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Mechanism of C-Terminal Fragments of Amyloid β -Protein as $A\beta$ Inhibitors: Do C-Terminal Interactions Play a Key Role in Their Inhibitory Activity?

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Abstract

Targeting the early oligomerization of amyloid β -protein ($A\beta$) is a promising therapeutic strategy for Alzheimer's disease (AD). Recently, certain C-terminal fragments (CTFs) derived from $A\beta_{42}$ were shown to be potent inhibitors of $A\beta$ -induced toxicity. The shortest peptide studied, $A\beta_{(39-42)}$, has been shown to modulate $A\beta$ oligomerization and inhibit $A\beta$ toxicity. To understand the mechanism of these CTFs, especially $A\beta_{(39-42)}$, is of significance for future therapeutic development of AD and peptidomimetic-based drug development. Here, we used ion mobility spectrometry-mass spectrometry to investigate the interactions between two modified $A\beta_{(39-42)}$ derivatives, VVIA-NH₂ and Ac-VVIA, and full-length $A\beta_{42}$. VVIA-NH₂ was previously shown to inhibit $A\beta$ toxicity whereas Ac-VVIA did not. Our mass spectrometry analysis reveals that VVIA-NH₂ binds directly to $A\beta_{42}$ monomer and small oligomers while Ac-VVIA only binds to $A\beta_{42}$ monomer. Ion mobility studies show that VVIA-NH₂ modulates $A\beta_{42}$ oligomerization by not only inhibiting the dodecamer formation but also disaggregating preformed $A\beta_{42}$ dodecamer. Ac-VVIA also inhibits and removes preformed $A\beta_{42}$ dodecamer. However, the $A\beta_{42}$ sample with the addition of Ac-VVIA clogs the nanospray tip easily, indicating there are larger aggregates formed in the solution in the presence of Ac-VVIA. Molecular dynamics simulations suggest that VVIA-NH₂ binds specifically to the C-terminal region of $A\beta_{42}$ while Ac-VVIA binds

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Supporting Information:

Additional information of starting structures of $A\beta_{42}$ and VVIA analogs for MD simulations, representative complex structures of $A\beta_{42}$ and VVIA analogs from MD simulations, ATDs and cross sections of $A\beta_{42}$ monomer and monomer complexes. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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dispersedly to multiple regions of A β 42. This work implies that C-terminal interactions are most important for C-terminal fragment inhibitors.

Abstract

Keywords

Alzheimer's disease; amyloid β -protein; C-terminal fragment; Mass spectrometry; Ion mobility spectrometry; Molecular dynamics simulation

Introduction

Amyloid β -protein (A β) has been shown to play a significant role in the pathology of Alzheimer's disease (AD).¹ A β is produced from the amyloid precursor protein (APP) via proteolytic cleavages by β - and γ -secretases.² A β , in reality, consists of a group of peptides containing 37-43 amino acid residues, among which A β 40 and A β 42 are the two primary alloforms (A β 42 sequence: DAEFRHDSGY¹⁰EVHHQKLVEF²⁰AEDVGSNKG³⁰IIGLMVGG-VV⁴⁰IA). Although A β 40 is more abundant than A β 42 in vivo (A β 40 constituting ~90% of all A β peptides), the latter is much more toxic and more aggregation prone. Recently, an increased body of evidence shows that the early oligomer states of A β , rather than the final fibrillar products, are the primary toxic agents in AD pathology.³⁻⁶ In solution, A β 42 forms dimer, tetramer, paranuclei (pentamer and hexamer), decamer and dodecamer, protofibrils and eventually fibrils.^{4,7-8} The 56-kDa dodecamer has been identified as a proximate toxic agent for AD pathology.⁹⁻¹⁰ Therefore, to target A β early oligomers, especially dodecamer, is a promising therapeutic strategy for AD treatment.

Short peptides derived from the A β sequence, and their derivatives have been shown to disrupt A β assembly and inhibit its toxicity.¹¹⁻¹² The C-terminal region of A β 42, which is highly hydrophobic, has been shown to play an important role in controlling A β structure stability and self-assembly.¹³⁻¹⁵ Thus researchers hypothesized that peptides derived from the C-terminus of A β 42 can serve as A β inhibitors as they may interact with C-terminal hydrophobic region of A β and be coassembled into A β 42 oligomers, and thereby disrupt their structures and inhibit their toxicity.¹⁶ Indeed this C-terminal interaction hypothesis has led to the discovery of several effective C-terminal fragment (CTF) inhibitors for A β 42 neurotoxicity, including peptides ranging from A β (29-42) to A β (39-42).¹⁶

A β (39-42), the shortest peptide studied, was shown to modulate A β oligomerization and inhibit A β neurotoxicity.¹⁷⁻¹⁹ It is particularly interesting because it is a small molecule which can easily penetrate into the membrane barriers. Thus it is very important to understand the mechanism of its inhibitory activity for its future use in drug development and other peptidomimetics based drug discovery. Previous theoretical study showed that A β (39-42) binds to several regions of A β 42, including the N-terminal, central hydrophobic core and the C-terminal regions.¹⁷ However, the key interaction region responsive for its inhibition activity remains unclear. To better understand the mechanism of action for A β (39-42), two terminally modified A β (39-42) analogs, N-terminal acetylated Ac-VVIA and C-terminal amidated VVIA-NH₂, were designed to test the charge effect on the interactions of A β (39-42) and A β 42. Previous studies have shown that modifications at the termini have very different effects on A β toxicity: VVIA-NH₂ inhibits A β -induced toxicity while Ac-VVIA does not.¹⁸

Here, ion mobility spectrometry coupled mass spectrometry (IMS-MS) and all-atom molecular dynamics (MD) simulations were used to investigate the interactions between these two A β (39-42) analogs and the full length A β 42. IMS is capable of separating species that have the same mass-to-charge ratio but different conformation or oligomer sizes.²⁰ It has been successfully applied in the past to study A β structure and assembly and the effects of small molecules.^{8,17,21-29} In this work, we use IMS to examine the effects of these two A β (39-42) derivatives on the early assembly of A β 42. MD simulations were performed to understand the details of the binding interactions between the A β 42 and CTF molecules. This study provides an example of ion mobility spectrometry combined with theoretical modeling as a powerful tool to understand the mechanism of A β C-terminal fragments as small molecule inhibitors for A β assembly and sheds light on future use of a peptidomimetic-based therapeutic strategy for AD and other diseases.

Experimental Procedures

Peptides and Sample Preparation

Full-length A β 42 was synthesized by *N*-9- fluorenylmethoxycarbonyl (Fmoc) chemistry.³⁰ A β (39-42) derivatives were prepared using a microwave-assisted peptide synthesizer as described previously¹⁸. The peptides were purified by reverse-phase HPLC and their integrity validated by mass spectrometry and amino acid analysis.

Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS)

Lyophilized A β 42 protein was dissolved in 10 mM ammonium acetate buffer (pH 7.4) with a final protein concentration of 10 μ M. Mass spectra were recorded on a home-built nanoESI instrument, which has been described in detail elsewhere.³¹ Briefly, ions are generated continuously by a nanoelectrospray ionization source, captured and guided through an ion funnel, injected into a temperature-controlled drift cell filled with 3-5 torr helium gas, mass analyzed with a quadrupole mass filter, detected by a conversion dynode and channel electron multiplier allowing a mass spectrum to be obtained.

For ion mobility measurements, the ions are stored in the ion funnel and pulsed into the drift cell. The injection energy of the ions can be varied from ~20 to ~150 V, but it is usually kept as low as possible to minimize thermal heating of the ions during the injection process. The ions gently pass through the cell under the influence of a weak electric field. The velocity of the ions in the drift cell, v_d , is proportional to the electric field E :

$$v_d = K \cdot E \quad (1)$$

The ions exiting the drift cell are mass selected and detected by the detector to allow an arrival time distribution (ATD) to be recorded. The arrival time, t_A , can be related to the time the ions spend in the drift cell, $t_D = t_A - t_0$, where t_0 is the time between exiting the cell and arriving at the detector. The time in the cell is directly related to the ion mobility and collision cross section of the analyte ion.³²

$$\sigma = 1.3 \left(\frac{q^2 E^2 T}{\mu k_B p^2 N^2 l^2} \right)^{1/2} (t_A - t_0) \quad (2)$$

Here, q is the ion charge, E is the voltage across the drift cell, T is the temperature, μ is the reduced mass of the ion–He collision, k_B is the Boltzman constant, p is the pressure, N is the buffer gas (Helium) number density at STP, and l is the length of the drift cell (4.503 cm). All of these quantities are either known constants or are measured for each experiment. The width of the ATD can be compared to the width calculated for a single analyte ion structure,³³ which gives information on the oligomer structural distribution in the ATD. The measured ion mobility and collision cross section provide information about the three-dimensional configurations of the ions.⁸

Transmission Electron Microscopy (TEM)

Microscopic analysis was performed by using a FEI T-20 transmission electron microscope operating at 200 kV. The A β 42 samples were prepared using the same procedure as for mass spectrometry analysis. The samples were kept in a refrigerator (~4 °C) for 2 weeks. For TEM measurements, 10 μ L aliquots of the samples were spotted on glow-discharged, carbon-coated copper grids (Ted Pella, Inc). The samples on grids were stained with 10 mM sodium metatungstate for 10 min and gently rinsed twice with DI-water. The sample grids were then dried in room temperature before TEM analysis.

Molecular Dynamics Simulations

System preparation: Our simulation systems contain one A β 42 peptide and one A β (39–42) derivative (VVIA-NH2 or Ac-VVIA), ~8000 water molecules, and several Na⁺ ions to neutralize the system. The initial peptide structures are taken from the previous study by Garcia and coworkers¹³ for A β 42, and from our own previous study³⁴ for A β (39–42). The A β (39–42) derivative is initially placed ~15 Å away from the A β 42 surface. The solute is immersed in a truncated octahedral box ($a = b = c = \sim 69$ Å, $\alpha = \beta = \gamma = 109.47^\circ$) filled with water molecules. The Duan *et al.* all-atom point-charge force field (AMBER ff03)³⁵ is used to represent the peptides. This force field has been successfully used to model the binding of A β (39–42) to A β 40/ A β 42 peptides¹⁷, the binding among A β protofibrils,³⁶ and the binding

of fluorescent dyes to A β protofibrils.³⁷ The water solvent was explicitly represented by the TIP3P³⁸ model.

Binding Simulations: The AMBER 9 simulation suite³⁹ is used in molecular dynamics simulations and data analysis. After an initial energy minimization, a total of eight simulations (four runs for each system) were performed with different initial random velocities. The random velocities of atoms are generated according to the Maxwell-Boltzmann distribution at 500 K. A 10 ps run at 500 K is used to further randomize the orientations and positions of the two peptides. The production run (150 ns) is at 310 K, including a short, 1-ns molecular dynamics in the NPT ensemble mode (constant pressure and temperature) to equilibrate the solvent and 149-ns dynamics in the NVT ensemble mode (constant volume and temperature). Periodic boundary conditions are imposed on the system. The particle-mesh Ewald method⁴⁰ is used to treat the long-range electrostatic interactions. SHAKE⁴¹ is applied to constrain all bonds connecting hydrogen atoms, enabling a 2-fs time step used in the dynamics. To reduce computation time, non-bonded forces are calculated using a two-stage RESPA approach⁴² where the short-range forces within a 10 Å radius are updated every step and the long range forces beyond 10 Å are updated every two steps. The Langevin dynamics is used to control the temperature (310K) using a collision frequency of 1 ps⁻¹. The center of mass translation and rotation are removed every 500 steps, which removes the “block of ice” problem.⁴³⁻⁴⁴ The trajectories were saved at 10-ps intervals for analysis. In total, 128 Opteron CPU cores (2.3 GHz) were used for ~50 days to complete the 8 binding simulations (a cumulative MD time of 1.2 μ s for the two systems).

Clustering analysis: To gain a better understanding of the binding interactions, the stable complexes (atom contacts >20) are grouped into different structural families based on the C α Root Mean Square Deviation (RMSD) of the complex (cutoff of 5 Å) using the GROMACS protocol.⁴⁵ Representative structures (centroids) of the top abundant clusters from the combined 4 runs of each system are shown in the Supplementary Material (Figures S2-S3).

Collision Cross Section Calculation: The centroids of the top abundant clusters are also used to calculate their collision cross sections by a projected superposition approximation (PSA) method⁴⁶⁻⁴⁹. To correlate better with the solvent-free experiments, these solution-phase structures are converted to ‘dehydrated’ structures via a 500,000-step energy minimization in vacuum prior to cross-section calculations. This “dehydration” generally reduces the overall size of the structures, while maintaining their solution structural features and in this paper these structures are referred to as “dehydrated solution structures”.

Binding energy calculation: The binding energy is evaluated on the centroid structure of a structural family using the Molecular Mechanics-Generalized Born/Surface Area (MM-GBSA) module in the AMBER package. The solvation energy is represented by the Generalized Born term (the polar part of the solvation) plus a Surface Area term (the hydrophobic part of the solvation free energy). Due to lack of solute entropy, the MM-GBSA binding energy tends to overestimate the absolute binding affinity. But when the

solute entropies in different binding modes are comparable, the relative binding affinities can be estimated from the relative MM-GBSA binding energies.⁵⁰

Results

Mass spectrometry: VVIA-NH₂ binds directly to A β 42 monomer and oligomers

The mass spectra of A β 42 samples with and without VVIA analogs were recorded and shown in Figure 1a-c. The mass spectrum of A β 42 sample without VVIA analogs (Figure 1c) shows three peaks corresponding to the $z/n = -4, -3, -5/2$ charge states, where z is charge and n is oligomer number. In the mass spectrum of a 1:5 mixture of A β 42 and VVIA-NH₂ (Figure 1a), there are more peaks in addition to the three A β 42 peaks. These peaks correspond to $z/n = -4$ and -3 complexes of A β 42 with one and two VVIA-NH₂ molecules bound (labeled with stars). Moreover, there is a peak tailing the $z/n = -5/2$ A β 42 peak which corresponds to a $-5/2$ complex of A β 42 oligomers with VVIA-NH₂ molecules bound. This indicates VVIA-NH₂ binds directly not only to A β 42 monomer but also to A β 42 oligomers. The mass spectrum of a 1:5 mixture of A β 42 and Ac-VVIA (Figure 1b) shows only one additional monomer complex peak, suggesting only one Ac-VVIA binds directly to A β 42 monomer. These results suggest that VVIA-NH₂ binds directly to A β 42 with relatively higher affinity than Ac-VVIA. Unfortunately A β 42 aggregates so fast that we are not able to quantify this statement but qualitatively the larger intensity and greater number of adducts of VVIA-NH₂ relative to Ac-VVIA in mixtures with A β 42 under identical conditions supports it.

Ion mobility studies: VVIA-NH₂ and Ac-VVIA modulate the early assembly of A β 42

To probe the effects of the two VVIA analogs on the early oligomer formation of A β 42, an ion mobility study was performed and the results are shown in Figure 1d-f. The ATD of the $-5/2$ peak for A β 42 alone (Figure 1f) shows four features with arrival times at $\sim 710, 680, 610$ and $540 \mu\text{s}$, which were previously assigned as A β 42 dimer, tetramer, hexamer and dodecamer, respectively, based on their cross sections (see ref. 8 for a detailed discussion of $-5/2$ ATD assignment). The dodecamer was previously identified as a proximate toxic agent for AD pathology.⁹⁻¹⁰ The ATD of the $-5/2$ peak for the A β 42 sample with VVIA-NH₂ (1:5 ratio, Figure 1d) shows only three features with arrival times at $\sim 720, 680$, and $620 \mu\text{s}$, which can be assigned as dimer, tetramer and hexamer, respectively, based on their cross sections. There is no feature with shorter arrival times, indicating there is no dodecamer or other larger oligomer formed with the presence of VVIA-NH₂. The ATD of the $-5/2$ peak for the A β 42 and Ac-VVIA mixture (1:5 ratio, Figure 1e) shows three similar features corresponding to dimer, tetramer and hexamer, suggesting AC-VVIA also inhibits the formation of A β 42 dodecamer. However, the relative intensity of hexamer in presence of VVIA-NH₂ is lower than that in presence of Ac-VVIA or A β 42 alone, suggesting the VVIA-NH₂ not only inhibits dodecamer formation, but also partially inhibits hexamer formation.

As shown in Figure 1a, an additional $z/n = -5/2$ oligomer complex peak was observed for the mixture of A β 42 and VVIA-NH₂, therefore its ATD was also recorded to better understand the effect of VVIA-NH₂ on the A β 42 oligomer distribution and the result is

shown in Figure 2. The ATD shows three features with arrival times at ~750, 710 and 640 μ s, which can be assigned as dimer, tetramer and hexamer complexes, respectively, based on their cross sections. This is of significance and suggests there are one, two and three VVIA-NH₂ molecules binding to A β 42 dimer, tetramer and A β 42 hexamers, respectively, which is not observed for the sample with Ac-VVIA. The arrival times of tetramers and hexamers for adducts of A β 42 with VVIA-NH₂ were 4 $\pm$ 0.5% longer than in pure A β 42 which is about what is expected from the percent increase in the number of residues in the adducts relative to neat A β 42 (4.7%). Hence no size difference is noted. This doesn't mean there are not structural differences just that the cross sections don't dramatically change when VVIA-NH₂ is added to these oligomers.

Taken together, these mass spectrometry and ion mobility results suggest that VVIA-NH₂ and Ac-VVIA both bind to A β 42 monomer and modulate dodecamer formation. However, VVIA-NH₂ binds not only to A β 42 monomer but also to A β 42 small oligomers (dimers, tetramers and hexamers), while Ac-VVIA only binds to A β 42 monomer. These results of VVIA-NH₂ are similar to previous results of VVIA, which bound to A β 42 monomer and small oligomers and modulated dodecamer formation.¹⁷ This is of significance since both VVIA and VVIA-NH₂ inhibit the A β 42- induced toxicity whereas Ac-VVIA does not.¹⁷⁻¹⁸

Disaggregation of preformed A β 42 dodecamer by VVIA analogs

To test whether these VVIA analogs can dissociate preformed A β 42 oligomers, concentrated VVIA-NH₂ or Ac-VVIA was added to a pre-aggregated A β 42 sample and the ATDs of $z/n = -5/2$ A β 42 were recorded at different time periods (Figures 3a-g). As shown in Figure 3a, after ~5 hours' incubation, the wild type A β 42 forms dimer, tetramer, hexamer and dodecamer. Immediately after the addition of VVIA-NH₂, the ATD of $z/n = -5/2$ A β 42 (Figure 3b) shows only three features corresponding to dimer, tetramer and hexamer, but not the dodecamer feature. The disappearance of dodecamer suggests that VVIA-NH₂ can disassociate preformed dodecamer. The ATD of the $-5/2$ peak for the A β 42 sample with the addition of Ac-VVIA (Figure 3e) also shows only dimer, tetramer and hexamer. However, the A β 42 sample with Ac-VVIA shows a noisy and broader ATD than that of VVIA-NH₂ sample, which suggests that there may be more families of oligomer structures for the A β 42 sample with Ac-VVIA. This is important and consistent with observation that the A β 42 sample with the addition of Ac-VVIA was more difficult to work with. The sample with Ac-VVIA became difficult to spray and the signal-to-noise of the $z/n = -5/2$ ATD decreased over time (see Figure 3e-g). After three hours, it became impossible to get the sample with Ac-VVIA to spray and no further data could be collected. In contrast, the A β 42 sample with the addition of VVIA-NH₂ worked smoothly during the whole experiment time, and even after several days (see Figure 3b-d). The ATD of the $-5/2$ peak recorded on the 2nd day (Figure 3d) still shows only dimer, tetramer and hexamer. These results and observation suggest that VVIA-NH₂ not only inhibits the formation of A β 42 dodecamer but also dissociates preformed dodecamer. On the other hand, Ac-VVIA, which appears to inhibit dodecamer formation, may actually facilitate formation of other larger oligomers or aggregates which clogged the spray tips. This qualitative observation of tip clogging suggests much faster formation of large aggregates for Ac-VVIA mixtures than for VVIA-

NH₂ mixtures²¹ and may well be the reason that Ac-VVIA does not reduce the toxicity of A β 42 but VVIA-NH₂ mixtures do.

VVIA-NH₂ and Ac-VVIA do not inhibit A β 42 fibril formation

VVIA was previously shown to modulate the A β 42 oligomer formation but did not inhibit A β 42 fibril formation. In this work, the effects of VVIA-NH₂ and Ac-VVIA on fibril formation were examined by TEM and the results are shown in Figure 4. After two weeks' incubation at 4 °C, the A β 42 samples form abundant long fibrils, regardless of the presence of VVIA-NH₂ or Ac-VVIA molecules (Figures 4a-c). This suggests that neither VVIA-NH₂ nor Ac-VVIA inhibits A β 42 fibril formation, which is similar to that of VVIA.

Modeling the interactions of A β 42 with VVIA-NH₂ or Ac-VVIA

To probe the interaction of these two VVIA analogs with the full-length A β 42 at an atomic level, a system consisting of one A β 42 and one VVIA-NH₂ or Ac-VVIA molecule was constructed for all-atom molecular dynamics (MD) simulations. The most populated conformation of A β 42 from the study of Sgourakis et al.¹³ was used as the initial conformation in our simulations, which enables efficient sampling of the most important conformations.

The overall binding was revealed by superimposing the most stable complexes identified from the trajectories and shown in Figure 5 (A-B). A β 42 in both complexes shows great flexibility as indicated by the widespread cloud of the overall peptide backbones (grey), which is expected as A β 42 is a natively disordered peptide. However, the binding of VVIA-NH₂ to A β 42 is more specific than that of Ac-VVIA. As shown in Figure 5A, the VVIA-NH₂ molecules bind exclusively to only one specific region of A β 42, the hydrophobic C-terminal region. The representative structure of the most populated structural family (57% of total population) from our clustering analysis shows that VVIA-NH₂ binds to the edge of the C-terminal β -hairpin (Figure 5C). On the other hand, Ac-VVIA molecules are observed to bind to several regions of A β 42, including the C-terminal, central hydrophobic core and the N-terminal regions (Figure 5B and Figure S3). Clearly, the binding of Ac-VVIA to A β 42 is more disperse and the most populated structural family only contains 28% of total population (Figure 5D). Moreover, the A β 42:VVIA-NH₂ complexes appear to be more rigid and display a uniform conformation, whereas the A β 42:Ac-VVIA complexes show more flexible and extended structures with slightly larger collision cross sections.

VVIA was previously shown to bind to A β 42 at several regions, including the C-terminal, central hydrophobic core and the N-terminal regions.¹⁷ Here our binding simulations of VVIA derivatives reveal significant differences in their binding interactions with A β 42. While the binding of Ac-VVIA to the C-terminal part of A β 42 is slightly reduced in comparison to that of VVIA,¹⁷ the binding of VVIA-NH₂ to the C-terminal part of A β 42 is significantly increased. Electrostatic interactions contribute to these changes, as the negatively charged C-terminus of Ac-VVIA repels the negatively charged C-terminus of A β 42, while the positively charged N-terminus of VVIA-NH₂ is attracted to the negatively charged C-terminus of A β 42. This attraction is manifest in a strong binding energy of VVIA-NH₂ to A β 42 of -105 kcal/mol which is dramatically larger than the computed

binding energy of Ac-VVIA of -20 kcal/mol (The binding energy for each representative structure of the complexes is noted in Figures S2 and S3). The correlation between the ability of VVIA-NH₂ to inhibit A β 42 oligomerization and toxicity and binding of this peptide specifically to the C-terminal hydrophobic region of A β 42 implies an important role of the C-terminal region in A β 42 structure stability, assembly, toxicity and its inhibition by C-terminal fragments.

A β 42 monomer complexes

The ATDs of $z/n = -3$ A β 42 monomer and monomer complexes for A β 42 and VVIA analogs were recorded and the results are shown in Figure S4-5 and Table S1. The ATDs of the $z/n = -3$ monomer and monomer with 1 VVIA analog complexed are shown in Figure 6. The ATD of A β 42 monomer shows two features which were previously assigned as a gas-phase-like compact conformer (M1) and a solution-like conformer (M2) (Figure 6a).⁵¹ The ATD of A β 42 monomer with one VVIA-NH₂ or Ac-VVIA bound shows two similar features (Figures 6b and c). By analog, they can be assigned as a gas-phase-like conformer and a solution-like conformer. The cross sections were measured and showed ~ 5 -6% increase in size after the addition of one VVIA analogy (Table 1), which is probably due to adding the four residues of the CTFs. The experimental cross section for the solution-like structure is similar to the theoretical values for the most populated structure (Table 1).

Discussion and Conclusions

Previous study has shown that VVIA-NH₂ inhibits A β toxicity while Ac-VVIA does not.¹⁸ Our mass spectrometry and ion mobility studies reveal that VVIA-NH₂ and Ac-VVIA have different effects on the A β 42 early assembly. VVIA-NH₂ binds directly not only to A β 42 monomer (up to two VVIA-NH₂ molecules bound to A β 42 monomer were observed) but also to A β 42 small oligomers (dimers, tetramers and hexamers). VVIA-NH₂ importantly inhibits dodecamer formation and removes preformed dodecamers. On the other hand, only one Ac-VVIA molecule bound directly to A β 42 monomer was observed. Ac-VVIA also appears to modulate dodecamer formation. However, the A β 42 sample with addition of Ac-VVIA shows broader ATDs indicating more complicated structures and oligomer distributions and the sample clogged the nanospray tip easily. The nanoESI completely stopped working after three hours' incubation indicating that large aggregates formed in the solution, likely through pathways that bypass dodecamer formation. These differences reflect the different binding of VVIA-NH₂ and Ac-VVIA to A β 42 and are consistent with their different abilities to inhibit A β 42 toxicity. A summary of the differences in assembly between the various systems is given in Figure 7. It is worth noting that uncapped VVIA reacts similarly to VVIA-NH₂ in that they both bind to A β 42 monomer and oligomers and both strongly reduce the toxicity of A β 42.¹⁷ Hence, it appears important for the CTF to bind not only to A β 42 monomer but also to the small oligomers to be effective.

Our MD binding simulations show significantly different binding interactions for VVIA-NH₂ and Ac-VVIA with A β 42. Ac-VVIA binds dispersedly to A β 42 at multiple sites, including the C-terminal, central region and the N-terminal regions. In contrast, VVIA-NH₂, the effective A β 42 inhibitor, binds specifically to only the C-terminal β -hairpin region of

A β 42. This is of significance and implies that the interaction at the C-terminal region, rather than at other regions, may be the key for the inhibition activity of A β (39-42).

The C-terminal hydrophobic region of A β 42 has been considered to play an important role in the structure stability and assembly of A β 42.^{7,52} A recent study of A β 42 fibrils showed that the C-terminal Ala42, absent in A β 40, formed a unique salt bridge with Lys28, to create an A β 42- selective self-recognizing and self-replicating amyloid-propagation machinery in AD pathology⁵³. Moreover, previous studies of prefibrillar A β 42 showed that Ile41 and Ala42 residues stabilize the C-terminal turn conformation which results in a more rigid C-terminus for A β 42 than A β 40.¹³ The increased conformation stability of C-terminus is correlated with the formation of more toxic oligomers in A β 42, which explains how the difference of only two residues between A β 40 and A β 42 can significantly change the toxicity and aggregation properties of A β proteins.¹³ These C- terminal hydrophobic residues in A β 42 have been considered to be the driving force for protein folding and self-assembly, and stabilize neurotoxic low-order oligomers.¹⁴ Therefore the hypothesis that peptides derived from the C-terminus of A β 42 may be coassembled into A β 42 monomer and oligomers, and disrupt their structures and thereby inhibit their toxicity, led to the successful discovery of effective C-terminal fragment inhibitors.¹⁶ However, recent studies using intrinsic tyrosine fluorescence and NMR methods suggested that A β (39-42) might primarily interact with the N-terminus of A β 42.¹⁸ Our earlier simulations on the binding of A β (39-42) to A β 42 corroborated this picture, with A β (39-42) observed to bind to multiple sites of A β 42. These regions included the C-terminal, central hydrophobic core and the N-terminal regions.¹⁷ These studies were intriguing, because they did not seem in line with the original hypothesis that the C-terminal peptide inhibitors would specifically target the C-terminus of A β 42. The peptide inhibitor, VVIA-NH₂, binds specifically to the C-terminus of A β 42 monomers and oligomers resulting in effective inhibition of A β 42 toxicity. However VVIA is also an effective inhibitor of toxicity and an indiscriminate binder to A β 42 so C-terminal binding cannot be the exclusive determining factor. What VVIA and VVIA-NH₂ have in common is strong binding to both monomers and oligomers of A β 42 whereas Ac-VVIA binds more weakly to A β 42 monomer and not at all to its oligomers. Thus, binding to A β 42 oligomers appears to be crucial for inhibition of toxicity, but more research is needed to fully understand the mechanism at play.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

a-c) Mass spectra of Aβ42 samples with and without CTF molecules. The charge states of each species are labeled with z/n , where z is charge and n is oligomer number. The Aβ42 peaks are denoted with rectangles, CTF molecules are denoted with circles and the complexes of Aβ42 with CTF molecules are denoted with asterisks. d-f) ATDs of the $z/n = -5/2$ peak for Aβ42 samples with and without CTF molecules. The oligomer number (n) is noted for each feature. The dashed lines represent the peak shape expected for a single structure in the ATD.

Figure 2.

ATD of the $z/n = -5/2$ complex peak for A β 42 sample with VVIA-NH₂. The oligomer number (n) is noted for each feature.

Figure 3.

A time-dependent study of dissociation of preformed A β 42 oligomers by VVIA derivatives.

a) ATD of the $z/n = -5/2$ peak for A β 42 sample which was preincubated on ice for five hours. b-d) ATDs for the $z/n = -5/2$ peak for A β 42 sample after the addition of VVIA-NH₂ recorded at 10 min, 2 hr and after 1 day. e-g) ATDs of the $z/n = -5/2$ peak for A β 42 sample after the addition of Ac-VVIA recorded at 10 minutes, 2 hours and 3 hours. The oligomer number is noted for each feature in the ATDs. In Figure 3g, the peak at 840 μ s is a noise peak.

Figure 4.
a-c) TEM images for Aβ42 samples incubated for 2 weeks without and with VVIA derivatives. The scale bar is 200 nm.

Figure 5. Interactions of Aβ42 with VVIA-NH₂ (**A** and **C**) or Ac-VVIA (**B** and **D**). The N-termini and C-termini of Aβ42 are indicated by blue and red balls respectively. First row(**A-B**): superposition of the complexes where the protein backbones are represented by the grey lines and the VVIA derivatives are noted by the larger cyan balls. Second row (**C-D**): representative bound complexes of the most populated structural families from the clustering analysis. The abundance and collision cross section are noted. Only the side-chains in contact with VVIA-NH₂/Ac-VVIA are shown (blue: positive charged, red: negative charged, black: hydrophobic, and green: hydrophilic). 3-10-helical, β-extended, turn and coiled conformation are colored in blue, yellow, cyan and white.

Figure 6.

The ATDs of $z/n = -3$ monomer and complex: a) A β 42; b) A β 42-VVIA-NH₂ complex; and c) A β 42-Ac-VVIA complex. M1 and M2 represent two monomer conformers for A β 42.

Figure 7.

Aggregation mechanism of Aβ42 in the absence or presence of VVIA-NH₂ or Ac-VVIA. VVIA-NH₂ binds directly to Aβ42 monomer and small oligomers (dimer, tetramer and hexamer), inhibiting the formation of dodecamer and driving the formation of nontoxic oligomers that eventually form fibrils. Ac-VVIA binds only to Aβ42 monomer, inhibiting the formation of dodecamer, however, Ac-VVIA binding may lead to other pathways bypassing dodecamer and form other toxic oligomers or aggregates which eventually form fibrils.

Table 1

Experimental and theoretical cross sections (σ) of $z/n = -3$ A β 42 monomer and complexes. The error of the experimental cross sections is within 1%.

Species	A β 42		+ 1 VVIA-NH2		+ 1 Ac-VVIA	
	M ₁	M ₂	M ₁	M ₂	M ₁	M ₂
Experimental σ ($\text{\AA}^2$)	631	707	668	748	670	742
PSA σ ($\text{\AA}^2$)				750		759