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New and Notable

Toward Understanding Amyloid Aggregation

Dorothea Kominos
Neuroscience Therapeutic Domain, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, New Jersey 08876 USA

(This paper refers to the article by Shen and Murphy in the August issue of *Biophysical Journal*.) The presence of amyloid plaques in Alzheimer's disease (AD) suggests that amyloid formation plays a major role in neurodegeneration. The major constitutive component of amyloid plaques is the Aβ peptide, a 40- to 43-residue polypeptide that is a cleavage product of a larger precursor protein. Aβ, as found in plaques, is assembled into characteristic amyloid fibrils, which exhibit birefringent staining with Congo red and give an x-ray fiber diffraction pattern typical of the cross-β conformation. Under the proper conditions of pH and concentration, synthetic Aβ also self-assembles into fibrils in vitro, making it relatively easy to isolate fibrillar peptide for study. The ease with which Aβ self-assembles into fibrils, rather than crystals, makes the study of the structure of the monomeric peptide by high-resolution methods such as NMR and x-ray crystallography essentially impossible. Several recent studies suggest strongly that aggregated (fibrillar) Aβ is toxic to neurons, while monomeric peptide is not. While it was once believed that Aβ was produced solely from an abnormal cleavage of its precursor protein, it is now known that soluble, monomeric Aβ is a normal constituent of blood and cerebral spinal fluid. Thus, an understanding of the processes by which soluble Aβ is converted into insoluble, potentially neurotoxic fibrils is crucial to understanding the genesis of the amyloid plaques of AD. To date, little progress has been made in understanding the process of self-assembly of Aβ, but the article in the August issue of *Biophysical Journal* (Shen and Murphy, 1995) makes a significant contribution to the current knowledge of the conformational and kinetic states Aβ goes through in the process of forming fibrils.

Anyone who has ever attempted to study the aggregation and toxicity of Aβ realizes how frustrating this peptide is to deal with because of the variability of its properties. Batch-to-batch variation even from the same source is very common, largely because different batches of Aβ come with different histories associated with them. In reality, the pretreatment of these peptides does a great deal toward affecting their aggregation behavior. Shen and Murphy (1995) contribute to the understanding of some of this erratic and irreproducible behavior by studying the effects of solvents on the aggregation state of the peptide using circular dichroism (CD), Fourier transform infrared spectroscopy (FTIR), and light-scattering techniques. The solvent that the peptide is exposed to before dilution in PBS profoundly affects the kinetics of aggregation. Additionally, the rate of increase of fibril formation is directly correlated to the secondary structure of the peptide in a stock solvent. There is considerable evidence from x-ray diffraction that Aβ fibrils derived from various Aβ fragments (Inouye et al., 1993) exist in an antiparallel β-sheet conformation, while NMR studies on Aβ fragments, Aβ(1–28) in aqueous trifluoroethanol (Zagorski and Barrow, 1992), suggest that the monomeric peptide is largely helical or random coil. Thus, understanding the transition of Aβ from helical to β-sheet is vital to understanding its self-assembly. Shen and Murphy (1995) find that a stock solution of Aβ in 35% ACN/0.5% TFA aggregates quickly and exhibits a significant amount of β-sheet character (as measured by CD), leading them to surmise that ACN acts to stabilize β-sheet secondary structure. The predissolution in the ACN solution causes fibrils to form quickly and to a greater extent as measured by light scattering. From FTIR studies, they find DMSO to be the best at maintaining Aβ in a non-β-sheet structure in that, upon dilution into PBS, the Aβ forms fibrils at a slower rate and to a lesser extent. Predissolution in 0.1% trifluoroacetate has an intermediate affect between the DMSO and ACN results.

A major contribution of these studies is the process of amyloid aggregation that is elucidated from these solvent studies. To date, the concept of a nucleation event (Jarrett and Lansbury, 1993) is a prevalent theory of the process of aggregation. However, many of the details in the nucleation theory remain to be elucidated. For example, just how many monomers are necessary to form a nucleus from which fibril formation occurs quickly? The contribution of the findings of the Shen and Murphy (1995) paper to this question is illustrated in Fig. 1. A monomer converts into a β-sheet structure, perhaps as a dimer or β-crystallite intermediate (Inouye et al., 1993). Upon dilution in PBS, the structure "remembers" the β-sheet structure and forms multimeric structures. Addition of this dimeric form onto this multimer results in fibrillar growth. Association of these fibrils can cause fibrillar extension and, finally, these fibrils may self-associate to form lateral aggregates or tightly packed deposits.

Many questions still need to be resolved about amyloid fibrillogenesis. Of critical importance is how aggregation in vitro compares with events that form amyloid plaques in vivo. In this regard, the effects of other proteins associated with AD and amyloid plaques such as apolipoprotein E and glycosaminoglycans need to be further investigated. Perhaps some of these questions will be answered by the emerging transgenic mice models of

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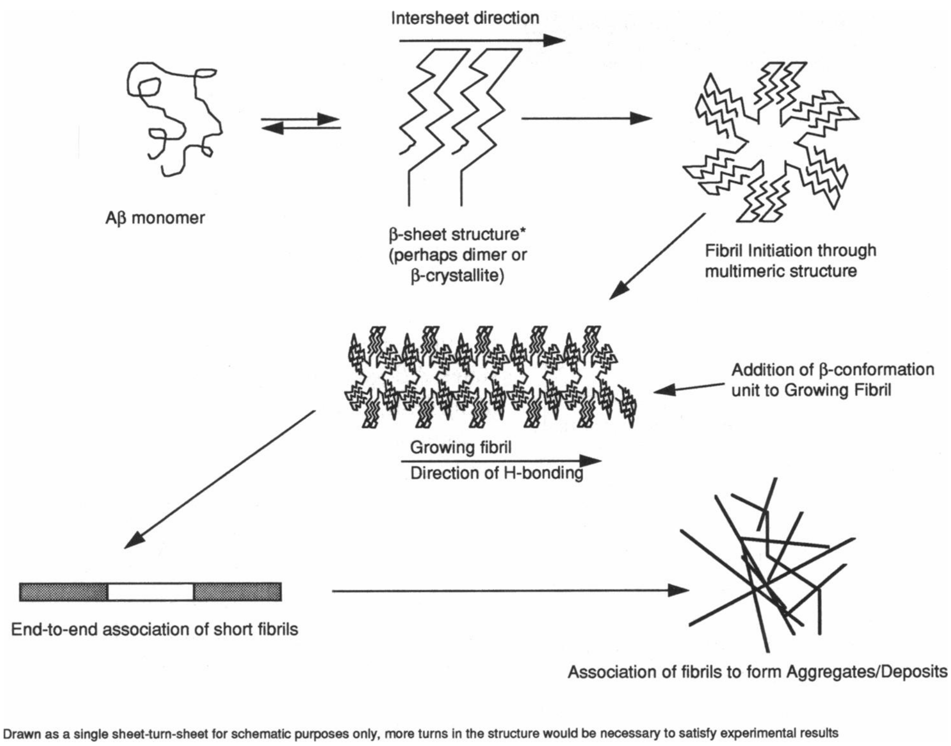

FIGURE 1 Schematic diagram illustrating the association of β-amyloid peptide into fibrils as discussed by Shen and Murphy (1995).

amyloid aggregation (Games et al., 1995). While it is true that the kinetics of the aggregation are very complicated, the Shen and Murphy (1995) study lays some important groundwork necessary to understand the process.

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