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Structural basis for A β _{1–42} toxicity inhibition by A β C-terminal fragments: Discrete molecular dynamics study

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Abstract

Amyloid β -protein (A β) is central to the pathology of Alzheimer's disease (AD). Of the two predominant A β alloforms, A β _{1–40} and A β _{1–42}, the latter forms more toxic oligomers. C-terminal fragments (CTFs) of A β were recently shown to inhibit A β _{1–42} toxicity *in vitro*. Here we studied A β _{1–42} assembly in the presence of three effective CTF inhibitors and an ineffective fragment, A β _{21–30}. Using a discrete molecular dynamics approach that recently was shown to capture key differences between A β _{1–40} and A β _{1–42} oligomerization, we compared A β _{1–42} oligomer formation in the absence and presence of CTFs or A β _{21–30} and identified structural elements of A β _{1–42} that correlated with A β _{1–42} toxicity. CTFs co-assembled with A β _{1–42} into large heterooligomers containing multiple A β _{1–42} and inhibitor fragments. In contrast, A β _{21–30} co-assembled with A β _{1–42} into heterooligomers containing mostly a single A β _{1–42} and multiple A β _{21–30} fragments. The CTFs, but not A β _{21–30}, decreased the β -strand propensity of A β _{1–42} in a concentration-dependent manner. CTFs and A β _{21–30} had a high binding propensity to the hydrophobic regions of A β _{1–42} but only CTFs were found to bind the A β _{1–42} region A2–F4. Consequently, only CTFs but not A β _{21–30} reduced the solvent accessibility of A β _{1–42} in the region D1–R5. The reduced solvent accessibility of A β _{1–42} in the presence of CTFs was comparable to the solvent accessibility of A β _{1–40} oligomers formed in the absence of A β fragments. These findings suggest that the region D1–R5, which was more exposed to the solvent in A β _{1–42} than in A β _{1–40} oligomers, is involved in mediating A β _{1–42} oligomer neurotoxicity.

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Supplementary Material Available: Description of the DMD approach and its limitations; simulation conditions; methods of structural analysis; supporting results on size distributions, tertiary, and quaternary structures of A β _{1–42} and A β _{X–Y} at different A β _{X–Y} concentrations within the A β _{1–42}/A β _{X–Y} heterotypic assemblies. In total: 7 figures and 1 table.

1. Introduction

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disorder that is the dominant cause of dementia in the elderly. One of the hallmarks of AD is accumulation of extracellular senile plaques, which contain fibrillar aggregates of amyloid β -protein ($A\beta$). Genetic, pathologic, and biochemical evidence strongly supports the hypothesis that low-order oligomeric assemblies of $A\beta$, rather than fibrils, are the proximate neurotoxic agents in AD [1, 2, 3, 4, 5, 6, 7]. The majority of $A\beta$ oligomers were found to be neurotoxic [8] and certain oligomers decreased neuronal viability 10–100-fold more strongly than $A\beta$ fibrils [9]. $A\beta_{1-40}$ and $A\beta_{1-42}$, the major $A\beta$ alloforms in the brain, differ by the presence of two amino acids, I41 and A42, at the C-terminus of the latter. $A\beta_{1-42}$ aggregates faster [10, 11], forms more toxic assemblies [5], and is genetically linked to aggressive, early-onset familial forms of AD [12]. Cross-linking studies showed that $A\beta_{1-42}$ forms pentamers and hexamers (paranuclei) and multiples of paranuclei, including dodecamers and octadecamers, whereas $A\beta_{1-40}$ exists as a mixture of monomers through tetramers [13]. These observations have been confirmed independently by ion-mobility spectrometry/mass spectrometry [14, 15]. Interestingly, $A\beta$ dodecamers, which putatively form by self-association of two paranuclei have been detected *in vivo* in several independent studies [16, 17, 18, 19].

In recent years, an *ab initio* discrete molecular dynamics (DMD) approach [20] using a four-bead protein model and residue-specific hydrophobic interactions offered important insights into the folding and assembly of $A\beta_{1-40}$ and $A\beta_{1-42}$ [21, 22, 23, 24, 25]. This approach recapitulated the essential experimentally observed differences between the folding and oligomerization of $A\beta_{1-40}$ and $A\beta_{1-42}$ and demonstrated that $A\beta_{1-42}$ but not $A\beta_{1-40}$ oligomerization was driven primarily through intermolecular interactions involving the C-terminal region (I31-A42) [22, 23, 25]. The DMD approach also predicted a quasi-stable turn at the C-terminus of $A\beta_{1-42}$, which does not occur in $A\beta_{1-40}$ [22], a prediction that was supported by several experimental studies [26, 27, 28, 29, 30]. In a most recent study, Streltsov *et al.* reported the first X-ray structure of the $A\beta_{18-41}$ tetramer encapsulated in a shark Ig new antigen receptor, which resembles the DMD-derived oligomeric structures [31].

The DMD findings have led to a hypothesis that C-terminal fragments (CTFs) of $A\beta_{1-42}$ may interfere with $A\beta_{1-42}$ oligomerization. Recently, we reported that $A\beta_{1-42}$ CTFs ranging from $A\beta_{29-42}$ through $A\beta_{39-42}$, as well as $A\beta_{30-40}$, attenuated $A\beta_{1-42}$ neurotoxicity in neuronal cell culture [32, 33]. We also investigated the aqueous solubility, aggregation kinetics, and morphology of CTFs [33], and found that their aggregation propensity correlated with previously reported [34] tendency to form β -hairpin structures whereas their ability to inhibit $A\beta_{1-42}$ -induced neurotoxicity correlated with a tendency to form an irregular “coil-turn” structure [33]. Dynamic light scattering (DLS) data revealed that two $A\beta_{1-42}$ oligomer populations which were scarcely populated in the absence of inhibitors, were enhanced by CTFs in inhibitor-specific manner. In particular, stabilization of the smaller of the two $A\beta_{1-42}$ /CTF heterotypic assembly populations with a hydrodynamic radius 8–12 nm correlated with the degree of toxicity inhibition [35]. Stabilization of non-toxic $A\beta_{1-42}$ assemblies might thus be a promising strategy for designing $A\beta_{1-42}$ toxicity inhibitors [35]. A similar mechanism was found for several other inhibitors, including scylloinositol [36, 37, 38] benzothiazole derivatives [39], and the polyphenols epigallocatechin-3-gallate (EGCG) [40, 41], resveratrol [42], myricetin, and nordihydroguaiaretic acid [39]. However, the mode of interaction of inhibitors with $A\beta_{1-42}$ and the structural changes in $A\beta_{1-42}$ that are required for a successful toxicity inhibition are unknown.

Our preliminary DMD study of A β ₁₋₄₂ assembly in the presence of A β ₂₉₋₄₂, A β ₃₁₋₄₂, or A β ₃₉₋₄₂, using A β ₁₋₄₂:CTF molar concentration ratios of up to 1:2, demonstrated that these CTFs inserted themselves among A β ₁₋₄₂ peptides, reducing their intermolecular contacts [32]. Inhibition of A β ₁₋₄₂ toxicity by CTFs in a cell culture was concentration dependent and most efficient at the A β ₁₋₄₂:CTF molar concentration ratio of ~1:10. Here, we applied the DMD approach to examine assembly of A β ₁₋₄₂ in the presence of three CTFs that efficiently inhibited A β ₁₋₄₂ toxicity and a control peptide A β ₂₁₋₃₀, which had no effect on A β ₁₋₄₂ toxicity [33], at several A β ₁₋₄₂:A β ₂₁₋₃₀ concentration ratios, including 1:10. We explored the effects of A β ₃₁₋₄₂, A β ₃₉₋₄₂, and two additional A β fragments (A β ₃₀₋₄₀ and A β ₂₁₋₃₀) which were not included into our prior DMD study [32], using an improved, recently reported parametrization of the DMD approach [24, 25]. The aim of the present work was to explore structural elements involved in A β ₁₋₄₂ toxicity inhibition by CTFs. To achieve that, we analyzed the A β ₁₋₄₂ structures formed in the presence of effective inhibitors and compared them to A β ₁₋₄₂ oligomers formed in the absence of inhibitors and in the presence of ineffective A β ₂₁₋₃₀ fragments. We also compared the A β ₁₋₄₂ assembly structures formed in the presence of A β fragments to A β ₁₋₄₀ oligomer populations (formed in the absence of A β fragments). Based on the present computational results and previously reported toxicity data [32, 33], we propose a mechanism, in which CTFs inhibit A β ₁₋₄₂ toxicity by binding to specific regions in of A β ₁₋₄₂, reducing its ability to form a β structure, and interrupting putative interactions of A β ₁₋₄₂ with its cellular targets.

2. Results

We selected four A β _{X-Y} fragments to study their effect on A β ₁₋₄₂ assembly. Of all the experimentally examined CTFs [33], A β ₃₁₋₄₂ was chosen because it was the strongest inhibitor of neurotoxicity [32, 33]. A β ₃₉₋₄₂, the shortest of all the CTFs under study, showed surprisingly high inhibition of neurotoxicity [32]. A β ₃₀₋₄₀ was selected because its degree of A β ₁₋₄₂ toxicity inhibition was comparable to the other two CTFs [33], and A β ₂₁₋₃₀, which did not inhibit A β ₁₋₄₂ toxicity in cell culture, was chosen as a control peptide.

We simulated A β ₁₋₄₂ assembly in the presence of CTFs or A β ₂₁₋₃₀ using a four-bead protein model with backbone hydrogen bonding and amino acid-specific interactions, as described in the Methods section (see Supplementary Methods in Supplementary Material). We used the implicit solvent parameters $E_{HP} = 0.3$ and $E_{CH} = 0$ and physiological temperature estimate $T = 0.13$, which recently has been shown to match well the *in vitro* temperature dependence of the average β -strand in A β ₁₋₄₀ and A β ₁₋₄₂ monomers [24] and the distinct oligomer size distributions of A β ₁₋₄₀, A β ₁₋₄₂, and their Arctic mutants, [E22G]A β ₁₋₄₀ and [E22G]A β ₁₋₄₀ [25]. The total number of A β ₁₋₄₂ and fragment peptides, and simulation box sizes (Table I in Supplementary Material) were chosen to correspond to a total molar concentration of ~3–4 mM as used in the previous DMD studies [22, 25]. Typical molar concentrations in *in vitro* studies are 10- to 100-fold lower than the total molar concentration in our computational study. Due to limitations in the total number of atoms and time scales that can currently be efficiently studied by computer simulations [43], millimolar concentrations are required for proteins to interact with each other and thus form assemblies, and to obtain sufficient statistics on the assembled structures. The effect of A β fragments on A β ₁₋₄₂ assembly and the resulting structures were quantified and compared to unaltered A β ₁₋₄₂ oligomer structures using data from the recently published work [25].

In the description of our results below, the following abbreviations for specific A β regions are used: central hydrophobic cluster CHC (L17-A21), mid-hydrophobic cluster MHR (I31-M35), and C-terminal region CTR (V39-A42).

2.1. $A\beta_{X-Y}$ and $A\beta_{1-42}$ associate into $A\beta_{1-42}/A\beta_{X-Y}$ heterooligomers

Initially separated monomeric $A\beta_{1-42}$ and toxicity inhibitors: $A\beta_{30-40}$, $A\beta_{31-42}$, or $A\beta_{39-42}$ (Figure 1a) associated first into small $A\beta_{1-42}$ /CTF heterooligomers of various sizes and compositions (Figure 1b), then into larger heterotypic assemblies (Figure 1c), and finally, all $A\beta_{1-42}$ and CTF peptides in each trajectory converged into a single large heterotypic assembly (Figure 1d–e). These results are in qualitative agreement with the DLS observations of increased abundance of $A\beta_{1-42}$ oligomers in the presence of these CTFs relative to $A\beta_{1-42}$ alone [35]. Thus, the three toxicity inhibitors ($A\beta_{30-40}$, $A\beta_{31-42}$, or $A\beta_{39-42}$) acted as a “glue” capturing $A\beta_{1-42}$ molecules into an amorphous heterotypic assembly. In contrast, the control peptide, $A\beta_{21-30}$, had the opposite effect. In the presence of this fragment, both $A\beta_{1-42}$ and $A\beta_{21-30}$ remained predominantly monomeric. In addition, small heterotypic assemblies comprising a $A\beta_{1-42}$ molecule surrounded by 7–8 $A\beta_{21-30}$ peptides also were observed (Figure 2a–e, see also Figure II in Supplementary Material). The size distributions of $A\beta_{1-42}/A\beta_{X-Y}$ assemblies (Figure II in Supplementary Material) evolved within $5\text{--}10 \times 10^6$ simulation steps into a quasi steady state, after which the temporal changes in the oligomer size distributions were no longer statistically significant up to 20×10^6 simulation steps.

2.2. CTFs inhibit β -strand formation in $A\beta_{1-42}$

The structural basis for $A\beta_{1-42}$ neurotoxicity is as yet unknown. Several studies addressed the relevance of β -strand formation in $A\beta$ oligomer-mediated toxicity. Neurotoxic pre-fibrillar $A\beta$ assemblies lacking the cross- β structure (which is characteristic of $A\beta$ fibrils with ~45–55% of β -sheet structure) were identified by several studies [44, 13, 45, 19]. In contrast, Chimon *et al.* described neurotoxic $A\beta$ intermediates with parallel β -sheet structures [46] and Wu *et al.* found fibrillar oligomers that nucleated formation of toxic oligomers but did not form fibrils [47]. Our DMD-derived oligomer structures [25] are consistent with *in vitro* data by Kirkitadze *et al.* who found $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers with a relatively low β -strand of 10–20% [44] and no cross- β structure. Here, we asked whether changes in the β -strand propensity in $A\beta_{1-42}$ due to the presence of CTFs and $A\beta_{21-30}$ at the stage of initial hydrophobic collapse may correlate with their reported ability to inhibit $A\beta_{1-42}$ toxicity.

To examine the effect of $A\beta$ fragments on β -strand formation in $A\beta_{1-42}$, we calculated the average β -strand content, $\langle\beta\text{-strand}\rangle$, of $A\beta_{1-42}$ within $A\beta_{1-42}/A\beta_{X-Y}$ assemblies and compared them to $\langle\beta\text{-strand}\rangle$ in $A\beta_{1-42}$ oligomers formed in the absence of fragments (Figure 3a, solid curves). $\langle\beta\text{-strand}\rangle$ in $A\beta_{1-42}$ decreased from 19% in the absence of fragments to ~16% at relative molar concentrations of $A\beta_{X-Y}$ of ~0.5 for all toxicity inhibitors. At the relative $A\beta_{X-Y}$ molar concentrations of ~1, $A\beta_{30-40}$ and $A\beta_{31-42}$ further reduced $\langle\beta\text{-strand}\rangle$ in $A\beta_{1-42}$ to ~15%. At this $A\beta_{31-42}$ concentration, $\langle\beta\text{-strand}\rangle$ in $A\beta_{1-42}$ was at its lowest value and at the relative $A\beta_{31-42}$ molar concentration above 2 and up to 10, $\langle\beta\text{-strand}\rangle$ remained at a value of ~16% (~16% reduction). $\langle\beta\text{-strand}\rangle$ in $A\beta_{1-42}$ above the relative $A\beta_{30-40}$ molar concentration of ~2 decreased with the $A\beta_{30-40}$ concentration and reached a value of ~12% at the relative molar concentration 10 (37% reduction). Thus, of the two longer toxicity inhibitors, $A\beta_{30-40}$ was significantly more efficient in suppressing the formation of β -strand structure in $A\beta_{1-42}$ than $A\beta_{31-42}$. The shortest toxicity inhibitor, $A\beta_{39-42}$, had the most prominent effect on $\langle\beta\text{-strand}\rangle$ in $A\beta_{1-42}$ at all relative molar concentrations >0.5 and reduced $\langle\beta\text{-strand}\rangle$ to ~10% (48% reduction). In contrast to the three toxicity inhibitors, the control peptide $A\beta_{21-30}$ increased $\langle\beta\text{-strand}\rangle$ in $A\beta_{1-42}$ (Figure 3a, grey solid curve) and this increase was strongly $A\beta_{21-30}$ concentration-dependent.

$\langle\beta\text{-strand}\rangle$ propensity in the $A\beta$ fragments themselves was variable. All three toxicity inhibitors showed similar concentration dependencies of $\langle\beta\text{-strand}\rangle$ (Figure 3a, black, red,

and green dashed curves), experiencing a slight $A\beta_{1-42}$ -induced increase in $\langle\beta\text{-strand}\rangle$ about or just below the relative molar CTF concentrations of 0.5, followed by a decrease at higher CTF concentrations. $A\beta_{31-42}$ had the highest $\langle\beta\text{-strand}\rangle$ values at all concentrations under study, reaching ~12% at the highest relative $A\beta_{31-42}$ molar concentration of 10, followed by $A\beta_{30-40}$ with ~7%, and $A\beta_{39-42}$ with ~2%. The control peptide $A\beta_{21-30}$ was characterized by the highest $\langle\beta\text{-strand}\rangle$ (ranging from 12.8% at the lowest to 17.6% at the highest $A\beta_{21-30}$ concentration) of all four $A\beta$ fragments. $\langle\beta\text{-Strand}\rangle$ in $A\beta$ fragments was at all concentrations *lower* than $\langle\beta\text{-strand}\rangle$ in $A\beta_{1-42}$ oligomers formed in the absence of fragments (19.1%) and significantly lower than that of $A\beta_{1-42}$ assembling in the presence of $A\beta_{21-30}$ at the 1:10 molar concentration ratio (27.9%). As shown in Figure 3b, β -strand propensity of $A\beta$ fragments correlated with the average β -strand in $A\beta_{1-42}$ and also with the $A\beta$ fragment-induced change in the $A\beta_{1-42}$ β -strand propensity. To our knowledge, there are currently no experimental data on the effect of $A\beta$ fragments on the secondary structure of $A\beta_{1-42}$, thus direct comparison between *in silico* and *in vitro* data cannot be made. Nonetheless, our analysis suggests that the $A\beta_{1-42}$ secondary structure changes may be relevant to the $A\beta$ fragment-induced changes in $A\beta_{1-42}$ toxicity.

In addition, we compared β -strand propensities per residue in $A\beta_{1-40}$ and $A\beta_{1-42}$ hexamers formed in the absence of fragments (Figure 3c, blue and orange curves) with the β -strand propensities per residue in heterotypic assemblies comprising $A\beta_{1-42}$ and $A\beta$ fragments (Figure 3c, black, red, green, and grey curves). None of the three toxicity inhibitors changed the relative distribution of β -strand propensities along the $A\beta_{1-42}$ sequence. The β -strand propensity per residue in $A\beta_{1-42}$ hexamers was significantly different from the one found in $A\beta_{1-40}$ hexamers, as reported and discussed in our prior work [25]. For example, the region A2-F4 in $A\beta_{1-40}$ but not in $A\beta_{1-42}$ hexamers had a high β -strand propensity (Figure 3c, blue and orange curves). All three toxicity inhibitors reduced the β -strand propensities in the region Q15-L17 of $A\beta_{1-42}$, but only $A\beta_{30-40}$ and $A\beta_{39-42}$ reduced these propensities at R5-V12, A21-S26, and I31-V36. The control peptide $A\beta_{21-30}$, on the other hand, substantially *increased* the β -strand propensity in several $A\beta_{1-42}$ regions: D7-E11, F19-F20, E22-V24, G29-I32, and L34-V36 (Figure 3c, grey curve).

2.3. $A\beta_{X-Y}$ fragments alter the tertiary and quaternary structure of $A\beta_{1-42}$

Here, we quantified structural changes in $A\beta_{1-42}$ due to the presence of each fragment and identified the specific peptide regions involved in the interaction between $A\beta_{1-42}$ and $A\beta_{X-Y}$. For this purpose, simulation data corresponding to the $A\beta_{1-42}:A\beta_{X-Y}$ molar concentration ratio 1:10, which was needed for an efficient toxicity inhibition, were used³ [32]. In these simulations, six $A\beta_{1-42}$ molecules co-assembled with 229 $A\beta_{30-40}$, 210 $A\beta_{31-42}$, 630 $A\beta_{39-42}$, or 252 $A\beta_{21-30}$ peptides using a simulation box size 318 Å.

2.3.1. Effect of $A\beta$ Fragments on the Tertiary Structure of $A\beta_{1-42}$ —The tertiary structures of $A\beta_{1-42}$ in the absence or presence of $A\beta$ fragments are shown in Figure 4a–e. All three toxicity inhibitors slightly reduced the number and strength of intramolecular contacts relative to $A\beta_{1-42}$ hexamers formed in the absence of inhibitors, mostly in the N-terminal region (Figure 4a–e, box 1). Almost no change in the tertiary structure was observed at the C-terminal region (Figure 4a–e, box 4). Among the three inhibitors, $A\beta_{39-42}$ reduced the intramolecular contacts in $A\beta_{1-42}$ the most (Figure 4d). The control peptide $A\beta_{21-30}$, inhibited the $A\beta_{1-42}$ intramolecular contacts at the N-terminal region (Figure 4e, box 1), but at the same time *increased* the number and strengths of intramolecular contacts in all other regions of $A\beta_{1-42}$ (Figure 4e, boxes 2–5) relative to $A\beta_{1-42}$ hexamers (Figure

³A similar structural analysis for $A\beta_{1-42}$ and $A\beta$ fragments using simulation data obtained at the four lower $A\beta_{1-42}:A\beta_{X-Y}$ concentration ratios is described in Supplementary Results in Supplementary Material (Figures III–VII).

4a). Overall, the three CTFs decreased, whereas A β _{21–30} increased, the stability of the A β _{1–42} tertiary structure. The exception was the N-terminal region (Figure 4a–e, box 1), where all A β _{X–Y} reduced intramolecular contacts in A β _{1–42}.

2.3.2. Effect of A β Fragments on the Quaternary Structure of A β _{1–42}—The quaternary structures of A β _{1–42} in the absence or presence of A β fragments are shown in Figure 4f–j. All four A β fragments reduced both the number and the strengths of intermolecular contacts among A β _{1–42} molecules in a concentration-dependent way (Figure IV, Supplementary Material). The remaining intermolecular A β _{1–42} contacts were A β _{X–Y}-specific. An almost complete absence of specific intermolecular contacts among A β _{1–42} was observed in the presence of A β _{30–40} and A β _{21–30}. The interpretation of this reduction was, however, different for the two peptides. In the case of A β _{21–30}, A β _{1–42} molecules did not interact because they belonged to different heterotypic assemblies, whereas in the case of A β _{30–40}, all six A β _{1–42} molecules belonged to the same A β _{1–42}/A β _{30–40} heterooligomer, but were spatially separated from each other, resulting in the reduction of all intermolecular contacts. Similar to A β _{30–40}, A β _{31–42} and A β _{39–42} spatially separated individual A β _{1–42} molecules within heterooligomers. Interestingly, none of the four fragments completely inhibited the intermolecular A β _{1–42} contacts. In the presence of A β _{31–42} or A β _{39–42}, the intermolecular contacts among A β _{1–42} molecules were more abundant than in the presence of A β _{30–40}. In the presence of A β _{39–42}, the intermolecular contacts among the CHC, MHR, and CTR regions of A β _{1–42} were more abundant than in the presence of A β _{31–42}. A β _{31–42} induced a few new intermolecular contacts involving the N-terminal region G9–V12 of A β _{1–42} (Figure 4h, box 5).

2.3.3. Interaction regions between A β _{1–42} and A β fragments—Intermolecular contacts between A β _{1–42} and each fragment are shown in Figure 4k–n. Boxes 1–4 indicate the regions of A β _{1–42} that most strongly interacted with all four fragments: the A2–F4 region, CHC, MHR, and CTR. Of the four fragments, A β _{39–42} formed the most frequent contacts with A β _{1–42}, likely due to its highly hydrophobic nature its short length (Figure 4m). A β _{30–40} and A β _{31–42} displayed comparable average numbers and strengths of intermolecular contacts (Figure 4k,l), though the strengths were slightly increased in A β _{30–40} relative to A β _{31–42}. The intermolecular contacts between the control A β _{21–30} peptide and A β _{1–42} were comparable to those observed for A β _{30–40} and A β _{31–42} in the CHC, MHR, and CTR (Figure 4n, boxes 2–4). There were no contacts between A β _{21–30} and the A2–F4 region of A β _{1–42} (Figure 4n, box 1). Instead, the region R5–V12 of A β _{1–42} formed and antiparallel β -sheet with A β _{21–30} (Figure 4n, region between boxes 1 and 2). These contacts were specific to A β _{21–30} interaction with A β _{1–42} and were not observed for any other A β fragment. Thus, the interaction between A β _{21–30} and A β _{1–42} was stronger than would have been expected based on the strongly hydrophilic nature of A β _{21–30} relative to CTFs.

2.3.4. Binding propensity of A β _{X–Y} to A β _{1–42}—To quantify the degree of the interaction between A β _{1–42} and A β _{X–Y}, we calculated the binding propensity by averaging the intermolecular A β _{1–42}/A β _{X–Y} contact maps shown in Figure 4k–n over the specified regions. The results are shown in Table 1. For comparison, the binding propensity of A β _{1–42} to itself in hexamers was included. We found that A β _{39–42} had ~2–4-fold higher binding propensity than the other three fragments to the four regions of A β _{1–42} under study (Table 1). A β _{21–30} had a similar binding propensity to the CHC, MHR, and CTR as A β _{30–40} and A β _{31–42} despite its predominant hydrophilic nature. Importantly, A β _{21–30} had at least 5-fold lower binding propensity to the A2–F4 region of A β _{1–42} relative to the CTFs, highlighting a possible relevance of the A2–F4 region of A β _{1–42} in mediating toxicity.

2.4. CTFs reduce solvent exposure of the N-terminal region of A β ₁₋₄₂

A β ₁₋₄₂ oligomers presumably mediate neurotoxicity through interaction with cell membranes and/or other cellular components [48, 49, 50, 51]. Important insights into the specific A β ₁₋₄₂ regions participating in these interactions may be gleaned from studying which regions are most exposed to the environment within homotypic and heterotypic assemblies. In our recent study, we showed that the SASA in the N-terminal region was significantly lower in A β ₁₋₄₀ hexamers than in A β ₁₋₄₂ hexamers (see Figure S5b in Supporting Information of Ref. [25]). To check whether this difference between A β ₁₋₄₀ and A β ₁₋₄₂ was specific to hexamers or a more general feature, we used here data from our previous study [25] to calculate SASA per residue using the entire A β ₁₋₄₀ and A β ₁₋₄₂ populations of monomers and oligomers. The results demonstrated that the distinct SASA profiles in the N-terminal region D1-R5 between A β ₁₋₄₀ and A β ₁₋₄₂ were characteristic not only for hexamers but for entire A β ₁₋₄₀ and A β ₁₋₄₂ populations (Figure 5, blue and orange curves). The regions of A β that on average were most exposed to the solvent were mostly hydrophilic residues: D1, R5-S8, H13-Q15, E22-D23, and S26-K28 whereas the hydrophobic regions (CHC, MHR, and CTR) had low SASA values.

To quantify the effect of A β fragments on the solvent exposure of A β ₁₋₄₂, we calculated SASA per residue using the simulation data acquired at the A β ₁₋₄₂:A β _{X-Y} molar concentration ratio 1:10 (Figure 5). For A β ₁₋₄₂ assembled in the presence of the three toxicity inhibitors, the SASA in the region D1-R5 was reduced relative to A β ₁₋₄₂ assembled in the absence of inhibitors. For all three CTFs, the SASA values almost overlapped with the SASA values derived for A β ₁₋₄₀ assemblies formed in the absence of A β fragments (Figure 5). In contrast, A β ₂₁₋₃₀ induced an *increased* SASA in the region D1-R5 of A β ₁₋₄₂ (Figure 5, grey curve). The observed correlation between solvent exposure of the N-terminal region of A β ₁₋₄₂ and the degree of toxicity suggests that the N-terminal region D1-R5 of A β ₁₋₄₂ is involved in A β ₁₋₄₂-mediated toxicity, likely through interaction with cellular targets.

3. Discussion

Our recent *in vitro* studies of a series of CTFs (A β _{X-42}, X = 28 – 39; A β ₃₀₋₄₀) and the control peptide A β ₂₁₋₃₀ were studied for aqueous solubility, aggregation propensity, and morphology characteristics. None of these properties directly correlated with the ability of CTFs to inhibit toxicity [33]. Rather, the degree of toxicity inhibition by CTFs, in particular A β ₃₁₋₄₂, A β ₃₉₋₄₂, and A β ₃₀₋₄₀, correlated with stabilization of the smaller and attenuation of the larger of the two oligomer populations with hydrodynamic radii of 8 – 12 nm and 20 – 60 nm, respectively, as measured by dynamic light scattering [35]. These findings are difficult to reconcile using a simple explanation of how the inhibitors affect A β ₁₋₄₂ assembly but rather demonstrate that: (i) CTFs do not prevent A β ₁₋₄₂ self-assembly; (ii) CTFs bind to A β ₁₋₄₂ and subtly affect its assembly; and (iii) CTFs and A β ₁₋₄₂ co-assemble into oligomeric structures that are not grossly different from those formed in the absence of inhibitors, i.e., they have similar hydrodynamic radii [35] and similar morphologies (H. Li and G. Bitan, unpublished results).

Two plausible mechanisms by which inhibitors could reduce A β ₁₋₄₂ toxicity are: (a) CTFs modulate A β ₁₋₄₂ oligomer structure by inducing subtle structural changes or (b) CTFs mask A β ₁₋₄₂ groups or regions that interact with cellular targets. These mechanisms are not mutually exclusive and may be operating together. Our purpose in the present study was to gain insight into these mechanisms from the relatively high-resolution data provided by the DMD approach. Thus, we examined the structures and structural changes occurring during early stages of A β ₁₋₄₂ assembly in the presence of three selected toxicity inhibitors (A β ₃₀₋₄₀, A β ₃₁₋₄₂, and A β ₃₉₋₄₂) or a control peptide A β ₂₁₋₃₀. A β ₁₋₄₂ was found to co-assemble with all three CTFs under study into large heterooligomers. In contrast, A β ₁₋₄₂ and

A β_{21-30} formed small heterotypic assemblies comprising mostly one A β_{1-42} and 7–8 A β_{21-30} peptides. Thus, in our simulations, toxicity inhibitors but not control peptides induced formation of large heterooligomers.

Mechanisms by which toxicity inhibitors interact with A β to reduce toxicity are not well understood. Liu *et al.* showed that a simple disaccharide, trehalose, inhibited oligomer formation and reduced toxicity of A β_{1-40} but not of A β_{1-42} [52]. This study suggests that trehalose induced alloform-specific structural changes that disrupted specific interactions of A β_{1-40} , but not of A β_{1-42} , with its environment. Similarly, here, we found specific interactions of different inhibitors with A β_{1-42} . Though all three CTFs decreased the tendency of A β_{1-42} to form β -strands, A β_{39-42} was the most, and A β_{31-42} the least, efficient among the three. The ability to disrupt β -strand formation was negatively correlated with the β -strand content in CTFs themselves. In contrast to CTFs, A β_{21-30} significantly increased the propensity of A β_{1-42} to form the β -strands.

Of the four A β fragments, A β_{39-42} interacted most strongly with A β_{1-42} . Interestingly, the predominantly hydrophilic A β_{21-30} interacted with A β_{1-42} as strongly as A β_{30-40} or A β_{31-42} , though the location and nature of these interactions were distinct from those between A β_{1-42} and the two CTFs. These results suggest that peptide length affects the A β_{1-42} -A β_{X-Y} interaction more than its hydrophobic versus hydrophilic nature. As expected, the self-assembly of A β_{X-Y} was strongly affected by the hydrophobic versus hydrophilic character of the fragments. In contrast to the CTFs, A β_{21-30} did not self-assemble. Importantly, unlike the three toxicity inhibitors, A β_{21-30} did not interact with the A2-F4 region of A β_{1-42} .

An involvement of the A2-F4 region in A β_{1-40} but not A β_{1-42} folding and oligomer formation was demonstrated in previous DMD studies [22, 24, 25]. In A β_{1-40} , the A2-F4 region had a relatively high β -strand propensity [22, 24, 25], resulting in less favorable A β_{1-40} hexamer formation, which included a slow dock-and-lock intermolecular interaction involving the A2-F4 β strand regions [25]. Intermolecular contacts among the A2-F4 regions caused the N-terminal region to be more shielded from the solvent in A β_{1-40} than in A β_{1-42} oligomers.

Similar to the differences between A β_{1-40} and A β_{1-42} , our present analysis showed that the region D1-R5 of A β_{1-42} was significantly less exposed to the solvent in heterooligomers formed in the presence of toxicity inhibitors than in A β_{1-42} oligomers formed in the absence of A β fragments. In contrast, in the presence of A β_{21-30} , the solvent exposure of the N-terminal region D1-R5 of A β_{1-42} significantly increased relative to unaltered A β_{1-42} oligomers.

Our hypothesis that the N-terminus of A β_{1-42} mediates A β_{1-42} -induced toxicity is consistent with findings of two recent studies. Luheshi *et al.* who used a *Drosophila* model of AD demonstrated that (a) an A2F substitution in A β_{1-42} increased its toxicity and (b) whereas an E22G substitution in A β_{1-42} dramatically increased its toxicity, a double substitution E22G/F4D led to a significantly reduced toxicity [53]. In addition, Jin *et al.* used A β dimers, isolated from the cortex of AD patients, and showed that they mediate toxicity by directly inducing Tau phosphorylation. They further demonstrated that the monoclonal antibodies that bind to the N-terminal D1, but not the antibody that binds to the C-terminus of A β_{1-42} , inhibited this A β -mediated toxicity [54].

In summary, our present results offer mechanistic insights into processes involved in A β_{1-42} assembly in the presence of A β fragments and provide an insight into the putative mechanism(s) by which the CTFs inhibit the toxicity. We identified two structural elements, increased β -strand propensity and increased solvent exposure at the N-terminus of A β_{1-42} ,

that correlated with A β ₁₋₄₂-induced toxicity (Table 2). This work delineates plausible structure—toxicity relationships amenable to *in vitro* and *in vivo* testing and provides structural information of potential importance for drug design.

4. Methods

4.1. The DMD approach

DMD is a form of MD that utilizes interparticle potentials in a form of a single or a combination of square wells, which simplifies calculations of individual trajectories and makes DMD orders of magnitude faster than MD [55]. Here, DMD is combined with a four-bead protein model, in which each amino acid is described by up to four beads (representing the amino *N*, β -carbon *C_α*, carboxyl *C*, and β -carbon *C_β* group) [56, 57]. This four-bead protein model is based on geometric properties and the backbone hydrogen bonding introduced by Ding *et al* [57]. The lengths of bonds and the angular constraints are determined phenomenologically by calculating their distributions using the known folded protein structures of ~7,700 proteins from the Protein Data Bank (PDB) [58, 57]. Effective backbone hydrogen bonds are implemented between the nitrogen atom *N_i* of the *i* – *th* amino acid and the carbon atom *C_j* of the *j* – *th* amino acid [57]. The absolute value of the potential energy of the backbone hydrogen bond interaction, *E_{HB}*, represents a unit of energy and the temperature is given in units *E_{HB}*/*k_B*. We implemented the amino acid-specific interactions due to hydrophathy and charge of individual side chains that are critical [21] to distinguish between A β ₁₋₄₀ and A β ₁₋₄₂ folding and assembly pathways [22, 20, 25]. The *ab initio* DMD approach with a four-bead protein model and implicit solvent used in this study is the same as that used in the previous studies of A β folding and assembly [22, 23, 24, 25] and has been described in detail by Urbanc *et al.* [20]. Relative to earlier DMD studies [22, 23, 32], a present DMD parametrization includes a more accurate estimate of physiological temperature and two-times longer simulation time, which was recently shown to best account for the distinct oligomer size distributions of A β ₁₋₄₀, A β ₁₋₄₂, and their Arctic mutants [24, 25].

4.2. The DMD simulation protocol

Keeping the number of A β ₁₋₄₂ molecules fixed (either 16 or 6), the number A β _{*X-Y*} molecules was varied to obtain a desired A β ₁₋₄₂:A β _{*X-Y*} concentration ration. The total molar concentration was kept constant across simulations and equal to the one used in our previous study of oligomer formation of full-length A β peptides [25]. Initially, the centers of mass of unstructured monomeric A β ₁₋₄₂ and A β _{*X-Y*} molecules were arranged in the simulation box into a cubic lattice. Distinct initial configurations for multiple trajectories per each A β ₁₋₄₂:A β _{*X-Y*} concentration were obtained by performing short, high-temperature DMD simulation runs and saving configurations every 0.1×10^6 simulation steps. This protocol resulted in randomly-placed and mixed peptides of each type that were used as initial populations for production runs (Figure I in Supplementary Material). Multiple trajectories at each A β ₁₋₄₂:A β _{*X-Y*} concentration ratio were acquired at physiological temperature (*T* = 0.13), using the implicit solvent parameters *E_{HP}* = 0.3 and *E_{CH}* = 0 that best described the initial hydrophobic collapse into globular A β ₁₋₄₂ oligomers. For each of the four A β _{*X-Y*}, five different molar concentration ratios were studied, resulting in 20 systems. For each system, eight trajectories of 20×10^6 simulation steps were acquired. The resulting A β ₁₋₄₂ structures formed in the presence of A β _{*X-Y*} molecules were quantified in terms of their secondary, tertiary, quaternary structures, solvent exposure, and compared to unaltered A β ₁₋₄₂ oligomer structures derived previously [25]. The section Supplementary Methods in Supplementary Material provides a more detailed description of our computational approach and its limitations.

Supplementary Material

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Figure 1. Populations of 16 A β_{1-42} and (a–d) 256 A β_{39-42} molecules at four different time frames: (a) $t = 0$ (b), $t = 0.1 \times 10^6$, $t = 10^6$, and $t = 20 \times 10^6$ simulation steps. A β_{39-42} molecules are displayed in yellow and A β_{1-42} molecules are represented in dark blue with the N-terminal amino acid D1 marked as red spheres. (e) A magnified and rotated final A β_{1-42} /A β_{39-42} heterooligomer obtained at $t = 20 \times 10^6$ simulation steps. The figure was created using the VMD software package [59].

Figure 2. Populations of 16 A β_{1-42} and (a–d) 128 A β_{21-30} molecules at four different time frames: (a) $t = 0$ (b), $t = 0.1 \times 10^6$, $t = 10^6$, and $t = 20 \times 10^6$ simulation steps. A β_{21-30} molecules are displayed in yellow and A β_{1-42} molecules are represented in dark blue with the N-terminal amino acid D1 marked as red spheres. (e) Two magnified A β_{1-42} /A β_{21-30} heterooligomers obtained at $t = 20 \times 10^6$ simulation steps. The figure was created using the VMD software package [59].

Figure 3.

(a) Average β -strand of $A\beta_{1-42}$ (solid lines) versus the relative molar concentration of each $A\beta$ fragment: $A\beta_{30-40}$ (black lines), $A\beta_{31-42}$ (red lines), $A\beta_{39-42}$ (green lines), or $A\beta_{21-30}$ (grey lines), as well as the average β -strand propensities of $A\beta$ fragments the within heterooligomers (dashed lines). Heterooligomer populations of 11 time frames between 19×10^6 and 20×10^6 simulation steps of each of 8 trajectories per concentration and per the CTF type were used in the analysis. (b) The average β -Strand propensity in $A\beta_{1-42}$ as a function of the average β -Strand propensity in the $A\beta$ fragment. (c) β -Strand propensity per residue. The error bars correspond to SEM.

Figure 4.

(a–e) Intra- and (f–j) intermolecular contact maps of $A\beta_{1-42}$ assemblies formed in the absence and at 1:10 molar concentration ratio of the three toxicity inhibitors ($A\beta_{30-40}$, $A\beta_{31-42}$, and $A\beta_{39-42}$) and the control peptide $A\beta_{21-30}$. The intra- and intermolecular contact maps of $A\beta_{1-42}$ hexamers are shown in (a) and (f), respectively. The intermolecular maps that elucidate regions of contact between $A\beta_{1-42}$ and each of the four $A\beta$ fragments are shown in (k–n). The contact maps are oriented such that the average number of contacts among the N-terminal amino acids is displayed at the top left corner and the average number of contacts among the C-terminal amino acids is at the bottom right corner. The boxes mark regions of concentrated contacts.

Figure 5.

Average SASA per residue of $A\beta_{1-40}$ and $A\beta_{1-42}$ monomer and oligomer populations assembled in the absence and presence of the three CTFs at the molar concentration ratio 1:10, relevant to toxicity inhibition, and the control peptide $A\beta_{21-30}$ at the same molar concentration ratio.

Binding propensity of Aβ₃₀₋₄₀, Aβ₃₁₋₄₂, Aβ₃₉₋₄₂, and Aβ₂₁₋₃₀ to specific regions of Aβ₁₋₄₂ at Aβ₁₋₄₂:Aβ_{X-Y} molar concentration ratio of 1:10. Binding propensities of Aβ₁₋₄₂ to itself in unaltered Aβ₁₋₄₂ hexamers are given for comparison.

Table 1

Aβ ₁₋₄₂ regions	Aβ ₃₀₋₄₀	Aβ ₃₁₋₄₂	Aβ ₃₉₋₄₂	Aβ ₂₁₋₃₀	Aβ ₁₋₄₂ hexamers
A2-F4	0.59 ± 0.03	0.51 ± 0.03 [‡]	1.69 ± 0.19 [‡]	0.09 ± 0.02 [*]	0.07 ± 0.01
CHC	0.66 ± 0.04	0.65 ± 0.03	2.45 ± 0.16 [‡]	0.64 ± 0.09	0.14 ± 0.01
MHR	0.70 ± 0.04	0.57 ± 0.03 [‡]	1.89 ± 0.13 [‡]	0.62 ± 0.09	0.15 ± 0.01
CTR	0.74 ± 0.05	0.72 ± 0.05	2.27 ± 0.18 [‡]	0.74 ± 0.14	0.18 ± 0.01

[‡] Aβ₃₁₋₄₂ binding propensities that were significantly different from Aβ₃₀₋₄₀ binding propensities (non-overlapping error bars).

[‡] Aβ₃₉₋₄₂ binding propensities that were 2–4 times larger than for any other fragment.

^{*} Aβ₂₁₋₃₀ binding propensity to the A2-F4 region more than 5 times lower than for any other fragment. The error bars correspond to SEM.

Table 2

Summary of the cell viability data for A β ₁₋₄₂-induced toxicity in cell cultures extracted from Fradinger et al. [32] and for A β ₁₋₄₂ in the presence of A β fragments extracted from Li et al. [33] (column 2) alongside the main structural changes in A β ₁₋₄₂ during its assembly at the highest concentration of A β fragments as observed here (columns 3–5). H-O stands for a heterooligomer, M for a monomer.

A β _{X-Y}	Cell Viability[%]	Assembly State	$\Delta\langle\beta\text{-strand}\rangle$ [%]	ΔSASA
A β ₁₋₄₂	62 $\pm$ 4	oligomers	0	$\equiv$ 0
+ A β ₃₀₋₄₀	98 $\pm$ 7	large H-O	-7	< 0
+ A β ₃₁₋₄₂	105 $\pm$ 5	large H-O	-3	< 0
+ A β ₃₉₋₄₂	89 $\pm$ 5	large H-O	-9	< 0
+ A β ₂₁₋₃₀	63 $\pm$ 7	Ms / small H-Os	9	> 0