amyloid fibrils become detectable by microscopy as the disease progresses. One proposed therapeutic strategy involves developing small molecules that stabilize the circulating tetramer, hence reducing (or eliminating) dissociation, oligomer formation, and tissue toxicity [18].

5. Pathologic consequences of amyloid formation

As indicated by **Table 1**, many disorders can be associated with amyloid deposition. In many cases, the precise mechanism of pathologic dysfunction is unknown. Nevertheless, several notions are important.

First, as noted, some polypeptide hormones are apparently stored in amyloid-like conformations. These differ from most of the other types in being reversible upon hormone release. Only in rare situations do these protein accumulations persist as amyloid deposits and become associated with disease.

Second, evolution has often minimized protein sequences that are particularly prone to nucleate amyloid fibrils [19]. Many proteins are located in intracellular regions or are associated with chaperones that reduce their likelihood of assuming alternative conformations. Degradation mechanisms including proteasomes can minimize intracellular aggregation.

In so-called "secondary" amyloid disease, the bulk of accumulated fibrils likely interferes with cellular and organ function. For example, remarkably large

quantities of AA protein were isolated from the kidneys, liver, and spleen in earlier studies [20, 21]. Examining affected tissues by light microscopy clearly shows large interruptions in organ structural integrity due to massive amyloid deposits. Similar accumulations often accompany "primary" (i.e., immunoglobulin-derived) amyloid disease where fragments of immunoglobulin light chains may be found in large deposits.

Evidence from other types of amyloid disease indicates that large, microscopically visible deposits of amyloid fibrils may not always be the initial cause of cell/organ dysfunction. While fibrils are often seen later in study of affected tissues (and, hence, appear as classic "amyloid" by staining), they may be late consequences. Earlier, oligomeric forms may be more disruptive and lead to organ dysfunction well before large deposits are detectable by microscopy.

Figure 4 (above) presents a simple scheme for oligomer formation. This is likely to occur as a basic pathway in various types of amyloid-related diseases. As described above, destabilization of the TTR tetramer occurs extracellularly and can lead to oligomers that are relatively small. Various TTR mutations can destabilize the tetramer and accelerate this process although even the normal protein also appears susceptible in some situations. This likely is a problem for various intracellular neurotoxic fibrils and their predecessors (e.g., β-protein and α-synuclein) as well [22]. Thus, finding substantial amounts of extracellular Congo Red staining deposits may be a late feature of the basic disorder rather than the primary cause of dysfunction. As noted above, such material is generally quite stable and often resistant to dissociation. Hence, extracellular deposits (or even substantial accumulations within organelles) may be the end point of the toxic oligomer pathway.

6. Therapeutic approaches to amyloid pathophysiology

As described above, once formed, amyloid fibrils are intrinsically quite stable due to intra- and intermolecular bonds and relative inaccessibility to proteases. Ideally, disrupting the fibrils themselves would be an appropriate approach to treating at least some amyloid disorders. In this regard, chaotropic molecules (e.g., urea, guanidinium, etc.), often used in the laboratory, are not options for treatment due to their toxicity. Alternative agents, compatible with in vivo use have not yet been identified. Thus, successful intervention(s) for amyloid disorders must either prevent formation of the precursor(s) or stabilize the protein predecessors of oligomers (or their proteolytic cleavage). Several approaches have been introduced, and these depend upon the *specific* type of amyloidosis (recall **Table 1**).

Immunoglobulin light chain overproduction ("primary" amyloidosis) is basically a clonal disorder of plasma cell proliferation/overexpression. Here, treatment generally depends on suppression or elimination of the responsible cell population. This falls into the spectrum of treatment of myeloma and related disorders and depends on oncologic approaches.

Amyloid A (SAA) disorders ("secondary" amyloidosis) generally reflect overproduction of the SAA precursor. These usually are related to chronic or periodic and recurrent infection/inflammation. The incidence of these has been reduced by successful treatment of conditions such as tuberculosis and osteomyelitis. However, these and other types of infections and inflammation remain prominent in certain parts of the world and can be accompanied by amyloidosis. Genetic disorders such as familial Mediterranean fever with recurrent, self-limited inflammatory episodes can usually be controlled with agents such as colchicine [23], minimizing SAA synthesis and AA amyloid accumulation.

For TTR-related disorders several approaches are recognized. First, because the liver is the main site for the synthesis of TTR, liver transplantation can eliminate production of the mutant TTR protein. Although this has been used successfully, it is a complex process with its own intrinsic short- and long-term complications [24]. Second, synthesis of the responsible mutant protein can be suppressed. Several approaches based on specifically interrupting stability of TTR messenger RNA through RNAi have been developed. (Carriers of TTR mutations are usually heterozygotes, so these approaches generally reduce levels of both mutant and normal TTR—apparently, a reduced concentration of TTR is well-tolerated.) Third, the circulating tetramer can be stabilized by exogenous agents (e.g., Tafamidis™) to shift the equilibrium away from dissociation (and, hence, minimize oligomer formation). These approaches show promise [25, 26].

For neurologic disorders where the underlying problem is likely intracellular (e.g., α-synuclein and β-protein), the above approaches have not been feasible. It is more likely that clarification of complex intracellular pathways will be required in order to develop approaches to prevent and/or interrupt fibril accumulation in these and related conditions.

7. Conclusions

Amyloid formation and related disorders present examples of situations where there is fundamental dependence on protein structure, its variation(s), abundance, and the consequences of alternative conformation(s). Because the molecules involved are quite different for each type of amyloid disease, different organs may be involved, and stabilizing or eliminating the precursors likely will vary. Successful therapeutic intervention will likely be unique to each type of amyloidosis. The basic concerns are similar, however, and considerable progress has already clarified both the problem(s) and the options.

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Conflict of interest

The author declares no conflict of interest.

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