When Conjugated Polymers Meet Amyloid Fibrils

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Alzheimer’s disease (AD) is characterized by the extracellular deposits of the 39–42 amino acid amyloid-β (Aβ) peptides along with neurofibrillary tangles that stain with Congo red in the brains of patients. The Aβ peptides arise from cleavage of the extracellular portion of the transmembrane amyloid-precursor protein (APP) by β- and γ-secretases (1–3). The toxicity of the processed Aβ peptide and its aggregates (Figure 1, panel a) may result from a combination of apoptosis, disrupted Ca²⁺ homeostasis, toxic radicals, and complement formation. Over the past decade, Selkoe, Lansbury, Teplow, Kelly, Dobson, Prusiner, and many others have helped establish a nucleation-dependent paradigm for fibril formation that appears to be general for most proteins tested (Figure 1, panel a). Numerous experiments have also demonstrated that the rate of fibrillization and the morphology of the final fibrillar state of “amyloidogenic” proteins are strongly influenced by environmental factors (pH, salt, temperature, agitation, etc.), by chemicals (proteins, lipids, cholesterol, metals, etc.), and by the nature of the seeding agent (4).

Recent evidence strongly suggests that soluble oligomeric intermediates rather than the final fibrils are responsible for neurological toxicity (5–7), though equilibrium between these different states is certainly possible (Figure 1, panel a) (6, 8). Perhaps most important, a stronger correlation exists between soluble Aβ (monomer and oligomers of Aβ) in the brain and early cognitive dysfunction than between the Aβ deposits stained by Congo red and the clinical severity of AD (9–12). Even with this growing knowledge, the origin and progression of AD remain a complex and formidable challenge because no genetic markers, diagnostic agents, or drugs directly address the progress of AD. A recent review by Kodali and Wetzel discusses the numerous structural polymorphisms possible for Aβ (13).

Clearly, it would be an important breakthrough to provide new chemically tuned reagents that are selective for different classes of oligomers and fibers and to correlate them to disease outcome. Thus, Nilsson et al.’s (14) recent identification of a class of thiophene-based polymers that distinguish between different classes of fibrils is very significant.

Current Clinical Status of AD. The diagnosis of AD-associated dementia is currently based on clinical diagnosis rather than chemical or biological tests. Pathological findings have until recently been postmortem through the selective staining of senile plaques, primarily β-sheet fibrillar aggregates. Postmortem diagnosis unfortunately provides little relief for those afflicted with AD and perhaps surprisingly does not provide a measure of the severity of the disease; similarly stained plaques have also been observed in normal aging. Current medications for AD, primarily cholinesterase inhibitors (15) or N-methyl D-aspartate antagonists (16), treat some of the symptoms of the disease. However, new avenues are being actively explored that include secretase inhibitors as well as direct Aβ

ABSTRACT In the early 1900s, Alois Alzheimer diagnosed one of his patients with a devastating neurological impairment, and this form of dementia became known as Alzheimer’s disease (AD). Much research over the past century has clearly established that numerous human diseases, ranging from AD and Parkinson’s disease to dialysis-related amyloidosis, are best characterized by the abnormal aggregation of specific proteins. However, in the case of AD, the true toxic molecular species is still debated. Thus, the recent development of new diagnostic agents capable of distinguishing between different morphologies of aggregated proteins is of much interest.
targeting therapeutics (17). Therapeutics go hand-in-hand with diagnostics, and recent advances in SPECT probes (18, 19) for in vivo brain imaging are based on analogues of the classic Congo red and thioflavin T (ThT) dyes. However, this method will likely suffer from intrinsic problems because of its inability to distinguish between plaques from healthy and diseased patients. Thus, new chemical reagents that may be truly useful in the early diagnosis of AD can perhaps distinguish between normal and disease-associated fibrils or by directly imaging the soluble “toxic” oligomers. Any advance in these areas has the potential to significantly impact how AD is diagnosed and treated. Moreover, such reagents would be of immense benefit in testing new therapeutic modalities both in vivo and in vitro if they can be shown to correlate specific Aβ species with the clinical symptoms of AD.

Congo red was the first small molecule shown to bind to amyloid in tissue sections and exhibited a yellow-green birefringence under cross polarizers (20, 21). Several decades later, ThT and ThS were also shown to characteristically stain amyloid deposits (22, 23). These dyes have remained the classic reagents for determining β-sheet-mediated fibrilization, although they bind different sites (24). In the case of Congo red, beautiful polarized light microscopy studies have shown that Congo red is oriented along the long axes of fibrils in Aβ plaques and can be utilized to recognize plaques from different protein aggregates (25). Despite the knowledge gained from many experiments with these dyes, a facile method for distinguishing between Aβ aggregates has yet to emerge.

**Polymers and Fibrils.** This brings us to the current work from Nilsson et al., which describes a new class of conjugated polythiophene (PT)-based dyes that resolve differences between fibril conformations (Figure 1, panel b). This study is exciting because it connects the conducting polymer field (26) popularized by Heeger, MacDiarmid, and Shirakawa with protein-misfolding diseases. PTs are polymers of conjugated sulfur heterocycles and possess novel electrical and optical properties resulting from electron delocalization along the polymer backbone. The synthesis of PTs was first reported in the late 1980s (27); these polymers have since been exploited in various sensor applications (28, 29). Of interest is that Swager and coworkers synthesized 2,5-diphenyldithiophene derivatives that bind Aβ with high affinity and have demonstrated that they can be used to image amyloid deposits in the brain of a transgenic mouse model of AD by multiphoton spectroscopy (30). More recently, Kung and coworkers have also synthesized numerous thiophene derivatives to target Aβ with high affinities, which they hope to develop as novel positron emission tomography tracers in patients with AD (31). Although these studies are promising, neither reported on the use of thiophene derivatives for the selective recognition of different types of amyloid fibers.

Nilsson and coworkers now provide a new reagent for the direct fluorescence imaging of amyloid fibrils, which can parse them into either “straight” or “twisted” species (14). Several classes of thiophene derivatives were investigated, and the most useful analogue, poly[(5,5′)-terthiophene-(2S,3R)-2-amino-3-[2-[(1R,2S)-2-amino-2-carboxy-1-methyl-ethoxy]-ethyl]-[2,2′,5′,2″-terthiophen-3-yl]-ethoxy]-butyric acid] (tPTT), contains chiral amino acid “side chains” (Figure 1, panel b) that likely aid in both solubility and its amyloid recognition properties. They clearly observe in in vitro experiments that tPTT stains fibrils obtained from unstirred Aβ solutions orange (639–661 nm), whereas it stains fibrils obtained from agitated Aβ solutions green (543–564 nm). It is likely that the polymer chains of tPTT are aligned along the fibrillar axis as documented for a related polymer, PT acetic acid (32). Thus, the orange luminescence likely arises from a linear arrangement of the dye in straight fibers, whereas the green luminescence arises from binding twisted fibers that disrupt conjugation and dye alignment. Even more interesting were the results when these dyes were used to investigate brain sections of transgenic mouse (tg-Aβswe) models of AD. The fluorescence microscopy images showed two distinct classes of amyloid plaque, one the authors dub a compact-core plaque that primarily

![Figure 1. Model of fibrillogenesis and illustration of tPTT dye activity.](image-url)
stained orange, whereas a more frequently found plaque form stained orange at the edges and green at the center (Figure 1, panel b). These data agree with those from Jin and others (25, 33), who have also shown that Aβ plaques contain a diffuse center and fibrillar exterior. However, this is the first example where two different classes of amyloid plaque in an AD mouse model have been directly identified, and it opens many new doors into investigating AD pathophysiology. Numerous questions arise from this work, some of which will certainly be addressed in the near future. For example, are the two plaque isoforms observed in vitro interconvertible, or are they kinetically trapped (Figure 1, panel b)? Can seeding experiments with one fibrillar isoform result in the selective enrichment of that particular species? Are different classes of fibrillar species also observed for other amyloidogenic proteins if those proteins are stained with tPTT? Does tPTT differentially stain the alternative nontoxic amyloid isoform reported by Kiesling, Murphy, and coworkers (34, 35)? Can the two types of in vivo and ex vivo plaques be isolated and tested for their toxicity against neuronal cells? And finally and perhaps most important, can this approach be utilized to demonstrate a correlation between the type of plaque and the severity of AD?

Future work in this area will certainly involve combinatorial optimization studies on the thiophene core, which may provide more effective binders for different classes of fibrils. It may even be possible to develop reagents that selectively stain soluble oligomeric species that have been shown to be sequestered by antibodies (36) and small proteins (37). It would also be of interest to establish the maximum effective conjugation length necessary for selective luminescence for TPT-like molecules. Smaller, appropriately functionalized, but still selectively luminescent thiophenes may cross the blood–brain barrier and be utilized in powerful multiphoton imaging modalities for in vivo imaging that can guide AD treatment. Clearly, much can be gained in the chemistry and biology of amyloid imaging by going afIELD and borrowing from the rich chemistry and physics available in the conducting polymer area.

REFERENCES


