Structure of amyloid-β (20-34) with Alzheimer’s-associated isomerization at Asp23 reveals a distinct protofilament interface

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Amyloid-β (Aβ) harbors numerous posttranslational modifications (PTMs) that may affect Alzheimer’s disease (AD) pathogenesis. Here we present the 1.1 Å resolution MicroED structure of an Aβ20–34 fibril with and without the disease-associated PTM, L-isoaaspartate, at position 23 (L-isoAsp23). Both wild-type and L-isoAsp23 protofilaments adopt β-helix-like folds with tightly packed cores, resembling the cores of full-length fibrillar Aβ structures, and both self-associate through two distinct interfaces. One of these is a unique Aβ interface strengthened by the isoaspartyl modification. Powder diffraction patterns suggest a similar structure may be adopted by protofilaments of an analogous segment containing the heritable Iowa mutation, Asp23Asn. Consistent with its early onset phenotype in patients, Asp23Asn accelerates aggregation of Aβ20–34, as does the L-isoAsp23 modification. These structures suggest that the enhanced amyloidogenicity of the modified Aβ segments may also reduce the concentration required to achieve nucleation and therefore help spur the pathogenesis of AD.
A prevalent theory for the biochemical basis of Alzheimer’s disease (AD) is the amyloid cascade hypothesis, which describes the aggregation of the Aβ peptide into oligomeric or fibrous structures that then trigger the formation of neurofibrillary tangles1–3. The Aβ peptide is subject to a number of posttranslational modifications (PTMs) that may affect its aggregation in vivo4. Specifically, Aβ phosphor-ylation (Ser8, Ser26), pyroglutamylation (Glu3, Glu11), nitration (Tyr10), and racemization/isomerization (Asp1, Asp7, Asp23, Ser26) have been shown in vitro to increase the aggregation propensity or neurotoxicity of the Aβ 1–42 peptide5–11, while other modifications, such as dityrosine crosslinking (Tyr10), have been shown to increase the stability of the Aβ aggregates12.

Isomerized products of aspartic acid residues perturb protein structure by rerouting the peptide backbone through the side chain β-carbonyl. This age-dependent modification introduces a methylene group within the polypeptide backbone and thus may have a significant effect on the structure of Aβ oligomers or fibrils13–15. In addition, the isopeptide bond is resistant to degradation, potentially increasing the concentration of the iso-merized Aβ form with respect to the native peptide. Despite the presence of a repair enzyme in the brain, the L-isoaspartate (o-aspartate) O-methyltransferase (PCMT1) for L-isoa-spartate, the isomerization of Aβ Asp1, Asp7, and Asp23 has been identified within AD brain parenchyma16,17. In the cases of the heritable early-onset AD Iowa mutation (Asp23Asn), 25–65% of Asn23 residues have been shown to be isomerized in frontal lobe tissues18, consistent with the increased rates of spontaneous deamidation/isomerization of asparagine relative to aspartate19.

In vitro studies demonstrate that L-isoa-spartate at Asp23 (L-isoAsp23) significantly accelerates Aβ 1–42 fibril formation, while L-isoAsp7 alone does not11,20. Subsequent studies using peptides with multiple sites of isomerization showed only minor accelerated aggregation of the tri-isomerized species (1, 7, and 23), over the di-isomerized species (7 and 23)18. Taken together, these results suggest that among the known sites of Asp isomerization in Aβ, L-isoAsp23 is primarily responsible for the increase in aggregation propensity in vitro.

Given the relevance of the isomerization of Asp23 to both sporadic and hereditary Iowa mutant forms of AD, we sought to discover the structural basis for its acceleration of fibril formation10,17,18. As a platform for evaluating this modification, we chose synthetically generated 15-mer peptides encoding residues 20–34 of the Aβ peptide (Aβ20–34) with and without an L-isoAsp modification at position 23 and spanning the core of known Aβ fibril structures21–29. Challenged by the small size of crystals formed by this segment, we employed the cryo-electron microscopic (cryo-EM) method microcrystal electron diffraction (MicroED) to determine the structures. The structures of Aβ20–34 and Aβ20–34, isoAsp23, determined to 1.1 Å resolution by direct methods, reveal with atomic detail conserved kinked β-helix-like-turns with complex features similar to those observed previously at lower resolution in the cores of fibrillar Aβ 1–42, as well as a distinct pair of protofibril interfaces. Our results suggest that the L-isoAsp23 residue facilitates the formation of a more stable form of this unique interface, promoting enhanced fiber formation and stability. The length of these peptide segments, four residues longer than any other crystallographically determined amyloid structures30–33, is key in facilitating their complex fold—a conformation more representative of the full-length Aβ fibrils.

Results

Fibril formation and characterization of Aβ20–34 peptides. Six early-onset hereditary Alzheimer’s mutations and two PTMs, including the isomerized Asp23, are localized in the Aβ 1–42 peptide to a region spanning six residues from Ala21 to Ser26 near the center of the peptide (Fig. 1a, b). 17,34,35. The amyloid-forming propensity of segments in this region of Aβ was assessed by using a computational method of predicting steric zippers by a threading protocol (ZipperDB36). This method highlights a region of Aβ from Asn27 to Gly37 with high aggregation propensity near the site of Asp23 isomerization (Fig. 1b). To characterize segments containing an isomerized Asp residue at position 23, we utilized synthetic 15 residue peptides spanning the Aβ residues 20–34 (Aβ20–34) in which Asp23 was substituted with either an L-Asn residue (Iowa mutant; Aβ20–34, Asp23Asn) or an L-isoAsp residue (Aβ20–34, isoAsp23).

To evaluate the effect of these variations on this 15-residue segment of Aβ, we assayed its capacity to form fibrils as measured by light scattering at 340 nm (Fig. 1c). Both the peptide based on the Iowa mutant (Aβ20–34, Asp23Asn) and the peptide based on L-isoAsp23 (Aβ20–34, isoAsp23) demonstrated significantly enhanced fibril formation over that of Aβ20–34, with the Iowa mutant peptide displaying the fastest initial rate of fibril formation (Fig. 1c). Fibers of the native peptide at this concentration (1.6 mM) were not observed by light scattering or EM. We further discovered that only 34% of these Aβ20–34, isoAsp23 aggregates could be methylated by the L-isoAsp repair protein carboxyl methyltransferase (PCMT1) in vitro (Supplementary Fig. 1). These data suggest that a majority of the L-isoAsp sites are occluded from the normal repair pathway once in this aggregate form.

To determine the ability of these modified forms to accelerate the aggregation of native peptide, seeding of 3.2 mM Aβ20–34 was performed using 10 μM final concentrations of pre-aggregated seeds of Aβ20–34, Aβ20–34, Asp23Asn, and Aβ20–34, isoAsp23 (Fig. 1d). The addition of each of the preformed aggregates caused significant acceleration in the onset of fiber formation. The largest shift occurred with the native Aβ20–34 seed, followed by isomerized Aβ20–34, isoAsp23 and Aβ20–34, Asp23Asn. Powder diffraction performed on the final aggregates revealed nearly identical sets of reflections, suggesting that the three seeds have similar enough structures to template wild-type (WT) Aβ20–34 aggregates whose diffusion resembles unseeded fibrils (Supplementary Fig. 2a). Fibrillization experiments of full-length Aβ 1–40 with and without the L-isoAsp modification at residue 23 revealed that the isomerized species displays a shorter lag time, consistent with the results obtained with the corresponding Aβ20–34 peptides (Supplementary Fig. 2b; Fig. 1c). Thus, while the isomerized form may be only a minor component of the in vivo Aβ population, it aggregates at a faster rate and can cross-seed the native form efficiently in vitro.

In contrast to the results obtained with 1.6 mM Aβ20–34, increasing the concentration to 3.2 mM Aβ20–34 did yield aggregates ~77 nm in width (Fig. 1c, d). Importantly, light scattering under these conditions for this native peptide is not detected until 3.5 h at the earliest, while shifts in light scattering for the 1.6 mM isomerized and mutated peptides were detected by 1.5 and 0.5 h, respectively (Fig. 1d). Direct comparisons of formation rates were complicated by the insolubility of the Aβ20–34, Asp23Asn peptide at high concentrations, but the delayed onset of even the 3.2 mM Aβ20–34 incubation compared to the 1.6 mM Aβ20–34, Asp23Asn and Aβ20–34, isoAsp23 incubations also support the increased rates of aggregation of the mutated and isomerized peptides (Fig. 1c, d).

Fibrils of each segment were also investigated for their resistance to dissociation by dilution into increasing concentrations of sodium dodecyl sulfate (SDS) at 70 °C as measured by light scattering at 340 nm (Fig. 2). Fibrils of the native 15-residue Aβ segment appeared to partially dissolve upon dilution into the SDS-free buffer, although remaining aggregates were found by
EM. However, these were completely dissolved upon incubation with 1% SDS and higher concentrations (Fig. 2). In contrast, the isomerized peptide showed increased resistance to dissolution compared to the native peptide and still showed light scattering at a concentration of 2% SDS, though no more aggregates were seen at 5% SDS (Fig. 2b). The fibrils of the Iowa mutant appeared to be largely unaffected by dilution even at the highest concentrations of SDS, with no significant changes observed in the levels of light scattering. However, the aggregates in 5% SDS seen by EM appeared to be less bundled than at lower concentrations (Fig. 2b). These results show that alterations of the structure at Asp23 strongly contribute to fibril formation and stability.

Crystallization and data collection of the Aβ20–34 segments. To understand the atomic structural basis for changes in the properties of the isomerized peptide, we sought to crystallize it in the amyloid state. Vapor diffusion screening yielded no crystals large enough for analysis by conventional X-ray crystallography for either the Aβ20–34 or the Aβ20–34, isoAsp23 segment. Instead ordered nanocrystals of the native segment were obtained with continuous shaking at 1200 rpm, and ordered nanocrystals of the isomerized segment were generated with constant mixing using an acoustic resonant shaker for analysis by MicroED as described in the "Methods" section. Nanocrystals obtained in varying buffer conditions were evaluated by morphology and diffraction via light and EM, respectively. Those formed under
the most promising conditions were used as seeds for additional rounds of batch crystal formation. The optimal crystallization condition for the isomerized segment was 50 mM Tris, pH 7.6, 150 mM NaCl, and 1% dimethyl sulfoxide (DMSO) for 48 h with 1% SDS. Isomerized crystal trials produced densely bundled nanocrystals conditioned for the isomerized segment was 50 mM Tris, pH 7.6, and 1% DMSO for 30 h without seeding. This interface buries approximately 130 Å² per chain in the native form and 131 Å² in the isomerized form.

Unlike the dry steric zipper interface A, six water molecules line the second \( \beta^{20–34} \) interface, which we designate the “L-Asp Interface B” (Fig. 3b, e). Here the protofilaments are also related by a two-fold screw symmetry axis. Nearest this central axis, Gly25 and Ser26 contact their symmetry partners across the interface, separated by only 3.5 Å. Furthest from the axis, Asp23 and Lys28 from opposing protofilaments form charged pairs. In between each of these two regions is a solvent channel with the three ordered waters, yielding low shape complementarity (\( S_c = 0.43 \)) to this interface overall. In contrast, in the \( \beta^{20–34,isoAsp23} \) L-isoAsp interface B the truncated side chain of the L-isoAsp23 residue no longer forms a charged pair with Lys28 and instead the isomerized protofilaments form a completely dry interface containing the methylene group of L-isoAsp23, Val24, Gly25, and Ser26 with high surface complementarity (\( S_c = 0.81 \); Fig. 3e). This interface is tightly mated over its entire surface with an average distance of 4.0 Å between the backbones. Interface B buries approximately 139 and 122 Å² per chain for the native and 0.62 in the isomerized. This interface buries approximately 130 Å² per chain in the native form and 131 Å² in the isomerized form.

Each protofilament self-associates with neighboring protofilaments in the crystals through two distinct interfaces. Interface A in both structures resembles a canonical steric zipper—

\[ \text{Fraction (XRD)} \]

Patterns revealed that the fibrils of \( \beta^{20–34} \) segments appear largely isomorphous, sharing major reflections at ~4.7, 10, 12.2, 14, and 29–31 Å (Fig. 4a, b). The similarity among the powder diffraction patterns of \( \beta^{20–34, isoAsp23} \), \( \beta^{20–34, Asp23Asn} \), and \( \beta^{20–34} \) indicates that \( \beta^{20–34, Asp23Asn} \) mimics the
structures of the native and isomerized segments. We modeled an L-Asn residue at position 23 of the Aβ20–34, isoAsp23 structure to see if the native L-amino acid could be accommodated in the dry L-isoAsp interface B (Fig. 4c, right panel). The L-Asn residue was integrated into the Aβ20–34, isoAsp23 interface B scaffold without significant clashes. However, this Asn model lacks a backbone hydrogen bond extending between the isoAsp23 amide carboxyl to the Val24 amide nitrogen of the adjacent protofilament that is present in our Aβ20–34, isoAsp23 structure (Fig. 4c). The residue at site 23 has to adopt an allowed, but unusual left-handed helical conformation to form the L-isoAsp interface B. Both the methylene of the isoAsp residue and the isoAsp23 to Val24 main chain hydrogen bond may help stabilize this structure. This backbone hydrogen bond is present in the native Aβ20–34 structure (Fig. 4c). In this native structure, the Asp main chain adopts a more canonical β-sheet conformation, but the side chain...
protrudes toward the opposite protofilament, prohibiting a tight, dry interface along residues Asp23-Ser26 as in the L-isoAsp interface B.

The L-Asn side chain in the L-isoAsp interface B model may be able to compensate for the loss of this dry interface by forming another ladder of hydrogen bonds along the protofilament axis (Fig. 4c, d right panel). Thus this second interface packing may be achievable for a Aβ20–34, Asp23Asn structure as shown in the L-isoAsp interface B model; however, the XRD patterns reveal that the native Aβ20–34 and mutated Aβ20–34, Asp23Asn peptides share more similarities than the isomerized Aβ20–34, isoAsp23 and the Aβ20–34, Asp23Asn peptide. Both the native and heritable Iowa mutant forms lack more defined peaks at 22.9, 24.7, 29.4, and 32.5, while both have more broad peaks at 30.9 Å (Fig. 4a, b). These similarities between the Aβ20–34 and Aβ20–34, Asp23Asn fiber diffractions patterns, and the lack of a methylene group in the normal L-residues, may suggest that the Iowa mutant peptide will assume a structure more similar to the native Aβ20–34 structure, as modeled in Fig. 4c, d (left panels).

This model maintains the backbone hydrogen bond between Asn23 and Val24, the ordered core of the Aβ20–34 structure, and allows for the additional polar zipper between stacked Asn23 residues. The added network of hydrogen bonds along the asparagine side chain may explain in part the increased fiber formation rates and stability of Aβ20–34, Asp23Asn against SDS and heat denaturation. While the isomorphous powder diffraction patterns seen between Aβ20–34, isoAsp23, Aβ20–34, Asp23Asn, and Aβ20–34, isoAsp23 do support the models in which Aβ20–34, Asp23Asn mimics the native and isomerized structures, it cannot be ruled out that Aβ20–34, Asp23Asn forms a distinct structure, perhaps lacking either the L-Asp or the L-isoAsp novel interface B, with the ordered core simply stabilized further by the Asn polar ladder.

Importantly, the powder diffraction of full-length Aβ and the shorter peptide segments all display cross-β patterns with strong reflections at ~4.7 and 9–10 Å (Fig. 4a), and the crystal structures of Aβ20–34 and Aβ20–34, isoAsp23 form parallel, in-register β-sheets similar to other full-length Aβ structures. Thus we hypothesized that the Aβ20–34, isoAsp23 structure could form the core of a distinct isomerized Aβ polymorph. To visualize a potential full-length fiber with the Aβ20–34, isoAsp23 structure as its core, we added the remaining residues of Aβ 1–42 onto the ends of the Aβ20–34, isoAsp23 protofilaments and energy minimized the entire model as described in the "Methods" section. The resulting model demonstrates that the remainder of the residues of Aβ 1–42 can be accommodated in a favorable conformation with the isomerized segment as a core with interface A or B as the primary interface (Fig. 5).

Table 1 Data collection and refinement statistics

|                | Aβ20–34 | Aβ20–34, isoAsp23 |
|----------------|---------|------------------|
| Data collection| P2_1    | P2_1             |
| Space group    | P2_1    | P2_1             |
| Cell dimensions| a, b, c (Å) | 33.17, 4.78, 30.33 | 33.17, 4.78, 30.33 |
|                | α, β, γ (°) | 90.00, 111.10, 90.00 | 90.00, 110.90, 90.00 |
| Resolution (Å) | 1.10 (1.13–1.10) | 1.05 (1.20–1.05) | 1.05 (1.20–1.05) |
| Rsym or Rmerge (%) | 18.9 | 19.7 |
| Completeness (%) | 85.2 | 82.7 (53.0) |
| Redundancy      | 6.67 (6.14) | 4.19 (3.10) |
| Resolution (Å) | 7.74–1.10 (1.26–1.10) | 5.96–1.05 (1.20–1.05) |
| No. of reflections | 3544 (1141) | 3943 (1167) |
| Rwork/Rfree (%) | 19.4/21.3 (21.3/26.9) | 19.7/24.6 (27.0/32.4) |
| No. of atoms    | Protein 210 | 204 |
|                | Ligand/ion 0 | 0 |
|                | Water 7 | 4 |
| B-factors      | Protein 6.50 | 8.29 |
|                | Ligand/ion 0 | 0 |
|                | Water 20.78 | 27.70 |
| R.m.s. deviations | Bond lengths (Å) 0.56 | 1.04 |
|                | Bond angles (°) 0.68 | 0.90 |

*Ten crystals were used in determining the Aβ20–34 structure

Five crystals were used in determining the Aβ20–34, isoAsp23 structure

Values in parentheses are for the highest-resolution shell

**Comparison of segment structures to known Aβ structures**

The structures presented here are the longest segments of an amyloid peptide determined by crystallography—four residues longer than the previous amyloid spines determined by MicroED (9–10 Å). This extension is significant due to the fact that, as the number of residues in a segment grows, the packing of idealized β-strands in a lattice becomes more difficult owing to the strain created by the natural twist of the β-sheet/strand. This strain hypothesis is consistent with observations that, as the number of residues in an amyloid segment grows, the crystals that can be grown are correspondingly smaller15. In the literature to date, the crystal structures of shorter segments of amyloid proteins have revealed that the dominant forces stabilizing protofilaments occur between different peptide chains16. In the native and modified Aβ20–34 structures, we are not only able to see interactions between protofilaments, such as the interfaces A and B, but we also see folding of the peptide to produce a β-helix-like...
A putative model of the heritable Iowa mutation in interface B. a Fiber diffraction patterns of Aβ1–42, Aβ20–34, Aβ20–34, isoAsp23, and Aβ20–34, Asp23Asn. All fibers including Aβ1–42 were prepared in 50 mM Tris, pH 7.6, 150 mM NaCl, and 1% dimethyl sulfoxide (DMSO), except Aβ20–34, Asp23Asn, in which the DMSO concentration was raised to 5%. b Intensities of reflections from fiber diffraction of the segments were plotted against D spacing. Radial intensity values are vertically staggered for visibility of peaks. c From left to right, interface B down the fibril axis of Aβ20–34 structure, a model Aβ20–34, Asp23Asn, on the backbone of the Aβ20–34 structure, Aβ20–34, isoAsp23, and a model Aβ20–34, Asp23Asn on the backbone of the Aβ20–34, isoAsp23 structure. d A view perpendicular to the fibril axis of residues 23–24 of each structure. Yellow dashed lines represent measured distances in Å between the amide carboxyl of residue 23 and the amide nitrogen of Val24 on the adjacent strand. Source data are provided as a Source Data file.

Fig. 4

Turn with a hydrophobic core of interacting residues within the same chain.

While not all full-length native structures contain β-arches, such as the peptide dimer structure shown in Schmidt et al.47 (PDB code: 5AEF), all do include ordered cores involving steric zippers similar to those found in shorter amyloid peptide structures, and a majority of the known Aβ structures do display β-helix-like turns as seen in the segment structures (Fig. 6 and Supplementary Fig. 5). The native Aβ20–34 structure aligns well with a number of these full-length Aβ structures, and both the native and isomerized structures presented here have the lowest total atom root-mean-square deviation (RMSD) with a structure of the Aβ Osaka mutant29, E22Δ, at 2.741 and 2.963 Å, respectively. A tree representing the structural relationships between residues 20 and 34 of eight full-length Aβ structures and our Aβ20–34 structure based on total atom RMSD values shows that 6 of the 8 structures contain turns about the Gly25 and Gly29 residues21,23,26,28,29, creating interfaces which align well with interface B of our L-Asp Aβ20–34 structure. Four21,23,26,29 of these structures correspond to both the Aβ20–34 segment structures with regard to the placement of charged residues Glu22, Asp23, and Lys28 outside the hydrophobic core and yield total atom RMSD values of ≤4 Å with Aβ20–34 (Fig. 6 and Supplementary Fig. 5). These strong overlaps between our segment structure and other full-length Aβ structures support the validity of this segment as an atomic resolution structure of an Aβ core. Importantly, in each of the full-length structures shown here, the putative interface B is accessible as a possible secondary nucleation site (Fig. 6). This interface is stabilized within our structures by the L-isoAsp modification, which mates more tightly between protofilaments than the L-Asp interface B and excludes waters. Thus a full-length structural polymorph with this interface may be isolated more readily with the modification.

The increased structural complexity afforded by extending from 11 to 15 residues is appreciated best in comparing the crystal structures of Aβ20–34 to the shorter Aβ24–34 crystal
structure, 5VOS32 (Fig. 7). The four extra N-terminal residues in both native and modified Aβ20–34 facilitate formation of kinks at Gly25 and Gly29, creating an internal core, whereas the Aβ 24–34 peptide assumes a linear β-strand. Despite Aβ 24–34 lacking these kinks, there is remarkable alignment between residues Gly29 to Leu 34 and interface A of the Aβ20–34 crystals, yielding a total atom RMSD of 0.70 and 0.68 Å with the native and isomerized forms, respectively (Fig. 7). An inhibitor was previously developed to the human islet amyloid polypeptide (hIAPP) steric forms, respectively (Fig. 6). The length of these peptides facilitates their similar overall fold to previous WT Aβ fibril structures and demonstrates that amyloid cores are rigid and ordered enough to form crystals. These structures reveal a previously unseen profolilament interface (B) involving residues Asp23-Lys28 in the native structure, and residues L-isoAsp23-Ser26 in the isomerized structure. The native interface (L-Asp interface B) has low surface complementarity and contains six water molecules encased between charged residue pairs Asp23 and Lys28 on opposing sheets. In contrast, the isomerized interface (L-isoAsp interface B) is a dry tightly mated sheet with high surface complementarity. Our data suggest that the changes in the structure along this interface, namely, the exclusion of water molecules and van der Waals attractive forces associated with the high Sα, are likely responsible in part for the increases in fiber formation rate and stability of the aggregate observed for the isomerized peptide. The modified interface may provide a better site for secondary nucleation of amyloid formation resulting in the observed enhancements in aggregation. However, it cannot be ruled out from the data presented here that the flexibility imparted by the methylene group of the L-isoAsp residue promotes amyloid formation by allowing an ordered nucleus for primary nucleation to form at a faster rate than the native peptide.

Our models of Asn23 in the Aβ20–34, isoAsp23 “L-isoAsp interface B” indicate that the completely dry interface may be possible for native residues (Fig. 4). However, the native Aβ20–34 structure did not preferentially adopt this interface and instead forms a hydrated L-Asp Interface B. Similar to our Aβ20–34 peptide structure, alignments of previous Aβ structures onto the Aβ20–34 and Aβ20–34, isoAsp23 protofilaments show the native Aβ23 side chain carboxyl group protruding into the putative interface B region (Supplementary Fig. 5). The hereditary Iowa mutant nuclear magnetic resonance (NMR) structure (Fig. 6 and Supplementary Fig. 5 (PDB: 2MPZ22)) kinks at Gly25 and Asn27, rather than at Gly25 and Gly29, and thus there is no equivalent interface A. Yet, our preparations of crystals in TBS of the Aβ20–34, Aβ20–34, isoAsp23, and Aβ20–34, Asp23Asn constructs appear nearly identical by XRD, suggesting that the structure of an Iowa mutant protofilament would resemble the native and isomerized structures presented here (Fig. 4), barring minor differences due to packing polymorphisms or different environmental conditions.

It is clear that both the isomerization and Iowa mutation at residue 23 accelerate aggregation and increase stability of Aβ fibrils. Our structures of Aβ20–34 and Aβ20–34, isoAsp23 reveal a potential mechanism for the increases in fiber formation rate and fiber stability within the isoAsp23 form: the addition of a completely dry interface with high surface complementarity. This analysis leads to the hypothesis that the Asp23 isomerization in vivo could lead to the accelerated formation of Aβ fibrils, thereby contributing to the aggregation of Aβ and AD pathology. The hereditary Iowa mutation Asp23Asn may work in a similar manner either by forming the same fold as the isomerized Asp23 or, since Asn undergoes isomerization more rapidly relative to Asp, may also produce an isomerized Aβ with accelerated aggregation and increased stability. The isomerized structure may have demonstrated accelerated amyloid formation of the isoAsp23 Aβ 1–40 and 1–42 peptides compared to native Aβ10,11,17,18,22. These results suggest that the change in the structure of Aβ accompanying isomerization at Asp23 may represent a route to the pathogenesis of AD.

In this work we present the 1.1 Å structures of segments spanning residues 20–34 of the Aβ peptide containing either an Asp or an isoAsp residue at site 23. These 15-residue segments, crystallized at physiological pH, maintain a topology seen in the core of Aβ fibrils, a β-helix-like turn (Fig. 6). The length of these peptides facilitates their similar overall fold to previous WT Aβ fibril structures and demonstrates that amyloid cores are rigid and ordered enough to form crystals. These structures reveal a previously unseen profolilament interface (B) involving residues Asp23-Lys28 in the native structure, and residues L-isoAsp23-Ser26 in the isomerized structure. The native interface (L-Asp interface B) has low surface complementarity and contains six water molecules encased between charged residue pairs Asp23 and Lys28 on opposing sheets. In contrast, the isomerized interface (L-isoAsp interface B) is a dry tightly mated sheet with high surface complementarity. Our data suggest that the changes in the structure along this interface, namely, the exclusion of water molecules and van der Waals attractive forces associated with the high Sα, are likely responsible in part for the increases in fiber formation rate and stability of the aggregate observed for the isomerized peptide. The modified interface may provide a better site for secondary nucleation of amyloid formation resulting in the observed enhancements in aggregation. However, it cannot be ruled out from the data presented here that the flexibility imparted by the methylene group of the L-isoAsp residue promotes amyloid formation by allowing an ordered nucleus for primary nucleation to form at a faster rate than the native peptide.

**Discussion**

The typical age of onset for sporadic AD is after 65 years, suggesting that slow spontaneous processes such as the accumulation of age-dependent PTMs in Aβ may be contributing factors to aggregation and toxicity4. The spontaneous isomerization of aspartate (isoAsp) has been identified at all three aspartate residues within the Aβ 1–42 peptide—1, 7, and 23. However, immunohistochemical studies have shown that, while native Aβ and isoAsp7 Aβ are present in senile plaques from four non-disease patient controls, isoAsp23 Aβ was identified only in one of the four non-disease patient controls, as well as in the senile plaques from all AD patient samples, indicating that the isoAsp23 may be more specifically associated with AD pathology than native Aβ and the L-isoAsp7 form30. This implied pathogenicity of isoAsp23 correlates with in vitro studies, which have
also provide insight into the mechanisms behind the A21G, E22G, and E22Δ hereditary mutations that introduce flexibility into the same region of the backbone. Importantly, we have also found that the only known repair pathway for L-isoAsp, the enzyme PCMT1, is unable to fully methylate and repair aggregates of Aβ20–34, isoAsp23 in vitro, thus once the modified aggregates have formed in vivo they may be difficult to repair and clear (Supplementary Fig. 1).

Recent structures of tau isolated from AD patients have revealed distinct structural polymorphs. Both the paired helical filaments and the straight filaments of tau display β-arches in their sheets, which is a feature also shared by the native and isomerized Aβ20–34 structures (Fig. 6 and Supplementary Fig. 4). This similarity not only suggests that our structure’s β-helix-like turn may be a common amyloid motif but also identifies a potential cross-seeding site between Aβ and the tau protein of AD. This discovery emphasizes the need for atomic-resolution structures of disease-associated amyloid, as these core segments are critical for structure-based drug design and protein prediction efforts.

These crystal structures can be used in conjunction with full-length cryo-EM structures to obtain a high-resolution view of the interactions mediating amyloid fiber formation. High-resolution structures are also valuable when looking at the effect PTMs may have on amyloid structure as seen here and elsewhere. Therefore, the combination of increasing peptide length and high resolution makes the Aβ20–34 and Aβ20–34, isoAsp23 structures an important step forward for the structural characterization of amyloid proteins and their role in disease.

**Methods**

**Materials.** Aβ20–34 peptides corresponding to the human sequence were purchased from and validated by Genscript at a purity of >98% as the trifluoroacetic acid salt and were stored at −20 °C. Peptides were validated by electrospray ionization–mass spectrometry (ESI-MS) performed by Genscript. Aβ1–42 was purchased from Bachem Americas, Inc. (Catalog #, H-1368).

**Aggregation of Aβ20–34 peptides for fibril-formation rates.** Peptides were dissolved at 1.6 mM in 50 mM Tris-HCl, pH 7.6, 150 mM NaCl (TBS) with 2.5% DMSO unless otherwise designated in the figure legend. Peptides were validated by electrospray ionization–mass spectrometry (ESI-MS) performed by Genscript. Aβ 1–42 was purchased from Bachem Americas, Inc. (Catalog #, H-1368).
Seeding of Aβ20–34 segment. Seeds were formed shaking continuously on an acoustic resonant shaker at 37 °C at a frequency setting of 3733.34. Seeds of Aβ20–34 were formed at 5 mg/mL in 50 mM Tris-HCl, pH 7.6, 150 mM NaCl (TBS) with 1% DMSO; seeds of Aβ20–34, isoAsp23 were formed at 2.5 mg/mL in 50 mM Tris-HCl, pH 7.6, 150 mM NaCl (TBS) with 1% DMSO; and seeds of Aβ20–34, Asp23Asn were formed at 2.5 mg/mL in 100 mM Tris-HCl, pH 7.5, 10% isopropanol, and 200 mM sodium acetate. All seeds were diluted to 200 μM stocks and 5 μL were added to 3.2 mM Aβ20–34 in a final volume of 100 μL. Not all wells of the unseeded 3.2 mM Aβ20–34 condition aggregated within the time course of this assay (Fig. 1d).

Synthesis and purification of native Aβ 1–40. The syntheses of Aβ (1–40) WT and Aβ (1–40) IsoAsp23 were completed in a CEM Liberty BlueTM Microwave Peptide Synthesizer. The crude peptides were purified using an Interchim puriFlash® 4125 Preparative Liquid Chromatography System. Details of the syntheses and purifications are available in the Supplementary Methods section (Supplementary Tables 1–3, Supplementary Figs. 6–22).

The purified Aβ (1–40) WT has an estimated purity of 93% by high-performance liquid chromatography (HPLC, Supplementary Figs. 14 and 15) and was characterized by ESI-MS via direct injection into a Q-Exactive™ Plus Hybrid Quadrupole-Orbitrap TM Mass Spectrometer (Supplementary Figs. 16 and 17). The calculated average mass for C$_{194}$H$_{295}$N$_{53}$O$_{58}$S: 4327.148 g/mol, m/z calculated: [M + 3H]$_{+}^{3+} = 1443.39$; [M + 4H]$_{+}^{4+} = 1082.79$; [M + 5H]$_{+}^{5+} = 866.44$; [M + 6H]$_{+}^{6+} = 722.20$. Observed: 1443.3913; 1082.7955; 866.4374; 722.1991. The purified Aβ (1–40) IsoAsp23 has an estimated purity of 97% by HPLC (Supplementary Figs. 19 and 20) and was characterized by ESI-MS via direct injection into a Q-Exactive™ Plus Hybrid Quadrupole-Orbitrap TM Mass Spectrometer (Supplementary Figs. 21 and 22). The calculated average mass for C$_{194}$H$_{295}$N$_{53}$O$_{58}$S: 4327.148 g/mol, m/z calculated: [M + 3H]$_{+}^{3+} = 1443.39$; [M + 4H]$_{+}^{4+} = 1082.79$; [M + 5H]$_{+}^{5+} = 866.44$. Observed: 1443.3912; 1082.7959; 866.4373.

Crystallization of the segments. Aβ20–34 was resuspended at a concentration of 3.2 mM in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl (TBS) with 1% DMSO in a final volume of 100 μL. The peptide solution was then shaken continuously for 30 h at 1200 rpm at 37 °C. Aβ20–34,isoAsp23 was resuspended at a concentration of 1.6 mM in 50 mM Tris-HCl, pH 7.6, 150 mM NaCl (TBS) with 1% DMSO in a final volume of 200 μL. The filtered peptide solution was then shaken for 2 days on an acoustic resonant shaker at 37 °C at a frequency setting of 3735.23. Four microcrystals of this suspension was then used to seed 196 μL of a second peptide solution (1.6 mM) as a 2% seed on the acoustic resonant shaker at 37 °C. Crystals were obtained within 48 h. The presence of crystals was verified by EM, using a standard holder, with no negative stain. Crystals of the native and isomerized segments were on average ~77 and ~71 nm in width, respectively, and were typically ~2 μm in length.

MicroED sample preparation. Quantifoil R1.2/1.3 cryo-EM grids (Electron Microscopy Sciences, product # Q325CR1.3) were glow discharged for 30 s on either side, and 1.5 μL of a 1:1 dilution of Aβ20–34 crystals in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl (TBS) with 1% DMSO was pipetted on both sides. Twenty microliters of Aβ20–34,isoAsp23 crystal suspensions were spun down at 5000 × g for 5 min, the supernatant was removed, and pelleted crystals were resuspended in 50 μL TBS + 0.75% (w/v) β-octyl-glucoside (VWR, P-1110), and rotated at 4 °C for 1 h. These detergent-treated crystals were then spun down a second time. Pelleted crystals were resuspended in 50 μL water. A total of 1.5 μL of the washed crystal solution was then applied on both sides of a glow discharged Quantifoil R1.2/1.3 cryo-EM grid (Electron Microscopy Sciences, product # Q325CR1.3). All grids were plunge frozen into supercooled ethane using a Vitrobot Mark 4 instrument.

MicroED data collection and processing. MicroED data was collected in a manner similar to previous studies. Briefly, plunge-frozen grids were transferred to an FEI Talos Arctica electron microscope and diffraction data were collected using a bottom-mount CetaD 16M CMOS camera with a sensor size of 4096 × 4096 pixels, each 14 × 14 μm. Diffraction patterns were recorded by operating the detector in continuous mode with 2 × 2 pixel binning, producing datasets with frames 2048 × 2048 pixels in size. The exposure time was set to 0.01 e~ A²/s. The exposure time per frame was set at 3 s while the rotation speed was set to 0.3 deg/s resulting in a final oscillation range of 0.9 deg/exposure for the Aβ20–34 data collection and to 0.443 deg/s resulting in a final oscillation range of 1.329 deg/exposure for the Aβ20–34,isoAsp23 data collection. This rotation rate was optimized to allow a maximum amount of reciprocal space to be sampled before crystal decay.
was observed while also slow enough to prevent overlapping diffraction spots in the diffraction images. Diffraction movies typically covered a 50–140 deg wedge of reciprocal space and were taken of crystals randomly oriented on the grid with respect to the incident beam. These crystals had a highly preferred orientation on the grid, resulting in a systematic missing cone and hence lower completeness along the e∗ axis; however, this did not preclude structure determination, with a high overall completeness of >80% for both structures (see Table 1).

Structure determination. Diffraction datasets were converted to SMV format to be compatible with the X-ray data processing software. Data were indexed and integrated using XDS. The parameters controlling the raster size during indexing and integration were optimized to reduce contributions by background and to exclude intensities that conform poorly to the lattice determined during indexing. The number of diffraction images per crystal was aggressively pruned to maximize I/σ. The resulting outputs from XDS were sorted and merged in XSCALE. To produce a final merged dataset, partial datasets were selected based on their effects on the Rmerge values. In total, for the Aβ20–34 structure, 10 partial datasets, containing 404 diffraction images, were merged to produce a final dataset with high completeness up to 1.1 Å. An ab initio solution was achieved using SHELXD. The phases obtained from both Aβ20–34 coordinates produced by SHELXD were used to generate maps of sufficient quality for subsequent model building in Coot. The resulting models were refined with Phenix, using electron scattering form factors, against the measured data.

Powder diffraction sample preparation and data collection. Designated aggregates of Aβ 1–42 and Aβ20–34 peptides were prepared in buffers as described in the figure legends. Aggregates were spun at 20,000 × g for 5 min. The pellet was resuspended in water and resuspended in 5 μl water and pipetted between two facing glass rods that were 2 mm apart and allowed to dry overnight at room temperature. These glass rods with ordered fibrils were secured to a brass pin and mounted for diffraction at room temperature using a 1.54 Å X-rays produced by a Rigaku FR+ rotating anode generator equipped with an HTC imaging plate. Patterns were collected at a distance of 200 mm and analyzed using the ADXV software package.

SDS dissolution of aggregates. Aggregates of Aβ20–34, Aβ20–34, isoAsp23, and Aβ20–34, Asn23Iowa were all prepared in TBS, with 1%, 2.5%, and 2.5% DMSO, respectively. Both Aβ20–34, isoAsp23, and Aβ20–34, Asn23Iowa were prepared at a peptide concentration of 2.5 mg/ml, while Aβ20–34 was prepared at 5 mg/ml, shaking at 1200 rpm at 25 °C. The Aβ20–34 was diluted to 2.5 mg/ml prior to the denaturation assay. Suspensions of Aβ20–34 aggregates were diluted 1:1 in 2, 3, 4, and 10% SDS stocks in TBS and heated for 15 min at 70 °C in a PTC-100 Peltier thermal cycle as described by Guenther et al. Measurements at 340 nm were recorded on a Cary 123 photometer, measuring absorbance at 595 nm. The Aβ20–34 samples were prepared in TBS, with 1%, 2.5%, and 2.5% DMSO, respectively. Both Aβ20–34, isoAsp23, and Aβ20–34, Asn23Iowa were prepared at a peptide concentration of 2.5 mg/ml, while Aβ20–34 was prepared at 5 mg/ml, shaking at 1200 rpm at 25 °C. The Aβ20–34 was diluted to 2.5 mg/ml prior to the denaturation assay.

Analysis of Sα and surface Sα in Aβ20–34 structures. The structures of Aβ20–34 and Aβ20–34, isoAsp23 were used to measure buried surface area (Sα) and (Sα) from an assembly consisting of two sheets generated by translational symmetry each consisting of ten stacked β-strands. Sα was calculated as the average of the buried surface area per chain and the difference between the sum of the solvent accessible surface area of the two sheets and the solvent accessible surface area of the entire complex, divided by the total number of strands in both sheets using the CCP4 suite.

Modeling modified and full-length Aβ and RMSD calculations. RESIDUES 1–42 of Aβ were modeled onto the N- and C-termini of the Aβ20–34, isoAsp23 structure using Coot, and the resulting structures were energy minimized using the Crystalllography & NMR System (CNS) suite of programs. Distance matrices for RMSD relationships between Aβ20–34, isoAsp23 residues 20–34 from native structures were generated in the LSQKAB program of CCP4, and resulting matrices were used to generate the tree shown in Fig. 4.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Atomic coordinates and structure factors for the Aβ20–34 structure have been deposited in the Protein Data Bank under accession code 6OIZ. The map for this structure has been deposited in the EMDDB with accession code EMD-20082. Atomic coordinates and structure factors for the Aβ20–34, isoAsp23 structure have been deposited in the Protein Data Bank under accession code 6NB9. The map for this structure has been deposited in the EMDDB with accession code EMD-0405. The source data underlying Figs. 1c, d, 2, and 4b and Supplementary Figs. 1 and 2 are provided as a Source Data file. Other data are available from the corresponding author upon reasonable request.

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Author contributions

R.A.W. and S.G.C. designed the project and wrote the manuscript with input from all other authors. R.A.W. conducted fibril growth experiments, stability assays, and crystalization of the AJβ(34–36) peptide. R.A.W. and D.R.B. performed fibril diffraction studies. Samples were prepared for MicroED and data were collected by R.A.W. and D.R.B. with advice from T.G. Data were processed by C.-T.Z. and R.A.W. and re

Additional information

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