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Mechanistic Investigation of the Inhibition of A β 42 Assembly and Neurotoxicity by A β 42 C-terminal Fragments

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Abstract

Oligomeric forms of amyloid β -protein (A β) are key neurotoxins in Alzheimer's disease (AD). Previously, we found that C-terminal fragments (CTFs) of A β 42 interfered with assembly of full-length A β 42 and inhibited A β 42-induced toxicity. To decipher the mechanism(s) by which CTFs affect A β 42 assembly and neurotoxicity, here, we investigated the interaction between A β 42 and CTFs using photo-induced cross-linking and dynamic light scattering. The results demonstrate that distinct parameters control CTF inhibition of A β 42 assembly and A β 42-induced toxicity. Inhibition of A β 42-induced toxicity was found to correlate with stabilization of oligomers with hydrodynamic radius (R_H) = 8–12 nm and attenuation of formation of oligomers with R_H = 20–60 nm. In contrast, inhibition of A β 42 paranucleus formation correlated with CTF solubility and the degree to which CTFs formed amyloid fibrils themselves but did not correlate with inhibition of A β 42-induced toxicity. Our findings provide an important insight into the mechanisms by which different CTFs inhibit the toxic effect of A β 42 and suggest that stabilization of non-toxic A β 42 oligomers is a promising strategy for designing inhibitors of A β 42 neurotoxicity.

Keywords

Amyloid β -protein; aggregation; neurotoxicity; inhibitor; dynamic light scattering

Alzheimer's Disease (AD) is the most common neurodegenerative disease, affecting over 35 million people worldwide (1). Abundant evidence suggests that oligomeric forms of amyloid

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SUPPORTING INFORMATION AVAILABLE Figures S1–S3 presenting examples of PICUP–SDS–PAGE analysis, A β (30–40) inhibitory assays, and correlation analysis are available free of charge via the Internet at <http://pubs.acs.org>.

β -protein ($A\beta$) are the main neurotoxins causing AD (2,3). Two main forms of $A\beta$ exist *in vivo*, containing 40 ($A\beta_{40}$) or 42 ($A\beta_{42}$) amino acid residues. $A\beta_{42}$ plays a central role in the pathogenesis of AD (4,5). Compared to $A\beta_{40}$, $A\beta_{42}$ is substantially more toxic, forms higher-order oligomers, and follows a different oligomerization pathway (6,7). Peptide inhibitors of $A\beta$ assembly and neurotoxicity reported previously mainly targeted $A\beta$ fibril formation (8). The sequences of such inhibitors were based on random selection (9), self-recognition of the central hydrophobic cluster (CHC) of $A\beta$ (10–13), or structural modifications of sequences from the CHC or C-terminal regions (14–17). As the hypothesis of the cause of AD shifted from $A\beta$ fibril formation and deposition to oligomeric $A\beta$, the design strategy for peptide inhibitors for treatment of AD was adjusted to target $A\beta$ oligomerization. In view of the important role of the C-terminus in $A\beta_{42}$ assembly and toxicity, we hypothesized that the C-terminal fragments (CTFs) of $A\beta_{42}$ might disrupt $A\beta_{42}$ assembly and inhibit its neurotoxicity. In a previous study, we confirmed this hypothesis and found that $A\beta_{42}$ CTFs, [$A\beta(x-42)$, $x = 28-39$], with the exception of $A\beta(28-42)$, inhibited $A\beta_{42}$ -induced neurotoxicity (18). Among these CTFs, $A\beta(31-42)$ and $A\beta(39-42)$ displayed outstanding inhibitory effects. In addition to inhibiting $A\beta_{42}$ -induced neuronal death, these CTFs were found to rescue disruption of synaptic activity by $A\beta_{42}$ oligomers at micromolar concentrations (18). Initial biophysical assessment suggested that these two CTFs exerted their inhibitory activity via distinct mechanisms (18). However, to gain further mechanistic understanding, additional investigation of the characteristics of the CTFs themselves, and of their interaction with $A\beta_{42}$, including comparison of $A\beta(31-42)$ and $A\beta(39-42)$ to other CTFs with low or high activity, was necessary.

Recently, we reported a systematic characterization of biophysical properties of all the CTFs in the original series (19), to which we added for additional structural insight two $A\beta_{40}$ CTFs, $A\beta(34-40)$ and $A\beta(30-40)$, and a fragment derived from the putative folding nucleus of $A\beta$, $A\beta(21-30)$ (20). We found that most of $A\beta_{42}$ CTFs longer than 8 residues readily formed β -sheet-rich fibrils, whereas the shorter CTFs did not. The two $A\beta_{40}$ CTFs were substantially less prone to aggregation than their $A\beta_{42}$ CTF counterparts (19). Surprisingly, $A\beta(30-40)$ was found to be a strong inhibitor of $A\beta_{42}$ -induced toxicity. Importantly, we found that the capability of the CTFs to inhibit $A\beta_{42}$ toxicity did not correlate with their propensity to aggregate or form β -sheet-rich amyloid structures. Rather, inhibition of toxicity appeared to correlate with a coil-turn structure identified by molecular dynamics simulations using experimental ion-mobility spectrometry/mass-spectrometry data as structural constraints (21).

To better understand the mechanism(s) by which CTFs inhibit $A\beta_{42}$ -induced toxicity, we asked whether they inhibited $A\beta_{42}$ assembly, and if so, to what extent inhibition of $A\beta_{42}$ assembly correlated with inhibition of $A\beta_{42}$ -induced toxicity. Here we used photo-induced cross-linking of unmodified proteins (PICUP) and dynamic light scattering (DLS) to study $A\beta_{42}$ assembly in the absence or presence of CTFs and control peptides and correlated the findings with our previously-published data on inhibition of $A\beta_{42}$ toxicity. The sequences of all the peptides used are shown in Table 1.

MATERIALS AND METHODS

Peptide preparation

$A\beta_{42}$, CTFs and control peptides were synthesized by solid-phase techniques (22), using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry, as described previously (19,23,24), purified by high-performance liquid chromatography and analyzed by mass spectrometry and amino acid analysis (AAA).

Cell viability assays

Previously, a cell-viability screen showed that all A β 42 CTFs, except A β (28–42), which was highly toxic itself, inhibited A β 42-induced toxicity (18). Under similar conditions, A β (30–40) showed strong inhibitory activity, whereas A β (34–40) and A β (21–30) were inactive (19). Here we used dose-dependent lactate dehydrogenase (LDH) experiments to evaluate the inhibition of A β (30–40) dose-dependently. The method was described previously (18). Briefly, A β 42:A β (30–40) mixtures of concentration ratios 1:0, 1:1, 1:2, 1:5, 1:10 were added to the differentiated rat pheochromocytoma (PC-12) cells and incubated for 48 h. Cell death was assessed by the CytoTox-ONE Homogenous Membrane Integrity Assay (LDH assay; Promega, Madison, WI). Three independent experiments with six replicates ($n = 18$) were carried out, and the results were averaged and presented as mean \pm SEM.

Electrophysiological studies

Spontaneous miniature excitatory postsynaptic currents (mEPSCs) of mouse primary hippocampal neurons in the presence of A β 42 and in the absence or presence of A β (31–42) or A β (39–42) were reported previously (18). Here we used the same method to measure A β 42 in the presence of A β (30–40). Briefly, cells were perfused at a flow rate of 0.4–0.5 mL/min with peptide samples of 3 μ M A β 42 and A β 42:A β (30–40) at 1:10 concentration ratio, or vehicle control (extracellular solution with equal volume of DMSO to that used to dissolve the peptide). To calculate mEPSC amplitude and frequency, events were analyzed for 1 min before, and every 5 min during the application of peptide samples. mEPSC frequency data are presented as mean \pm SEM.

Photo-induced cross-linking of unmodified proteins (PICUP)

PICUP experiments for A β (31–42) and A β (39–42) were described previously (18). Here we expanded these experiments to the entire series of A β 42 CTFs, the two A β 40 CTFs, and A β (21–30). Briefly, peptides were dissolved in 60 mM NaOH and diluted with 10 mM sodium phosphate, pH 7.4. Low molecular weight (LMW) A β 42 was prepared by filtration through a 10-kDa molecular weight cut-off filter (MWCO) (25) or by centrifugation at 16,000 g for 10 min (because some batches of A β 42 had low solubility and yielded insufficient concentration for PICUP experiments). Solutions of A β fragments were sonicated for 1 min and then filtered through an Anotop 10 syringe filter with 20-nm pore size (Whatman, Florham Park, NJ). The final concentration of each peptide was determined by AAA. The solution of LMW A β 42 was mixed with different nominal concentrations of A β fragments and the mixtures were cross-linked immediately, fractionated by SDS-PAGE, silver stained, and subjected to densitometric analysis using ONE-Dscan (Scanalytics, Fairfax, VA). Three replicates were measured for each peptide. The abundance of A β 42 hexamer was normalized to the entire lane, and reported as mean \pm SEM. IC₅₀ values were calculated by fitting of hexamer abundance vs. logarithm of CTF concentration using Prism (GraphPad, La Jolla, CA)

Dynamic light scattering (DLS)

Solutions of A β 42 in the absence or presence of CTFs were measured using an in-house-built optical system with a He-Ne laser (wavelength 633 nm, power 50 mW; Coherent, Santa Clara, CA) as a light source and using either PD2000DLS or multitaue PD4047 Precision Detectors correlators. The size distribution of scattering particles was reconstructed from the scattered light correlation function using PrecisionDeconvolve software (Precision Detectors, Bellingham, MA) based on the regularization method by Tikhonov and Arsenin (26).

Peptides were prepared in UCLA by dissolution in 60 mM NaOH and diluted with 10 mM sodium phosphate (pH 7.4). A β 42:CTF mixtures contained 30 μ M each of A β 42 and A β (x-42), with x = 29, 30, 31, 32, 35 or 39, or A β (30-40). For transportation from UCLA to MIT for measurements, two hundred μ L samples were lyophilized stored at -20°C, and shipped. The samples then were reconstituted in 200 μ L water. The solutions were sonicated for 1 min and filtered through an Anotop 10 syringe filter (20-nm pore size) prior to DLS measurements. The hydrodynamic radius and intensity of particles were recorded. The particle growth rate (dR_H/dt), i.e., the increase of hydrodynamic radius over time, was determined using at least 20 measurements of 10 min each taken immediately after sample preparation and no less than 10 measurements taken on the next day. The data are presented as mean \pm SEM. Three replicates were measured for each peptide.

RESULTS

A β 42 oligomerization in the presence of CTFs

Previously, using PICUP (27) the oligomer size distribution of A β 42 was found to contain abundant pentamers and hexamers, which were termed “paranuclei” (6). The abundance of these paranuclei, particularly the hexamers, was found to be a sensitive probe for testing inhibition of A β 42 oligomerization (28). Importantly, because all A β fragments used here contained only residues that have low reactivity in PICUP chemistry (18,28), cross-linking of CTFs to A β 42 or to themselves was not observed, facilitating unhindered analysis of A β 42 oligomer size distributions. LMW A β 42 was prepared by filtration through a 10-kDa MWCO filter (25) at ~30 M, mixed with increasing concentrations of each peptide, and cross-linked. The cross-linked mixtures were analyzed by SDS-PAGE and densitometry. Examples are shown in Supplementary Figure S1.

Previously, we found that A β (31-42) inhibited A β 42 hexamer formation dose-dependently, whereas A β (39-42) did not (18). Here, all other A β 42 CTFs, A β 40 CTFs, and A β (21-30) were tested. Analysis of densitometric data showed that A β (36-42) and shorter A β 42 CTFs at concentrations above 100 μ M did not inhibit A β 42 hexamer formation (Figure S1C). Similarly, A β 40 CTFs and A β (21-30) had no effect on A β 42 hexamer formation (Figure 1). In contrast, A β (35-42) and longer A β 42 CTFs caused a dose-dependent decrease of A β 42 hexamer abundance (Figures 1, S1A and S1B). The inhibitory activity increased with peptide length from A β (35-42) through A β (33-42), whereas additional elongation to A β (32-42) and A β (31-42) had little effect on activity. Remarkably, further elongation to A β (30-42) and A β (29-42) resulted in ~2 orders of magnitude increase in inhibitory activity, yielding nanomolar IC₅₀ values.

A β 42 particle growth in the presence of CTFs

To further evaluate the interaction between CTFs and A β 42, we used DLS. PICUP and DLS are complementary methods for investigation of A β assembly. PICUP offers high-resolution detection of low-order oligomers, whereas DLS enables non-invasive detection of high-order assemblies with high sensitivity (6,29). Out of the twelve A β 42 CTFs, we selected six for DLS characterization of their interactions with full-length A β 42. A β (30-42), A β (31-42), and A β (39-42) were studied because they were the strongest inhibitors of A β 42-induced neurotoxicity (18). A β (29-42) was included as the most potent inhibitor of A β 42 hexamer formation (Figure 1). A β (32-42) was selected because it stood out in the CTF series. This CTF was slightly toxic itself, was less efficient than A β (31-42) or A β (33-42) as an inhibitor of A β 42-induced toxicity, and displayed unusual aggregation behavior, namely forming predominantly amorphous, rather than fibrillar, aggregates (19). A β (35-42) was selected as a negative control with relatively low inhibitory activity in both the toxicity (18) and the

oligomerization (Figure 1) assays. Finally, A β (30–40) was studied as a potent (IC₅₀ = 29 ± 4 μ M in the LDH assay) A β 40-derived inhibitor of A β 42-induced neurotoxicity (Figure S2).

Similar to previous observations (6), in the absence of CTFs, immediately after preparation, A β 42 comprised predominantly two populations of particles. Particles with hydrodynamic radius R_{H1} = 8–12 nm, which remained largely unchanged during the measurements, and particles with R_{H2} = 20–60 nm, which were observed in some measurements and tended to fluctuate substantially. We name these oligomer populations *P1* and *P2*, respectively. Note that the large oligomers comprising the *P2* fraction cannot possibly pass through a 20-nm pore size filter and therefore must form immediately after filtration. After several days, large particles, presumably fibrils, formed (Figure 2, bottom panel). The long CTFs themselves, e.g., A β (29–42), A β (30–42), A β (31–42), and A β (30–40) showed aggregation in DLS measurements (19). However, because in the equimolar mixtures of CTF and A β 42 used here the CTF mass is several times lower than that of A β 42, the contribution of CTF to the observed scattering is negligible.

In the presence of the seven CTFs we tested, immediately after preparation, a significant enrichment of population *P2* was observed (Figure 2, first column). It is important to consider, however, that the scattering intensity is proportional to the square of the average mass of particles in each fraction. Thus, although the relative contribution of *P2* particles to the observed scattering was large, their weight fraction in the A β 42:CTF mixtures was still no more than a few percent. During the experiments, the size of *P1* remained relatively unchanged, whereas *P2* appeared to grow in size. Notably, substantial differences in *P2* growth rate, dR_{H2}/dt , were observed in the presence of different CTFs (Figure 3A). In fact, the strongest toxicity inhibitor, A β (31–42), decreased dR_{H2}/dt substantially by 60±13% relative to A β 42 alone. A β (39–42), had a weaker effect on dR_{H2}/dt decreasing the rate by 35±28%. Other CTFs had little or no effect.

Interestingly, on day 1, smaller R_H values were observed in the presence of A β (39–42) (R_{H1} = 6 ± 3 nm, R_{H2} = 30 ± 10 nm) relative to other CTFs. Similarly, in the presence of A β (30–40) *P1* particles had R_{H1} = 6 ± 3 nm on day 1, though *P2* particles were larger than in the presence of other CTFs. Because both peptides were among the strongest inhibitors of A β 42-induced toxicity, these data suggested a correlation between inhibition of toxicity and smaller size of oligomers corresponding to *P1* particles.

The relative abundance of *P1* and *P2* particles showed substantial differences among mixtures of A β 42 with different CTFs. *P2* particles appeared to be less abundant in the presence of the CTFs that had been found to be effective inhibitors of toxicity (18). For example, in the presence of A β (30–40), *P2* particles contributed 20% of the scattering on day 2 and 4% on day 7. In contrast, in the presence of A β (29–42), which showed weak inhibition of A β 42-induced toxicity, *P2* particles contributed 74% of the scattering on day 2 and 87% on day 7. Notably, in the presence of A β (29–42), the scattering intensity, but not the particle size, grew ~5-times as fast as in all other samples (data not shown), suggesting that aggregates of similar size had larger mass, i.e., were more dense compared to aggregates of A β 42 alone or in the presence of other CTFs.

In addition to measuring particle size, we also measured the frequency of intensity spikes that occur when large particles cross the laser beam (Figure 3B). This measurement is a convenient proxy of formation of very large particles, presumably fibrils, before they become so large that they precipitate out of solution. A β (29–42), A β (30–42), A β (31–42), and A β (30–40) showed inhibition of fibril growth relative to A β 42 in the absence of CTFs or in the presence of the shorter CTFs, A β (32–42), A β (35–42), and A β (39–42).

DISCUSSION

Inhibition of A β assembly is an attractive pathway to developing reagents that will block A β toxicity and potentially will lead to treatment for AD. Because the assembly process of A β is complex and the relationship between assembly size and structure and toxicity are not well understood, it is important to understand the mechanisms by which inhibitors affect A β assembly and how the resulting structures correlate with inhibition of toxicity. Such structure–activity analysis may lead eventually to the ability to predict factors necessary for successful inhibition of A β toxicity.

Here, we used two complementary methods, PICUP and DLS, for studying the interaction of peptide inhibitors with A β 42, and compared the data with our previous characterization of inhibition of A β 42-induced toxicity by these peptides (18) and the biophysical features of the peptides themselves (19). The PICUP data showed that A β (35–42) and longer CTFs interrupted A β 42 paranucleus formation, whereas the shorter peptides did not. The order of activity of the CTFs in inhibiting hexamer formation in this assay roughly followed CTF length and did not explain the relatively high potency with which A β (30–42), A β (31–42), A β (39–42), or A β (30–40) inhibited A β 42-induced toxicity.

DLS measurements showed that CTFs interacted with A β 42 and stabilized two oligomer populations. The data suggested several lines of correlation between inhibition of A β 42-induced toxicity and the assembly behavior of different CTFs. The two previously-characterized toxicity inhibitors, A β (31–42) and A β (39–42) showed the strongest reduction of growth rate of *P2* particles, dR_{H2}/dt . However, slow growth rate alone did not explain the behavior of other CTFs, such as A β (30–42) or A β (30–40), which showed strong inhibition of A β 42-induced toxicity but had little effect on dR_{H2}/dt . A decrease in the size of *P1* particles was observed in the presence of A β (39–42) or A β (30–40), but not other CTFs. Inhibition of formation of putative fibrils measured by the effect of CTFs on the frequency of intensity spikes correlated only partially with inhibition of toxicity and did not provide satisfactory mechanistic explanation for the toxicity results. These analyses suggested that more than one mechanism might be responsible for inhibition of A β 42-induced toxicity by CTFs.

To gain additional mechanistic insight, we examined potential sets of correlation among the different data sets, including inhibition of paranucleus formation (Figure 1), abundance of *P2* particles (Figure 2), inhibition of toxicity ((18) and Figure S2A), CTF solubility (19), CTF conversion to β -sheet-rich fibrils (19), and CTF particle growth (19). We calculated linear correlations among these data sets, which, depending on the parameter and the availability of the data, ranged from 4–7 data points. The analysis confirmed a poor correlation between inhibition of paranucleus formation and inhibition of A β 42-induced neurotoxicity ($r^2 = 0.01$, Figure 4A). Inhibition of paranucleus formation showed relatively high correlation with CTFs solubility ($r^2 = 0.72$, Figure 4B), β -sheet formation ($r^2 = 0.96$, Figure S3A), and particle size increase ($r^2 = 0.94$, Figure S3B). The error bars of the solubility and particle growth rate of the CTFs alone in Figure 4 and Figure S3 are inherently quite large due to the large variability in amyloid peptide samples (30). These errors are reflected in the calculated r^2 and p values for the linear correlations. The correlation calculated might raise a concern regarding precipitation of A β 42 in the presence of the least soluble CTFs. However, neither SDS-PAGE analysis of cross-linked oligomers (Figure S1) nor the DLS measurements (Figure 2) showed such precipitation or reduced solubility. Thus, our analysis suggests that the same forces that reduce aqueous solubility and promote fibrillogenesis of CTFs in the absence of A β 42 also facilitate the interaction of the CTFs with A β 42 leading to inhibition of paranucleus formation.

Out of the different parameters we measured in the DLS experiments (dR_{H2}/dt , the abundance of $P2$ particles, and intensity), we found that inhibition of $A\beta_{42}$ -induced toxicity correlated with a low abundance of $P2$ particles on day 2 ($r^2 = 0.90$, Figure 4C) and on day 4–7 ($r^2 = 0.75$, data not shown). Thus, although the particle distribution initially had increased contribution of $P2$ particles in the presence of all CTFs relative to $A\beta_{42}$ alone, on subsequent days, the relative contribution of $P2$ particles was small for strong inhibitors of toxicity and large for weak inhibitors. For reasons that are not entirely clear, $A\beta_{(30-42)}$ was an outlier and therefore was not included in this analysis.

Though the DLS experiments were done under conditions that differ from those of toxicity experiments, the high correlation between low abundance of $P2$ and $A\beta_{42}$ -induced toxicity at 48 h provides important insights into the mechanism(s) by which CTFs might inhibit the toxicity. This putative mechanism is summarized in Figure 5. In the absence of CTFs (Figure 5, top path), $A\beta$ monomers rapidly self-assemble into small oligomers ($P1$ particles). Association of these oligomers into larger assemblies ($P2$ particles) is relatively slow, whereas the conversion of $P2$ assemblies into fibrils or their disassembly back into $P1$ oligomers is fast. As a result, little accumulation of $P2$ particles is observed. In the presence of CTFs (Figure 5, bottom path), $A\beta_{42}$ and the CTFs co-assemble into heterooligomers, the size of which is generally similar to that of the oligomers formed in the absence of CTFs. The CTFs stabilize both $P1$ and $P2$ oligomers and retard the conversion of $P2$ assemblies into fibrils. However CTFs vary in their effect on the conversion of the small $P1$ oligomers into the larger $P2$ oligomers. Effective inhibitors slow down this process and give rise to predominantly $P1$ oligomers, whereas less effective inhibitors allow for a relatively fast $P1 \rightarrow P2$ conversion. Thus, the anti-correlation between $P2$ abundance and inhibition of toxicity suggest that a predominant mechanism by which CTFs inhibit $A\beta_{42}$ toxicity is by stabilizing $P1$ heterooligomers.

Taken together, our data indicate that CTFs affect $A\beta_{42}$ assembly in different ways, including disruption of paranucleus formation by $A\beta_{(35-42)}$ and longer $A\beta_{42}$ CTFs, stabilization of $P1$ and $P2$ particles by all CTFs, alteration of the size and abundance of $P1$ and $P2$ assemblies, and co-assembly with $A\beta_{42}$ into heterooligomers. Inhibition of $A\beta_{42}$ toxicity by CTFs correlates with accumulation of $P1$ heterooligomers suggesting attenuation of $P1 \rightarrow P2$ conversion. Stabilization of non-toxic $A\beta$ oligomers is a mechanism shared by other inhibitors of $A\beta$ assembly and toxicity, including *scyllo*-inositol (31) and (-)-epigallocatechin gallate (32). Thus, we propose that efforts geared towards designing inhibitors of protein self-assembly should focus on diversion of the process towards formation of non-toxic oligomers (or heterooligomers of $A\beta$ and the inhibitor) that can be degraded by cellular clearance mechanisms rather than attempting to prevent monomer self-association.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AAA	amino acid analysis
$A\beta$	amyloid β -protein

AD	Alzheimer's disease
CHC	<u>central hydrophobic cluster</u>
CTF	C-terminal fragment
DLS	dynamic light scattering
LDH	lactate dehydrogenase
LMW	low molecular weight
mEPSC	miniature excitatory postsynaptic currents
MWCO	molecular weight cut-off filter
PICUP	photo-induced cross-linking of unmodified proteins

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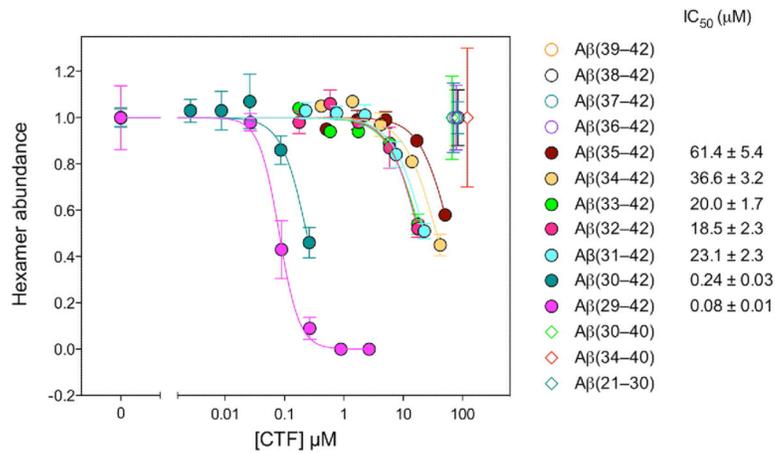


Figure 1.

Inhibition of Aβ₄₂ hexamer formation. Aβ₄₂ was cross-linked in the absence or presence of increasing concentrations of each CTF and analyzed by SDS-PAGE and silver staining. The amount of Aβ₄₂ hexamer was determined densitometrically and normalized to the protein content in the entire lane. IC₅₀ values are the CTF concentration required for 50% inhibition of Aβ₄₂ hexamer formation.

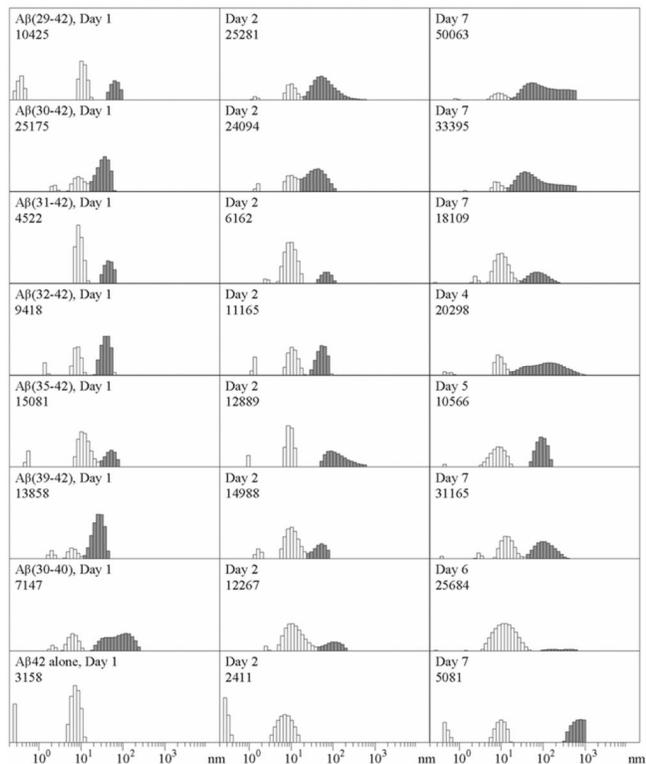


Figure 2. CTF effect on Aβ42 particle size distribution. Representative distributions of Aβ42 in the absence or presence of CTFs immediately after preparation (*Left*), on the next day (*Center*), and after 4–7 days (*Right*). White bars represent P1 particles. Gray bars represent P2 or larger particles (in the case of Aβ42 alone). Days of measurement and the total scattering intensities in counts per second are shown in the upper left corner of each panel. Only intensities within the same row are directly comparable with each other.

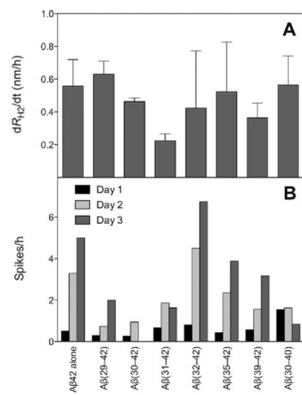


Figure 3. DLS monitoring of Aβ42 aggregation in the absence or presence of CTFs. A) Growth rates (dR_{H2}/dt) of particles with initial $R_{H2} = 20-60$ nm. The data for Aβ42 alone could not be obtained consistently (see text). B) Intensity spikes per hour indicating fibril development.

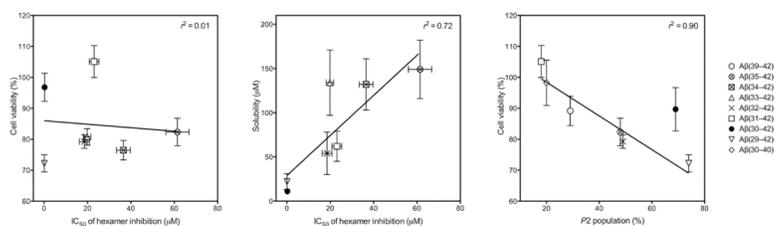


Figure 4. Correlation analysis. A) Linear regression analysis correlating inhibition of paranucleus formation for A β (29–42)–A β (35–42) with inhibition of A β 42-induced toxicity (19) ($r^2 = 0.01$, $p = 0.8$). B) Linear regression analysis correlating inhibition of paranucleus formation for A β (29–42)–A β (35–42) with CTFs solubility (19) ($r^2 = 0.72$, $p = 0.02$). C) Linear regression analysis correlating the population of P2 on day 2 for A β (29–42)–A β (32–42), A β (35–42), A β (39–42), and A β (30–40) with inhibition of A β 42-induced toxicity ($r^2 = 0.90$, $p = 0.004$). A β (30–40) is an outlier in this correlation, which is not included in the calculation.

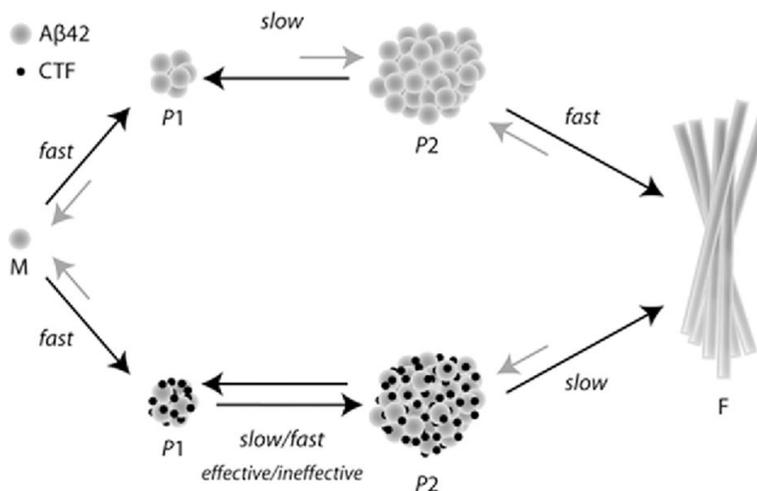


Figure 5. Schematic representation of a putative mechanism by which CTFs affect Aβ42 assembly. Monomer (M) assembly into P1 particles is a fast process in the absence (top path) or presence (bottom path) of CTFs. CTFs may accelerate the conversion of P1 into P2 oligomers, but effective inhibitors of Aβ42-induced toxicity induce slower acceleration than ineffective ones, shifting the population towards P1. All CTFs slow down the maturation of P2 assemblies into fibrils (F).

Table 1

Peptide code and sequence.

Peptide	Sequence
A β (39–42)	VVIA
A β (38–42)	GVVIA
A β (37–42)	GGVVIA
A β (36–42)	VGGVVIA
A β (35–42)	MVGGVVIA
A β (34–42)	LMVGGVVIA
A β (33–42)	GLMVGGVVIA
A β (32–42)	IGLMVGGVVIA
A β (31–42)	IIGLMVGGVVIA
A β (30–42)	AIIGLMVGGVVIA
A β (29–42)	GAIIGLMVGGVVIA
A β (28–42)	KGAIIGLMVGGVVIA
A β (34–40)	LMVGGVV
A β (30–40)	AIIGLMVGGVV
A β (21–30)	AEDVGSNKGA