Efficient reversal of Alzheimer’s disease fibril formation and elimination of neurotoxicity by a small molecule

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The Aβ1-42 peptide that is overproduced in Alzheimer’s disease (AD) from a large precursor protein has a normal amino acid sequence but, when liberated, misfolds at neutral pH to form “protofibrils” and fibrils that are rich in β-sheets. We find that these protofibrils or fibrils are toxic to certain neuronal cells that carry Ca-permeant α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Disrupting the structure of the Aβ1-42 fibrils and protofibrils might lead to the discovery of molecules that would be very useful in the treatment of AD. A high-throughput screen of a library of >3,000 small molecules with known “biological activity” was set up to find compounds that efficiently decrease the β-sheet content of aggregating Aβ1-42. Lead compounds were characterized by using thioflavin T (ThT) as a β-sheet assay. The most effective of six compounds found was 4,5-dianilinophthalimide (DAPH) under the following conditions: DAPH at low micromolar concentrations abolishes or greatly reduces previously existing fully formed Aβ1-42 fibrils, producing instead amorphous materials without fibrils but apparently containing some protofibrils and smaller forms. Coincubation of the Aβ1-42 peptide with DAPH produces either amorphous materials or empty fields. Coincubation of DAPH and Aβ1-42 greatly reduces the β-sheet content, as measured with ThT fluorescence, and produces a novel fluorescent complex with ThT. When the Aβ1-42 peptide was coincubated with DAPH at very low micromolar concentrations, the neuronal toxicity mentioned above (Ca2+ influx) was eliminated. Clearly, DAPH is a promising candidate for AD therapy.

Ca ions | amyloid peptide | aggregation | toxicity | aggregation

The pathology of Alzheimer’s disease (AD) was first defined by Alois Alzheimer in 1907. He saw “senile plaques” and also “tangles” in stained sections from the postmortem brain of his first diagnosed patient. It turned out (1, 2) that the plaques were composed of fibrils formed by the aggregation of short peptides, which were mostly 40 and 42 aa long. These peptides had a perfectly normal amino acid sequence (common to both peptides) and represented a part of a much larger common transmembrane “amyloid precursor protein” (APP) of unknown function. For various reasons, some of which are genetic and some of which are unknown, these peptides are greatly overproduced in AD. The two overproduced peptides, Aβ1-40 and Aβ1-42, when liberated from their parent APP, refold (“misfold”) and aggregate spontaneously to a neurotoxic, β-sheet-containing fibrillar form. This structure attacks particular neurons in the CNS, causing them to become dysfunctional and, eventually, die in large numbers. This neurotoxicity (for Aβ1-40) was first described by Yankner et al. (3). Shortly afterward, Lorenzo and Yankner (4) described an organic compound, Congo red, that inhibits Aβ fibril formation and neurotoxicity. The involved peptides, Aβ1-40 and Aβ1-42, will hereafter be referred to as Aβ40 and Aβ42, respectively.

The plaques are extracellular. One result of the consequent dysfunction is thought to be the hyperphosphorylation of the microtubule-associated tau protein (5), which then aggregates to form the tangles that fill the cell body and neurites, making them dysfunctional.

A currently popular view is that the misfolded (6–8) Aβ peptides produce a novel conformation with new and toxic properties. The notion that the cytotoxicity of Aβ peptides involves the disturbance of cytosolic Ca2+ ion homeostasis has been stated (9, 10). It is thought that the misfolded Aβ peptide, especially Aβ42, is an oligomer of that peptide (11–13). Our proposed mechanism (6) (Fig. 1) involves a deleterious influx of external Ca2+ ions. The consequences are severe: dysfunction and cell death. Our molecular mechanism for immediate Ca2+ influx involves the activation of Ca-permeant α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the neurons that have these particular receptors (i.e., it is not due to release of Ca2+ ions from internal storage). This influx is inhibited completely by 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[1]quinoxaline (NBQX), a specific AMPA-receptor antagonist (see Results). Earlier experiments with 6-cyano-7-nitroquinoxaline-2,3-dione (CNOX), another AMPA antagonist, confirm this observation. These observations prove the direct and crucial involvement of Ca2+-permeant AMPA receptors in this mechanism. This mechanism requires the β-sheet-containing fibrils and protofibrils formed by Aβ42; it is the molecular basis of our proposed AD therapy.

In our experience, Aβ42 must be preincubated for at least 24 h before the preparation will promote Ca2+ ion influx, as described in Results. By this time, the early-stage oligomers, also called “donuts,” have turned into “protofibrils” and fibrils. This transition into protofibrils and fibrils is expected to occur in vitro. Donuts, the early oligomeric forms, do not contain β-sheet structures, as do protofibrils and fibrils (13). Such a scheme (Fig. 1) could explain the neurotoxicity (deleterious Ca2+ ion influx) as well as the singular distribution of cell loss. Only certain neurons have the particular receptors that are sensitive to Aβ42 fibrils and that conduct Ca2+ ions. It is known that the distribution of, for example, Ca-permeant AMPA-receptors is quite restricted. Our proposed therapy is based on this model.

A high-throughput screen of a library of >3,000 small molecules with known “biological activity” was set up to find compounds that efficiently decrease the β-sheet content of aggregating Aβ42. Lead compounds were characterized by using thioflavin T; E

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Abbreviations: AD, Alzheimer’s disease; Aβ, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DAPH, 4,5-dianilinophthalimide; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[1]quinoxaline; CNOX, 6-cyano-7-nitroquinoxaline-2,3-dione; ThT, thioflavin T; EM, electron microscopy/microscope.

See accompanying Biography on page 14323.

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Fig. 1. Schematic representation of neurotoxicity. Misfolding of peptide Aβ42 produces a neurotoxic molecule.

Materials and Methods

Peptide and Compound Preparation. Stock solutions of Aβ42 (special TFA preparation, catalog no. 03-112, BioSource International, Camarillo, CA) were prepared in autoclaved water with the addition of 1 M NaOH to pH 10–11.

Aβ42_total is unfraccionated synthetic Aβ42 that was dissolved in water at pH 8–9 by using NH₃ or at pH 10–11 by using dilute NaOH and stored at −40°C.

Aβ42_GSK is seedless Aβ42 made according to the protocols of Fezoui et al. (15), except that we needed to filter the dissolved peptide through a 30,000-kDa spin filter instead of the 10,000-kDa filter that they used. However, they had described only the procedure for the shorter peptide Aβ40. The method, as used by us, involves dissolving the total Aβ42 peptide in water at pH 10.5 by using NaOH. There was considerable loss of peptide; the concentration of peptide in the filtrate was determined by the addition of 1 M NaOH to pH 10.5 and Eₚ/×₀₄₅ nm; Eₚ/×₄₄₄ nm and Eₚ/×₅₁₀ nm or a FLUOstar Optima plate-reader (BMG Lab Technologies, Durham, NC) at Eₚ/×/₄₄₅ nm and Eₚ/×₄₈₀ nm. The ThT fluorescence spectrum was measured in an f4500 spectrophotometer (Hitachi, Tokyo) at Eₚ/×/₁₄₃₅ nm and Eₚ/×₄₅₀–₅₅₀ nm.

DiBAC₄(3) Fluorescence. To measure membrane potential of cultured neuronal cells in the original high-throughput screen, the voltage-sensitive dye DiBAC₄(3) was added to solutions containing Aβ42_Total in 96-well plates and measured in a Fluoroskan II at Eₚ/×/₄₄₄ nm and Eₚ/×₅₁₀ nm or FLUOstar Optima plate-reader (BMG Lab Technologies) at Eₚ/×/₄₈₀ nm and Eₚ/×₅₀₅ nm. It turned out later that compounds that lowered the DiBAC₄(3) fluorescence did so by reducing the fluorescent signal for β-sheet content of aggregated Aβ42_Total; the cells were not involved.

Electron Microscopy (EM). Negative-staining EM was used to visualize the kinetics and morphology of Aβ42 fibrillation over time, with and without the presence of inhibitors. Samples of 10 μM seedless Aβ42 (Aβ42_GSK) were incubated for the indicated amount of time at 37°C and vigorously vortex mixed immediately before and after incubation. Very small volumes, typically 5 μl, of each sample were absorbed for 2–4 min onto glow-discharged, carbon-coated, Formvar-filmed 400 mesh copper grids and gently wicked away with filter paper. A total of 5 μl of freshly filtered 2% uranyl acetate staining solution was then absorbed for 2 min onto the grid and gently wicked off. Grids were allowed to dry in a light-protected environment overnight before being viewed in a 1200 EXII EM (JEOL) operated at 80 kV. Images were captured on EM film, and positives were printed.

EM Image Analysis. Images were scanned for fiber measurement in NIH IMAGE 1.62. Fiber measurements were calibrated by comparison with the pixel lengths of T4 phage tails, which are known to be 100 nm long (16). Measurements were analyzed, and width-distribution histograms were produced by using EXCEL 98 (Microsoft).

Results

Aggregation Kinetics of the Aβ42 Peptide. The longer of the two AD peptides, Aβ42, is thought to be involved more directly in the neurotoxicity underlying the disease. Certainly, the Aβ42 peptide aggregates more readily to form fibrils than Aβ40 (17). However, it is not clear whether that property correlates with the ability of the peptide to form toxic oligomers. Our experiments used two different preparations of the synthetic AD Aβ42 peptide, which we called Aβ42_Total and Aβ42_GSK (see Materials and Methods). The synthetic Aβ42 was dissolved in water at a pH
of ~8 and then stored at ~20°C until diluted into Tyrode’s solution/2 mM Ca (pH 7.4). We refer to this preparation as Aβ42Total. For our experiments, it was preincubated for 24 or 48 h at 37°C without stirring. The seedless Aβ4230K peptide, prepared as described in Materials and Methods, was also preincubated before experiments at pH 7.4.

Fig. 2A and B shows the time course of aggregation of Aβ4230K (10 µM in Tyrode’s solution/2 mM Ca, pH 7.4) by using negative-staining EM. Under these conditions, several stages can be distinguished. At 0 h, many very small objects are seen, but characteristically, we also see many small circular structures of ~5 nm in diameter (so-called donuts). They are made up of five globular subunits, as reported in ref. 8. At 6 h, we see only flexible fibrous structures, consisting of globular structures similar to those seen at 0 h (“beads-on-a-string”). These beads-on-a-string are referred to as protofibrils. Next, at 16 h, these globular units form the beginning of a long smooth fibril, still somewhat flexible. They appear to be growing in one direction, as judged by the globular subunits seen at one end of nascent fibrils. Finally, at 24 and 48 h, the fibrils are smooth, very long, and straight, but intertwined to form “nests.” These images emphasize the role of small globular subunits forming donuts at 0 h and forming protofibrils at slightly later times. During this time, there is very little β-sheet structure detectable by ThT; it is the lag phase if one follows β-sheet content (18). However, the description does not allow us to deduce the possible structure of the neurotoxic oligomers.

A High-Throughput Screen Yielded Compounds, for Example, DAPH, That Destabilize β-Amyloid Fibrils (see Fig. 10). A library of 3,780 compounds with known biological activity was kindly provided by Brent Stockwell (Columbia University, New York), as well as robotic equipment. We were interested in the possible effect of aggregated Aβ42 on the membrane potential of neuronal cells because we knew that aggregated Aβ42 causes large Ca2+ influx into certain neuronal cells. We used PC12 cells and exposed them to preincubated Aβ42Total (10 µM, 48 h at 37°C, pH 7.4) in the presence of the slow polarization-sensitive dye DiBAC4 (3), which fluoresces strongly when membranes are depolarized. It is known that this (anionic) dye can enter depolarized cell membranes and forms reversible fluorescent complexes with intracellular proteins. The PC12 cells, when exposed to preincubated Aβ42Total, fluoresced strongly at 530 nm (Ex = 485 nm).

*Emission spectra of fluorescent complexes of aggregated Aβ42 are as follows: DiBAC4 (3), Ex = 485 nm and Em = 530 nm; and ThT, Ex = 435 nm and Em = 485 nm.
We looked for, and occasionally found, a strong decrease in fluorescence in the presence of certain compounds. The compound DAPH gave the strongest decrease, but we found five other compounds with robust decreases (data not shown). Further experiments showed that the observed fluorescence had nothing to do with the cells but was caused by the formation of a fluorescent complex between the aggregated peptide and the dye (21). We found that the fluorescent behavior of DiBAC₄ (3) and its decrease by certain compounds was seen also when ThT was used. ThT is a well known reagent used to measure a fluorescent complex between the aggregated peptide and the fibril formation of Aβ. The IC₅₀ value for the inhibition of β-sheet content. Reversal of the fibrils, as detected by the ThT assay, takes only very few minutes and depends on the concentration of DAPH (Fig. 4).

DAPH reverses the fibrils formed by Aβ42_total or Aβ42_total+. A very striking effect of DAPH is the ability to reverse completely the fibril formation of Aβ42_total (Fig. 3). The peptide was allowed to aggregate under the usual conditions at pH 7.4 for 24 h and form fibrils. The incubation was either continued for another 24 h in the presence of the vehicle (1% DMSO) or in the presence of equimolar DAPH that had been dissolved in DMSO. The usual fibrils are formed in the control and are seen with negative staining in the EM, albeit not as crisply as without DMSO.

DAPH blocks fibril formation by Aβ42_total+. Coincubation of Aβ42_total+ with equimolar DAPH (10:10 μM) resulted in almost complete elimination of the usual fibrils, as seen by EM (Fig. 5). The vehicle DMSO (1%), the solvent for the water-insoluble DAPH, was present in the test and control experiments. EM images (Fig. 5) reveal a nearly complete elimination of higher-order structure upon 24 h incubation with DAPH; very rarely, small “protofibrillar” and amorphous structures appear against a backdrop of tiny, 2- to 3-nm objects, which are not observed in control samples without DAPH.

DAPH profoundly alters the β-sheet conformation of aggregated Aβ42_total+. When Aβ42_total+ (10 μM) is preincubated with equimolar DAPH for 48 h at 37°C pH 7.4, the usual sharp maximum emission peak of the ThT complex is greatly reduced, indicating a profound structural change in β-sheet conformation (Fig. 6). In addition, a second and new emission peak appears at ~510–530 nm; we do not know what the structure of the new ThT complex is. Aβ42_total behaves similarly.

The IC₅₀ value for the inhibition of β-sheet content of aggregated Aβ42_total by DAPH is ~15 μM (Fig. 7) when ThT is the fluorescent reagent. However, we have reported (19) that the dye DiBAC₄ (3) also forms a highly fluorescent complex with aggregated (preincubated) Aβ42_total. In fact, this was the dye used by us to conduct the high-throughput screen that led to the characterization of DAPH as an effective inhibitor of β-sheet conformation. We tested the inhibition of β-sheet formation by using DiBAC₄ (3, 23), and we found a much lower IC₅₀ value (4.5 μM) than we found with ThT (Fig. 7). Apparently, less...
DAPH is required to displace Aβ from DiBAC₄(3) than from ThT because Aβ binds less avidly to DiBAC₄(3).

Treatment of Neuronal Cells with Preincubated Aβ₄₂ Causes Ca Influx via Ca-Permeant AMPA Receptors. Preincubated Aβ₄₂Total was applied to CATH.a neuronal cells that had been loaded with fura-2 (see Materials and Methods). We measured cytosolic Ca²⁺ concentration as a function of time and saw an immediate and dramatic influx of Ca (Fig. 8A). This Ca influx decayed spontaneously and slowly, but not to zero. In the presence of CNQX, an AMPA receptor antagonist with some specificity toward NMDA receptors, a peak of Ca influx was prevented. When NBQX was present (Fig. 8B), there was also no new Ca spike, but the level of cytosolic Ca dropped abruptly to a value close to and somewhat below the value found in the Tyrode’s solution control. This inhibition by NBQX was at least partially reversible. NBQX is a highly specific antagonist for AMPA receptors. Its effective shut down of Ca influx argues very strongly for the involvement of a Ca-permeant AMPA receptor, activated by preincubated Aβ₄₂, as one important mechanism for the disruption of neuronal Ca homeostasis in AD.

Ca Influx Depends on Preincubation and on the Concentration of Aβ₄₂. The Aβ₄₂ peptide must be preincubated at pH 7.4 and 37 °C for 24–48 h to modulate the immediate Ca influx into these neuronal cells maximally. This Ca influx is true for both Aβ₄₂Total (Fig. 9A) and Aβ₄₂₃₀k (data not shown). Apparently, unincubated Aβ₄₂Total already contains some Aβ₄₂ oligomer/protofibril that is able to activate the AMPA receptors to a limited extent; further incubation increases the amount of this component or, perhaps, allows another, more active form to appear.

The rate of immediate Ca²⁺ influx (i.e., the peak height in Fig. 9A) depends on the concentration of Aβ₄₂. The Aβ₄₂ peptide must be preincubated at pH 7.4 and 37 °C for 24–48 h to modulate the immediate Ca influx into these neuronal cells maximally. This Ca influx is true for both Aβ₄₂Total (Fig. 9A) and Aβ₄₂₃₀k (data not shown). Apparently, unincubated Aβ₄₂Total already contains some Aβ₄₂ oligomer/protofibril that is able to activate the AMPA receptors to a limited extent; further incubation increases the amount of this component or, perhaps, allows another, more active form to appear.

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Inhibition of Ca Influx by DAPH. The immediate Ca\(^{2+}\) ion influx caused by preincubated A\(\beta_42\)\(_{\text{Total}}\) (10 \(\mu\)M) is shown clearly in Fig. 10A. Dramatically, the Ca\(^{2+}\) ion influx is abolished by the presence of DAPH, also at 10 \(\mu\)M, during the preincubation. This effect is at least partly reversible when the cells are next exposed to preincubated A\(\beta_42\)\(_{\text{Total}}\) alone. Similar inhibition is seen at 10, 5, 2.5, and 1 \(\mu\)M DAPH, but it is greatly reduced at 0.5 and 0.25 \(\mu\)M (Fig. 10B). The suggested IC\(_{50}\) value for this part of the curve is \(\approx 0.7 \mu\)M, which is much less than the IC\(_{50}\) value for the effect of DAPH on the \(\beta\)-sheet content of aggregated A\(\beta_42\)\(_{\text{Total}}\) (\(\sim 15 \mu\)M ThT). This value suggests that the neurotoxic “oligomer” of A\(\beta_42\)\(_{\text{Total}}\) is much more sensitive to DAPH than the \(\beta\)-sheet-containing mature fibrils seen in the EM.

At higher DAPH concentrations (20 or 30 \(\mu\)M), the cytosolic Ca\(^{2+}\) concentrations in treated CATH.a cells dips well below the control values seen in buffer alone (see Discussion). This observation is not due to the increased concentrations of DMSO in these experiments, because the net Ca\(^{2+}\) concentrations are recorded in Fig. 10. We have no explanation for this effect.

Discussion

We have reported (7) the development of “decoy peptides” that complex with the aggregated misfolded A\(\beta_42\) peptide and, thereby, prevent their toxic interaction with neuronal cells. The decoy peptides remain promising candidates for AD therapy\(^7\) and are ready for testing in animals.

As we pursue an approach to AD therapy that is designed to inhibit the mechanism of neuronal toxicity described above, we are aware that others are pursuing different paths to therapy (see ref. 14 for review). These approaches are very welcome but are described as being only partially effective in correcting cognitive deficits in mice or humans. Clearly, there is a need for an additional and different therapeutic approach, such as the decoy peptides mentioned above and the approach described here. Ultimately, the AD patient will benefit from a combination of different drugs.

Our high-throughput screen discovered a small molecule that possessed the potential to eliminate the neurotoxic characteristic of the disease. Whereas other research groups have concentrated their efforts on developing an AD therapy that prevents or at least reduces the production of the neurotoxic A\(\beta\) peptides A\(\beta_40\) and A\(\beta_42\), we have chosen to develop small molecules that block the toxic activities of these misfolded peptides.

Our hypothesis states (Fig. 1) that, in the first instance, the misfolded peptide aggregates spontaneously, eventually forming fibrils. One or several stages in this process possess new properties that allow them to interact with particular neurons, which then become dysfunctional and eventually die. Our experiments indicate that the first event in this toxicity is the activation by aggregated A\(\beta_42\) peptide of Ca-permeant AMPA receptors on particular sets of neurons (Fig. 1). We hypothesize that those neurons that possess these particular receptors will be affected, perhaps explaining the very particular distribution of cell-type specificity in early AD. The dysfunction and death of such neurons causes the disease.

Our aim is to study in vitro the molecular mechanism of this interaction, especially in the first few seconds, and then to find means of preventing this interaction. We believe that we have found such a molecule, which has become a candidate for AD therapy.

We find that during incubation, an increase in \(\beta\)-sheet content of the A\(\beta_42\) peptide parallels the increase in neurotoxicity, as expressed in Ca\(^{2+}\) ion influx (Fig. 9A). Here, we report on a class of small molecules discovered in a robotic high-throughput screen that searched for compounds that are effective in altering the \(\beta\)-sheet conformation that is typical of aggregated misfolded A\(\beta_42\). We had observed that preincubation of the A\(\beta_42\) peptide was necessary before neurotoxicity (Ca\(^{2+}\) influx) was seen (Fig. 9A) and that this effective change was paralleled by an increase in \(\beta\)-sheet content (18). The particular representative of this class that we describe here is DAPH, which shows considerable promise.

The set of libraries that we searched contained \(\sim 3,780\) molecules with known biological functions. It was put together in another purpose by our colleague Brent Stockwell. For historical reasons, the original screen used the dye DiBAC\(_4\) (3) as an indicator for the degree of \(\beta\)-sheet conformation in the aggregated misfolded A\(\beta_42\) peptide. Half a dozen compounds from the library showed a great reduction in fluorescence when aggregated A\(\beta_42\) at 10 \(\mu\)M was exposed to unknown compounds at 5 \(\mu\)g/ml. The use of DiBAC\(_4\) (3) to measure \(\beta\)-sheet confor-

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\(^{7}\)The fact that they are made entirely of D-amino acids makes them physiologically stable.

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mation was validated with the usual ThT fluorescence test for β-sheet content. DAPH was the most effective at lowering the fluorescence associated with binding to β-sheets.

Three in vitro assays have been used to investigate and quantitate the effect of DAPH on the properties of aggregated Aβ42. These are (i) the ability of DAPH to block the toxic influx of Ca$^{2+}$ ions into neuronal cells, (ii) the great reduction in β-sheet content of aggregated Aβ42 by using ThT, and (iii) the dramatic inhibition and reversal of fibril formation when Aβ42 aggregates.

DAPH was included in the library of biochemically active compounds because it is a tyrosine kinase inhibitor with specificity for the epidermal growth factor receptor kinase. The additional activities described for DAPH are likely to reside in different structural aspects of this molecule, but where these are remains to be discovered.

It is unlikely that the kinase-inhibitor property of DAPH plays a role in its effect on Aβ fibrils and neurotoxicity because cells are not present in our in vitro assays. Moreover, seven other tyrosine kinase inhibitors that we tried were inactive, with one exception (data not shown). Coincubation of DAPH with the peptide Aβ42 eliminates the Ca$^{2+}$-influx potential, presumably because it changes the β-sheet conformation of the fibrils or protofibrils dramatically. This observation by itself does not solve the question of whether the neurotoxicity of Aβ42 aggregates resides in protofibrils that are intermediates or final fibrils or some other oligomeric aggregate that is formed as a side product in vitro (and in vivo). In our experience, the decrease in neurotoxicity correlates with loss of β-sheet conformation. The stoichiometry of the process is interesting; it seems that complete reversal of fibril formation, as seen in the EM, occurs at equimolar peptide/DAPH ratio (for example, 10:10 μM (Figs. 3 and 5) and that the IC$_{50}$ value for β-sheet elimination is ~15 μM (Fig. 7). We can assume that we are looking at the behavior of the bulk peptide. However, much less DAPH is needed to eliminate the neurotoxicity; the Ca$^{2+}$-influx effect, when Aβ42 is 10 μM, reduces the effect with an IC$_{50}$ of ~0.7 μM DAPH (Fig. 10B).

This observation supports the notion that the active Aβ oligomer species has a high-affinity binding site that controls the Ca$^{2+}$-influx effect and a low-affinity site that is involved in the reversal of β-sheet-containing fibrils (Fig. 7). In our experience, preincubation of Aβ42 for >24 h is needed for the full Ca$^{2+}$-influx effect (Fig. 9D), suggesting that the active oligomer might not be an early intermediate, as suggested by Wang et al. (20) but is perhaps a novel product that is formed during aggregation. This observation raises the interesting possibility, discussed by Lansbury and coworkers (17), that there might be an equilibrium between mature fibrils (as in plaques) and active oligomers. If such oligomers existed around mature plaques, they might account for the heavily stained neuritis surrounding the plaques. The plaques themselves might indeed be “harmless.”

DAPH is an interesting compound for studying the stability and interactions of β-sheet-containing fibrils. Structural analogs of DAPH would enable us to define the function of each part of the molecule. We already know from Traxler et al. (21) the effect that substitutions in different parts of DAPH have on its ability to inhibit tyrosine kinase and other protein kinases. We will eliminate from the DAPH molecule its kinase-inhibitor capacity to avoid possible toxicity complications.

DAPH is a remarkably efficient molecule for eliminating the neuronal cell toxicity that lies at the root of AD pathology. It is also extraordinarily efficient at destroying the underlying β-sheet structure of the characteristic AD fibrils (plaques). It has not escaped our notice that DAPH has the potential to reverse the β-sheet-containing fibrils that are characteristic of other protein-misfolding diseases.

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