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Protein Amyloidose Misfolding

Mechanisms, Detection, and Pathological Implications

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Summary

A variety of diseases result because of misfolded protein that deposits in extracellular space in the body. These deposits can be amorphous (disordered) or fibrillar (ordered). Inclusion bodies are an example of amorphous aggregates, and amyloid fibril is an example of fibrillar or ordered aggregates. In this chapter, we discuss a class of diseases caused by fibrillar aggregate deposits or amyloid fibrils called amyloidosis. We also review mechanisms by which different proteins misfold to form amyloid fibrils. Each amyloid fibril formed from a different protein causes a different disease by affecting a different organ in the body. However, the characteristics of different amyloid fibrils, namely structure and morphology, observed by electron microscopy and X-ray fiber diffraction appear to be quite similar in nature. We present therapeutic strategies developed to eliminate amyloid fibril formation. These strategies could possibly avert a whole class of fatal diseases caused by amyloid fibril deposition owing to similar characteristics of the amyloid fibrils.

Key Words: Amyloid fibril formation; protein folding; transthyretin; lysozyme; immunoglobulin; Alzheimer disease; prions.

1. Introduction

Protein folding whereby the protein acquires its native and active structure starting from a linear inactive state (in which the information for its coding is contained in its amino acid sequence) is a central problem in biology. During folding, certain proteins fail to acquire the native and active state and precipitate as an insoluble "mass." In vivo, the insoluble mass deposits in extracellular spaces in various organs in the human body, leading to lethal diseases that initially result in organ failure and eventually lead to organ death. The deposits could be either amorphous aggregates, such as inclusion bodies or disordered

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aggregates or fibrillar aggregates, such as amyloid fibrils. The diseases caused owing to deposition of amyloid fibrils are called amyloidosis. The precursor proteins that misfold to form amyloid fibrils are amyloidogenic proteins.

Sixteen different amyloidogenic proteins have been identified to form cross-β fibril components owing to misfolding. They result in different clinical syndromes ***(1,2)***. The most familiar include Alzheimer disease and transmissible spongiform encephalopathies (TSEs), prion diseases such as bovine spongiform encephalopathy or mad cow disease (consumption of tainted meat causes Creutzfeldt-Jacob disease in humans; in cows it is called mad cow disease). Molecular chaperones such as chaperonins that include heat-shock proteins, groEL, hsp60 family, and the hsp90 family are proteins that help in the proper assembly of proteins while folding to attain the native state. The excess unfolded proteins and misfolded proteins are removed by proteolytic degradation. This is the *quality control* mechanism. The amyloid fibrils that are formed overcome all the chaperone-mediated folding and degradation mechanisms to deposit in extracellular spaces in the human body, causing fatal diseases.

This chapter reviews different mechanisms by which amyloid fibrils are formed from precursor proteins. Amyloid fibrils formed from different amyloidogenic proteins have been characterized to have similar structural and morphological characteristics, as observed from Congo red staining, electron microscopy, and X-ray fiber diffraction data. Therapeutic strategies to inhibit or eliminate fibril formation for different precursor proteins have been reviewed. These strategies include surgical gene therapy, genetic engineering, artificial chaperones, and stabilizing ligands. The therapeutic strategies developed eliminate the formation of fibrils. This could possibly lead to a decline in the lethal class of diseases such as amyloidosis owing to the similar characteristics of these types of diseases.

2. Mechanisms of Amyloid Fibril Formation

The fundamental model for protein aggregation is shown in **Fig. 1**. The native forms, ordered aggregates (amyloid fibrils), and amorphous aggregates (inclusion bodies) are formed from the unfolded protein through intermediates. The intermediate can be considered a building block, and the native state is formed by intermolecular interaction of hydrophobic faces of such building blocks ***(3)***. Aggregates are formed when hydrophobic surfaces interact in an intermolecular manner, and three-dimensional (3D) propagation of such intermediates forms larger aggregates. The aggregates precipitate once the solubility limit is exceeded. The intermediates are prone to aggregation because they have large patches of contiguous surface hydrophobicity. The hydrophobic surfaces are relatively sealed within the structure in the final folded state.

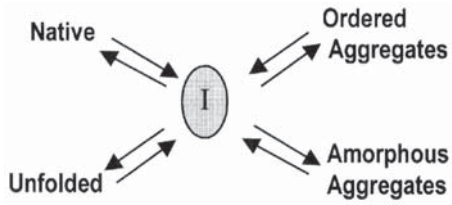

Fig. 1. Basic protein-folding mechanism ***(3)***.

2.1. Concept of Crowding

The various stages of protein folding may take place either in a single fluid medium or in a series of compartmental fluid media. In both cases, macromolecules occupy a large fraction of the total volume of the fluid. Such fluid media are generally referred to as "crowded" rather than concentrated because no macromolecule may be present at a high concentration ***(4,5)***. Crowding would enhance the rate and equilibrium constants for formation of amyloid-like aggregates. Competitive steps exist in protein folding to prevent it from forming amyloid fibrils. Nevertheless, a sustained condition of overcrowding would enhance any preexisting propensity of partially unfolded protein to form amyloid and/or inclusion bodies ***(4)***.

2.2. Conformational Change Hypothesis

The conformational change hypothesis indicates that tertiary structural changes under partially denaturing conditions convert 1 of the 16 normally soluble and functional human proteins into an alternative conformation. This alternative conformation subsequently undergoes self-assembly into amyloid fibril. This is the putative causative agent in amyloid diseases ***(6)***.

2.3. Fibril Characteristics

Amyloid fibril formation refers to an in vivo process in which 1 of the 16 human amyloidogenic proteins abnormally self-assembles into a fibril 60 to 100 Å in width and of variable length. Amyloid plaque is characterized by three features ***(3)***: A characteristic birefringent staining using Congo red, fibrous morphology observed by electron microscopy, and a distinctive X-ray fiber diffraction pattern consistent with a high β-sheet content.

Amyloid fibrils are generally 7 to 12 nm in diameter and can be dissociated by a high concentration of denaturant. Fiber diffraction studies have suggested that the core structure of amyloid fibrils is a cross-β structure, involving β strands running perpendicular to the axis of the fibril. The strands interact to form β sheets with their planes parallel to the axis of the fibril ***(3)***. Each amyloid fibril is considered to be made up of a bundle of fibrous "protofilament."

Each protofilament is a cytoplasmic microtubule, with a 25-nm od and 5-nm-thick walls, packed in a helix and associated with various proteins. Depending on the protein or peptide, four to five protofilaments intertwine to form a fibril, with a characteristic diameter of approx 10 nm *(3)*.

The ability to form an amyloid is a generic property of polypeptide chains. An increase in the concentration of precursor and subsequent amyloid formation may be brought about by mutation, and improper posttranslational modification *(4)*. The increased incidence of amyloid disease in the aged may be a direct consequence of increased total intracellular protein concentration crowding (or, equivalently, a decrease in cell water content) in the cells of aging tissues. This is supported by evidence for a significant decrease in water content in brain and liver cells with advancing age *(4)*. Amyloid fibrils are an apparently inert, insoluble, mainly extracellular protein polymer that kills the cell without tissue necrosis but by the activation of the apoptotic mechanism *(6)*.

Although there are 16 different amyloidogenic precursor proteins involved in amyloid fibrils formation, the following section restricts discussion regarding protein function, the mechanism of folding, and the characteristics of amyloid aggregate formation to the clinically important proteins transthyretin (TTR), immunoglobulin, Aβ peptide involved in Alzheimer disease, lysozyme, and prions. A few characteristics of β-2 microglobulin and apolipoprotein are also discussed.

3. Clinically Important Proteins Involved in Amyloidic Diseases

3.1. Transthyretin

TTR is involved in two amyloid diseases: familial amyloidotic polyneuropathy (FAP) and senile systemic amyloidosis. A partially folded monomeric intermediate, populated at a low pH, has been implicated in in vitro amyloid formation from TTR *(3)*.

TTR transports thyroxine by direct binding and transports retinol indirectly by binding to the retinol-binding protein. In certain individuals, wild-type (WT) TTR is converted to amyloid, which appears to cause senile systemic amyloidosis around the age of 80. This type of amyloid is characterized by heavy amyloid deposits in the heart, leading to congestive heart failure. Alternatively, 1 of 54 TTR mutants deposited systemically as amyloid can cause peripheral neuropathy or organ dysfunction. This typically has a much earlier onset. These diseases are broadly referred to as FAPs *(7)*. The FAP mutations appear to function by destabilizing the native protein-folded structure, and by making the amyloidogenic intermediate more accessible relative to the folded state under mildly denaturing conditions. These mutations do not alter the native folded structure. Destabilization increases the steady-state concentration of the monomeric amyloidogenic intermediate. This, in turn, increases the mass of

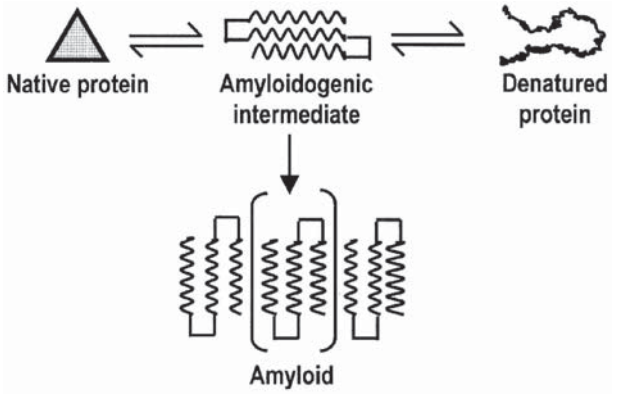

Fig. 2. Mechanism of TTR misfolding to form amyloid ***(1)***.

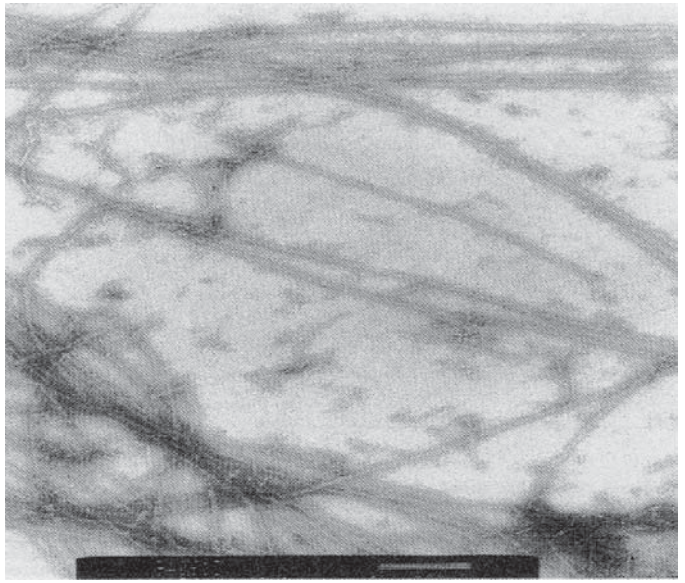

Fig. 3. Electron micrograph of TTR amyloid fibrils ***(11)***.

the amyloid formed, thereby lowering the age of disease onset ***(1)***. The amyloidogenic intermediate undergoes self-assembly into several quaternary structures that lead to TTR amyloid fibril formation at physiological TTR concentrations ***(8)***. The mechanism is shown in **Fig. 1**.

FAP variants denature to the amyloidogenic intermediate much more quickly than the WT TTR. This facilitates an increased concentration of the amyloidogenic intermediate, thus predisposing individuals with these muta-

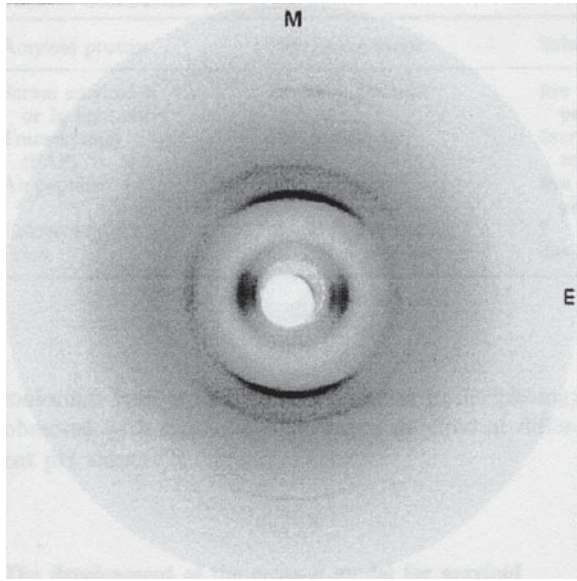

Fig. 4. X-ray fiber diffraction of TTR amyloid fibril ***(9)***. M represents meridional reflection; E represents equatorial reflection.

tions to fibril formation and disease. The increased rate of formation of the amyloidogenic intermediate for the FAP variants could be especially important, since the duration of TTR's residence in the lysosome (the likely intracellular site for the amyloid conversion process) could be relatively short. The self-assembly process may not be a nucleated condensation polymerization but, instead, appears to involve the self-assembly of quaternary structures of increasing complexity ***(7)***.

The high-resolution pattern of the structure of amyloid fibrils derived from TTR variants was in agreement with the cross-β structure and revealed a new repeat of 115 Å. **Figures 2** and **3** represent high resolution electron micrograph and X-ray fiber diffraction patterns of TTR amyloid fibril. This can be interpreted as a repeating unit of 24 β strands that forms a complete helical turn of the β sheet about an axis parallel to the fiber axis ***(3)***. At present, the thyroxine-binding site appears to present the most promising target for pharmacological inhibition of the conformational changes responsible for fibril formation. In addition, flufenamic acid and diclofenac ***(9)*** act as a powerful inhibitor for fibril formation ***(8)***.

3.2. Lysozyme

Lysozyme is known to have a protective effect against bacterial infection through its capacity to hydrolyze the bacterial cell wall. Lysozyme catalyzes

the hydrolysis of natural and synthetic polymers of β(1-4)-linked units of *N*-acetylglucosamine and *N*-acetylmuramic acid by binding to the substrate ***(8)***. Lysozyme synthesis occurs in all human tissues. This protein is present in all biological fluids, from tears to plasma, where it is mainly sustained by neutrophils, leukocytes, and monocytes. Lysozyme is rapidly removed from plasma by kidney filtration, and it has been calculated that in 1 h approx 76% of plasmatic lysozyme is excreted in the urine. Lysozyme distribution data have been analyzed, and it is suggested that there is a certain discrepancy between the synthesis and tissue localization of radiolabeled lysozyme in the liver and spleen. However, the two organs are able to take up more than 25% of the circulating enzyme. This suggests that the circulating lysozyme is physiologically taken up by the liver and spleen structures. Note that the two organs are heavily involved in this type of amyloidosis.

Two naturally occurring variants of human lysozyme, Ile56Thr and Asp67His, have been found to be amyloidogenic, apparently causing hereditary nonneuropathic systemic amyloidosis. Both mutants are significantly less stable than the WT protein and have populated partially folded intermediates at 37°C that are assumed to be the precursors of amyloid ***(1,3)***. Both of these species have single amino acid mutations, either threonine for isoleucine at position 56 or histidine for aspartate at position 67 ***(8)***. Unlike TTR amyloidosis, WT lysozyme appears incapable of amyloid fibril formation ***(7)***. In the case of the two known disease-related human lysozyme variants, fibrils form most readily at low pH or at slightly elevated temperatures ***(10)***. The decreased protein stability, rather than the altered folding kinetics, is a common feature of the two variants. The lower stability of the native state results in the population of a partially folded state that is very similar to the major intermediate populated on the folding pathway of the WT protein ***(10)***.

The unfolded region of the intermediate is primarily in the β domain of the protein. This indicates that the aggregation process might be initiated by the intermolecular association of the β strands that are involved in the intramolecular interactions in the native structure ***(10)***. The key to both amyloidogenic mutations in human lysozyme apparently lies in the effect that they produce at the interface between the α and β domains. Residue 56 acts as an anchor of the β domain onto the α domain ***(11)***. The formation of the native state for the major fraction of the lysozyme is retarded compared with the direct folding process. Partially structured intermediates that transiently populate seem to be kinetically trapped in a conformation that can only slowly reach the native state ***(12)***. Stabilization of the intermediate, rather than the equilibrium instability, is a crucial factor in amyloidogenicity of human lysozyme ***(13,14)***. The mechanism for amyloid formation in lysozyme is shown in **Fig. 5** ***(13)***.

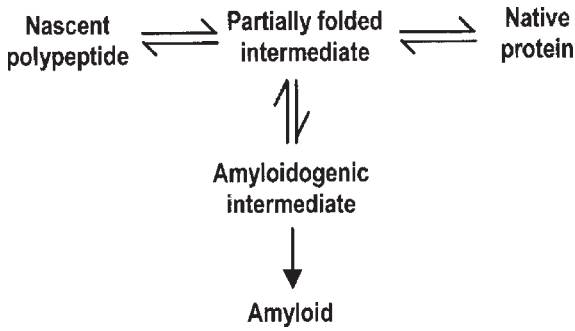

Fig. 5. Lysozyme folding mechanism and amyloid fibril formation ***(9)***.

3.3. Immunoglobulin

Light-chain amyloidosis is observed in approx 10% of those individuals experiencing monoclonal light-chain overproduction. This leads to malfunction of the peripheral nervous system, organ dysfunction, and death, usually within 5 yr. The conversion of immunoglobulin (Ig) light chain into an amyloid under acidic and partially denaturing conditions proceeds through at least one conformational intermediate that is readily accessible to some light chains but not to all. Domain stability is inversely proportional to the extent of amyloid fibril formation. It is the conformational intermediates rather than the native state that render the protein capable of amyloid fibril formation ***(1)***.

Some mutations leading to amyloid fibril formation in TTR, lysozyme, and Ig variable light (V_L)–chain domains are also observed to result in a decreased stability of the native state. In such cases, there is differential destabilization of the native conformation compared with aggregating intermediate conformation that results in the population of the aggregating conformation ***(3)***.

3.4. Aβ Peptide and Alzheimer's Disease

Alzheimer's disease, which leads to dementia and eventual death, is characterized by the presence of amyloid fibrils surrounded by dead and dying neurons in the brain tissue ***(15)***. The principal component of the fibrils is the β-amyloid peptide, a 39- to 43-residue peptide of known sequence but unknown function, which is found in normal human tissue and is derived from a larger protein precursor ***(1,8)***. The Aβ amyloid polypeptide is generated by two distinct proteolytic events referable to β and γ secretase. The Aβ aggregates can induce neurotoxicity, loss of presynaptic terminals, and development of dystrophic neuritis. The degree of toxicity is dependent on the level of aggregation ***(8)***. Aβ adopts a metastable structure in its soluble state that most likely becomes more structured on formation of the amyloidogenic intermediate. In fact,

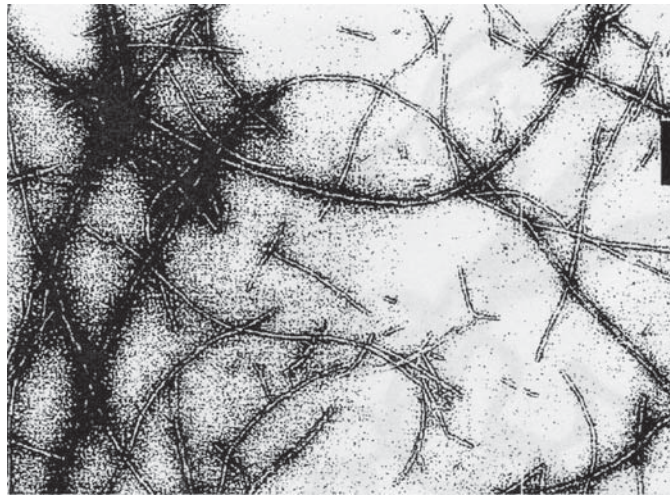

Fig. 6. Electron micrograph showing amyloid fibrils formed from lysozyme ***(10)***.

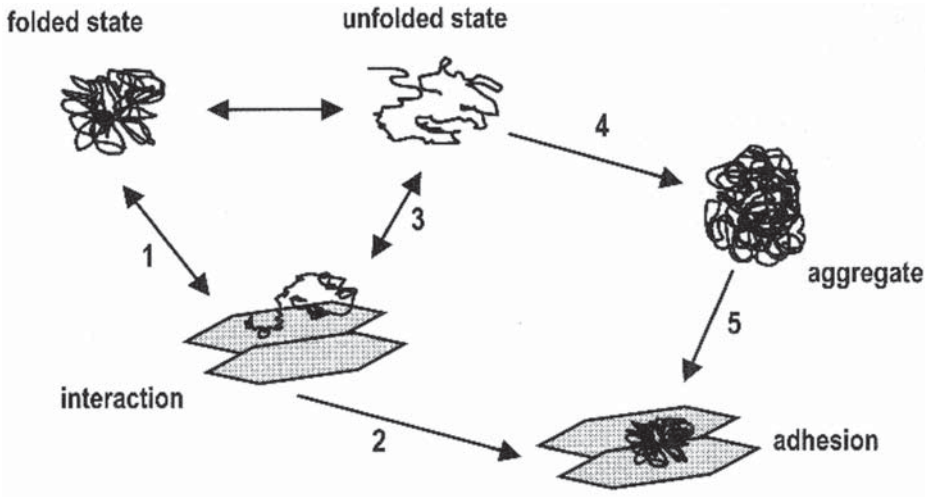

Fig. 7. Fibril formation mechanism owing to β (25-35) amyloid interaction with folded state ***(16)***.

the assembly of the Aβ helical structures may drive the increase in secondary and quaternary β-sheet structure ***(7)***. Aβ micelles form above a certain critical Aβ concentration. Fibrils nucleate within these micelles, and these fibrils grow through the irreversible binding of monomers to the fibril ends ***(7)***.

The native, soluble, and folded states interact with the β (25-35) amyloid (**step 1** in **Fig. 7**). The amyloid–protein complex forms aggregates and deposits

(**step 2**). In some cases, the amyloid exerts a force to unfold the protein on the surface of the amyloid fibril (**step 3**). The unfolded or partially unfolded state has a stronger propensity to aggregate. This results in amorphous aggregates, which adhere strongly to the surface of the amyloid fibril and undergo irreversible deposition. The β (25-35) amyloid interacts with the native protein and destroys its native conformation. This leads to the formation of pathological aggregates. The action of β (25-35) amyloid is exactly opposite that of a chaperone, which helps proteins fold correctly. Hence, β (25-35) is termed an *antichaperone **(16)***. Fibrillar Aβ that deposits in cerebral vessels can effectively localize and enhance the anticoagulant functions AβPP. This contributes to a microenvironment conducive to hemorrhaging ***(17)***. The demonstration that certain compounds such as Congo red, anthracyclines, and rifampicin can avidly bind to the fibrils and, without modifying their overall structure, annihilate their cytotoxic power suggests a useful therapeutic strategy ***(6)***.

3.5. Prions

Prions might represent a distinct class of infectious agents that are proteinaceous and devoid of nucleic acid. The prion is an abnormal form of a host-encoded protein that can interact with its normal counterpart and cause it to become abnormal. This adaptation of the word *prion* involves a broadening of the definition from a focus on the proteinaceous infectious agent of TSE diseases to include infectious proteins or protein-based genetic elements ***(18)***. The prion hypothesis asserts that an abnormal form of PrP, probably the aggregated, amyloidogenic protease-resistant form (PrP-res or PrP^{Sc}), is the infectious agent that propagates by inducing a change in the normal, protease-sensitive PrP (PrP-sen or PrP^{C}). PrP-res is usually correlated with TSE infectivity and can induce PrP-sen to convert to the protease-resistant, aggregated state in cell-free reaction ***(18)***. The aggregate formed in this case induces further aggregation of the normal form of the protein. Hence, prions are termed *infectious*, unlike other forms of amyloidogenic proteins. However, prionogenic aggregates possess fibrillar structure, as observed from electron micrographs and X-ray fiber diffraction ***(11,18)***. These aggregates stain with Congo red dye ***(18)***, suggesting that the prions are amyloidal in nature.

The "seeding" mechanism, also known as nucleated polymerization has been proposed as a model for prion aggregation. Studies involving these mechanisms are of utmost importance because the disease is transmitted across species barrier, from cattle (cow, lamb) to humans, hence the name TSE. PrP^{Sc} can adopt a fibrillar structure similar to an amyloid. A possible therapeutic approach is to design peptidomimetics that prevent the assembly of prionogenic intermediate into a prion particle ***(19)***. Two models of amyloidal prion formation are presented in **Figs. 8**,**9**.

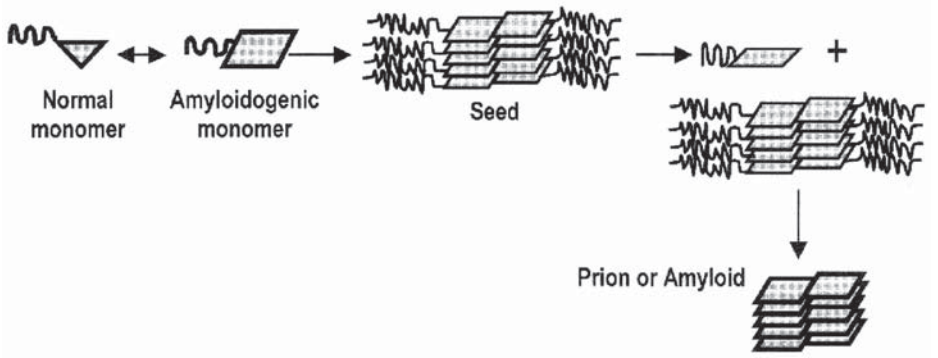

Fig. 8. Noncatalytic model for amyloidal prion formation ***(18)***.

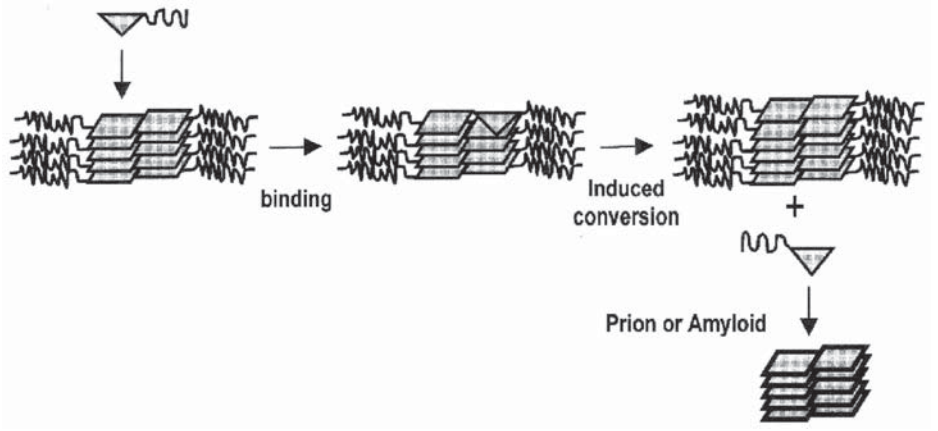

Fig. 9. Autocatalytic model for amyloidal prion formation ***(18)***.

4. Common Fibril Characteristics

Fibril diffraction data from amyloid fibrils composed of different precursor proteins are virtually identical. This suggests that the structure of amyloid is common, despite the lack of primary or tertiary structural homology among the 17 precursor proteins ***(7)***. A range of experiments, particularly X-ray fiber diffraction, indicates that the fibrils have extensive β-sheet character. These sheets run perpendicular to the fibril axis to generate what is described as a cross-β structure. This observation is remarkable in view of the fact that the soluble native forms of the proteins associated with these diseases vary quite considerably in their nature. This similarity of the fibrillar forms of the proteins prompted the proposal that there are strong similarities in the inherent structure of amyloid fibrils and in the mechanism by which they are formed ***(10)***.

Furthermore, there is a body of evidence indicating that the different amyloid fibrils that are composed of different proteins have the same molecular form. All amyloid fibrils seem to exhibit the following molecular properties, which are now considered to be diagnostic for amyloid ***(11)***:

1. *Appearance in the electron microscope*. Amyloid fibrils examined under the electron microscope show almost uniform fibrils about 100 Å in diameter. The fibrils are straight and unbranched and show a smooth surface lacking any very obvious surface features.
2. *Tinctorial properties*. All amyloid fibrils can be stained with the diazo dye Congo red and exhibit an apple-green birefringence, which is the most widely used diagnostic for amyloid. It is likely that the Congo red molecule is bound and ordered in a specific way by the amyloid fibrils. However, the interaction remains to be characterized.
3. *X-ray diffraction pattern*. Amyloid fibrils give rise to characteristic X-ray fiber diffraction patterns dominated by relatively sharp and intense 4.7-Å meridional and weaker and more diffuse 10-Å equatorial reflections.

The X-ray pattern is indicative of a particular type of β-sheet structure, known as cross-β structure. The β sheets are arranged parallel to the axis of the fibers with their constituent β strands perpendicular to the fiber axis. These same characteristics seem to occur in amyloid fibril from different amyloid diseases and from different precursor proteins. Each amyloid aggregate is a fibril, which is composed of several protofilaments whose subunit is termed a *subprotofilament*. Ex vivo fibrils formed from an Ig light chain are found to be 75 to 80 Å in diameter and are composed of five or six protofilaments each about 25 to 30 Å wide arranged parallel to one another. The protofilaments appear to be composed of two or three subunit strands 10 to 15 Å wide and are called subprotofilaments. They are helically arranged with a 30- to 50-Å repeat ***(11)***. The substructure of different amyloid fibrils with their dimensions are given in **Table 1**.

Amyloid fibrils derived from different polypeptide precursors may be composed of different numbers and arrangements of protofilaments. Variations can combine to produce variability in the transverse dimensions of amyloid fibrils. However, the degree of variability can be accommodated within a common molecular structure of amyloid fibril.

All amyloid fibrils contain β-sheet structure in which the peptide strands are aligned orthogonal to the direction of fibril growth ***(15)***. The core structure of the TTR amyloid fibril, which is a continuous β-sheet helix, can be extended to other amyloids formed by proteins as diverse as lysozyme and Ig light chain ***(18)***. Fibrils are an epiphenomenon linked to disease ***(15)***. The ability to form amyloid fibrils is not a characteristic associated wholly or primarily with those proteins found to be associated with amyloidoses. This is a property that could be common to many or indeed all proteins under appropriate conditions ***(10)***.

Table 1
Substructure of Amyloid Fibrils With Dimensions *(9)*

Amyloid protein	Fibril dimension	Substructure
Serum amyloid A or Ig light chain	75- to 80-Å diameter	Five 25- to 35-Å protofilaments in pentagonal array
TTR (FAP)	130-Å diameter	Four 50- to 60-Å protofilaments in square section array
Aβ peptide	90-Å diameter	Five to six 20- to 30-Å protofilaments in pentagonal or hexagonal array
Calcitonin	50- to 60-Å diameter	—
Prion	60- to 200-Å ribbons	Flat assembly of protofilaments

Thus, the fibrils from different peptides and proteins are, in reality, variations on a common theme.

The dimensions of the protofilaments, and the lengths of the β strands within them, may be determined simply by the physiochemical properties of an unfolded polypeptide chain ***(10)***. In other words, the potential to form an amyloid can be predicted based on the physiochemical properties of the proteins ***(20,21)***. The nature of the polypeptide of the fibrils is used to define the type of amyloidoses, and it affects the pathology of the disease state. Nevertheless, all amyloid fibrils, regardless of the character of the precursor or the site of deposition in the body, appear to have a related ultrastructure and a closely similar molecular core structure ***(11)***.

5. X-Ray Diffraction Studies of Amyloid Fibrils

Fibrous protein samples, like amyloid are usually exposed to the X-ray beam with the long axis of the fibrils more or less perpendicular to the direction of the beam. The X-ray reflections are then distinguished by their direction with reference to the fiber axis and their distance from the center of the pattern: meridional reflections are defined as those lying parallel to the fiber axis, and equatorial reflections are those positioned at right angles to the fiber axis.

The earliest reported fiber diffraction investigations of serum amyloid A and light-chain amyloid indicated an intense meridional reflection at 4.68 Å and an equatorial reflection at 9.8 Å. This is characteristic of the cross-β structure and occurs only if the amyloid fibrils are protofilaments composed of two or more β sheets. Purified amyloid fibril cores isolated from senile systemic plaques associated with Alzheimer disease show reflections at 4.76 Å and approx 10.6 Å. Prion rods exhibit a prominent interstrand spacing at 4.72 Å, and an equatorial spacing at 8.26 Å.

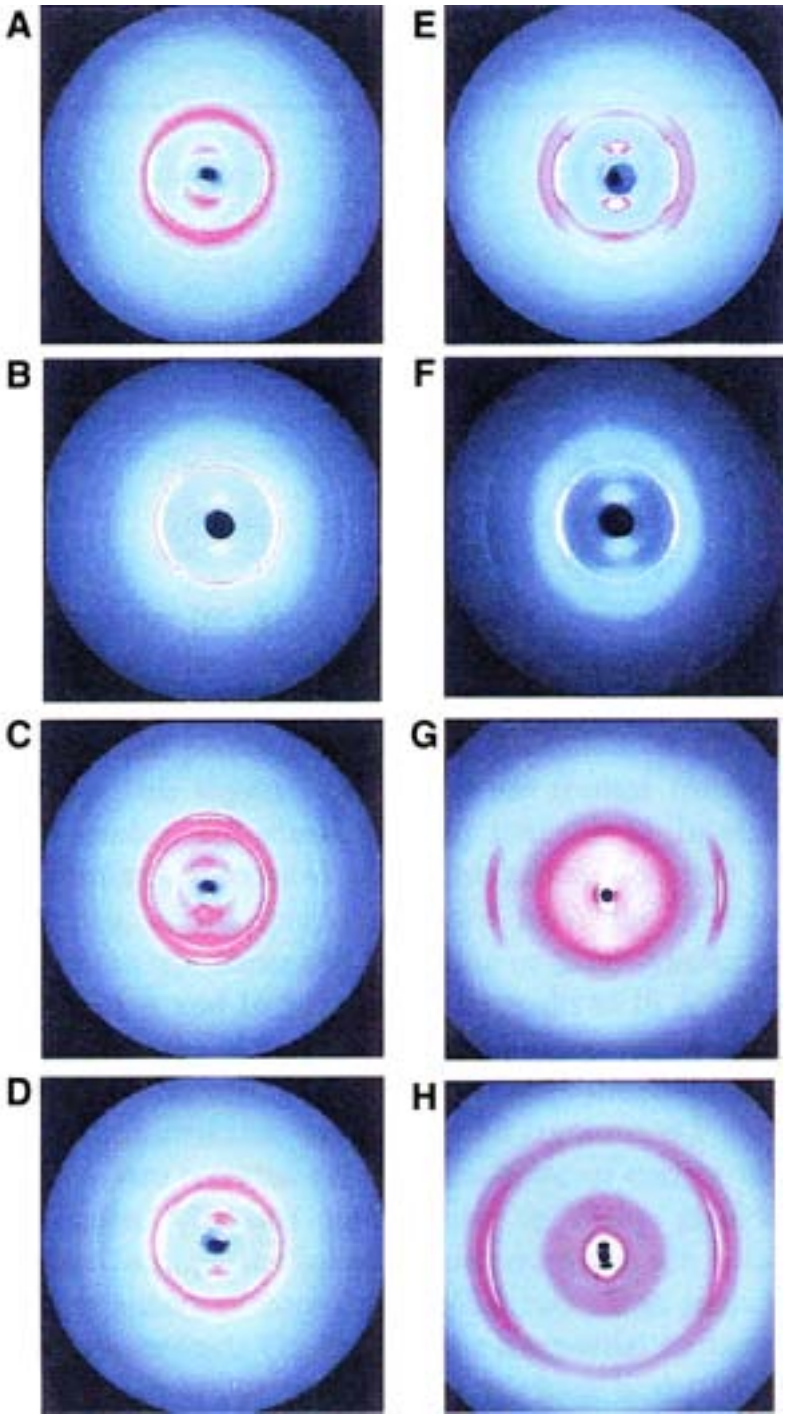

Fig. 10. X-ray fiber diffraction showing eight different amyloid fibrils from different proteins having the same core structure: **(A,B,E)** TTR; **(C)** apolipoprotein; **(D)** Ig; **(F)** islet-associated peptide; **(G)** serum amyloid A; **(H)** lysozyme ***(11)***.

The most thoroughly studied amyloid fibrils to date have been those extracted from Swedish patients with FAP. X-ray diffraction was used to study the molecular structure of various amyloid fibrils, including ex vivo fibrils from patients with Ig light chain, serum amyloid A, apolipoprotein A-1, TTR (two different variants), lysozyme amyloidoses, and synthetic amyloid fibrils formed from amyloidogenic fragments of TTR. The islet-associated polypeptide and the high-resolution images are shown in **Fig. 10**. It can be seen in **Fig. 10** that in spite of the large differences in the size and structures of the polypeptide precursors of the fibrils, the similarities in the diffraction patterns are quite marked. This indicates that different amyloid fibrils actually share a common molecular skeleton on the fibril axis, with the same atomic spacings ***(11)***.

The similarity in the diffraction over the medium- and high-angle regions of the meridional X-ray pattern can only occur if the fibrils have well-defined and closely similar molecular structures, at least insofar as their ordered core components are concerned. Thus, apparently most, if not all, amyloid fibrils have as their core structure the continuous β-sheet helix that has been described in detail for the TTR amyloid fibril. A model of the amyloid protofilament, containing helically twisted β sheets, gives reasonable agreement with the major features of the fiber diffraction patterns exhibited by all amyloid fibrils examined so far. Within this framework, there are many possibilities for fibrils to be composed of different numbers or arrangements of protofilaments. In addition, protofilaments may vary in diameter as a result of the need to incorporate different-sized loops linking the β strands.

It is also possible that some amyloids may be composed of only one pair of β sheets, rather than the two pairs of sheets thought to compose the TTR amyloid protofilament. In spite of the possibility of these considerable variations, the common core structure indicated by the diffraction patterns is evidence that the amyloid fibrils do have a well-defined generic molecular structure that accounts for their characteristic disease-forming properties ***(11)***. The closely similar cross-β fiber diffraction patterns exhibited by amyloid fibrils indicate that this underlying ordered structure is primarily a β sheet. Also, the constituent β strands lying at right angles to the fiber axis ***(11)***.

6. Unified Mechanism of Amyloid Formation

The conformational properties of all proteins should be considered in terms of the multiple steady states that are accessible to such structures ***(10)***. The unified mechanism in **Fig. 11** suggests the various structures awaiting a polypeptide chain after biosynthesis, which includes the mechanism of amyloid fibril formation ***(10,22)***. In addition, if the protein is able to fold rapidly, any partially folded species will have a short lifetime, reducing the probability that intermolecular interactions will occur. Moreover, once folded, the native state

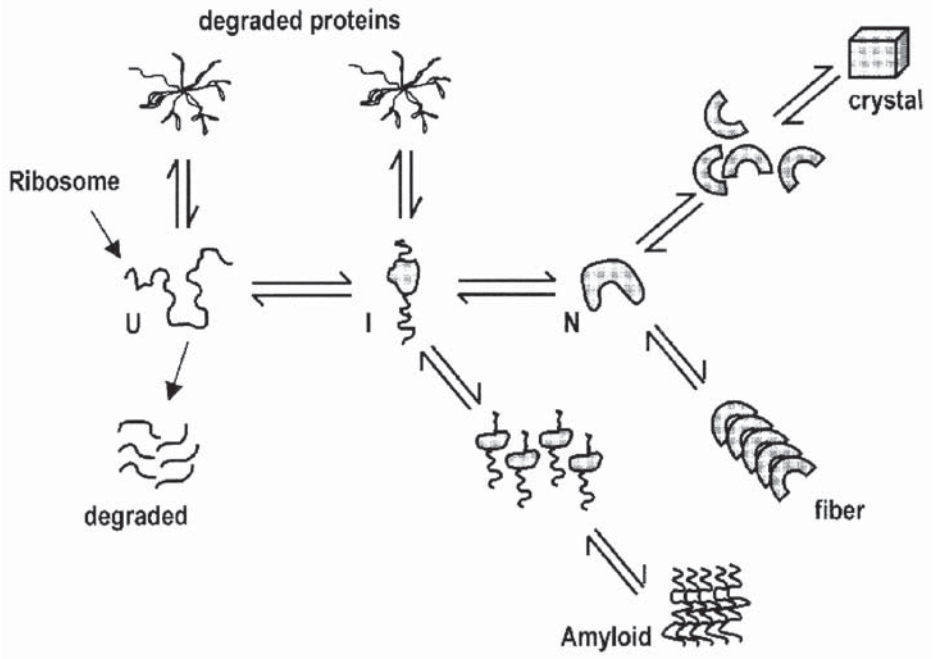

Fig. 11. Unified mechanism of amyloid fibril formation ***(10,22)***.

is generally a high-compact structure that conceals the polypeptide main chain within its interior. Such a state is protected from aggregation except through the interactions of surface side chains (as is the case of protein crystals). This state is unable to form the strong intermolecular hydrogen bonds associated with the polypeptide backbone. If the native state is maintained under appropriate folding conditions, then aggregation to amyloid fibrils will be resisted by the kinetic barrier associated with unfolding, even if the aggregated state is thermodynamically more stable ***(10)***.

Parkinson disease, caused by mutations in the gene encoding α-synuclein, is characterized by cytoplasmic neuronal deposits. These deposits are lewy bodies in the affected regions of the brain and resemble amyloid fibrils. This has been determined by morphological studies such as atomic force and electron microscopies, distinctive dye-binding properties (Congo red & thioflavin T), and antiparallel β-sheet structure (Fourier transform infrared spectroscopy and circular dichroism spectroscopy) ***(23)***.

7. Therapeutic Strategies

The generic picture of amyloid structure and the mechanism of its formation provide a conceptual framework for linking various pathological conditions

associated with the deposition of this material. This suggests possible general approaches to the prevention or treatment of the whole family of amyloid diseases ***(10)***. A system of artificial chaperones (detergents and cyclodextrin) has been successfully used to minimize aggregation in the renaturation of denatured-reduced lysozyme. Osmolytes and other stabilizing ligands may decrease the amount of aggregation. This probably reflects differential stabilization of the native state. Osmolytes have been shown to inhibit prion formation in the scrapie system, consistent with the involvement of protein conformational changes in the formation of prions ***(3)***.

Seeding as in the case of prion protein increases the rate of amyloid formation. Hence, reducing seeding, by introducing a ligand that probably binds and stabilizes the native state, would decrease aggregate formation. The surgical gene therapy method of treating FAP, in which the FAP mutation is replaced with the WT TTR, causes (1) cessation of fibril formation, (2) clearance of the amyloid already deposited, and (3) clinical improvement where complications from transplantation do not occur ***(7)***. This works by modification of the protein sequence ***(10)***. Aggregation is minimized, leading to elimination or a decrease in the clinical symptoms.

For example, small proteins, such as acylphosphatase, have shown that this is likely to be a general method of reducing the tendency to form amyloid fibrils under conditions in which the native state of the protein has low stability ***(10)***. In addition to these therapeutic strategies, other techniques are designed to inhibit amyloid formation either by blocking fibril growth or by stabilizing the α-helical or random coil structures. This prevents the β peptide from precipitating to an amyloid structure and becoming toxic to nerve cells ***(24)***. Novel therapeutic strategies like genetic engineering ***(25)*** to eliminate amyloid fibril formation could be promising for eliminating a whole class of fatal diseases caused by protein misfolding. This may be facilitated by the fact that fibrils have the same core structure and can be characterized by common properties.

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