Bioorganic & Medicinal Chemistry Letters 19 (2009) 4952–4957

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Ashley A. Reinke, Han Yiau Seh, Jason E. Gestwicki *

Departments of Biological Chemistry, Pathology and The Life Sciences Institute, University of Michigan, 210 Washtenaw Ave., Ann Arbor, MI 48109-2216, United States

ARTICLE INFO

Article history:
Received 3 May 2009
Accepted 16 July 2009
Available online 19 July 2009

Keywords:
Alzheimer's disease
Neurodegeneration
Protein misfolding
Thioflavin T
Fluorescence

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.

* Corresponding author. Tel.: +1 734 615 9537; fax: +1 734 615 1247.
E-mail address: gestwick@umich.edu (J.E. Gestwicki).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.07.082

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

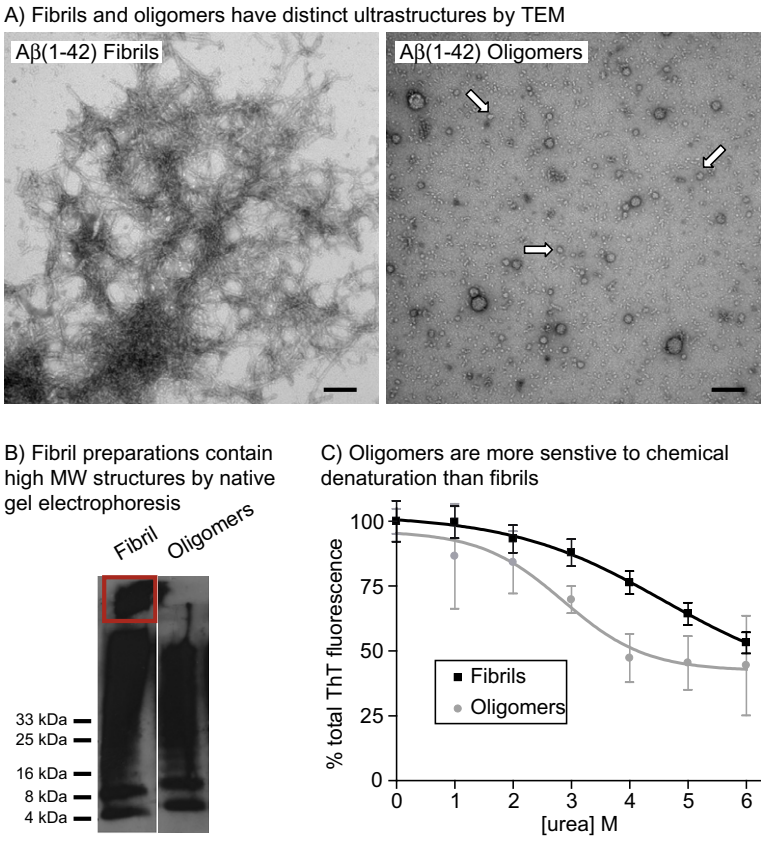

Figure 1. Characterization of the differences in shape, size, and stability of Aβ (1–42) fibrils and oligomers. (A) Analysis of 25 μM Aβ (1–42) fibrils (left) and oligomers (right) by transmission electron microscopy (TEM). Fibrils were prepared in PBS at 37 °C; oligomers were prepared in DMEM-F12 at 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.

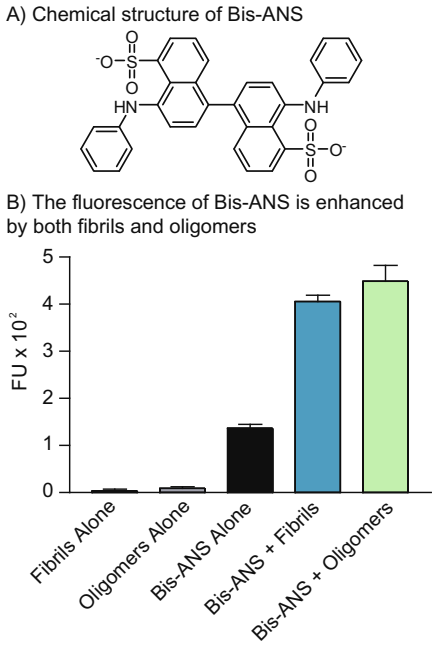

Figure 2. Bis-ANS fluorescence increases upon addition of both Aβ (1–42) oligomers and fibrils. (A) Chemical structure of Bis-ANS. (B) Fluorescence of 25 μM Bis-ANS (in 30 mM citrate pH 2.4) alone and in the presence of 25 μM Aβ fibrils or oligomers (Ex 385 nm, Em 520 nm). Fluorescence of fibrils and oligomers alone is shown for comparison. Error is expressed as the standard deviation of six replicates. (C) Fluorescence spectra of Bis-ANS alone and in the presence of oligomers or fibrils (Ex 350–450 nm, Em 520; Ex 385 nm, Em 450–600 nm). For each experiment, fluorescence was read 2 min after Aβ addition.

(Supplementary Fig. 1). These compounds were largely selected based on their reported ability to inhibit Aβ aggregation or bind Aβ fibrils,[23,24,36–38] in the hopes that these probes might be enriched for those with the potential to recognize features unique to Aβ structures. Importantly, in the selection of these compounds, we also favored low molecular mass (<500 Da) compounds because we were interested in understanding the response of amyloids to small, organic probes. Finally, many of the chosen compounds contained conjugated ring systems that, like Bis-ANS and ThT, might provide convenient fluorescence signatures.

Guided by these principles, we screened the chemical collection for fluorescence changes in the presence of preformed Aβ oligomers or fibrils (Fig. 3). For most of the compounds, we found that their fluorescence was insensitive to amyloids. This result is not interpreted as a failure to bind, only a failure of Aβ to impact the compound's fluorescence. Other members of the collection, such as **16** and **76**, displayed behaviors reminiscent of Bis-ANS; their fluorescence was indiscriminately altered in the presence of both oligomers and fibrils. However, we found that the fluorescence of nine compounds (**1**, **25**, **45**, **57**, **58**, **62–64** and **82**) was sensitive to either fibrils or oligomers. Interestingly, five of these compounds (**57, 58, 62–64**) contained an indole and their fluorescence was partially quenched in the presence of Aβ oligomers but not fibrils. To our knowledge, these are the first molecules shown to discriminate between pre-formed amyloid forms in vitro. Based on the structural similarity between five of the nine active compounds, we further explored the activity of indole (**58**).

Indole fluorescence is quenched by Aβ oligomers but not fibrils: To confirm the screening result, we performed a spectral scan on indole (**58**). Consistent with the finding at a single wavelength, no significant change in the indole spectra was observed upon addition of 25 μM Aβ (1–42) fibrils (Fig. 4A). However, the excitation and emission intensities decreased in the presence of 25 μM

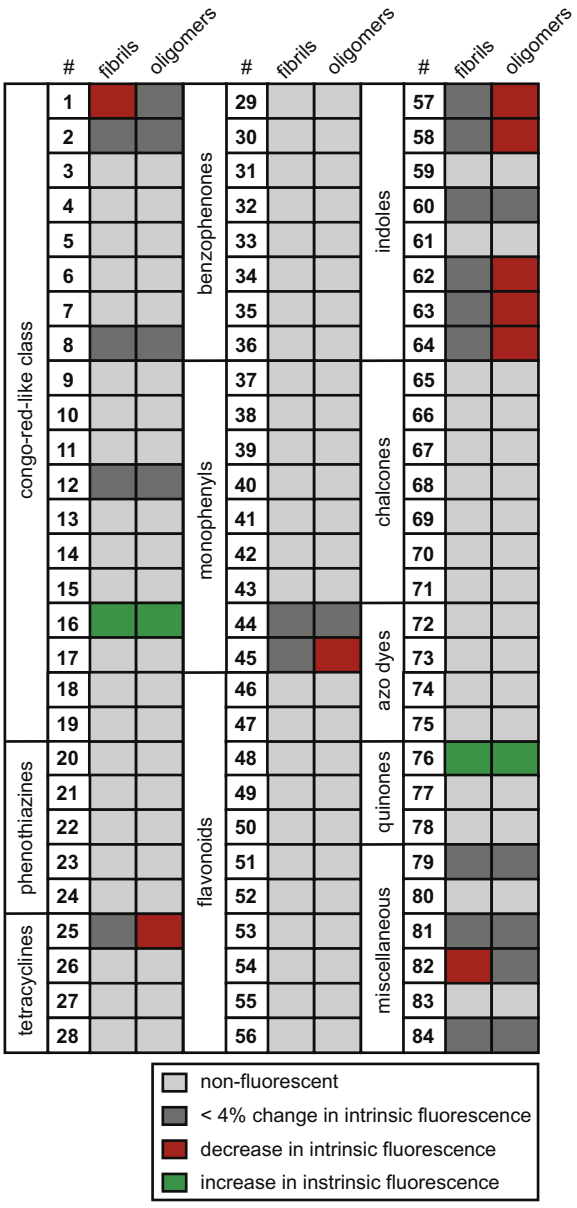

Figure 3. Screen for Aβ conformation-specific small molecules. The fluorescence of each compound (100 μM) was recorded alone or in the presence of 25 μM Aβ (1–42) fibrils or oligomers. Four wavelength ranges were used: Ex 290 nm, Em 320–520 nm; Ex 350 nm, Em 380–650 nm; Ex 400 nm, Em 430–650 nm; Ex 450 nm, Em 480–650 nm. Compounds were scored if changes in fluorescence behavior were observed in any of these regimes. Based on this analysis, compounds were catagorized as weakly fluorescent (light gray boxes), fluorescent, but with minimal (<4%) sensitivity for Aβ (dark gray boxes), or fluorescent and sensitive (>4%) for Aβ (red box for quenched signal; green box for increased signal). See the Supplementary data for compound names, chemical structures and raw values.

oligomers (Fig. 4B); excitation was quenched by approximately 8% at 280 nm and emission at 350 nm was reduced by 17%.

After exposure to aggregated Aβ, the fluorescence of other amyloid probes, such as ThT, requires a short incubation to achieve maximal signal.[26,27] To explore the kinetics of indole quenching, we monitored the fluorescence immediately after addition of Aβ (Fig. 4C). Specifically, indole was added to oligomers, fibrils, or low molecular weight Aβ peptide (LMW-Aβ) and fluorescence was recorded every 30 s for 1 h. LMW-Aβ contains no visible aggregates by TEM analysis (data not shown) and we used this preparation to exemplify pre-oligomeric species. To compare the samples, we expressed the results as the percent change in total indole

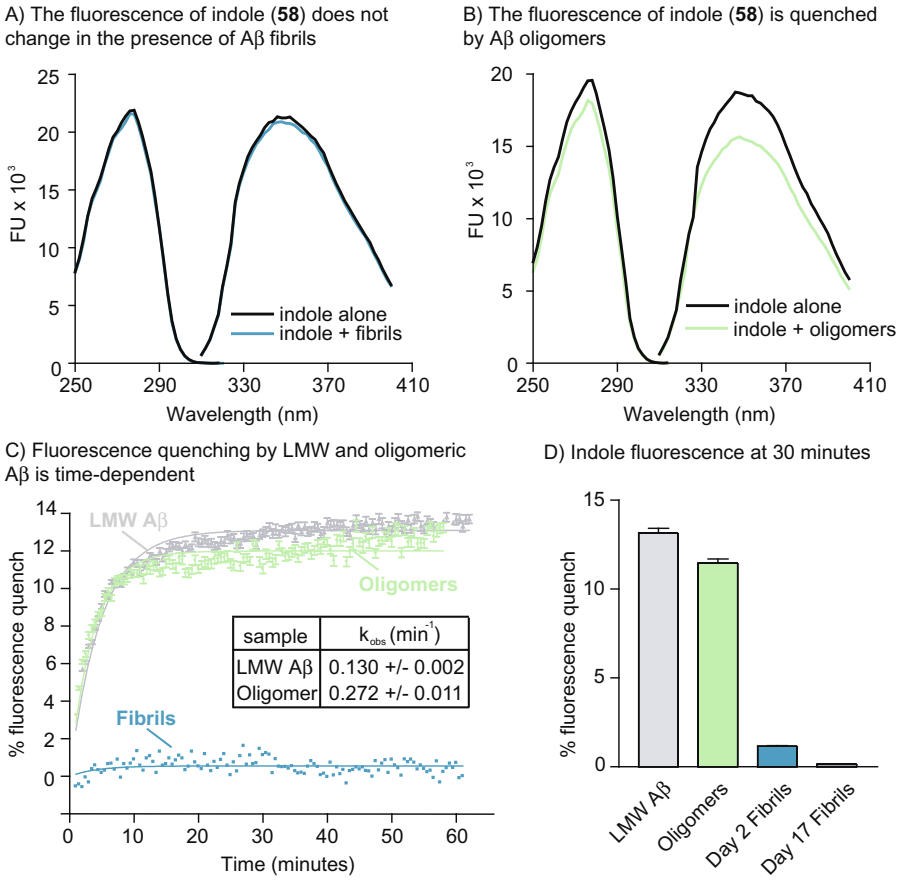

Figure 4. Indole fluorescence is quenched by Aβ (1–42) oligomers but not fibrils. Excitation and emission spectra of indole in the absence and presence of 25 μM Aβ (1–42) (A) fibrils and (B) oligomers (Ex 250–320 nm, Em 350; Ex 280 nm, Em 310–410 nm). Fluorescence was read 30 min after Aβ addition. (C) Indole fluorescence was monitored kinetically in the presence of low molecular weight (LMW) Aβ, oligomers, and fibrils (Ex 280 nm, Em 350). Results are plotted as percent quench in fluorescence. (D) Percent decrease in indole fluorescence following a 30 min incubation with LMW Aβ, oligomers, day 2 fibrils, or day 17 fibrils. In panels C and D, error is expressed as the standard deviation of six replicates.

fluorescence. Using this approach, we found that both oligomer and LMW-Aβ quenched fluorescence, and that this effect reached a maximum of 12% after 15 min. Consistent with our previous experiment, only a minor (0.5%) change was observed in the fibril sample. Using a one-phase exponential fit, we calculated the observed rate constants (k_{obs}) and showed that LMW-Aβ ($0.130 \pm 0.002\ min^{-1}$) and oligomers ($0.272 \pm 0.011\ min^{-1}$) share similar kinetics. Together, these findings suggest the presence of a binding site that is exposed in both oligomeric and LMW-Aβ architectures but inaccessible or otherwise unavailable in fibrils.

Because TEM analysis shows that fibril preparations contain a fractional amount of oligomers after two days (data not shown), we hypothesized that the minor quenching observed in the fibril sample might be due to contaminating oligomers or LMW-Aβ structures. Given that the prevalence of these species decreases with incubation time, we permitted fibril preparations to aggregate for 17 days prior to recording the change in indole fluorescence (Figs. 4D and 5D). Using this approach, we found that the quench is insignificant in the aged samples, consistent with minor LMW or oligomer contribution at earlier incubation times.

Characterization of indole quenching by Aβ oligomers versus fibrils: To further characterize the interaction between the parent compound indole (**58**) and Aβ conformations, we varied parameters of the fluorescence assay, including pH, indole concentration and structure, Aβ incubation time, and Aβ concentration (Fig. 5). In these experiments, we observed no significant trends over the pH range tested (pH 2.2–8.2), which is expected based upon the pK_a of indole (~21 in DMSO) (Fig. 5A).[39] When indole concentration was increased, an overall decrease in activity was observed, an effect that is likely caused by self-quenching interactions at high concentrations (Fig. 5B). Interestingly, the substituted indoles (**57, 58, 62–64**)) behaved similarly in the presence of oligomers, suggesting that oligomers are able to accommodate indoles decorated with various functional groups (Fig. 5C). To explore the sensitivity of the interaction, we determined the lowest concentration of oligomers at which we could detect quenching of indole (**58**) and found that this value was between 5 and 15 μM (Fig. 5E). Finally, amyloid experiments are often subject to heterogeneity in sample preparations, so we wanted to explore whether the observed indole activity was reproducible across independent samples. To test this idea, four separate aliquots of oligomers and fibrils were prepared and we found good reproducibility (less than 5% variance) between these trials (Fig. 5F). Finally, using TEM and ThT approaches, we found that indole has no affect on aggregation of fresh Aβ (Supplementary Fig. 2). Together, these experiments help define the experimental parameters for indole interactions with Aβ oligomers.

Small amounts of Aβ oligomer in fibril preparations can be detected by indole: Common amyloid probes, such as ThT, cannot distinguish between amyloid forms and, therefore, they cannot be used to quantify the percentage of oligomer content in a heterogeneous amyloid preparation. Towards that goal, we were interested in testing whether quenching of indole fluorescence by oligomers could be detected in a background of fibrils. Specifically, oligomers and fibrils were prepared separately and then mixed at known ratios, while maintaining the total protein concentration (Fig. 5G). Using this approach, we observed that even a minor percentage

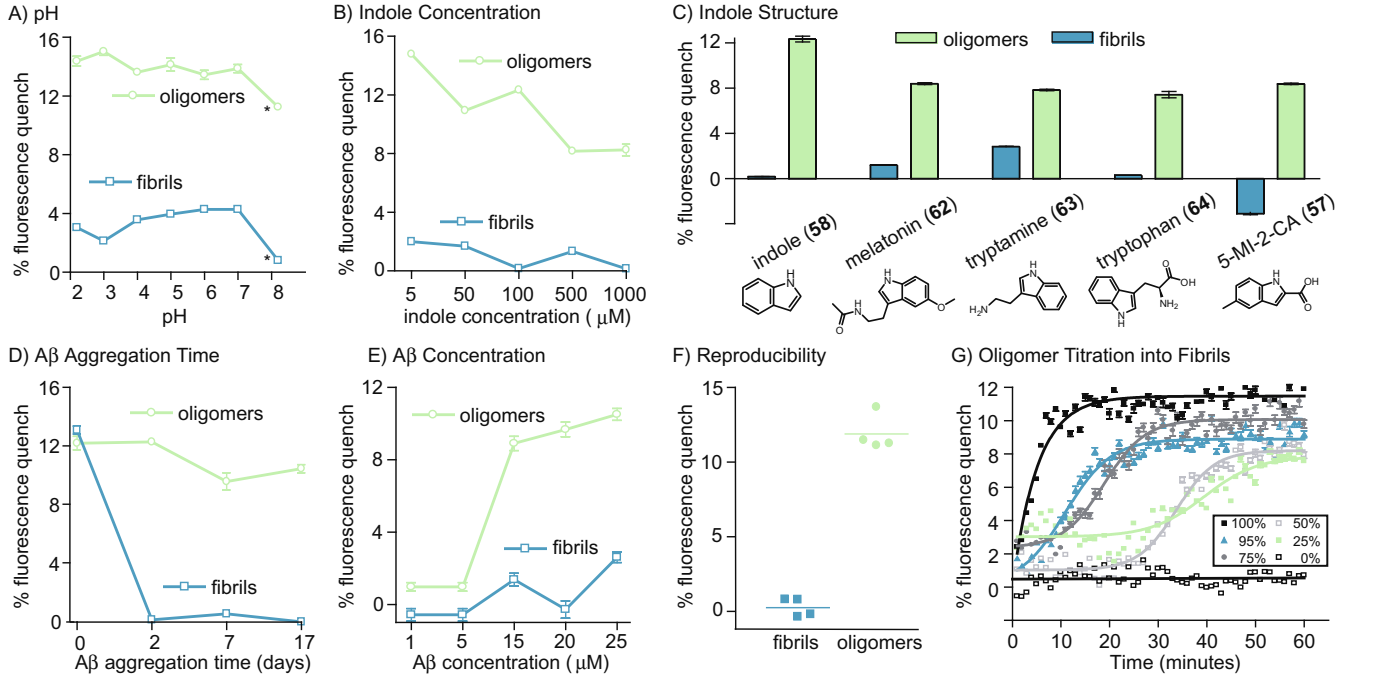

Figure 5. Characterization of indole quenching by Aβ oligomers versus fibrils. (A–E) Indole fluorescence was recorded following a 30 min incubation with Aβ (1–42) oligomers or fibrils (Ex 280 nm, Em 350 nm) and all results are expressed as percent decrease in indole fluorescence. Unless noted, indole and Aβ concentrations remained constant at 100 μM and 25 μM, respectively, in 50 mM glycine (pH 8.2). In panel A, indole was prepared in PBS or * 50 mM glycine. In panel F, four aliquots of Aβ fibrils and oligomers were prepared independently to test the reproducibility of the indole fluorescence quenching. In panel G, oligomers were titrated into fibril preparations and the percentages indicate the amount of oligomer added to fibrils (0% = fibril only; 100% = oligomer only). For all experiments, errors bars are expressed as the standard deviation of three replicates. (5-MI-CA = 5-methylindole-2-carboxylic acid).

of oligomer (~25%) could be measured in a mixed population. One interesting aspect of these results was that there was a delay in the quenching of samples that contained fibrils. Specifically, we found that the length of the lag phase was proportional to the percentage of fibrils present, suggesting that, despite failure to trigger a quench, indole might transiently interact with fibrils. Because ThT and indole do not compete for the same site (see Supplementary data), these probes might be used in concert to study amyloid formation. It is important to note that experiments in this area would likely be restricted to in vitro studies because indole is known to bind non-amyloid targets that would be present in biological settings.

Discussion: To date, different Aβ aggregates have been largely defined by their solubility, appearance in TEM, reactivity with select antibodies, and their differential neurotoxicity. The goal of this effort was to determine if small molecules could differentiate between these pre-formed structures. We expected that molecules with this property could be used in studies of amyloid formation in vitro—much like how ThT and its derivatives are used to monitor the extent of aggregation. Towards this end, we employed Aβ samples enriched for oligomers or fibrils and screened 68 structurally diverse small molecules. These studies revealed that five indole-containing compounds were sensitive to pre-fibrillar structures but not fibrils. In contrast, the majority of chemical probes, such as Bis-ANS and Congo Red, failed to differentiate between them under these conditions. These results suggest that indoles might be complementary to existing chemical probes for studying amyloid formation in vitro.

Experimental. Materials: Amyloid-β (1–40) and (11–40) peptides were purchased from Anaspec (San Jose, CA). Aβ (1–42) peptide was purchased from EZBiolab (Westfield, IN). DMSO and HFIP were purchased from Sigma–Aldrich. Compounds used in the fluorescence screen were synthesized internally[38] or purchased from Sigma–Aldrich, Fluka, Fisher Scientific, and Cayman Chemicals (Ann Arbor, MI). Anti-amyloid β antibody (6E10) was purchased from Calbiochem (San Diego, CA). Secondary antibodies were purchased from Bio-Rad (Hercules, CA). All fluorescence readings were taken on a SpectraMax M5 multi-mode plate reader (Molecular Devices, Sunnyvale, CA).

Amyloid-β stock preparation: One milligram samples of Aβ (1–42) peptide were dissolved in 200 μL hexafluoroisopropanol (HFIP) and aliquoted to obtain 0.1 mg stocks. HFIP was removed under nitrogen to provide a thin film and these stocks were stored at −30 °C until ready for use. Immediately prior to the start of each experiment, an aliquot was dissolved in DMSO (see below). Fibrils were obtained by adding phosphate buffered saline (PBS; pH 7.4) to a final amyloid concentration of 25 μM (1% final DMSO concentration). These solutions were vortexed, sonicated for 75 s, and agitated for 48 h at 37 °C. Oligomers were obtained by adding DMEM-F12 media (Gibco) to a final amyloid concentration of 25 μM (1% final DMSO concentration), followed by vortexing, sonicating for 75 s, and incubating for 48 h at 4 °C without agitation. LMW-Aβ was obtained by adding PBS (pH 7.4) to a final amyloid concentration of 25 μM (1% final DMSO concentration), vortexing, sonicating for 75 s and using these samples immediately.

Transmission electron microscopy: Freshly suspended Aβ or aggregated sample (25 μM; 5 μL) was added to glow-discharged, Formvar/carbon 300-mesh copper grids (Electron Microscopy Sciences, Hatfield, PA) and incubated for 1.5 min at room temperature. Excess sample was blotted off with filter paper and each grid was washed twice with ddH_2O. Uranylacetate (1%, 3 μL) was added to each grid and incubated for 1 min. Excess sample was blotted off and grids were then dried for 15 min. Samples were visualized on a Phillips CM-100 transmission electron microscope at 80 kV and 94,000× magnification.

Native gel electrophoresis and western blotting: Aβ fibrils and oligomers were prepared at 25 μM as described and cross-linked using gluteraldehyde (0.04% final concentration) for 40 min at room

temperature. The reaction was quenched with glycine pH 8.2 (final concentration of 10 mM) and SDS-loading dye (non-reducing) was added (25% final volume). Each sample (8 μg) was separated on a 10–20% gradient tris-tricine gel (Bio-Rad), and the gel was then transferred to a nitrocellulose membrane. The nitrocellulose was blocked in 10% non-fat dried milk in Tris-buffered saline (TBS) containing 0.1% Tween-20 (TBS-T) for 1 h at room temperature, incubated in anti-Aβ 6E10 antibody (1:1000) in 3% BSA in TBS-T for 1 h at room temperature, and probed with HRP-conjugated goat anti-mouse antibody (1:10,000) in 3% BSA in TBS-T for 1 h at room temperature. Protein bands were visualized using the Bio-Rad ECL kit according to the manufacturer's instructions.

Urea denaturation of preformed Aβ aggregates: Aβ fibrils and oligomers were prepared at (25 μM) as described and an aliquot (10 μL) dispensed into 96-well plate format (opaque, black Corning). Urea (30 μL) at varying concentrations (0–6 M) was added to these samples, mixed thoroughly, and the solutions were incubated for 40 min at room temperature. Following this treatment, 200 μL ThT (5 μM in 50 mM glycine, pH 8.2) was added and these samples were then incubated for 15 min and the fluorescence recorded (Ex 446 nm; Em 490 nm). Control experiments confirmed that urea did not impact the intrinsic fluorescence of the ThT reagent and TEM experiments confirmed a disruption in ultrastructure (not shown).

Bis-ans fluorescence: 4,4′-Dianilino-1,1′-binaphthyl-5,5′-disulfonate (Bis-ANS) (100 μL; 25 μM in 30 mM citrate pH 2.4, 1% DMSO) was added to 9 μL Aβ fibrils or oligomers (25 μM) or 9 μL PBS or DMEM-F12 (1% DMSO) in a black 96-well plate (Corning Costar). Fluorescence was measured after 2 min (Ex 385 nm, Em 520 nm, cutoff 515 nm). Experiments were performed using six replicates. Background fluorescence of fibrils, oligomers, PBS, and DMEM-F12 in the presence of 100 μL buffer alone (30 mM citrate pH 2.4, 1% DMSO) was subtracted.

Fluorescence screen: Each compound was dissolved in DMSO to a final concentration of 100 mM and then diluted to 50 μM with ddH_2O (1% final DMSO concentration). 100 μL of each compound was added to 9 μL Aβ fibrils or oligomers (25 μM) or 9 μL PBS or DMEM-F12 (1% DMSO) in triplicate to a black 96-well plate and incubated for 10 min. Fluorescence spectra were then recorded at four excitation and emission values: (a) Ex 290 nm, Em 320–520 nm; (b) Ex 350 nm, Em 380–650 nm; (c) Ex 400 nm, Em 430–620 nm; (d) Ex 450, Em 480–650. Background fluorescence of fibrils, oligomers, PBS, and DMEM-F12 in the presence of 100 μL ddH_2O only (1% DMSO) was subtracted. For indole time-course experiments, the fluorescence of six replicates was measured every 30 s for 1 h (Ex 280 nm, Em 350 nm, cutoff 325 nm) and this data was fit using a one-phase exponential association in GraphPad Prism software. For the detailed studies of the indoles (**57, 58, 62–34**) (5-methylindole-2-carboxylic acid, indole, melatonin, tryptamine, and tryptophan), each compound was dissolved in DMSO to a final concentration of 100 mM and diluted to 100 μM in 50 mM glycine pH 8.2 (1% final DMSO concentration). To determine pH effects, pH was varied in either PBS (pH 2.2–7.0) or 50 mM glycine (pH 8.2). For the oligomer titration experiment, fibrils and oligomers were prepared as described and mixed in varying percentages (0, 5, 10, 25, 50, 75, 95, and 100% oligomer), while maintaining a constant protein concentration.

Acknowledgements

The authors would like to thank D. Hicks, I. Graef, K. Wisser, J. Schuarte, and A. Gafni for helpful discussions and resources, and D. Sorenson for assistance with microscopy. A.A.R. was supported by a predoctoral fellowship from the Biogerontology NIA Training Grant (AG000114). This work was also supported by a grant from the Alzheimer's Association (NIRG-08-89471).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.082.

References and notes

1. Caughey, B.; Lansbury, P. T. *Annu. Rev. Neurosci.* **2003**, *26*, 267.
2. Selkoe, D. J. *Neuron* **1991**, *6*, 487.
3. Glabe, C. C. *Subcell Biochem.* **2005**, *38*, 167.
4. Dahlgren, K. N.; Manelli, A. M.; Stine, W. B., Jr.; Baker, L. K.; Krafft, G. A.; LaDu, M. J. *J. Biol. Chem.* **2002**, *277*, 32046.
5. Haass, C.; Selkoe, D. J. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 101.
6. Chromy, B. A.; Nowak, R. J.; Lambert, M. P.; Viola, K. L.; Chang, L.; Velasco, P. T.; Jones, B. W.; Fernandez, S. J.; Lacor, P. N.; Horowitz, P.; Finch, C. E.; Krafft, G. A.; Klein, W. L. *Biochemistry* **2003**, *42*, 12749.
7. Glabe, C. G.; Kayed, R. *Neurology* **2006**, *66*, S74.
8. Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Rowan, M. J.; Selkoe, D. J. *Biochem. Soc. Trans.* **2002**, *30*, 552.
9. Selkoe, D. J. *Behav. Brain Res.* **2008**, *192*, 106.
10. Barghorn, S.; Nimmrich, V.; Striebinger, A.; Krantz, C.; Keller, P.; Janson, B.; Bahr, M.; Schmidt, M.; Bitner, R. S.; Harlan, J.; Barlow, E.; Ebert, U.; Hillen, H. *J. Neurochem.* **2005**, *95*, 834.
11. Cleary, J. P.; Walsh, D. M.; Hofmeister, J. J.; Shankar, G. M.; Kuskowski, M. A.; Selkoe, D. J.; Ashe, K. H. *Nat. Neurosci.* **2005**, *8*, 79.
12. Chimon, S.; Shaibat, M. A.; Jones, C. R.; Calero, D. C.; Aizezi, B.; Ishii, Y. *Nat. Struct. Mol. Biol.* **2007**.
13. Kagan, B. L.; Azimov, R.; Azimova, R. *J. Membr. Biol.* **2004**, *202*, 1.
14. Kayed, R.; Sokolov, Y.; Edmonds, B.; McIntire, T. M.; Milton, S. C.; Hall, J. E.; Glabe, C. G. *J. Biol. Chem.* **2004**, *279*, 46363.
15. Lashuel, H. A. *Sci. Aging Knowledge Environ.* **2005**, *2005*, pe28.
16. Shankar, G. M.; Li, S.; Mehta, T. H.; Garcia-Munoz, A.; Shepardson, N. E.; Smith, I.; Brett, F. M.; Farrell, M. A.; Rowan, M. J.; Lemere, C. A.; Regan, C. M.; Walsh, D. M.; Sabatini, B. L.; Selkoe, D. J. *Nat. Med.* **2008**, *14*, 837.
17. Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Cullen, W. K.; Anwyl, R.; Wolfe, M. S.; Rowan, M. J.; Selkoe, D. J. *Nature* **2002**, *416*, 535.
18. Stine, W. B., Jr.; Dahlgren, K. N.; Krafft, G. A.; LaDu, M. J. *J. Biol. Chem.* **2003**, *278*, 11612.
19. Mastrangelo, I. A.; Ahmed, M.; Sato, T.; Liu, W.; Wang, C.; Hough, P.; Smith, S. O. *J. Mol. Biol.* **2006**, *358*, 106.
20. Yagi, H.; Ban, T.; Morigaki, K.; Naiki, H.; Goto, Y. *Biochemistry* **2007**, *46*, 15009.
21. Yu, L.; Edalji, R.; Harlan, J. E.; Holzman, T. F.; Lopez, A. P.; Labkovsky, B.; Hillen, H.; Barghorn, S.; Ebert, U.; Richardson, P. L.; Miesbauer, L.; Solomon, L.; Bartley, D.; Walter, K.; Johnson, R. W.; Hajduk, P. J.; Olejniczak, E. T. *Biochemistry* **2009**.
22. Qi, W.; Zhang, A.; Patel, D.; Lee, S.; Harrington, J. L.; Zhao, L.; Schaefer, D.; Good, T. A.; Fernandez, E. J. *Biotechnol. Bioeng.* **2008**, *100*, 1214.
23. Necula, M.; Breydo, L.; Milton, S.; Kayed, R.; van der Veer, W. E.; Tone, P.; Glabe, C. G. *Biochemistry* **2007**, *46*, 8850.
24. Necula, M.; Kayed, R.; Milton, S.; Glabe, C. G. *J. Biol. Chem.* **2007**, *282*, 10311.
25. Kayed, R.; Head, E.; Thompson, J. L.; McIntire, T. M.; Milton, S. C.; Cotman, C. W.; Glabe, C. G. *Science* **2003**, *300*, 486.
26. LeVine, H., 3rd *Protein Sci.* **1993**, *2*, 404.
27. LeVine, H., 3rd *Methods Enzymol.* **1999**, *309*, 274.
28. Klunk, W. E.; Pettegrew, J. W.; Abraham, D. J. *J. Histochem. Cytochem.* **1989**, *37*, 1273.
29. Wu, C.; Wang, Z.; Lei, H.; Zhang, W.; Duan, Y. *J. Am. Chem. Soc.* **2007**, *129*, 1225.
30. Maezawa, I.; Hong, H. S.; Liu, R.; Wu, C. Y.; Cheng, R. H.; Kung, M. P.; Kung, H. F.; Lam, K. S.; Oddo, S.; Laferla, F. M.; Jin, L. W. *J. Neurochem.* **2008**, *104*, 457.
31. Das, K. P.; Surewicz, W. K. *FEBS Lett.* **1995**, *369*, 321.
32. Shi, Y.; Fan, D. J.; Li, S. X.; Zhang, H. J.; Perrett, S.; Zhou, J. M. *Protein Sci.* **2007**, *16*, 1165.
33. LeVine, H., 3rd *Arch. Biochem. Biophys.* **2002**, *404*, 106.
34. Chen, Y. R.; Glabe, C. G. *J. Biol. Chem.* **2006**, *281*, 24414.
35. Lindgren, M.; Sorgjerd, K.; Hammarstrom, P. *Biophys. J.* **2005**, *88*, 4200.
36. Taniguchi, S.; Suzuki, N.; Masuda, M.; Hisanaga, S.; Iwatsubo, T.; Goedert, M.; Hasegawa, M. *J. Biol. Chem.* **2005**, *280*, 7614.
37. Masuda, M.; Suzuki, N.; Taniguchi, S.; Oikawa, T.; Nonaka, T.; Iwatsubo, T.; Hisanaga, S.; Goedert, M.; Hasegawa, M. *Biochemistry* **2006**, *45*, 6085.
38. Reinke, A. A.; Gestwicki, J. E. *Chem. Biol. Drug Des.* **2007**, *70*, 206.
39. Bordwell, F.; Drucker, G.; Fried, H. *J. Org. Chem.* **1981**, *46*, 632.