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Inhibiting protein–amyloid interactions with small molecules: A surface chemistry approach

Petra Inbar and Jerry Yang*

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, MC 0358, La Jolla, CA 92093-0358, USA

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Abstract—This paper presents a surface-based approach to inhibit the binding of proteins to Alzheimer's-related β-amyloid (Aβ) fibrils with small molecules. It reports the idea of using an intracellular, disease-related fibril as a material whose surface can be coated with small molecules. Using an ELISA-based assay, molecular surface coatings with thioflavin T are shown to inhibit 65 ± 10% of the binding of two different anti-Aβ IgGs to Aβ fibrils. A molecular surface coating with 3,6-diamino acridine was able to inhibit 76 ± 10% of the binding of an anti-Aβ IgG to Aβ fibrils. Maximal inhibition of these protein–amyloid interactions appears in the low to mid-micromolar range of small molecule. This demonstration that molecular surface coatings can be used to attenuate the interaction of proteins with these fibrils suggests a potentially new strategy for therapeutics in neurodegenerative amyloid diseases.

This paper describes the coating of Alzheimer's-related β-amyloid (Aβ) fibrils with small molecules as a method to inhibit the binding interactions between amyloid-binding proteins with these fibrils. Amyloid fibrils formed from misfolded proteins or peptides are a hallmark of many neurodegenerative diseases.[1] One possible cause for the development of Alzheimer's disease (AD), for instance, is the interaction of Aβ peptides, oligomers, and fibrils with cellular components in the brain.[2] The interactions between proteins (e.g., catalase,[3] ABAD,[4] and RAGE[5]) and aggregated Aβ, for example, have been reported[3–5] for their potential contribution to Aβ-induced neurotoxicity in the pathogenesis of AD, although there is still much debate as to which form of aggregated Aβ peptide (e.g., oligomers or fibrils) is most toxic.[6] These results suggest that therapeutic strategies that interfere with protein–amyloid interactions (especially amyloid in aggregated form) may be useful for the treatment of patients with AD. This paper presents a simple and general surface chemistry approach to inhibit the interaction of proteins with one form of aggregated Aβ—fibrils—using a small molecule.

Molecular coatings on metallic and polymeric surfaces are used frequently to attenuate interactions of proteins with artificial materials for biological studies and biotechnology applications.[7] Here, we extend this technology to natural, biological materials and demonstrate proof-of-concept for generating protein-resistive coatings on the surface of Aβ fibrils with small molecules. We hypothesize that the binding of molecules to Aβ fibrils can generate surface coatings that are capable of resisting the interaction of Aβ-binding proteins with these fibrils, resulting in a potentially new strategy to intervene in AD-related pathology. We demonstrate that the small molecule, thioflavin T (ThT), can inhibit the interaction of amyloid-binding proteins with AD-related Aβ fibrils by binding and coating the surface of these fibrils. In this research, we used Aβ-binding IgGs as simplified models for natural Aβ-binding proteins.

ThT—a fluorescent histological staining agent (Fig. 2A)—is used extensively for the characterization of Aβ fibrils[8] and for the detection of aggregation of Aβ in solution.[9] Several groups[10] have studied the interaction of ThT with Aβ fibrils by fluorescence and showed that ThT binds uniformly to the bulk of Aβ fibrils with high affinity (K_d's ranging from high nM to low μM[10b,d]). We propose that if ThT binds Aβ fibrils with high enough density to coat the bulk of the fibril, ThT will be able to block potential binding sites for

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* Corresponding author. Tel.: +1 858 534 6006; fax: +1 858 534 4554; e-mail: jyang@chem.ucsd.edu

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proteins and thus function as an inhibitor for protein-Aβ fibril (here, IgG-Aβ fibril) interactions (Fig. 1B).

We grew Aβ fibrils in vitro from synthetic AD-related Aβ peptides (residues 1–42). We characterized these fibrils by atomic force microscopy. Images indicated the presence of fibrils (Fig. 1A) that were consistent with literature reports[11] in terms of size (5–10 nm in diameter and >400 nm long) and in terms of morphology (single fibrils and bundles of fibrils). We coated the wells of commercial 96-well plates with freshly prepared Aβ fibrils and incubated them with solutions of ThT. After removal of excess ThT, we treated the ThT-coated fibrils in the wells with a monoclonal anti-Aβ IgG[†] (clone 6E10, derived from residues 3 to 8 of Aβ peptide as antigens). Finally, we quantified the interaction of the anti-Aβ IgG with the ThT-coated Aβ fibrils using an ELISA-based assay (see Supporting Information for details of this IgG-Aβ inhibition assay).

Figure 2A shows that ThT had an inhibition concentration corresponding to 50 percent inhibition (IC_{50}) of 5 μM for the binding of the anti-Aβ IgG (clone 6E10, 0.16 μg mL^{-1}) to the Aβ fibrils (deposited from solutions containing 1.3 μM Aβ peptide).[‡] We measured a total inhibition of ~65% of the interaction between this IgG and Aβ fibrils when we incubated the fibrils with a 50 μM solution of ThT.[§] Solutions of ThT with concentrations higher than 50 μM did not increase the total inhibition of the IgG-Aβ fibril interactions above ~65 %.[¶] Exposing the ThT-coated Aβ-fibrils to prolonged washing steps (from 0 to 4 h) with PBS buffer prior to incubation with primary anti-Aβ IgG did not affect the total amount of inhibition of the IgG–amyloid interactions, suggesting that the rate of unbinding of ThT from the Aβ fibrils is slow relative to the timescale of the binding assay.

To demonstrate that these surface coatings can inhibit other proteins that bind to Aβ fibrils, we tested the ability of ThT to inhibit the interaction of Aβ fibrils with an anti-Aβ IgG raised against a different epitope of Aβ peptide (clone AMY-33, derived from residues 1–28 of Aβ peptide as antigens). We measured an IC_{50} of 0.4 μM (Fig. 2B) for ThT with this IgG (clone AMY-33) under the same conditions we used to assay the first anti-Aβ IgG (clone 6E10). We observed a maximum inhibition of ~65% of this IgG (clone AMY-33)-Aβ fibril interaction with solutions of ThT having concentrations of 10 μM or higher.

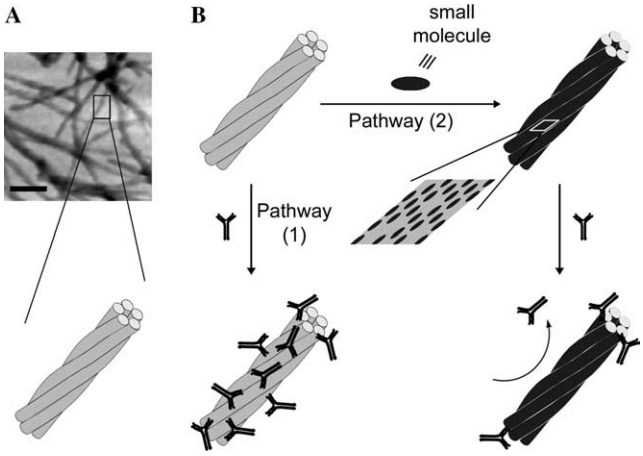

Figure 1. Illustration of the inhibition of IgG–amyloid interactions by coating surfaces of Alzheimer's-related Aβ fibrils with small molecules. (A) Aβ fibrils imaged by atomic force microscopy. The dark regions indicate the location of fibrils. The scale bar is 100 nm. The blownup region indicates a schematic diagram of a single Aβ fibril.[16] (B) Pathway (1) illustrates the binding of anti-Aβ IgGs to the fibrils in the absence of small molecules. Pathway (2) illustrates the proposed binding of small molecules to Aβ fibrils parallel to the direction of the fibril axis;[10e] the proposed surface coating on Aβ with small molecules prevents anti-Aβ IgGs from binding to the fibrils.

When we used 3,6-diamino acridine (DAA)—another histological staining agent for amyloid fibrils[12,13]—instead of ThT, we observed similar inhibition of the interaction between the anti-Aβ IgG (clone 6E10) and Aβ-fibrils (Fig. 2C). We measured an IC_{50} of 4.6 μM and a maximum inhibition of 76 ± 10% of the IgG–amyloid interactions when we used DAA. We observed maximum inhibition of the interaction of this anti-Aβ IgG (clone 6E10) with Aβ fibrils using concentrations of DAA of 67 μM and higher.

We did not observe inhibition of the interaction between the IgGs and Aβ fibrils in control experiments using 1-naphthol-4-sulfonate (Fig. 2D)—structurally similar molecules to 1-naphthol-4-sulfonate do not interact with Aβ fibrils[14]—suggesting binding of the small molecule to the Aβ fibrils is necessary for the observed inhibition with ThT and DAA.

In all cases shown in Figure 2A–C, we did not observe inhibition of the IgG–amyloid interactions above 76 ± 10% by small molecules. These results may be due to the inability of the small molecules to bind effectively to defects or the terminal ends of Aβ fibrils (ThT is known to bind only to the fibril form of Aβ[10c]). Figure 2E shows TEM images of the IgGs (clone 6E10) bound to Aβ fibrils; the IgGs are labeled with gold particles for clarity using a previously reported procedure.[15] When the Aβ fibrils are coated with ThT prior to incubation with a solution of anti-Aβ IgGs, significantly fewer IgGs appear bound to the Aβ fibrils (Fig. 2F). These images qualitatively support the results from the binding assays.

[†] We used monoclonal IgGs to minimize the chance for cross-reactivity of the IgGs with the other molecules used in our assay.

[‡] Since we do not know the final amount of the Aβ fibrils deposited in the wells, we also incubated the Aβ fibrils (1.3 μM) with solutions of ThT prior to depositing the coated fibers into the wells and measured an IC_{50} of 60 μM (See Supporting Information, Figure S1). For comparison, we observed an IC_{50} of ~1 μM when we used a 0.3 μM solution of Aβ peptide in the control procedure (data not shown). We would, therefore, expect the observed IC_{50}'s to be different using the two procedures.

[§] We defined 0 percent inhibition as the UV–vis signal observed when the assay is run in the absence of small molecule and define 100 percent inhibition as the UV–vis signal observed when the assay is run in the absence of both amyloid fibrils and small molecule.

[¶] Coating the fibrils in solutions of ThT (>100 μM of ThT) prior to deposition into wells resulted in a maximum inhibition of ~80% of the protein–amyloid interactions (See Supporting Information, Figure S1).

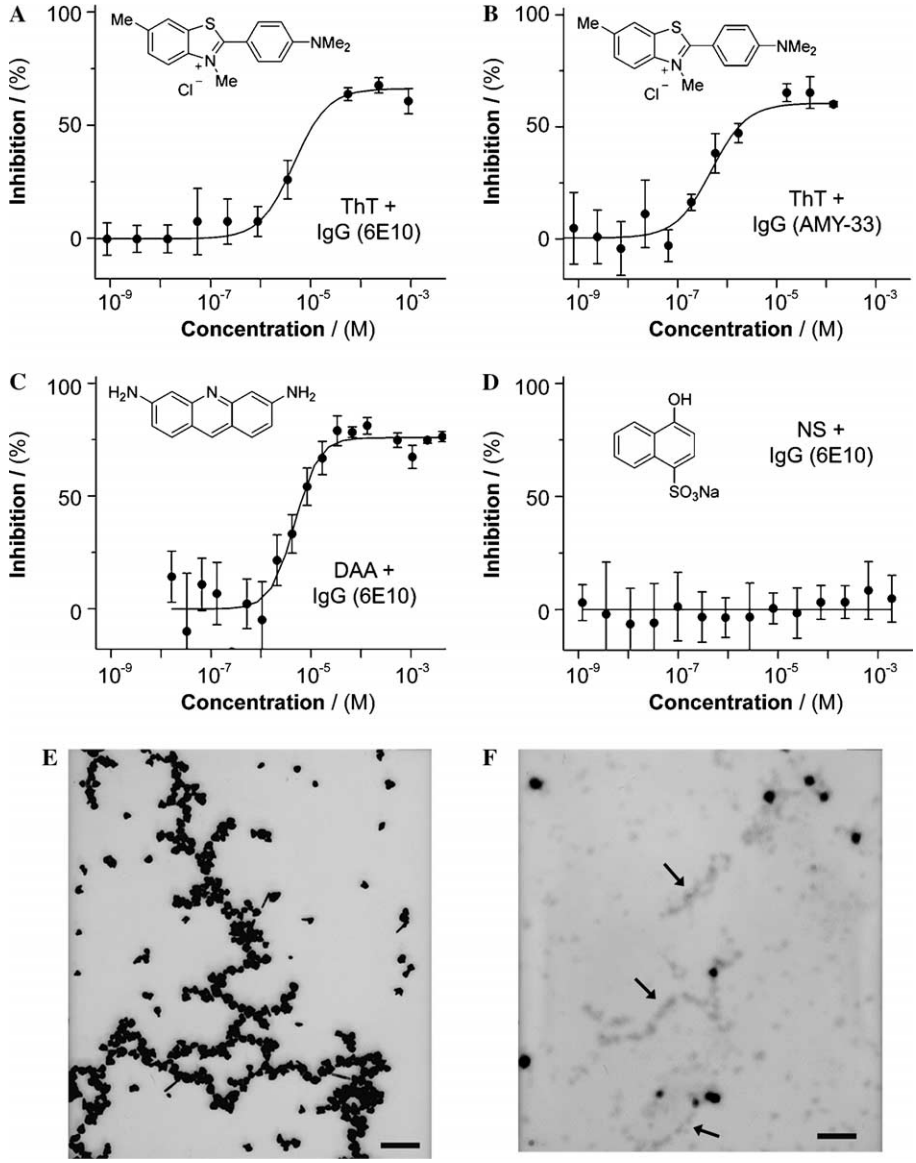

Figure 2. Inhibition of IgG-Aβ fibril interactions with ThT. (A) Aβ fibrils incubated with solutions of ThT and exposed to an anti-Aβ IgG (clone 6E10). (B) Same assay as in (A) but using an anti-Aβ IgG raised against a different binding epitope of Aβ peptide (clone AMY-33). (C) Same assay as in (A), except that the inhibition is plotted against the concentration of 3,6-diamino acridine (DAA) instead of ThT. (D) Same assay as in (A), except that the inhibition is plotted against the concentration of 1-naphthol-4-sulfonate (NS) instead of ThT. (E) TEM images of anti-Aβ IgGs (clone 6E10) bound to Aβ fibrils. For clarity, the antibodies are labeled with gold particles.[15] (F) TEM images of fibrils coated with ThT followed by incubation with anti-Aβ IgG. Again, IgGs were labeled with gold particles for clarity. ThT-coated fibrils (arrows) were stained positively with uranyl acetate. See Supporting Information for details and additional images. Scale bars are 200 nm.

We believe, therefore, that at least part of the surface of Aβ fibrils is accessible for binding by anti-Aβ IgGs even after coating the surface of Aβ fibrils with small molecules. Perhaps, molecules that more thoroughly coat the surface of Aβ fibrils compared to ThT or DAA will show improved total inhibition of protein-Aβ fibril interactions. The recent reports of small molecules that can bind to Aβ fibrils, can be tolerated in vivo, and can be permeable to the brain[17] suggest that protein-resistive molecular coatings on Aβ fibrils may also be generated in living patients.

This work demonstrates that ThT can inhibit $65 \pm 10\%$ of IgG-Aβ-fibril interactions, a result that is confirmed using two different Aβ-binding IgGs. DAA was shown to be slightly better than ThT by inhibiting 76 ± 10 % of these amyloid-protein interactions. A molecule that does not bind to Aβ fibrils—1-naphthol-4-sulfonate—had no inhibitory effect on the binding of anti-Aβ IgGs to Aβ fibrils. These results support the hypothesis that inhibition of the interaction between proteins and Aβ fibrils is due to the coating of Aβ fibrils with Aβ-binding molecules. The generation of protein-resistive surface coatings on amyloid fibrils with small molecules is a first step toward developing a new therapeutic strategy for the inhibition of harmful protein–amyloid interactions in neurodegenerative diseases.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.10.067.

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