ACS Chemical Neuroscience

Research Article

pubs.acs.org/chemneuro

Amyloid β-Protein Assembly: Differential Effects of the Protective A2T Mutation and Recessive A2V Familial Alzheimer's Disease Mutation

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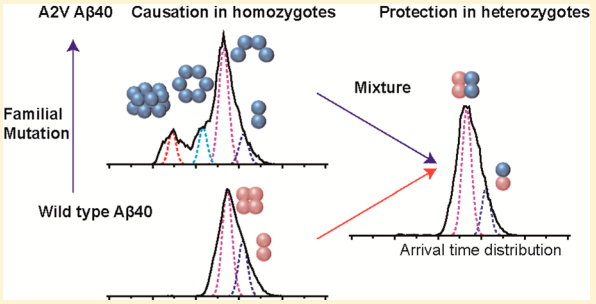
Supporting Information

ABSTRACT: Oligomeric states of the amyloid β-protein (Aβ) appear to be causally related to Alzheimer's disease (AD). Recently, two familial mutations in the amyloid precursor protein gene have been described, both resulting in amino acid substitutions at Ala2 (A2) within Aβ. An A2V mutation causes autosomal recessive early onset AD. Interestingly, heterozygotes enjoy some protection against development of the disease. An A2T substitution protects against AD and age-related cognitive decline in non-AD patients. Here, we use ion mobility-mass spectrometry (IM-MS) to examine the effects of these mutations on Aβ assembly. These studies reveal different assembly pathways for early oligomer formation for each peptide. A2T Aβ42 formed dimers, tetramers, and hexamers, but dodecamer formation was inhibited. In contrast, no significant effects on Aβ40 assembly were observed. A2V Aβ42 also formed dimers, tetramers, and hexamers, but it did not form dodecamers. However, A2V Aβ42 formed trimers, unlike A2T or wild-type (wt) Aβ42. In addition, the A2V substitution caused Aβ40 to oligomerize similar to that of wt Aβ42, as evidenced by the formation of dimers, tetramers, hexamers, and dodecamers. In contrast, wt Aβ40 formed only dimers and tetramers. These results provide a basis for understanding how these two mutations lead to, or protect against, AD. They also suggest that the Aβ N-terminus, in addition to the oft discussed central hydrophobic cluster and C-terminus, can play a key role in controlling disease susceptibility.



KEYWORDS: *Amyloid β-protein, familial Alzheimer's disease, A2T, A2V, oligomerization, ion mobility spectrometry, mass spectrometry*

Amyloid β-protein (Aβ) plays an important role in Alzheimer's disease (AD) pathogenesis.[1,2] Aβ is produced from amyloid precursor protein (APP) by successive endoproteolytic cleavages by β- and γ-secretase. Aβ exists in the body primarily in two forms, 40- (Aβ40) or 42-residues (Aβ42) in length. Although Aβ40 is present in the body at a concentration ≈10-fold that of Aβ42, the latter peptide is more toxic and is the primary component of amyloid plaques.[3] Although pathognomonic for AD, fibril-containing plaques do not appear to be the primary pathologic agents in AD: the primacy of Aβ oligomers has been suggested instead.[4,5] In solution, Aβ40 and Aβ42 monomers are both intrinsically disordered, yet they display distinct aggregation pathways on the way to fibril formation. Aβ40 initially forms small oligomers, including dimers and tetramers, whereas Aβ42 forms larger aggregates, including dimers, tetramers, hexamers, and dodecamers. These oligomeric states appear to be on-pathway for fibril formation.[6,7] Of these oligomers, the 56 kDa dodecamer has been shown to be a proximate toxic agent for AD pathology.[8,9]

Although most AD cases occur sporadically, ∼5% of AD cases are caused by mutations in the APP,[10,11] presenilin 1 (PS1),[12,13] or presenilin 2 (PS2)[14] genes. These familial AD (FAD) cases often lead to early onset of disease (<60 years of age). Numerous FAD-related mutations in the APP gene have been identified, and many of them are near β- or γ-secretase cleavage sites. This results most commonly in overproduction of Aβ or increases in the amount of Aβ42 that is produced relative to Aβ40.[15] However, as many mutations occur within the Aβ region, it is very likely that these substitutions would alter the structural and aggregation properties of the resultant Aβ42 and Aβ40 peptides. Notably, many mutations in the APP gene result in amino acid substitutions within the central region of Aβ, such as, for example, Flemish (A21G),[16] Arctic (E22G),[17] Dutch (E22Q),[18] Osaka (E22Δ),[19] Italian (E22K),[20] and D23N (Iowa)[21] mutations. The resulting peptides exhibit distinct aggregation propensities and toxicities.

Received: June 22, 2015
Revised: August 2, 2015
Published: August 5, 2015

DOI: 10.1021/acschemneuro.5b00171
ACS Chem. Neurosci. 2015, 6, 1732−1740

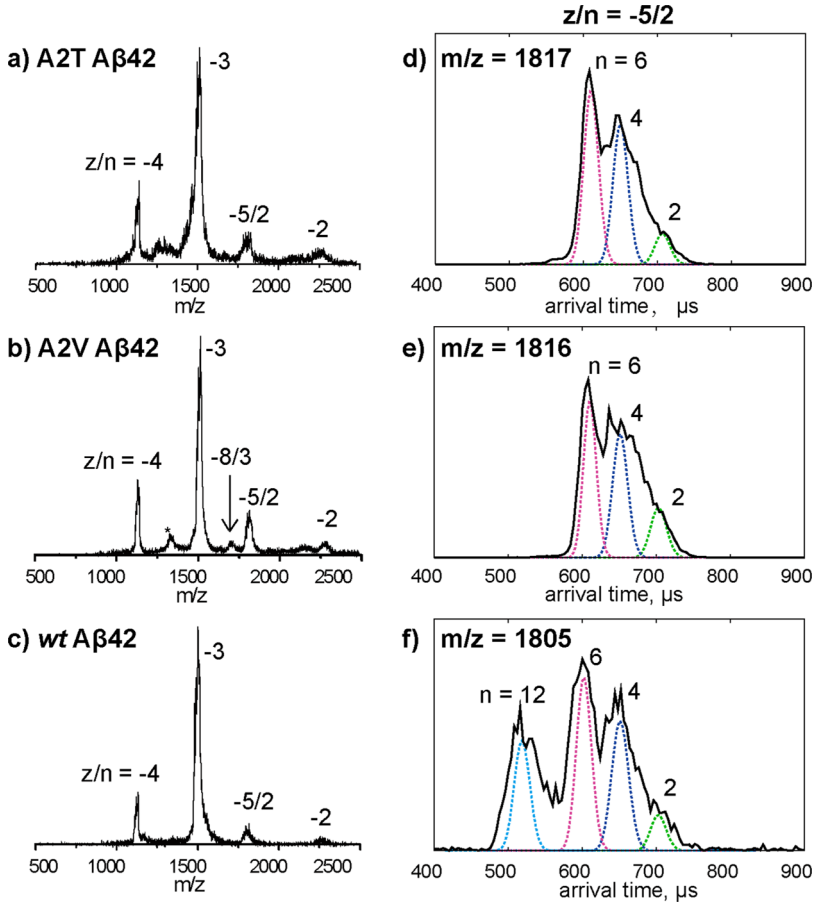


Figure 1. (a–c) Mass spectra of A2T, A2V, and wt Aβ42. The charge state of each species is noted with z/n, where z is the charge and n is oligomer number. The peak marked with an asterisk (*) in panel b is assigned as a fragment peak or impurity (see discussion in the Supporting Information). (d–f) ATDs of $z/n = -5/2$ peaks for A2T, A2V, and wt Aβ42. The oligomer order (n) is noted for each feature. The dashed lines represent the peak shape for a single conformation. Injection energy studies of the $z/n = -5/2$ A2T and A2V Aβ42 peaks are provided in Figure S1. The injection energy in panels d–f is 40 eV.

The central region of Aβ has been shown to be crucial for the initial nucleation of Aβ folding and assembly.[22] Mutations near this region may disrupt the conformation of Aβ, resulting in increased aggregation propensity and formation of toxic oligomers.[23] On the other hand, the role of the N-terminus in aggregation, toxicity, and pathology has been less thoroughly studied due to the fact that this region appears to be disordered in the fibril state.[24,25] However, as with the central region of Aβ, a number of APP mutations result in amino acid substitutions at the N-terminus, and these substitutions alter Aβ assembly. These include the English (H6R),[26,27] Tottori (D7N),[26−29] and Taiwanese (D7H)[30] mutations. The importance of the Aβ N-terminus in disease causation thus is clear. Most recently, two new APP mutations have been described that result in the substitutions A2T and A2V can be important in Aβ structure and assembly.[31,32] In the work presented here, we elucidate the effects on early Aβ assembly of these two recently discovered mutations.

The A2T substitution substantially decreases AD risk as well as protects against age-related cognitive decline in the elderly without AD.[31] It is thought to be the first example of a sequence variant that protects against AD. The A2T substitution occurs immediately adjacent to the β-secretase site, and, indeed, the mutation has been found to reduce Aβ production ~20% in heterozygous carriers. Such a reduction may be responsible for its protective function in AD pathology.[31] However, as the mutation is within the Aβ sequence, it is possible that the A2T mutation also changes the aggregation properties of Aβ proteins, thus contributing to its protective effect, a possibility that we investigate here.

The mutation causing the A2V substitution results in early onset AD in homozygotes, whereas some protection against AD is observed in heterozygotes.[32] In contrast to the A2T substitution, A2V increases Aβ production. Interestingly, coincubation of A2V Aβ42 and wt Aβ42 produced slower aggregation rates than those exhibited by either peptide alone, as well as decreased toxicity.[32] The A2V substitution accelerates Aβ42 oligomerization and also leads to the production of annular structures with a higher hydrophobicity than that of wt Aβ42.[33]

A consensus regarding the effects of the A2T and A2V substitutions on Aβ assembly has not been reached. Two recent studies of A2T and A2V peptides reported different aggregation kinetics by thioflavin T (ThT) fluorescence studies. Benilova et al. showed that the A2T substitution has little effect on Aβ42 aggregation, but it did affect its solubility.[34] Maloney et al., in contrast, showed that the A2T mutant had a lower aggregation propensity compared to that of the A2V mutant or wt Aβ42.[35] For Aβ40, the A2T mutant was shown to aggregate similarly to wt, whereas the A2V mutant exhibited faster aggregation and a shorter lag phase, making this peptide behave more Aβ42-like.[34,35]

DOI: 10.1021/acschemneuro.5b00171
ACS Chem. Neurosci. 2015, 6, 1732−1740

To improve our understanding of the A2T and A2V substitutions, we used ion mobility coupled to mass spectrometry (IM-MS) to examine the early assembly and subsequent aggregation of these mutant peptides. IM-MS can separate species with the same mass-to-charge (m/z) ratio but different shapes or sizes.[36] As a consequence, it has successfully revealed the structures on Aβ oligomers and the effects of small molecule inhibitors of Aβ assembly.[7,29,37−42] We examine here the early oligomer distributions of A2T- and A2V-containing Aβ40 and Aβ42 to understand how each assembles and whether the early assembly pathways are identical or different. We also examine the early oligomer distributions of mixtures of wt and mutant peptides to understand how each affects the other's assembly. This provides the means to model *in vitro* the homozygous and heterozygous states that exist in humans. These studies provide mechanistic insights into the etiology of FAD, mechanisms of protection from FAD, and potential targets for therapeutic agents.

RESULTS

Different Oligomer Distributions of wt and Mutant Aβ42. Mass spectra of wt Aβ42, A2T, and A2V were recorded individually and are shown in Figure 1a−c. Four common peaks were observed for each peptide, corresponding to z/n ratios of −4, −3, −5/2 and −2, where z is charge and n is oligomer size. The mass spectrum of A2V Aβ42 was interesting because, in addition to the four peaks, another peak was observed between z/n = −3 and −5/2 in the spectrum, corresponding to z/n = −8/3. This indicates the A2V mutant forms a trimer, which is not observed for wt or A2T Aβ42. Moreover, there is another peak between z/n = −4 and −3 for A2V, denoted by an asterisk (*), which is assigned as a fragment peak or impurity (see Supporting Information, Figure S3, for a detailed discussion of this peak assignment).

The arrival time distributions (ATDs) of the z/n = −5/2 peaks for all three Aβ42 alloforms are shown in Figure 1d,e. The ATD of wt Aβ42 shows four features, with arrival times at ∼710, 670, 610, and 540 μs, which were previously assigned as Aβ42 dimer, tetramer, hexamer, and dodecamer, respectively, based on their collision cross sections (see ref 7 for a detailed discussion of these assignments). However, the ATD of A2T or A2V Aβ42 (Figure 1d or e) shows only three features, with arrival times at ∼710, 670, 610 μs, which were assigned as dimer, tetramer, and hexamer, respectively, based on their cross sections. There is no feature at lower arrival time observed in either of the ATD for mutants, indicating that no other oligomers larger than hexamers are formed. These results suggest the formation of Aβ42 dodecamer is inhibited by both A2T and A2V mutations.

To assign the peaks in the ATDs unambiguously, and to better understand the oligomer distributions of the Aβ42 mutants, the −5/2 ATDs for Aβ42 mutants were measured at different injection energies. At low injection energy, the ions are rapidly thermalized by cooling collisions with the helium gas in the drift cell and therefore large complexes can be preserved through the process. At high injection energy, the ions are given sufficient energy to lead to internal excitation, which can cause isomerization into a low energy structure or dissociation of large noncovalent complexes into smaller species. As shown in Figure S1, the ATDs measured at intermediate injection energy (40 eV) are the same ones shown in Figure 1d,e. When the injection energy is lowered to 25 eV (Figure S1 top panel), the hexamer peak becomes especially prominent, whereas the tetramer and dimer features decrease. However, there are still no peaks with earlier arrival times observed, suggesting that oligomers of size dodecamer or larger are not formed in solution. At high injection energy (100 eV, Figure S1, bottom panel), the hexamer peak disappears, whereas the tetramer and dimer peaks dominate the spectrum. This suggests hexamer dissociation into smaller oligomers. These injection energy studies are fully consistent with the assignment of the three peaks in the ATDs as dimer, tetramer, and hexamer.

Ion Mobility Study of z/n = −2 and −8/3 Peaks: A2V Aβ42 Forms Trimers. The z/n = −2 Aβ42 is a relative low charge state of the Aβ42 alloforms and possibly consists of high order oligomers, making its ATD of interest. The signal of the z/n = −2 peak for wt Aβ42 is too low to obtain a reliable ATD; therefore, no data for it is shown. However, we were able to record ATDs for the −2 peaks of A2T and A2V Aβ42 (Figure 2).

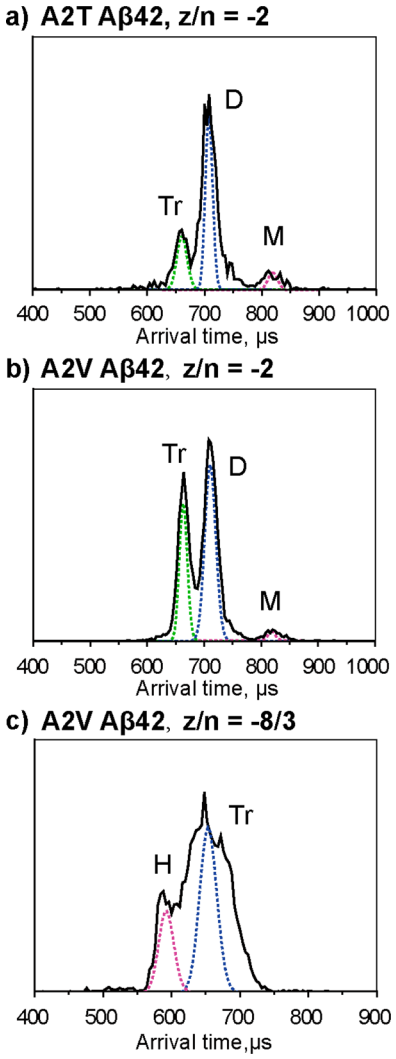


Figure 2. (a, b) ATDs of the z/n = −2 peaks for A2T and A2V Aβ42, respectively. (c) ATD of the z/n = −8/3 peak for A2V. The dashed lines represent the peak shape for a single conformation. The oligomer order is noted for each feature, where M represents monomer, D represents dimer, Tr represents trimer, and H represents hexamer. The injection energy is 40 eV. Summary of ATDs for each peak and their cross sections for Aβ42 alloforms is given in Figure S7.

DOI: 10.1021/acschemneuro.5b00171
ACS Chem. Neurosci. 2015, 6, 1732−1740

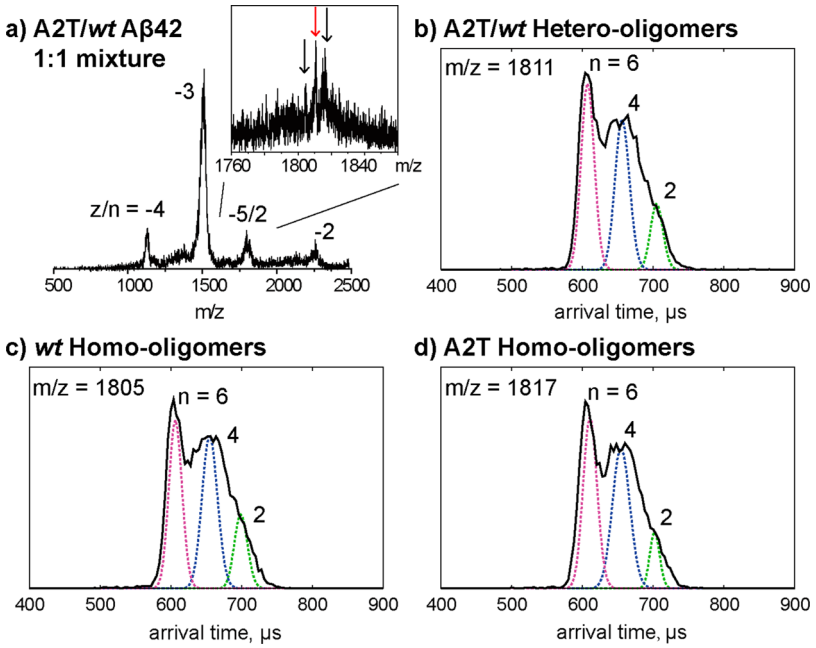


Figure 3. Ion mobility study of an equimolar mixture of wt and A2T Aβ42. (a) Full mass spectrum of a wt/A2T Aβ42 mixture and a zoomed-in spectrum of $z/n = -5/2$ peaks that contains three species, which correspond to wt Aβ42 homo-oligomers, wt/A2T Aβ42 hetero-oligomers, and A2T Aβ42 homo-oligomers. (b–d) ATDs of the three −5/2 oligomer peaks. The oligomer order (n) is noted for each feature. The dashed lines represent the peak shape for a single conformation. The injection energy in panels b–d is 40 eV.

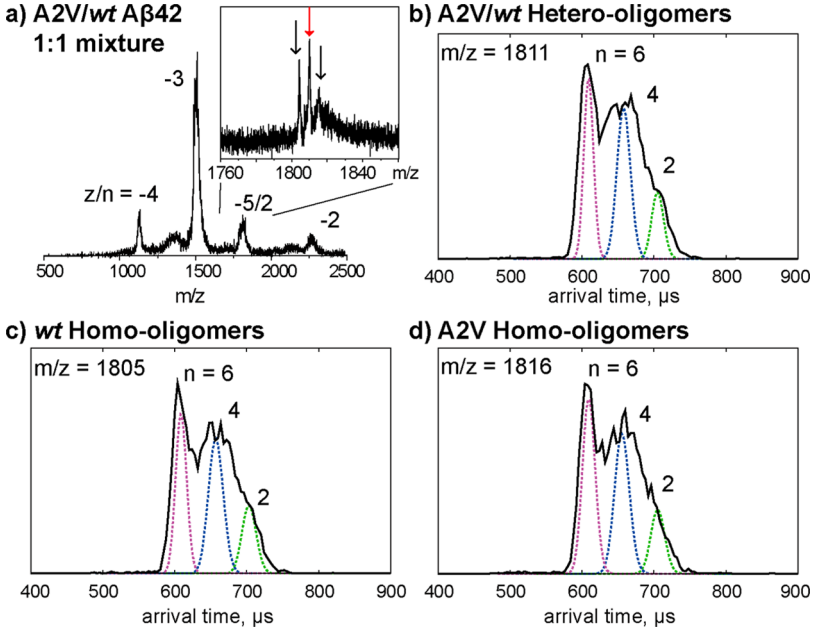


Figure 4. Ion mobility study of an equimolar mixture of wt Aβ42 and A2V mutant. (a) Full mass spectrum of A2V/wt Aβ42 mixture and a zoomed-in spectrum of $z/n = -5/2$ peaks that contains three species, which correspond to wt Aβ42 homo-oligomers, wt/A2V Aβ42 hetero-oligomers, and A2V Aβ42 homo-oligomers. (b–d) ATDs of the three −5/2 oligomer peaks. The oligomer order (n) is noted for each feature. The dashed lines represent the peak shape for a single conformation. The injection energy in panels b–d is 40 eV.

The ATD of −2 A2T Aβ42 shows three features, with arrival times at ~820, 720, 670 μs, which can be assigned as monomer, dimer, and trimer, respectively. Similarly, the ATD of the $z/n = -2$ A2V Aβ42 shows three features, corresponding to monomer, dimer, and trimer. However, the relative intensity of the A2T trimer is much lower than that of its dimer, whereas the relative intensity of the A2V trimer is comparable to that of its dimer, indicating that the formation of trimer is more favored for A2V Aβ42. Injection energy results (Figure S2b) support this trend. At low injection energies, the trimer of A2V Aβ42 is dominant, whereas the trimer of A2T Aβ42 remains minor, indicating that trimer in the A2V mutant is significant in solution.

The ATD of $z/n = -8/3$ A2V Aβ42 shows two features, with arrival times at ~660 and 590 μs, which correspond to an A2V trimer and hexamer, respectively (Figure 3c). The breadth of the trimer feature indicates that there is a family of trimer structures existing in the solution. The injection energy study of

DOI: 10.1021/acschemneuro.5b00171
ACS Chem. Neurosci. 2015, 6, 1732–1740

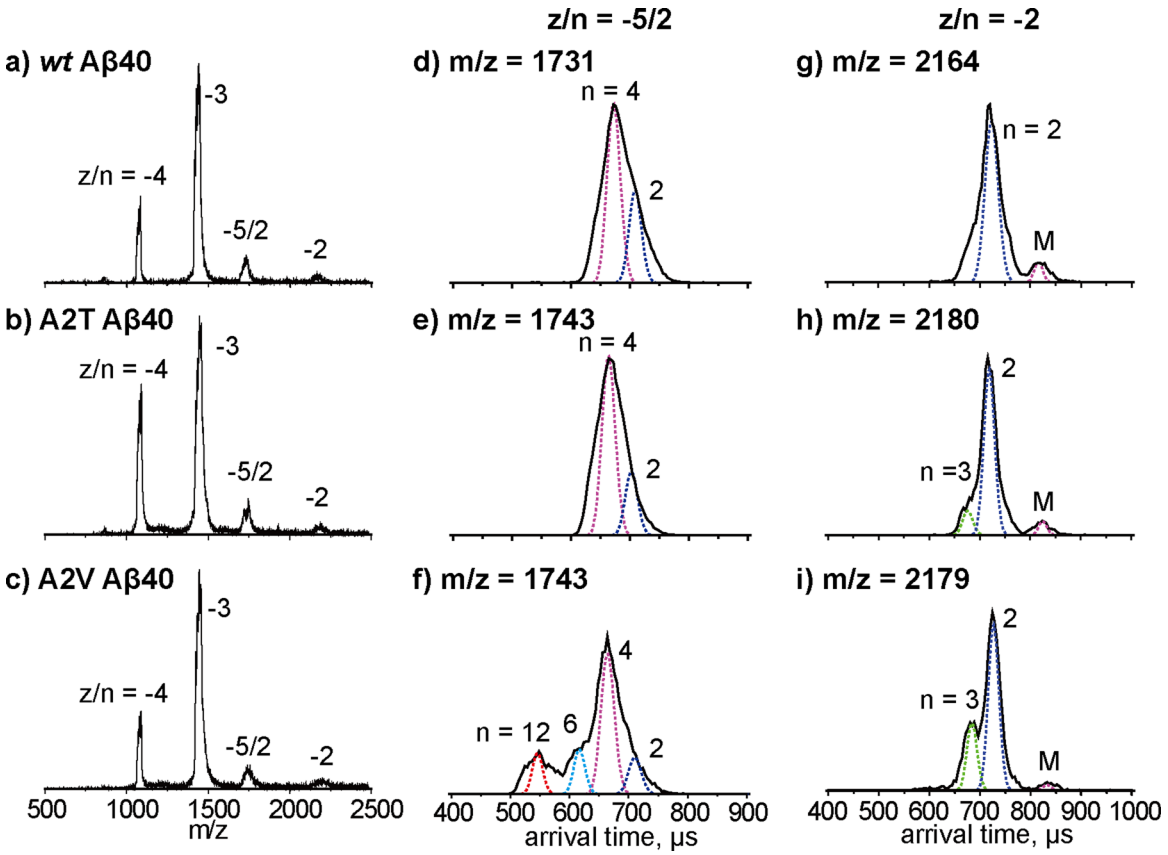


Figure 5. (a–c) Mass spectra of wt, A2T, and A2V Aβ40. The charge state of each species is noted with z/n, where z is the charge and n is oligomer number. (d–f) ATDs of $z/n = -5/2$ peaks for wt, A2T, and A2V Aβ40. (g–i) ATDs of $z/n = -2$ peaks for wt, A2T, and A2V Aβ40. The oligomer order (n) is noted for each feature. The dashed lines represent the peak shape for a single conformation. The injection energy in panels d–i is 40 eV. Summary of ATDs for each peak and their cross sections for Aβ40 alloforms is given in Figure S8.

−8/3 A2V Aβ42 (Figure S2c) indicates that the hexamer feature increases at lowest energies and the trimer peak gets sharper. At high energy (100 eV), the hexamer feature disappears and the broad trimer feature becomes the dominant peak.

Taken together, these ion mobility results reveal that the oligomerization pattern is different for each of the alloforms. wt Aβ42 forms dimer, tetramer, hexamer, and dodecamer. A2T and A2V Aβ42 form dimer, tetramer, and hexamer, without the formation of dodecamer, but A2V forms a significant trimer, which is only very minor in A2T and may not be present in wt Aβ42 at all.

Transmission electron microscopy (TEM) images were recorded for the same Aβ42 samples after 5 days of incubation at room temperature, and the results are shown in Figure S9. The wt Aβ42 forms long fibrils after 5 days of incubation, whereas A2T and A2V Aβ42 form some short fibrils or protofibrils along with some long fibrils. These results are consistent with those of a previous study[34] and suggest that the oligomers we detected are on-pathway.

Mixtures of wt and Mutant Aβ42: Effects on wt Aβ42 Oligomerization. The A2T mutation has been shown to protect carriers from AD or normal age-related cognitive decline.[31] To model the effects of this peptide in heterozygotes, we created an equimolar mixture of A2T and wt Aβ42 and then performed MS (Figure 3). Four sets of peaks were observed, corresponding to $z/n = -4$, −3, −5/2, and −2 charge states. A zoomed-in spectrum of the −5/2 region using the QTOF-MS was obtained and shows that there are three peaks with charge state of −5/2, which correspond to −5/2 wt Aβ42 homo-oligomers, wt/A2T hetero-oligomers (1:1 ratio), and A2T homo-oligomers. The ATDs of these three peaks (Figure 3b–d) display a similar oligomer distribution with three features, with arrival times of ∼710, 670, and 600 μs. We assign these features as dimers, tetramers, and hexamers, respectively. Note that no feature at shorter arrival time was observed, indicating that there is no homo/hetero-dodecamer or higher oligomer formation. These results indicate that the A2T mutant forms small hetero-oligomers (up to heterohexamers) with wt Aβ42 and inhibits the formation of wt Aβ42 dodecamer or higher oligomers.

Previous studies showed that A2V is a recessive mutation that causes early onset of AD in homozygotes but appears to be protective in heterozygotes.[32] To provide an insight into this these observation, we performed ion mobility studies on an equimolar mixture of wt and A2V Aβ42 (Figure 4). Similar to the A2T/wt mixture, the A2V/wt mixture shows three −5/2 peaks, corresponding to wt Aβ42 homo-oligomers, wt/A2V hetero-oligomers (1:1 ratio), and A2V homo-oligomers. The ATDs of these −5/2 peaks all show three features that can be assigned as dimer, tetramer, and hexamer, respectively. The data show that A2V Aβ42 forms small hetero-oligomers (only up to hexamers) with wt Aβ42 and prevents the formation of larger oligomers.

There is no −8/3 trimer peak observed in the equimolar mixture of wt and A2V Aβ42. Moreover, the ATD of the $z/n = -2$ peak for the wt/A2V mixture (Figure S4b) shows a dominant dimer peak and only a minor trimer peak, unlike that

DOI: 10.1021/acschemneuro.5b00171
ACS Chem. Neurosci. 2015, 6, 1732–1740

of A2V alone (Figure 2b). These results indicate that A2V trimer formation is inhibited by wt Aβ42.

Ion Mobility Study of Aβ40 Mutants: A2V Aβ40 Forms Hexamer and Dodecamer. We next examined the effects of the A2T and A2V substitutions on Aβ40 assembly (see Figure 5). The mass spectra for the A2T and A2V mutants (Figure 5b,c) showed four peaks, corresponding to $z/n = -4$, -3, $-5/2$, and -2, which is similar to that of wt Aβ40 (Figure 5a).

The ATD of $z/n = -5/2$ wt Aβ40 (Figure 5d) displays two features, with arrival times at ~710 and 670 μs, which were previously assigned as Aβ40 dimer and tetramer, respectively.[7] The ATD of the $z/n = -5/2$ A2T Aβ40 (Figure 5e) again shows two features, with arrival times at ~710 and 670 μs, corresponding to dimer and tetramer, respectively. This ATD was similar to that of wt Aβ40. However, the ATD of $z/n = -5/2$ A2V Aβ40 (Figure 5f) shows four features, with arrival times of ~710, 670, 620, and 550 μs, which can be assigned as dimer, tetramer, hexamer, and dodecamer, respectively. Hence, A2V Aβ40 forms hexamers and dodecamers, something not observed for wt or A2T Aβ40. This is consistent with previous ThT studies showing that A2V Aβ40 displays a shorter lag phase during aggregation, which is similar to that of wt Aβ42.[34,35]

The ATDs of $z/n = -2$ Aβ40 alloforms were recorded and are shown in Figure 5g–i. The ATD of wt Aβ40 shows a dominant dimer peak at ~720 μs and a small monomer peak at ~840 μs. The dimer peak is slightly broad at the bottom, which indicates that there might be a small amount of trimer formed. The ATDs of A2T and A2V Aβ40 (Figure 5h,i) show one additional peak, with a shorter arrival time at ~680 μs, which is assigned as trimer. The relative intensity of the A2V trimer is greater than that of the A2T trimer or wt Aβ40 trimer. This is consistent with the results of $z/n = -5/2$ peaks, which suggests that A2V Aβ40 is aggregating into larger oligomers than are A2T and wt Aβ40.

In summary, the A2T mutation does not significantly change Aβ40 oligomerization. The A2V mutation, in contrast, promotes Aβ40 oligomerization and causes it to undergo a more Aβ42-like aggregation process. Although the relative intensity of the A2V dodecamer is smaller than that observed for wt Aβ42 (Figure 1f), the Aβ40 isoform is 10 times more abundant than Aβ42 *in vivo*. Hence, this is a significant result and is fully consistent with the fact that homozygous carriers of the A2V mutation develop early onset AD.

The TEM results of Aβ40 samples after 5 days of incubation (Figure S9) showed that they all formed short fibrils along with some annuli-like aggregates. Interestingly, the Aβ40 A2V fibrils are thinner and tend to clump together to form plaques, indicating that the aggregation of Aβ40 A2V is faster than that of wt or A2T Aβ40.

The results of coincubation experiments using wt and mutant Aβ40 are shown in Figures S5 and S6. The A2T/wt Aβ40 mixture shows formation of homo/hetero-dimer and tetramer, which is similar to that of wt Aβ40, indicating no enhancement of aggregation by A2T. Similarly, the A2V/wt mixture shows only homo- and heterodimer and tetramer. This is important because it indicates wt Aβ40 inhibits formation of A2V hexamer or dodecamer. Hence, heterozygous A2V carriers are protected from dodecamer formation, whereas homozygous A2V carriers are not.

DISCUSSION

Our results show that amino acid substitutions at Ala2 of Aβ affect Aβ oligomerization (summarized in Figure 6). The

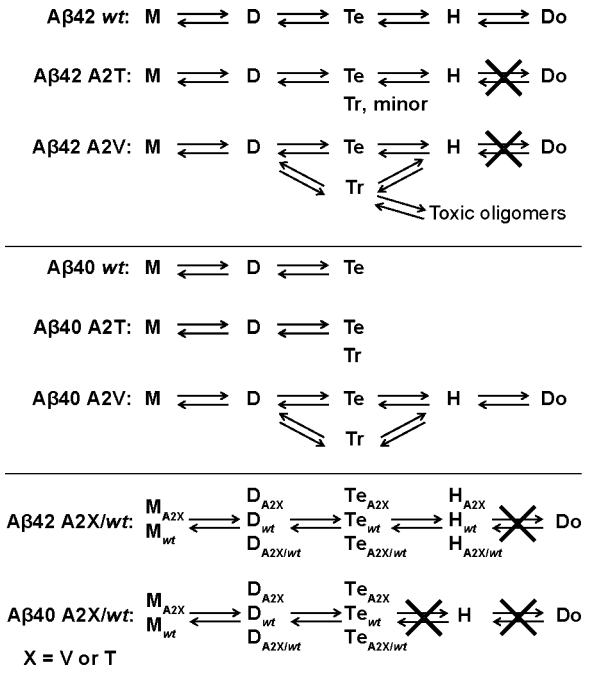


Figure 6. Different oligomerization patterns for wt Aβ, A2T and A2V alloforms, and mixtures. M, D, Tr, Te, H, and Do represent monomer, dimer, trimer, tetramer, hexamer, and dodecamer, respectively.

Iceland mutation A2T was observed to prevent the formation of Aβ42 dodecamer, which was previously identified as an important neurotoxin in AD.[8,9] These results are consistent with previous studies demonstrating that A2T is a protective mutation.[31] However, our ion mobility studies show that the A2T mutation does not have a significant effect on oligomerization of the less toxic Aβ40 isoform.

The A2V mutation was observed to inhibit the formation of Aβ42 dodecamer as well. However, the A2V mutation leads to a much greater fraction of Aβ42 trimer formation and observation of a unique $z/n = -8/3$ trimer peak that contains a significant fraction of hexamer not formed in A2T or wt Aβ42. This result implies that A2V Aβ42 may adopt another early assembly pathway through trimer that leads to toxic oligomers before going on to form fibrils. Even more interestingly, the A2V mutation shows significant effects on Aβ40 assembly, resulting in the formation of Aβ40 hexamer and dodecamer, which are not observed for A2T or wt Aβ40. Hence, the A2V mutation changes the Aβ40 aggregation pathway into a Aβ42-like pathway, which is consistent with a previous ThT fluorescence study showing that A2V has a shorter aggregation lag phase than that of wt Aβ40.[34] Although the relative intensity of dodecamer in A2V Aβ40 is smaller than that for wt Aβ42 (Figure 1f), this peptide is 10 times more abundant than Aβ42 *in vivo*; hence, the fact that it produces potentially toxic oligomer states will be strongly amplified *in vivo*. This result, while not proof, is entirely consistent with the fact the A2V mutant results in early onset AD in homozygous carriers.[32]

The effects of the A2T and A2V substitutions on wt Aβ42 oligomerization were evaluated by coincubating equimolar

DOI: 10.1021/acschemneuro.5b00171
ACS Chem. Neurosci. 2015, 6, 1732–1740

mixtures of the mutant and wt Aβ42 proteins. Both mutants formed small hetero-oligomers with wt Aβ42, including dimers, tetramers, and hexamers. However, no hetero- or homododecamers were observed, indicating that the formation of Aβ42 dodecamer is inhibited by the mutants. Co-incubation of A2T and wt Aβ40 shows formation of dimers and tetramers, which is similar to that of wt Aβ40, indicating no enhancement of aggregation by A2T (Figure S5). However, coincubation of A2V and wt Aβ40 shows only homo- and heterodimer and tetramer formation, indicating that hexamer and dodecamer formation is inhibited (Figure S6). This indicates that rapid A2V aggregation is inhibited by wt Aβ40. These results are consistent with previous studies suggesting that A2T protects against AD and that A2V heterozygous carriers are not affected by this mutation.[31,32]

The N-terminus of Aβ is relatively hydrophilic and appears to exist in a disordered state. It thus has been argued that it plays only a modest (or no) role in controlling Aβ assembly compared to that of the central hydrophobic cluster region or the C-terminus.[24] However, we find here that single A2T and A2V amino acid substitutions *do* affect Aβ oligomerization quite significantly, offering a mechanistic explanation for the phenotypes of humans expressing the cognate genes.

Threonine (T) and valine (V) have similar sizes but different hydrophobicities. The substitution of the neutral alanine (A) with a nucleophilic threonine or a hydrophobic valine will change the hydrophobicity of the N-terminus region and perhaps change the conformation of Aβ. A recent simulation study of A2T and A2V Aβ42 showed significantly different conformational landscapes of the Aβ42 monomer.[43] The A2T Aβ42 mutant makes the N-terminus more polar, which displays unusual long-range electrostatic interactions with residues such as Lys16 and Glu22.[43] Through such electrostatic interactions, the hairpin structure in the central hydrophobic region is disrupted, resulting in a population of unique conformations with only a C-terminal hairpin. In contrast, A2V Aβ42 shows an enhanced double-hairpin population due to hydrophobic interactions between the N-terminus and distant hydrophobic regions (central hydrophobic core and C-terminus hydrophobic region).[43] A previous simulation showed that the A2V mutation reduced the intrinsic disorder and increased the hairpin population in the Aβ(1−28) monomer.[44] In addition, a previous MD simulation study showed that the N-terminus of Aβ40 displayed a β-strand structure at Ala-2-Phe-4, which was not present in Aβ42.[45] The hydrophilic N-termini of Aβ proteins are on the surface of the oligomers; thus, the presence of an N-terminal β-strand in Aβ40 might prevent the hydrophobic core of the oligomers from adding additional Aβ40 molecules to form larger oligomers, which explains why Aβ40 aggregates slower and forms smaller oligomers than Aβ42. Therefore, the substitution of Ala with a hydrophobic Val may disrupt the formation of a hydrophilic N-terminal β-strand and make the hydrophobic core accessible for other Aβ40 molecules, shifting the A2V Aβ40 oligomerization toward those of Aβ42. Our ion mobility studies reveal different oligomerization for Aβ proteins with a single mutation in the N-terminus region and imply the importance of the N-terminus region for Aβ assembly, results consistent with previous studies.[43−45]

In this work, we have demonstrated that IMS-MS is becoming a powerful tool to carry out studies that lead to understanding AD familial mutations. This is of significance, as single mutations have been implied to be important in disease etiology. For instance, the G127V mutation in a prion variant has been shown recently to completely protect transgenic mice from prion disease.[46] Hence, understanding the mechanism of these positive substitutions becomes important for future therapeutic development. Thus, IMS-MS can be used as a new tool to study other systems of this kind and provide an insight into their structure−disease relationship.

CONCLUSIONS

(1) The A2T mutation prevents formation of Aβ42 dodecamer both in homo- and heterozygotes. The dodecamer has been implicated as a proximate toxic agent in AD. (2) The A2V mutation in homozygotes also prevents dodecamer formation in Aβ42 but promotes trimer formation, which may initiate a new pathway for early oligomer formation in Aβ42. (3) The A2V mutation in homozygotes promotes hexamer and dodecamer formation in Aβ40, whereas wt Aβ40 assembly terminates at the tetramer. Since Aβ40 is 10 times more prevalent than Aβ42 *in vivo*, facilitation of Aβ40 hexamer and dodecamer formation may well explain why the A2V mutation causes early onset AD in homozygotes. (4) Both the A2T and A2V mutations eliminate dodecamer formation in heterotypic mixtures with wt Aβ40 and Aβ42, consistent with the protective effects of these substitutions in heterozygotes. (5) Ion mobility methods are emerging as an important new tool in developing an understanding of the effect of familial mutations on Aβ assembly in AD and the assembly of other mutated protein systems.

METHODS

Peptide and Sample Preparation. Full-length Aβ and mutants were synthesized by *N*-9-fluorenylmethoxycarbonyl (FMOC) chemistry.[47] The peptides were purified by reverse-phase HPLC, and their quality was validated by mass spectrometry and amino acid analysis. Samples were prepared in 10 mM ammonium acetate buffer, pH 7.4, at a final peptide concentration of 10 μM. Equimolar mixtures of wt and mutant Aβ were prepared at a total peptide concentration of 10 μM (5 μM of each peptide).

Mass Spectrometry and Ion Mobility Spectrometry Analysis. Most data were recorded on a home-built ion mobility spectrometry-mass spectrometer[48] or a Micromass QTOF2 quadrupole/time-of-flight tandem mass spectrometer. The home-built instrument is composed of a nanoelectrospray ionization (nano-ESI) source, an ion funnel, a temperature-controlled drift cell, and a quadrupole mass filter followed by an electron multiplier for ion detection.

Briefly, for ion mobility measurements, ions are generated continuously by a nano-ESI source, focused, and stored in the ion funnel. A pulse of ions is injected into a temperature-controlled drift cell filled with 3−5 Torr helium gas, where they gently pass through under the influence of a weak electric field. The injection energy can be varied from ∼20 to ∼150 eV. At low injection energy, the ions are rapidly thermalized by cooling collisions with the helium gas in the drift cell. At high injection energy, the ions are given energy that can lead to internal excitation before reaching thermal equilibrium. Such internal excitation can cause isomerization into a low energy structure or dissociation of large noncovalent complexes into small species.[37] Usually, the injection energy is kept as low as possible to minimize thermal heating of the ions during the injection process. (The injection energy studies are provided in the Supporting Information, Figures S1 and S2.) The ions exiting the drift cell are mass analyzed with a quadrupole mass filter and detected by a conversion dynode and channel electron multiplier, allowing a mass spectrum to be obtained.

The pulse of ions into the drift cell starts a clock at $t = 0$ and ends at $t = t_A$ when the ions reach the detector. This allows an arrival time distribution (ATD) to be obtained. The ATD can be related to the time the ions spend in the drift cell, which is directly related to the ion

DOI: 10.1021/acschemneuro.5b00171
ACS Chem. Neurosci. 2015, 6, 1732−1740

mobility and collision cross section of the analyte ion.[49] The width of the ATD can be compared to the width calculated for a single analyte ion structure,[49] which gives information on the structural distribution favored in the ATD.

Transmission Electron Microscopy (TEM). Microscopic analysis was performed using a FEI T-20 transmission electron microscope operating at 200 kV. The Aβ samples were prepared using the same procedure as that for the mass spectrometry analysis. The samples were incubated at room temperature for 5 days. For TEM measurements, 10 μL aliquots of samples were spotted on glow-discharged, carbon-coated copper grids (Ted Pella, Inc.). The samples were stained with 10 mM sodium metatungstate for 10 min and gently rinsed twice with deionized water. The sample grids were then dried at room temperature before TEM analysis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.5b00171.

Additional IMS-MS data of injection energy studies for A2T and A2V proteins, discussion of peak assignment for possible pentamer for A2V Aβ42, ATDs of $z/n = -2$ for the A2T/wt and A2V/wt Aβ42 mixtures, IMS-MS data of mixtures of A2T or A2V and wt Aβ0, summary of ATDs and cross sections for A2T and A2V Aβ proteins, and TEM images for Aβ40 and Aβ42 proteins (PDF).

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Author Contributions

X.Z. and M.T.B. designed research; X.Z., D.L., and R.R. performed research; X.Z. and M.T.B. analyzed data; X.Z., D.B.T., and M.T.B. wrote the paper.

Funding

The work was supported by National Institutes of Health grant nos. AG047116 (to M.T.B.) and AG041295 (to D.B.T.). The Material Research Laboratory Shared Experimental Facilities are supported by the MRSEC Program of the NSF under award no. DMR 1121053 (a member of the NSF-funded Materials Research Facilities Network, www.mrfn.org).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Margaret Condron at UCLA for synthesizing and purifying the Aβ proteins used in this work and the mass spectrometry facility in the Department of Chemistry and Biochemistry at UCSB.

■ ABBREVIATIONS

FAD, familial Alzheimer's disease; Aβ, amyloid β-protein; IMS-MS, ion mobility spectrometry-mass spectrometry; ATD, arrival time distribution

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