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A chemical screening approach reveals that indole fluorescence is quenched by pre-fibrillar but not fibrillar amyloid-β

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ABSTRACT

Aggregated amyloid-β (Aβ) peptide is implicated in the pathology of Alzheimer's disease. In vitro and in vivo, these aggregates are found in a variety of morphologies, including globular oligomers and linear fibrils, which possess distinct biological activities. However, known chemical probes, including the dyes thioflavin T and Congo Red, appear to lack selectivity for specific amyloid structures. To identify molecules that might differentiate between these architectures, we employed a fluorescence-based interaction assay to screen a collection of 68 known Aβ ligands against pre-formed oligomers and fibrils. In these studies, we found that the fluorescence of five indole-based compounds was selectively quenched (~15%) in the presence of oligomers, but remained unchanged after addition of fibrils. These results suggest that indoles might be complementary to existing chemical probes for studying amyloid formation in vitro.

Introduction: Alzheimer's disease (AD) is a severe neurodegenerative disorder characterized by the age-dependent aggregation of amyloid-β (Aβ) peptide in the brain.[1,2] Aβ self-assembles into distinct conformations both in vitro and in vivo, giving rise to structures such as globular oligomers and linear fibrils.[1,3] Despite being composed of the same monomer peptide, these conformations are strikingly different in shape and size.[4–6] In addition to these architectural differences, evidence from both cell culture and animal models indicates that oligomers are more neurotoxic than fibrils.[7–12] For example, Aβ oligomers permeate lipid membranes more readily than fibrils, a function that is thought to be involved in the neurotoxicity in AD.[13–15] Further, oligomers disrupt long-term potentiation and impair memory in AD mouse models.[16,17] Although it is clear that different conformations of Aβ exert independent biological activities, the structural basis for these unique properties has not been established.

Conditions such as temperature, time, salinity, and pH, have been established that promote the progression of Aβ monomers into predominantly oligomers or fibrils in vitro.[4,18] The oligomers formed in this way share the properties of soluble Aβ preparations from AD patients, namely high levels of toxicity and spherical appearance by transmission electron microscopy (TEM) and atomic force microscopy (AFM).[6,19,20] One of the powerful uses of fabricated oligomers is in studies of their structure. For example, recent NMR and hydrogen-deuterium exchange (HDE) reports show that Aβ oligomers are predominantly composed of β-sheets, but that the exposure of side chains as well as the packing of this β-sheet character in these species is distinct from fibrils.[12,21,22] These studies also show that oligomers are less stable than fibrils, which are known to be more densely packed and resistant to denaturation. Collectively, these reports suggest that different Aβ conformations possess unique structural and biological properties. Yet, the molecular features that distinguish Aβ oligomers from fibrils have not been clearly established, and, surprisingly, reagents that discriminate between these structures are few.[23–25] However, it is clear that cellular components (e.g., proteins, lipids) are somehow able to distinguish between oligomers and fibrils and, thus, it is important to identify how differences in their molecular surfaces might be recognized.

The spectral properties of small molecules, such as thioflavin T (ThT) and Congo Red (CR), are influenced by aggregated Aβ and, thus, these probes are often employed to quantify Aβ self-assembly.[26–28] Recent models predict that these ligands interact with the pleated β-sheets of self-assembled Aβ.[29] Interestingly, although these compounds can readily detect the extent of aggregation, they do not distinguish between Aβ conformations.[30,28] This finding might be expected, based on the similarities in the secondary structure of Aβ oligomers and fibrils; however, Necula et al. recently reported that certain chemical inhibitors selectively block formation of Aβ oligomers.[24,23] These results suggest that small molecules might exploit subtle differences in architecture to differ-

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid-β peptide; HCR, hydrophobic core region; TEM, transmission electron microscopy; AFM, atomic force microscopy; LMW, low molecular weight; 5-MI-2-CA, 5-methylindole-2-carboxylic acid; CR, Congo Red; ThT, thioflavin T; PET, positron emission tomography.

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entially engage pre-fibrillar and fibrillar Aβ. To test this hypothesis, we screened 68 structurally diverse small molecules against preformed oligomers and fibrils in a fluorescence assay and, interestingly, discovered that the intrinsic fluorescence of five indole-containing compounds was sensitive to Aβ oligomers but not fibrils. To our knowledge, these are the first small molecules able to distinguish between preformed Aβ oligomers and fibrils.

Results. Preparation and characterization of Aβ (1–42) fibrils and oligomers: We were interested in testing whether small molecules could differentiate between oligomers and fibrils. Toward this goal, we employed known conditions to generate relatively homogeneous populations of Aβ oligomers and fibrils.[4,18,6] Briefly, Aβ (1–42) oligomers were prepared by incubation in DMEM-F12 at 4 °C, and fibrils were prepared by incubating in PBS at 37 °C (see Experimental procedures). After 48 h, these samples were analyzed by transmission electron microscopy (TEM), which confirmed that oligomer samples were free of the elongated, linear structures commonly observed in the fibril preparations (Fig. 1A). To independently confirm these findings, the samples were cross-linked using glutaraldehyde and analyzed by non-denaturing gel electrophoresis followed by Western blotting using an anti-Aβ antibody (6E10) (Fig. 1B). Consistent with previous reports,[6] oligomer solutions lack the high molecular weight species that are present in the fibril preparation. Finally, the relative stabilities of the structures were probed by denaturation. Because the Aβ (1–42) peptide lacks a convenient tryptophan for monitoring integrity, we employed ThT reactivity to follow the response of the amyloid structure to denaturant. These studies confirmed that Aβ oligomers are less stable than fibrils (EC_{50} = 3.1 ± 1.0 M for oligomers and 8.0 ± 2.0 M for fibrils; Fig. 1C), generally consistent with previous findings.[12,21]

Bis-ANS fluorescence is increased by both Aβ oligomers and fibrils: Although the common dyes, thioflavin T (ThT) and Congo Red (CR), are unable to differentiate between Aβ oligomers and fibrils,[28,26] we first considered whether another common fluorescent probe, Bis-ANS, might possess this activity (Fig. 2A). Bis-ANS fluorescence increases upon binding hydrophobic regions of proteins, and is therefore widely employed to probe this property.[31,32] Based on this literature, we reasoned that Bis-ANS may reveal differences between in Aβ oligomers and fibrils. To test this model, we measured changes in Bis-ANS fluorescence in the presence of preformed Aβ (1–42) structures. In these experiments, fluorescence increased fourfold upon addition to Aβ fibrils, consistent with previous studies.[33–35] However, we found that the fluorescence increase in the presence of Aβ oligomers was indistinguishable from the fibril-induced response (Fig. 2B and C). Thus, Bis-ANS does not discriminate between oligomers and fibrils, suggesting that it interacts with either a shared structural element or that it is otherwise insensitive to differences between Aβ structures.

Fluorescence screen for Aβ conformation-specific small molecules: Because Bis-ANS failed to distinguish between Aβ conformations, we turned to a screening approach. Specifically, we collected a library of 68 structurally diverse small molecules, which included more than 11 distinct chemical scaffolds, including sulfonated dyes, curcumins phenothiazines, tetracyclines, benzophenones, monophenyls, flavonoids, indoles, chalcones, azo dyes and quinones

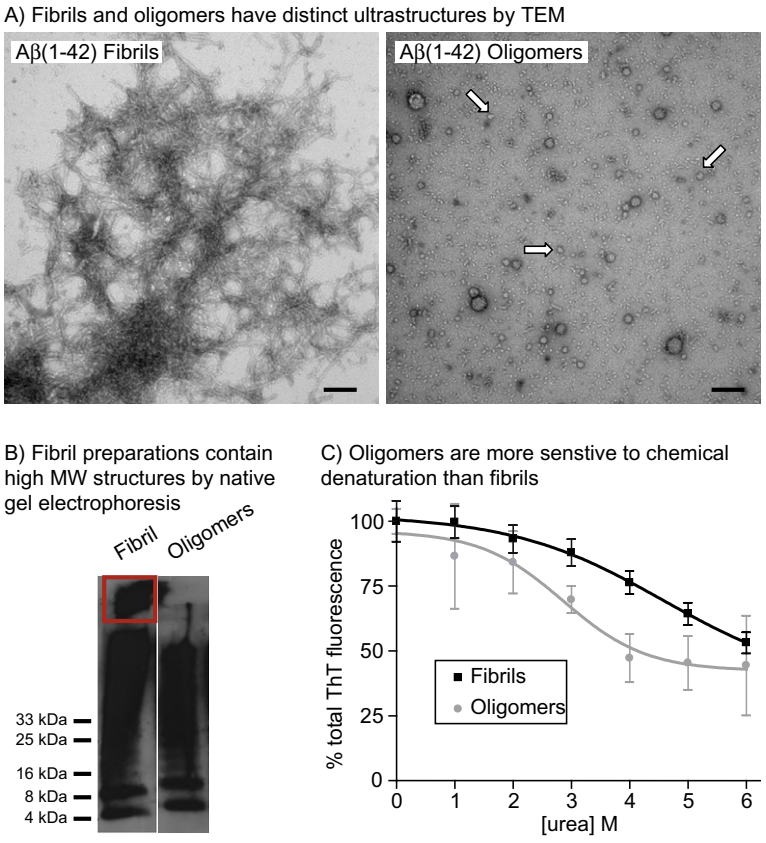

Figure 1. Characterization of the differences in shape, size, and stability of Aβ (1–42) fibrils and oligomers. (A) Analysis of 25 μM Aβ (1–42) fibrils (left) and oligomers (right) by transmission electron microscopy (TEM). Fibrils were prepared in PBS at 37 °C; oligomers were prepared in DMEM-F12 at 4 °C (see Experimental procedures). Samples were incubated for 48 h. Scale bar = 100 nm. (B) Native gel electrophoresis and Western blot of fibrils and oligomers using anti-Aβ antibody (6E10). Oligomer preparations lack the high molecular weight material present in fibrils (red box). (C) Oligomers are less stable than fibrils. Chemical denaturation of preformed aggregates with urea shows that Aβ oligomers are more sensitive to destabilizing conditions than fibrils.