

Seeding “One-Dimensional Crystallization” of Amyloid: A Pathogenic Mechanism in Alzheimer’s Disease and Scrapie? Minireview

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Alzheimer’s disease (AD) is a neurodegenerative disease characterized by the presence of cerebral amyloid plaque (reviewed by Selkoe, 1991), a highly ordered protein aggregate defined by its insolubility and fibrillar structure (Lansbury, 1992). The AD amyloid protein (β protein) is a secreted protein of unknown function that is overproduced in some but not all AD cases (Seubert et al., 1992; Shoji et al., 1992). A causal relationship between amyloid formation and AD has not been proven, but the slow onset of symptoms appears to parallel the gradual deposition of amyloid. Therefore, it is important to understand the molecular mechanism of amyloid formation and to explain why the β protein aggregates in diseased individuals (Citron et al., 1992; Cai et al., 1993). In this review, a simple chemical explanation is proposed, based on the observation that in vitro amyloid formation bears a mechanistic resemblance to processes involving ordered protein aggregation (such as protein crystallization and microtubule formation), which will be referred to as nucleation-dependent polymerizations.

Like AD, the human prion diseases, Creutzfeldt–Jakob disease and Gertsman–Sträussler–Scheinker disease, are characterized by the slow onset of neurodegeneration. Brain pathology in these diseases resembles that of AD (Prusiner, 1984; Baker and Ridley, 1992) and is also characterized by aggregation of a normal cellular protein, prion protein (PrP) (rather than the β protein), often in amyloid plaques (reviewed by Prusiner, 1991). In contrast with AD, the pathogenic nature of PrP aggregates has been established, thanks to extensive work on the transmissible prion disease, scrapie. The infective agent of scrapie may operate by accelerating the step in amyloid formation that is normally rate determining (Griffith, 1967; Prusiner, 1991). We propose that this step is mechanistically relevant to amyloid formation in human prion disease and in AD; it is the formation of an ordered nucleus, which is the defining characteristic of a nucleation-dependent polymerization. According to this hypothesis, the transmission of scrapie and the initiation of AD may both involve the seeding of amyloid formation.

Protein Solubility Is Normally Operationally Defined

The measurement of protein solubility often reflects a kinetic effect rather than true thermodynamic solubility. For instance, when a protein solution appears to be clear throughout the course of an experiment, the protein is defined as soluble, although precipitation may eventually occur. The rate at which a protein polymerizes and precipitates is not necessarily related to its thermodynamic solubility. However, both properties may be relevant to the pathogenesis and treatment of amyloid diseases.

Proteins can form different types of insoluble aggregates. Amorphous aggregates have multiple protein conformations and ill-defined intermolecular interactions. In contrast, protein crystals are often characterized by a single protein conformation and a single well-defined intermolecular packing arrangement. Ordered noncrystalline polymers such as amyloid share these properties. In fact, amyloid can be thought of as a one-dimensional crystal in which packing in the plane perpendicular to the direction of fibril growth is nonuniform (Lansbury, 1992). Amorphous aggregates can form rapidly when the protein concentration exceeds the solubility. However, protein crystal formation requires time, owing to the kinetic barrier imposed by nucleus formation, the rate-determining step. Ordered noncrystalline protein polymers such as amyloid share this requirement for nucleation.

Nucleation-Dependent Polymerization Is Common

Nucleation-dependent protein polymerization describes many well-characterized processes, including protein crystallization, microtubule assembly, flagellum assembly, sickle-cell hemoglobin fibril formation, bacteriophage procapsid assembly, and actin polymerization. A simple general mechanism is illustrated for the formation of a helical protein polymer in Figure 1. Nucleus formation requires a series of association steps that are thermodynamically unfavorable ($K_n \ll 1$) because the resultant intermolecular interactions do not outweigh the entropic cost of association (Chothia and Janin, 1975). Once the nucleus (P_n ; in this case, one turn of a helix) has formed, further addition of monomers becomes thermodynamically favorable ($K_g \gg 1$) because monomers contact the growing polymer at multiple sites, resulting in rapid polymerization/growth.

A distinctive feature of a nucleation-dependent polymer-

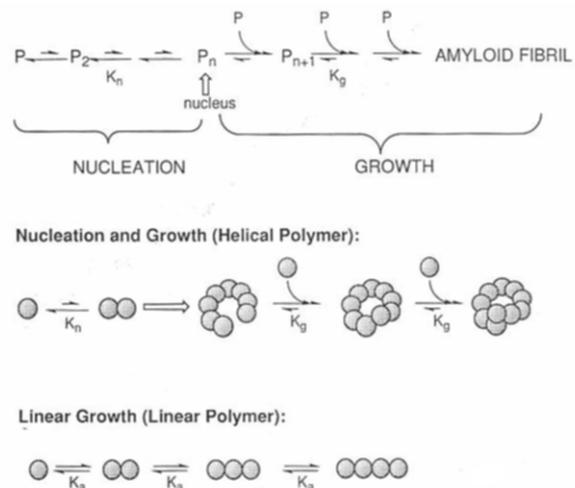


Figure 1. A Simple Mechanistic Scheme for Nucleation-Dependent Polymerization of an Amyloid Fibril

As an example, the formation of a helical polymer is illustrated. In contrast, linear polymerization (bottom) does not require nucleation and cannot be seeded.

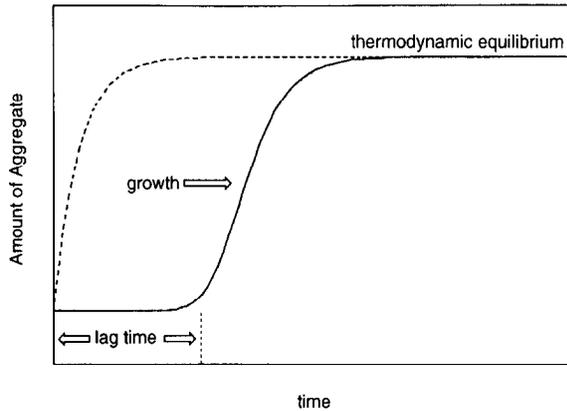


Figure 2. Experimentally Observable Formation of Aggregate for a Nucleation-Dependent Process above the Critical Concentration

Aggregate formation is indicated by a solid line. At high concentration, nucleation is so rapid that no lag time is observed (dashed line). Addition of a nucleus or seed eliminates the requirement for nucleation and also results in rapid polymerization.

ization is a lag time before aggregates are detectable (Figure 2). During this period, trace amounts of dimer, trimer, and eventually, nuclei (n -mers) are formed. We refer to this metastable situation as kinetic solubility. The length of the lag time is dramatically dependent on protein concentration (Hofrichter et al., 1974): if a protein requiring an octameric nucleus ($n = 8$) has a lag time of 1 hr at 10-fold supersaturation, its lag time at 2-fold supersaturation will be ~ 45 yr, and its lag time at 100-fold supersaturation will be ~ 36 μ s. With a larger nucleus ($n = 20$), the concentration dependence is even more marked (lag times of 1 hr, 1×10^{10} yr, and 3.6×10^{-17} s, respectively). Since lag times are observable over only a narrow concentration range, it can be difficult to recognize a nucleation-dependent protein polymerization (Figure 2).

In addition to the existence of a lag time, two other characteristics are diagnostic of a nucleation-dependent mechanism, critical concentration and seeding.

Lag Time: During the time required for nucleus formation, the protein appears to be soluble (i.e., is kinetically soluble). Since intermediates are not necessarily detectable during the lag time, a "fresh" supersaturated solution and one that is on the brink of polymerization may not be experimentally distinguishable.

Critical Concentration: At equilibrium, after polymerization is complete, the solution contains predominantly monomers and high polymers. The monomer concentration at this point is referred to as the critical concentration. Below the critical concentration, polymerization will not occur.

Seeding: Nucleation is rate determining at low supersaturation levels. Therefore, adding a seed or preformed nucleus to a kinetically soluble supersaturated solution results in immediate polymerization (Figure 2).

In contrast with nucleation-dependent polymerization, the growth of a linear polymer does not require nucleation (Figure 1). Linear polymerization is characterized by the sequential buildup of intermediates: no lag time is observed, and supersaturated solutions are unstable and

rapidly aggregate. This process can be difficult to distinguish from a nucleation-dependent process with a very short lag time or from seeded growth (Figure 2).

In Vitro Amyloid Formation Is Nucleation Dependent

The kinetic behavior of amyloidogenic peptides based on the sequences of scrapie PrP (Come et al., 1993) and the bacterial protein OsmB (Jarrett and Lansbury, 1992) has demonstrated that amyloid formation is nucleation dependent. Amyloid formation is seeded by preformed amyloid fibrils but not by fibrils prepared from closely related peptide analogs (Jarrett and Lansbury, 1992). The specificity of seeding suggests that complementarity between the seed and the amyloid peptide is required for growth (Come et al., 1993). Amyloid formation by the protein transthyretin also exhibits a lag time under certain conditions (Colon and Kelly, 1992).

Variants of the β protein of AD that differ only at their C-termini are found in vivo. β 1-40 seems to be the major AD amyloid plaque protein, whereas β 1-42, β 1-43, or both are minor components (Miller et al., 1993; Mori et al., 1992). Several studies have reported that β protein aggregation is a time-dependent process (e.g., Fraser et al., 1992; Barrow et al., 1993; Pike et al., 1993, and references therein). These studies used β protein concentrations that are at least 50-fold supersaturated and 10^4 -fold greater than the normal levels in the blood (Seubert et al., 1992; Shoji et al., 1992). Therefore, no lag time was observed, and the nucleation dependence was not recognized. However, at lower (~ 5 -fold) supersaturation levels, two β protein variants, β 1-39 and β 1-40, are kinetically soluble for several days, while β 1-42 immediately polymerizes into amyloid fibrils. β 1-40 amyloid formation can be seeded by trace amounts of fibrils of β 1-40 or β 1-42 (Jarrett et al., 1993).

Studies using amyloidogenic peptides based on the β protein C-terminus (β 26-39, β 26-40, β 26-42, β 26-43) have shown that the length of the C-terminus does not have a significant effect on the thermodynamic solubility (K_d) of these peptides (Jarrett et al., 1993). However, in part because of the exponential concentration dependence of nucleus formation, it has a dramatic effect on the rate of nucleation (kinetic solubility). These observations parallel those made for the naturally occurring proteins. Furthermore, supersaturated solutions of kinetically soluble peptides (e.g., β 26-40) seeded by kinetically insoluble peptides (e.g., β 26-43) result in immediate amyloid formation (Jarrett et al., 1993).

In Vivo Amyloid Formation Can Be Seeded

By striking analogy to the experimental transmission of scrapie (Prusiner, 1991), systemic amyloidosis can be induced in hamsters by intraperitoneal injection of a preparation derived from sonicated amyloid fibrils (Niewold et al., 1987). Although this preparation contains no amyloid fibrils detectable by electron microscopy or Congo red staining, infrared spectroscopy indicates the presence of small fragments of amyloid fibrils, which could seed in vivo amyloid growth. In experiments that may be related, cerebral amyloid has been induced by microinjection of β amyloid protein into rodent brains (Kosik and Coleman, 1992) or AD brain tissue homogenate into primate brains

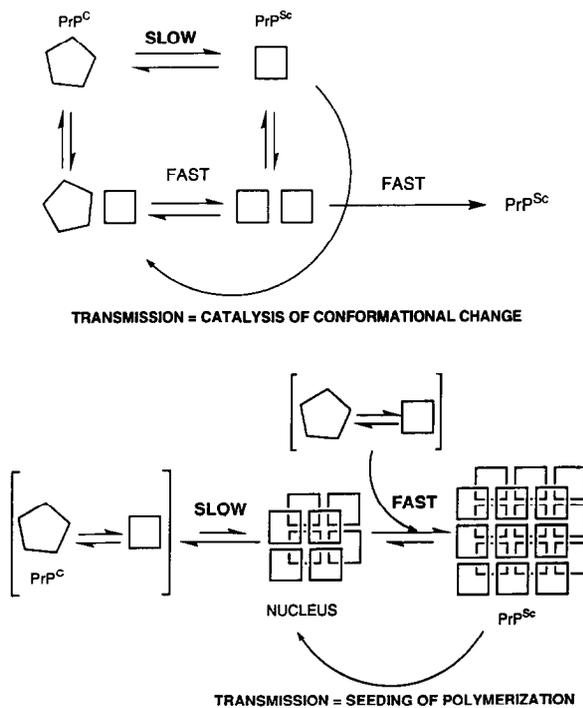


Figure 3. Two Models for the Infectivity of Scrapie

In the top model, the slow step in PrP^{Sc} formation is the conversion of PrP^C to PrP^{Sc}, and transmission would involve the catalysis of this conversion by PrP^{Sc} monomers. In the bottom model, PrP^{Sc} formation occurs by a nucleation-dependent polymerization mechanism in which the slow step is nucleus formation and transmission would involve seeding.

(Baker et al., 1993). These examples may involve the in vivo seeding of amyloid formation.

It is extremely difficult to characterize the seed directly (Brown et al., 1991; Prusiner, 1991), since it is inherently unstable. The aggregation state of the nucleus, however, can be deduced from kinetic studies (Hofrichter et al., 1974). In addition, prenucleus oligomers may be stabilized (e.g., Pike et al., 1993) under nonphysiological conditions.

Slow Nucleation and Seeding May Explain Sporadic and Transmitted Prion Disease

Scrapie can be transmitted by the prion, an insoluble aggregate of PrP. The prion may act by accelerating a very slow process that disfavors sporadic prion disease. It shows virus-like characteristics (self-replication and susceptibility to species barriers), but contains no nucleic acid (Prusiner, 1991). The cellular form of PrP (PrP^C) and the form found in the prion (PrP^{Sc}) are generally agreed to be conformational isomers (Griffith, 1967; Prusiner, 1991). There is no consensus yet as to the identity of the slow step in PrP aggregation or the role of the prion.

One model proposes that the slow step is the conformational change from PrP^C to PrP^{Sc} (Griffith, 1967; Prusiner, 1991), which could be catalyzed by the formation of a PrP^{Sc}-PrP^C heterodimer. Thus, the PrP^{Sc} monomer would be the infectious species (Figure 3, top). Alternatively, scrapie infectivity could be based on a nucleation-dependent polymerization mechanism (Figure 3, bottom) (Griffith, 1967; Brown et al., 1991; Come et al., 1993). The

conformational difference between soluble and insoluble PrP does not mean that their interconversion is slow; many nucleation-dependent polymerizations involve unstable protein conformers (Caspar, 1980). In this model, the slow step is nucleus formation, and the infectious species is an ordered aggregate of PrP^{Sc} that acts as a seed (Come et al., 1993). Thus, scrapie may be a form of transmissible amyloidosis (Niewold et al., 1987; Baker and Ridley, 1992).

The failure to detect amyloid fibrils in an infectious prion preparation (Prusiner, 1991) does not rule out this possibility, since the seed need not have the tinctorial or morphological properties of an amyloid fibril (Niewold et al., 1987). The potentially high concentration dependence of nucleation suggests that slight increases in cellular PrP levels could lead to sporadic prion disease. Similarly, PrP mutations that alter the PrP^C/PrP^{Sc} equilibrium could have a major effect on nucleation time.

Nucleation-dependent polymerization can explain the virus-like features of the prion. Prion self-replication would involve propagation of PrP^{Sc}, and amplification would arise from fragmentation of fibrils, exposing new growth faces. The strain dependence of scrapie incubation time may arise from imperfect complementarity between the interacting surface of the foreign seed and host PrP, leading to slower initial growth (and altered K_d) and delayed disease onset. Scrapie infectivity takes on the strain of the host, because after growth using host PrP, the face of the seed assumes the characteristics of the host. A similar effect has been observed in the seeded polymerization of sickle-cell hemoglobin (Eaton and Hofrichter, 1990) and bacterial flagellin (Asakura, 1970). Experimental proof of the seeded polymerization model is difficult in the absence of a cell culture model for PrP^{Sc} formation that would allow the required kinetic studies.

Nucleation-Dependent Polymerization Suggests Explanations for Accelerated Amyloid Formation in AD

The β protein variant β1-40 is present in normal and AD cerebrospinal fluids at comparable concentrations (Seubert et al., 1992; Shoji et al., 1992). Since seeded polymerization can be extremely concentration dependent, a small

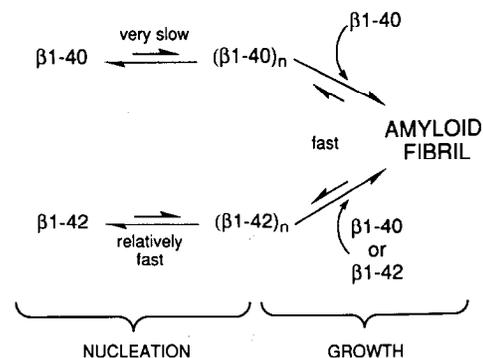


Figure 4. Scenario for Amyloid Formation in AD Based on In Vitro Aggregation Experiments

If β1-42, which has not been detected in healthy individuals, acts as an in vivo seed for amyloid formation, its production may be a pathogenic event (Jarrett et al., 1993).

increase in $\beta 1-40$ could significantly increase the rate of amyloid formation (Figure 4), which may explain some cases of early onset AD. Cells that are transfected with a mutant form of β amyloid precursor protein linked to early onset AD produce 6- to 15-fold more β protein (Citron et al., 1992; Cai et al., 1993). This increase could push the β protein concentration over the critical concentration and/or lead to an increase in the nucleation rate of 1,700,000-fold (assuming a 6-fold increase in supersaturation level and an octameric nucleus). These increases could reduce the lag time from 500 yr to 3 hr.

Some AD cases may involve the seeding of amyloid formation. Endogenous molecules such as proteoglycans or lipids could act as heterogeneous seeds for the polymerization of $\beta 1-40$. Alternatively, amyloid formation by the kinetically soluble variant $\beta 1-40$ could be seeded by small amounts of the variants $\beta 1-42$ or $\beta 1-43$, which nucleate very rapidly and seed aggregation of $\beta 1-40$ in vitro. $\beta 1-42$ and $\beta 1-43$ could be produced in trace amounts by an abnormal C-terminal proteolysis (Figure 4) (Jarrett et al., 1993). This model predicts that amyloid plaque from aged individuals who did not have AD (and presumably formed amyloid at a greatly reduced rate) would not contain the seed variants $\beta 1-42$ or $\beta 1-43$.

Ramifications for the Treatment of AD and Prion Diseases

Slowing the abnormally increased rate of amyloid formation could be a therapeutic strategy against AD and prion diseases. The nucleation-dependent polymerization mechanism suggests two approaches: reduction of amyloid protein concentration and interference with nucleus formation. The high concentration dependence of nucleation suggests that a slight decrease in amyloid protein could lead to a significant decrease in the rate of amyloid deposition. For example, a therapy that could reduce the levels of amyloid protein by 10% could lead to a 2-fold reduction in the rate of amyloid deposition, assuming an octameric nucleus (or 8-fold for a nucleus of 20).

Compounds that bind and stabilize the nucleus or its precursor(s) may also slow amyloid formation. Several examples of this effect are known: hydrophobic peptides that bind hemoglobin S inhibit red blood cell sickling (Eaton and Hofrichter, 1990), and colchicine inhibits microtubule formation by binding the tubulin dimer (Hastie, 1991). Finally, since in vitro amyloid formation is reversible (Maggio et al., 1992; Jarrett and Lansbury, 1992), compounds that inhibit monomer addition to the seed could increase the solubility of existing plaques (decrease K_G ; Figure 1), allowing clearance by normal mechanisms. The design of molecules that interfere with amyloid formation promises to be an active area of research.

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