Resveratrol Selectively Remodels Soluble Oligomers and Fibris of Amyloid Aβ into Off-pathway Conformers*§

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Misfolded proteins associated with diverse aggregation disorders assemble not only into a single toxic conformer but rather into a suite of aggregated conformers with unique biochemical properties and toxicities. To what extent small molecules can target and neutralize specific aggregated conformers is poorly understood. Therefore, we have investigated the capacity of resveratrol to recognize and remodel five conformers (monomers, soluble oligomers, non-toxic oligomers, fibrillar intermediates, and amyloid fibrils) of the Aβ1–42 peptide associated with Alzheimer disease. We find that resveratrol selectively remodels three of these conformers (soluble oligomers, fibrillar intermediates, and amyloid fibrils) into an alternative aggregated species that is non-toxic, high molecular weight, and unstructured. Surprisingly, resveratrol does not remodel non-toxic oligomers or accelerate Aβ monomer aggregation despite that both conformers possess random coil secondary structures indistinguishable from soluble oligomers and significantly different from their β-sheet rich, fibrillar counterparts. We expect that resveratrol and other small molecules with similar conformational specificity will aid in illuminating the conformational epitopes responsible for Aβ-mediated toxicity.

Despite the remarkable fidelity of protein folding in diverse cellular environments, defects do occur that are linked to an array of protein aggregation diseases. In many such disorders (e.g. Alzheimer (1–4), Parkinson (5, 6), Huntington (7–9), and Prion (10, 11) diseases) specific peptides of unrelated sequence aggregate into similar types of assemblies ranging from soluble, low molecular weight oligomers to insoluble, high molecular weight amyloid fibrils (1, 12).

A particularly intriguing aspect of protein misfolding is that a single polypeptide chain can adopt multiple aggregated conformations with unique biological activities (13). Such conformational diversity was first observed for the mammalian prion protein PrP (14–21). Different infectious prion conformations of PrP, known as strains or variants, encipher unique prion diseases through differences in their aggregate structure (14, 16, 19, 22–24). More recently, polymorphic aggregate structures have been formed in vitro and identified in vivo for many other proteins (25–39). However, the biological consequence of such conformational diversity and which conformers are most toxic remains poorly defined.

Aggregated Aβ conformers associated with Alzheimer disease also display such conformational diversity (30, 32, 33, 38, 40). The Aβ peptide self-assembles through multiple pathways in which several intermediates are transiently populated (41–46). These conformers, which range from dimers and soluble oligomers to fibrillar oligomers and protofibrils, are typically classified either by size or structure. Even though size is an important characteristic of different Aβ conformers, it is now clear that aggregates of the same size can have unique structures (44, 47). These recent findings have been illuminated primarily through the use of novel conformation-specific antibodies (47–51). Many of these antibodies display selective recognition of different aggregated conformers that form not only for Aβ but also for other disease-associated proteins (i.e. α-synuclein, huntingtin, and PrP) (47–50).

For example, one such antibody (A11) is capable of selectively recognizing prefibrillar intermediates larger than tetramers for many polypeptides (49). Importantly, this antibody does not recognize either monomers or fibrillar conformers (e.g. amyloid fibrils). More recently, another conformation-specific antibody (OC) has been isolated that recognizes soluble and insoluble fibrillar intermediates that form later in the aggregation process (44, 47, 52). In addition, the OC antibody also recognizes amyloid fibrils but not monomers or soluble prefibrillar oligomers. Importantly, both A11 and OC antibodies recognize many aggregated conformers that overlap in size on a Western blot (47), a finding that further emphasizes the importance of classifying such misfolded conformers in terms of structure.

Although many small molecules have been identified that antagonize the aggregation of Aβ and related polypeptides (for review, see Refs. 53–55 and references therein), little is known about their conformational specificity and whether they can target specific aggregated conformers. Reasons for this include the difficulty in preparing and detecting different aggregated conformers, the lack of suitable conformational controls to evaluate if small molecules are sequence- or conformation-specific, and the overreliance on amyloid-specific dyes (which can be misleading) to detect inhibition of protein aggregation.

Nevertheless, several insightful studies are emerging on the conformational specificity of small molecule antagonists of Aβ aggregation (40, 56–61). For example, Glabe and co-workers

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Thioflavin T (ThT) Assay—Aβ (25 μM) was diluted with ThT (44 μM) at a ratio of 1:19 by volume. The fluorescence was quantified in 384-well microtiter plates (Microfluor 1, Thermo Fisher Scientific) using a Tecan Safire plate reader (450/482 nm excitation/emission, 15-nm bandwidth). Seeding experiments were conducted using Aβ monomers (25 μM) and various aggregated Aβ conformers (5 wt % seed) without agitation.

AFM—Aβ samples (25 μM) were spotted on cut mica mounted on glass slides. The samples were adsorbed (30 min) and then washed with water and dried overnight. Images were taken using an Asylum Research MFP 3D AFM system with Olympus AC240TS cantilevers.

Cell Toxicity Assay—Rat adrenal medulla cells (PC12, ATCC) were cultured in modified Dulbecco’s modified Eagle’s medium (5% fetal bovine serum, 10% horse serum, and 1% penicillin-streptomycin). The cell suspension (110,000 cells/ml) was separated into aliquots (90 μl) in 96-well microtiter plates (CellBIND, Corning) and allowed to adhere for 24 h. Afterward, 10 μl of Aβ or control samples were added to the microtiter plate, and the cells were further incubated for 48 h at 37 °C. The medium was then removed, and the cells were washed with PBS. Next, Dulbecco’s modified Eagle’s medium (200 μl) and thiazolyl blue tetrazolium bromide (Sigma; 50 μl of 2.5 mg/ml) were added to each well for 3 h at 37 °C. Finally, these solutions were removed, 250 μl of DMSo was added, the plate was shaken (~75 rpm) for 20 min, and the absorbance was measured at 562 nm. The toxicity values were normalized to the PBS control without Aβ.

Gel Electrophoresis and Silver Staining—Aβ samples (25 μM) were diluted into sample buffer (Novex LDS, Invitrogen), sonicated, and analyzed using 10% Bis-Tris gels (Invitrogen) with MES running buffer (Invitrogen). Each gel was silver-stained using a SilverXpress kit (Invitrogen).

Antibody Dot Blot Analysis—Aβ conformers (25 μM) were spotted (1 μl) on nitrocellulose membranes (Hybond ECL, GE Healthcare). After the blots were dried, they were blocked overnight at 4 °C (10% nonfat dry milk in PBSt) and washed 3 times in PBST. The blots were then incubated with A11 (Invitrogen), OC, or 6E10 (Millipore) antibodies diluted 1:500 to 1:1000 (5% nonfat dry milk in PBST). The blots were washed three times (PBST) and then incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (goat-anti-rabbit for A11 and OC, goat-anti-mouse for 6E10). After washing, the blots were exposed to substrate (ECL Western blotting Substrate, Thermo Fisher) and developed.

Circular Dichroism—The secondary structures of Aβ conformers (25 μM in water; 300 μl) were evaluated using a Jasco 815 spectrometer (1-mm path length cuvette) at 25 °C. Each sample spectra is the average of at least 10 readings.

Results

Resveratrol Selectively Remodels Aβ-soluble Oligomers Relative to Non-toxic Oligomers and Monomers—We first established assembly conditions under which Aβ1–42 forms toxic

(40) recently performed a comprehensive study of how >40 small molecule compounds influence the aggregation of Aβ1–40 by adding various compounds to monomeric peptide and evaluating the resulting aggregation behavior. Their findings suggest that different small molecules can selectively inhibit formation of two unique aggregated conformers (soluble prefibrillar oligomers and amyloid fibrils). A limitation of this intriguing study is that it is difficult to determine whether a small molecule prevents the formation of a specific aggregated conformer or accelerates its conversion into another one (e.g. Ref. 56). Nevertheless, this and other related studies (57–59, 61–63) strongly suggest that small molecules can selectively target unique conformations of Aβ and related proteins.

We posit that analyzing the impact of small molecules on a panel of preformed Aβ conformational variants is the most direct approach for elucidating their conformational specificity. Therefore, we have formed four well characterized Aβ conformations (soluble oligomers, non-toxic oligomers, fibrillar intermediates, and amyloid fibrils) in addition to monomeric Aβ. For each of these conformations, we have evaluated the capacity of resveratrol, a small polyphenol of broad biological interest (64–67), to recognize and remodel them. Resveratrol (a well known constituent of red wine), like many other polyphenols, displays apparent anti-aggregation activity for Aβ (60, 68, 69), but its conformational specificity for different Aβ conformers is poorly understood. Herein, we report the discovery that resveratrol selectively remodels a subset of aggregated Aβ conformers with highly dissimilar biochemical properties into a common, off-pathway aggregated conformer that is non-toxic.

Experimental Procedures

Preparation of Aβ Conformers—Aβ1–42 (American Peptide) was dissolved in an aqueous 50% acetonitrile solution (1 mg/ml), separated into aliquots, dried under vacuum, lyophilized, and then stored at −20 °C. For preparing soluble prefibrillar oligomers and non-toxic oligomers under quiescent conditions and fibrillar intermediates under agitated conditions, aliquots of Aβ were first dissolved in 100% hexafluoroisopropanol (Fluka). The hexafluoroisopropanol was evaporated overnight at room temperature, and then the dried peptide was reconstituted in 50 mM NaOH for 30 min (1 mg/ml Aβ). Afterward, it was sonicated (30 s) and then diluted in PBS (25 μM Aβ). Next, the dissolved Aβ was subjected to centrifugation (22,000 × g for 30 min), and the pellet fraction (5% of starting volume) was discarded. The supernatant was sonicated (1 min) and then incubated at 25 °C for 0–6 days either with agitation (lateral mixing rate of 250 rpm for assembling fibrillar intermediates) or without it (for assembling soluble oligomers and non-toxic oligomers).

For preparing amyloid fibrils, aliquots of Aβ were dissolved in 50% hexafluoroisopropanol in water at a final concentration of 25 μM. The dissolved peptide was then sealed in a microcentrifuge tube with Parafilm, and a single pin hole was introduced to allow slow evaporation of hexafluoroisopropanol (25 °C) over 2 days with agitation (250 rpm). After 2 days, the remaining solution was diluted with water to achieve a final concentration of 25 μM Aβ and then mixed for an additional 5 days (25 °C, 250 rpm).
soluble oligomers recognized by the A11 antibody (49) in a reproducible and well defined manner. Based on other reports (40, 49) and our own experiments, we found that soluble, A11-positive oligomers formed after 1 day when assembled at 25 °C without agitation (Fig. 1A). Importantly, these oligomers remained stable for 2 days, after which time they mature into a conformer no longer recognized by the A11 antibody (Fig. 1A). We confirmed that these A11-positive conformers are SDS-soluble, ThT-negative, and toxic (supplemental Fig. S1).

Intriguingly, we found that soluble Aβ oligomers do not convert into fibrillar conformers under these conditions. Using a second conformation-specific antibody (OC) that selectively recognizes fibrillar intermediates and fibrils (47), we found that OC-positive conformers did not assemble either before or after formation of soluble prefibrillar oligomers (Fig. 1A). This suggests that soluble oligomers may mature into off-pathway aggregates. Indeed, we confirmed that Aβ conformers formed on days 4 and 5 were off-pathway conformers that possess similar morphologies to soluble oligomers, yet they were SDS-resistant, ThT-negative, and non-toxic (supplemental Fig. S1).

Moreover, using circular dichroism we found that freshly dissolved Aβ (day 0), A11-positive oligomers (day 1), and off-pathway aggregates (day 5) all possess random coil secondary structures (Fig. 1B). Thus, soluble and off-pathway oligomers display...
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![Diagram](https://example.com/diagram.png)

**FIGURE 2.** Dose dependence of resveratrol remodeling of soluble prefibrillar Aβ oligomers. Aβ-soluble oligomers (25 μM; formed after 1 day without agitation) were exposed to various concentrations of resveratrol (Res.) and control solutions for 4 h and monitored via SDS-PAGE (A), dot blots probed with conformation-specific (A11, soluble oligomers) and sequence-specific (6E10) antibodies (B), and cell toxicity analysis (2.5 μM, Aβ, n = 3) (C). Van, vanillin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

indistinguishable secondary structures and aggregate morphologies, yet they possess unique toxicities and are differentially recognized by the A11 antibody.

Given the subtle differences between soluble and off-pathway oligomers, we posited that resveratrol would either fail to remodel these conformers or modify them both in a similar manner. Therefore, we first performed SDS-PAGE analysis of the Aβ conformers that formed over 5 days before and after the addition of resveratrol and vanillin (a control phenolic compound) for 4 h at a near stoichiometric concentration (0.8:1 phenol:Aβ1–42; Fig. 1C). Strikingly, we found that resveratrol selectively remodels Aβ-soluble oligomers that form on days 1–3 (which are recognized by the A11 antibody) into high molecular weight aggregates. Moreover, resveratrol did not alter the aggregation behavior of freshly dissolved Aβ (day 0) or insoluble, off-pathway Aβ aggregates (day 5; Fig. 1C). We confirmed that the remodeled soluble oligomers are non-covalently associated by disassembling them with heat (100 °C for 10 min; Fig. 1C). We also confirmed that the resveratrol remodeling activity for A11-positive conformers is similar at 25 and 37 °C (supplemental Fig. S2) and is unaffected by the addition of non-ionic surfactant (0.1% Triton; supplemental Fig. S3). Finally, we confirmed that the A11-positive oligomer samples (days 1–3) contain little monomer, as when they were mixed with Aβ monomers, resveratrol failed to convert a significant fraction of the Aβ peptide into large aggregates (supplemental Fig. S4).

To confirm that the intriguing conformational specificity of resveratrol for remodeling soluble oligomers is not an artifact of the SDS-PAGE analysis, we analyzed the morphologies of the Aβ conformers using AFM. Indeed, we found that A11-positive oligomers formed on day 1 were remodeled into large aggregates after incubation with resveratrol (Fig. 1D). However, freshly dissolved Aβ (day 0) remained largely unaggregated after the addition of resveratrol (Fig. 1D). Strikingly, the sizes and morphologies of the off-pathway oligomers (day 5), which were visually indistinguishable from soluble Aβ oligomers (day 1), were unchanged after exposure to resveratrol (Fig. 1D).

Because small oligomers of Aβ are more toxic than their larger aggregated counterparts (i.e. amyloid fibrils), we assumed that the remodeled soluble oligomers would display low toxicity relative to untreated oligomers. Thus, we evaluated the relative toxicities of remodeled Aβ conformers when added to PC12 cell cultures (Fig. 1E). We first confirmed that resveratrol, vanillin, and the vehicle (1% DMSO) display little toxicity in the absence of Aβ (Fig. 1E). Notably, Aβ soluble oligomers formed on days 1–3 display enhanced toxicity relative to Aβ monomers and off-pathway oligomers (herein referred to as non-toxic oligomers) both before and after incubation with control solutions (1% DMSO and vanillin; Fig. 1E). However, resveratrol antagonizes this oligomer-induced toxicity and restores the cell viability to the level of the freshly dissolved peptide (day 0; Fig. 1E).

We confirmed that this protective effect of resveratrol is not mediated via an indirect mechanism by adding Aβ oligomers and resveratrol simultaneously to PC12 cell cultures; we observed toxicities indistinguishable from those obtained without resveratrol (supplemental Fig. S5). Thus, resveratrol remodels Aβ soluble oligomers, but not monomers or non-toxic oligomers, into large assemblies that are non-toxic.

Nevertheless, if the remodeling of Aβ oligomers into large aggregates is the key event by which resveratrol neutralizes their toxicity, then the resveratrol dose dependence for promoting off-pathway aggregation and antagonizing Aβ-mediated cell toxicity should be tightly linked. Thus, in Fig. 2 we have investigated the dose-dependent remodeling activity of resveratrol using biochemical and cell toxicity assays. First, using SDS-PAGE analysis, we find that concentrations of resveratrol ≥20 μM promote high molecular weight aggregation, whereas these same concentrations of vanillin or lower ones of resveratrol do not (Fig. 2A). Second, we evaluated the capacity of resveratrol to eliminate the conformational epitope recognized by the A11 antibody in soluble oligomers (Fig. 2B). For Aβ-soluble
oligomers formed on days 1–3, resveratrol eliminates this conformational epitope at concentrations ≥20 μM, in agreement with the SDS-PAGE analysis (Fig. 2A). Moreover, we find that resveratrol eliminates this conformational epitope without generating OC-positive fibrillar conformers, whereas a control molecule (methylene blue) does promote conversion of Aβ1–42-positive soluble oligomers to OC-positive fibrillar conformers (supplemental Fig. S6) (56). Finally, resveratrol neutralizes the toxicity of soluble oligomers relative to Aβ monomers also at ≥20 μM resveratrol (Fig. 2C). This strong concordance between the resveratrol dose dependence for modulating these three dissimilar properties strongly argues that the remodeling of Aβ-soluble oligomers is the key molecular event that leads to elimination of their toxic properties.

Resveratrol Remodels Aβ Fibrillar Conformers in a Manner Similar to Soluble Prefibrillar Oligomers—The fact that resveratrol remodeled soluble prefibrillar oligomers, but not insoluble non-toxic oligomers, led us to investigate whether it would remodel other insoluble Aβ conformers (e.g. fibrillar intermediates and amyloid fibrils). Thus, we established assembly conditions that would favor formation of fibrillar intermediates and fibrils. We found that mild agitation of Aβ1–42 at 25 °C led to reliable and reproducible formation of structures that were recognized by the OC antibody (Fig. 3A). Importantly, soluble oligomers recognized by the A11 antibody formed after 1 day and persisted for 2 days, after which OC-positive conformers formed (Fig. 3A). These OC-positive structures persisted for at least 10 days (longer times were not evaluated). We characterized this transition from SDS-soluble, unstructured conformers (days 1–2) to SDS-insoluble, β-sheet-rich ones (days 3–5) using a battery of biochemical and toxicity assays (Fig. 3B and supplemental Fig. S7). Indeed, Aβ conformers formed on days 3–5 are SDS-resistant, ThT-positive aggregates that possess β-sheet secondary structures akin to amyloid fibrils (Fig. 3B and supplemental Fig. S7). Moreover, the SDS-resistant conformers formed on days 3–5 sediment at high speed (100,000 × g for 1 h; supplemental Fig. S7), confirming that they are not soluble fibrillar intermediates (47, 52). Finally, we used AFM to confirm that these OC-positive conformers were fibrillar intermediates that did not mature into amyloid fibrils even after 20 days (supplemental Fig. S7; longer times were not evaluated).

To evaluate the remodeling activity of resveratrol for β-sheet rich fibrillar intermediates, we first conducted SDS-PAGE analysis of Aβ conformers that form over 5 days before and after incubation with resveratrol and control solutions (Fig. 3C). Surprisingly, resveratrol remodeled fibrillar intermediates (days 3 and 5) into large, SDS-resistant conformers that were indistinguishable from remodeled soluble oligomers (day 1; Fig. 3C). We also confirmed these results using AFM imaging and observed that resveratrol-remodeled fibrillar intermediates (days 3 and 5; Fig. 3D) were large aggregates similar to resveratrol-remodeled soluble oligomers (Fig. 1D). Finally, we confirmed that resveratrol reverses the toxicity of these fibrillar conformers (days 3–5; Fig. 3E) in the same manner it does for soluble oligomers (days 1 and 2; Fig. 3E). Thus, despite that soluble prefibrillar oligomers and fibrillar intermediates possess highly dissimilar biochemical properties, resveratrol remodeled them both into large, SDS-resistant aggregates.

Given the similarity in secondary structures of fibrillar intermediates and amyloid fibrils, we assumed that resveratrol would remodel them in the similar manner. To test this hypothesis, we established an alternative assembly method whereby the Aβ peptide was mildly agitated in the absence of salt (33). We found that A11-positive soluble oligomers formed on day 2 and persisted until day 4 (Fig. 4A). Moreover, OC-positive conformers formed on day 3 and persisted for at least 10 days (Fig. 4A; longer times were not evaluated). We confirmed by AFM that amyloid fibrils formed after 7 days that were indistinguishable from seeded fibrils formed in PBS (supplemental Fig. 8).

As expected, we found that resveratrol remodeled Aβ fibrils (after resuspension in PBS) in a manner similar to fibrillar intermediates (Fig. 4B and supplemental Fig. S9). In addition, A11-positive soluble oligomers did not form during the remodeling process (supplemental Fig. S10). Resveratrol-remodeled fibrils were large aggregates, as confirmed by AFM (Fig. 4B) and SDS-PAGE (supplemental Fig. S9), that were non-toxic relative to Aβ monomers (supplemental Fig. S9).

Importantly, we also characterized the structures of the resveratrol-treated amyloid fibrils as well as resveratrol-treated soluble prefibrillar oligomers and fibrillar intermediates. Circular dichroism analysis revealed that each Aβ conformer (25 μM) treated with resveratrol (20 μM for 4 h) was largely unstructured (supplemental Fig. S11). Thus, resveratrol remodeled a subset of Aβ conformers that possess random coil (soluble oligomers but not monomers or non-toxic oligomers) and β-sheet (fibrillar intermediates and fibrils) structures into SDS-resistant, unstructured conformers.

Aβ Fibrillar Intermediates and Amyloid Fibrils Possess Unique Conformations—Fibrillar intermediates and amyloid fibrils are similar in many ways as they both are insoluble, β-sheet rich and less toxic than soluble oligomers (44, 47, 52). In fact, the only indication from our results that these conformers were unique was AFM measurement of their sizes. Thus, we sought to determine whether fibrillar intermediates were simply small fibrils or if they possess different structures. Because fibrils are well known to seed monomers into the fibril conformation (70), we first evaluated if fibrillar intermediates lack such seeding activity (Fig. 5). Indeed, despite that fibrillar intermediates display ThT fluorescence, they are incapable of catalyzing the conversion of monomeric Aβ into ThT-positive conformers (Fig. 5A) or do so at a much slower rate, consistent with previous findings for other fibrillar intermediates (30, 52).

In contrast, Aβ fibrils are active for catalyzing Aβ monomers into ThT-positive conformers (Fig. 5B), as expected. Importantly, fibrils retain this seeding activity even in the presence of resveratrol when the amyloids were not preincubated with resveratrol before addition to Aβ monomers (Fig. 5B) (the residual resveratrol and fibril concentrations after dilution were 1 and 1.25 μM, respectively, relative to the Aβ monomer concentration of 25 μM). This finding confirms that resveratrol neutralizes the seeding activity of fibrils by remodeling their conformation instead of simply disrupting the interaction between fibrils and monomers. Finally, that fact that resveratrol-treated fibrils do not seed (Fig. 5B) provides further evidence that the remodeled Aβ conformers are not amyloid.
These seeding results provide strong evidence that the structures of fibrillar intermediates and fibrils are unique. Thus, we posited that the concentration of resveratrol required to remodel these conformers into large aggregates would also differ. To test this hypothesis, we evaluated the resveratrol dose dependence for remodeling Aβ fibrillar intermediates and fibrils into large, non-toxic conformers. We observed excellent concordance between four independent assays (SDS resistance, OC antibody dot blots, ThT fluorescence, and cell toxicity) that revealed concentrations of resveratrol ≥2 μM transformed fibrillar intermediates into OC-negative, SDS-resistant, ThT-negative, non-toxic conformers (Fig. 6). In contrast, these same four assays revealed that ≥20 μM resveratrol is required to transform Aβ fibrils into such non-toxic assemblies (Fig. 7). The fact that fibrils are more resistant to resveratrol remodeling than fibrillar intermediates confirms that the two fibrillar conformers possess unique structures.

**DISCUSSION**

A key finding of our work is that a subset of Aβ conformers (soluble prefibrillar oligomers, fibrillar intermediates, and

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**FIGURE 3. Resveratrol remodeling activity for Aβ fibrillar intermediates.** Aβ (25 μM) conformers were assembled via mild agitation (250 rpm) for 5 days (A–E) and then incubated with resveratrol (Res., 20 μM), vanillin (Van., 20 μM), or the vehicle (1% DMSO) for 4 h (C–E). A dot blot analysis of Aβ conformers is shown. B, circular dichroism spectra of Aβ conformers is shown. C, image of silver-stained, SDS-PAGE gel. D, AFM images of Aβ conformers before and after incubation with resveratrol. The scale bar is 1 μm. E, cell toxicity analysis of Aβ conformers (2.5 μM, 0.8:1 resveratrol:Aβ molar ratio, n = 3). MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
fibrils) elicit resveratrol to dramatically remodel their structures, whereas non-toxic oligomers and monomers do not. This is intriguing because soluble Aβ oligomers possess secondary structures similar to monomers and non-toxic oligomers and significantly different from their β-sheet rich, fibrillar counterparts. Moreover, resveratrol remodels soluble oligomers and both fibrillar conformers into a common, off-pathway aggregated form that possesses a combination of biochemical properties not found in the original conformers. Finally, resveratrol remodels soluble oligomers and fibrils with identical dose dependence despite their dissimilar biochemical properties.

We speculate that soluble prefibrillar oligomers and fibrillar conformers (fibrillar intermediates and fibrils) possess common structural features that are absent in non-toxic oligomers and monomers. Importantly, fibrils possess in-register, β-sheet structures in which side chains in the amyloid core self-stack (i.e. identical amino acids from different monomers stack on themselves) (71–76). Thus, one possibility is that aromatic residues in Aβ (e.g. Phe-4, Tyr-10, Phe-19, Phe-20) stack in both soluble prefibrillar oligomers and fibrils even though soluble oligomers are devoid of β-sheets, and this aromatic stacking may elicit resveratrol remodeling activity. In contrast, non-toxic oligomers that are insoluble and unstructured may lack such stacking interactions, which may explain why they (as well as Aβ monomers) are not remodeled by resveratrol. This hypothesis is also consistent with previous suggestions that polyphenols interact with aromatic side chains (77–79). Nevertheless, this structural hypothesis awaits experimental evaluation.

It is also possible that the conformational specificity of resveratrol is not due to structural similarities between soluble oligomers and fibrillar conformers of Aβ. Instead, resveratrol may promote conversion of soluble prefibrillar oligomers into either fibrillar intermediates or fibrils (or vice versa), and then remodel a common Aβ conformation into large, unstructured aggregates. This hypothesis is plausible as another small molecule (methylene blue) promotes conversion of soluble oligomers into amyloids (56). Despite that we do not observe resveratrol-mediated conversion between on-pathway Aβ conformers (e.g. soluble oligomers converted into fibrillar intermediates) before remodeling into off-pathway structures (supplemental Figs. S6 and S10), we are unable to eliminate this possibly because the kinetics of these processes may be faster than we can monitor.

Another important finding of our work is that A11-positive soluble oligomers can mature into at least two types of aggregated conformers (Fig. 8). Under quiescent conditions, soluble prefibrillar oligomers mature into aggregates that are SDS-resistant, morphologically indistinguishable from soluble oligomers, and negative for several conformation-specific probes (A11 and OC antibodies, ThT). In contrast, under agitated conditions, A11-positive oligomers mature into fibrillar intermediates that are SDS-resistant (as are non-toxic Aβ oligomers), yet they are more compact and positive for multiple conformation-specific probes (ThT and the OC antibody). The fact that soluble oligomers can sample multiple aggregation pathways is important given the conflicting reports of whether soluble oligomers are on-pathway structures for assembly of β-sheet rich conformers for Aβ and other polypeptides (42, 46, 80–90). Our results are consistent with both hypotheses depending on the conditions of Aβ assembly (Fig. 8). For agitated Aβ assembly, our results suggest that soluble oligomers are on-pathway structures as resveratrol remodels them in a manner indistinguishable from amyloid fibrils. In contrast, for quiescent Aβ assembly, soluble oligomers mature into off-pathway structures. Thus, A11-positive soluble oligomers are not committed to a single nucleation pathway and can mature into multiple aggregated conformers in a manner reminiscent of prion strains (24, 28).

That resveratrol promotes high molecular weight aggregation in-
stead of disassembling soluble and insoluble Aβ conformers into low molecular weight species is contrary to conventional wisdom. However, this observation is supported by a few disparate reports for resveratrol and other polyphenols (57, 58, 60, 63, 91, 92), as well as the broader literature demonstrating that many small molecules and peptides redirect the aggregation cascade instead of inhibiting it completely (40, 56, 62, 93–96). For example, the aggregation of monomeric Aβ and α-synuclein was studied in the presence of EGCG. This polyphenol was found to induce both polypeptides to aggregate into high molecular weight, SDS-resistant conformers that are non-toxic, ThT-negative, and lack the A11 conformational epitope (58). The authors conclude that EGCG recognizes unstructured conformers such as monomers of these natively unstructured polypeptides and promotes their off-pathway aggregation via alternative hydrogen bonding facilitated by the hydroxyl-rich polyphenol. Intriguingly, we find that resveratrol does not promote off-pathway aggregation for unstructured Aβ peptides, but instead, it selectively remodels on-pathway soluble and insoluble Aβ conformers (Fig. 8). More recently, EGCG was also shown to convert mature Aβ and α-synuclein fibrils into off-pathway aggregates (63).

Another report of the impact of polyphenols on the aggregation of a different protein (Tau) appears to support our findings (57). Two different polyphenols (trihydroxybenzophenone and exifone) promote high molecular weight aggregation of Tau fibrils and an uncharacterized form of Tau oligomers but not Tau monomers. Although no other conformational controls were performed (e.g. non-toxic oligomers) and it is unknown whether the high molecular weight Tau aggregates were remodeled into an unstructured conformation, these results suggest that polyphenols other than resveratrol may target specific aggregated conformers and convert...
them into large, non-toxic aggregates as well. Moreover, these results also suggest that our analysis of the conformational specificity of resveratrol for Aβ-aggregated conformers may be relevant to other misfolded proteins.

Importantly, several reports are emerging that cells in diverse organisms ranging from worms to mice mitigate the proteotoxicity of small protein aggregates by assembling them into larger, less toxic aggregates (97, 98). For example, reducing insulin growth factor signaling leads to hyperaggregation of Aβ in worms (98) and mice (97) and prevention of Alzheimer-like symptoms in mice (97). These findings suggest that promoting hyperaggregation is a compelling therapeutic strategy, and molecules with conformational specificity akin to resveratrol may be attractive therapeutic candidates.

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