Polymorphic Triple β-Sheet Structures Contribute to Amide Hydrogen/Deuterium (H/D) Exchange Protection in the Alzheimer Amyloid β42 Peptide*2

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Buyong Ma1 and Ruth Nussinov15

From the 4Basic Science Program, SAIC-Frederick, Inc., Center for Cancer Research Nanobiology Program, NCI, National Institutes of Health, Frederick, Maryland 21702 and the 5Sackler Institute of Molecular Medicine, Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Background: Experimental structural elucidation of polymorphic amyloid ensembles is difficult.

Results: We found a novel amyloid structural motif of a triple β-sheet by comparing computational and experimental H/D exchange.

Conclusion: The triple β-sheet Aβ42 has a minimal exposure of hydrophobic residues and is further stabilized by the E22Q (Dutch) mutation in Alzheimer disease.

Significance: The finding helps to understand the Dutch mutation in Alzheimer disease.

Characterization of the polymorphic structural range of Aβ oligomers is important to the understanding of the mechanisms of toxicity. Yet for highly polymorphic ensembles, experimental structural elucidation is difficult. Here, we use a combination of NMR solvent protection experiments and computational structural screening to identify major species in the amyloid conformational ensemble. We examined the polymorphic pentamer and fibril seeds of Aβ42 and its mutants and compared the theoretical backbone amide protection obtained from simulations with experimental hydrogen/deuterium (H/D) exchange protection ratio. We observed that highly flexible pentamers do not share structural similarities with fibril seed oligomers, except the turn regions. We found that a novel amyloid structural motif of a triple β-sheet, with the N-terminal residues interacting with the core (Lys17–Glu22) β-sheet region, correlates with H/D exchange protection. The triple β-sheet Aβ42 oligomer has a minimal exposure of hydrophobic residues and is further stabilized by the E22Q (Dutch) mutation in Alzheimer disease. The experimental H/D exchange solvent protection ratio implies that triple β-sheet fibrils and globulomers could coexist in the Aβ42 ensemble, pointing to a broad heterogeneous aggregate population. Our results suggest that an approach that combines computational modeling with NMR protection data can be a useful strategy for obtaining clues to the preferred conformational species of the assemblies in solution and help in alleviating experimental difficulties and consequently possible errors in the exchange data for Aβ42 fibrils.

Aβ peptide (with 40 or 42 residues) aggregates are observed in brain tissues of patients with Alzheimer disease, and soluble Aβ peptide oligomers are the major species leading to neuron death (1). Inhibition of oligomer formation is one possible therapeutic strategy to improve cognition (2, 3). Oligomer and fibril formation are sensitive to the environment. Under certain conditions, Aβ40 and Aβ42 may have similar protofilaments (4); however, they can also form different fibrils under identical conditions (5). Although typical Aβ fibrils display in-register parallel β-sheets (6), the D23N fibril can have anti-parallel β-sheets around the Lys16–Glu22 region (7). Complex polymorphism underlies difficulties in unveiling amyloid structures and their toxic mechanisms (8). For example, Alzheimer brain-derived fibrils can have distinct fibrils (9) that differ from those observed in test tubes. Polymorphism also relates to pathway kinetics; the E22Q (Dutch) mutation can accelerate amyloidosis. Overall, protein aggregates are likely to present different preferred architectures depending on their sequences (10) as well as their physical environment. The problem is to obtain the polymorphic range, preferred aggregate states, pathways leading to these, time scales, and their mechanisms of toxicity. Figuring these out is of crucial importance for understanding amyloid-related diseases and pharmacological strategies.

Experimental methods to elucidate amyloid structures include high resolution x-ray diffraction for short peptides (11), single molecule spectroscopy for oligomer size distribution (12), cryo-electron microscopy for fibril morphology (4), and solid state NMR (7) and hydrogen/deuterium (H/D)3 exchange NMR (1, 5) for organization detail. For highly polymorphic structures, especially in the absence of dominant populations, structural elucidation is difficult. Yet, in vivo, neurotoxic species are often mixed, with some forms able to relatively easily inter-transform (13) but not others (14, 15). Thus, delineating the observed and likely aggregation states is an important goal.

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1 The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1–3.

1 To whom correspondence should be addressed: Bldg. 469, Rm. 151, NCI-Frederick, Frederick, MD 21702. Fax: 301-846-5598; E-mail: mabuyong@mail.nih.gov.

2 The abbreviations used are: H/D, hydrogen/deuterium; MD, molecular dynamics.
H/D exchange experiments have the advantage to provide information about averaged residue-specific protections in amyloid oligomers, kinetic intermediates, and fibrils(1, 5, 13, 16–18). Simulations of β-sheet oligomers have been qualitatively compared with experimental H/D exchange protection to obtain clues to the amyloid fibril structure in prion protein fragment 106–126 (19). In this study, we focus on quantitatively comparing H/D exchange protection ratios for Aβ42 and amide solvation protection obtained from simulations of a range of potential oligomers. By systematically exploring polymorphic Aβ42 aggregates using molecular dynamics (MD) simulations, we are able to identify major species in the polymorphic ensemble observed in experimental H/D exchange NMR. Our analysis revealed a novel structural motif of triple β-sheet Aβ42 amyloid with minimum exposure of hydrophobic residues, offering new insights into the E22Q Dutch mutant in Alzheimer disease. Thus, our work indicates that combining experimental H/D exchange and simulations of amide solvation could be a powerful approach to solve structural mixtures of amyloid species.

EXPERIMENTAL PROCEDURES

Model Construction and Molecular Dynamics Simulations Procedure—We systematically explored possible structures for the polymorphic Aβ42 aggregates. Our structural ensemble includes several novel structural motifs, which are based on recent experimental data (5, 7, 13, 20). The structures have different β-strand conformations in the N and C terminus, with parallel or anti-parallel β-strand orientation, and different associations between protofibrils. The core structures of the models are based on experimental NMR models (residues 11–40 from Refs. 21, 22 or residues 17–42 from Ref. 1); the overall structures of Aβ42 are then fit to recent experimental observations (5, 7, 13, 20). Each constructed model is subjected to explicit solvent molecular dynamics simulations for 60 ns to test its structural stability, and the relative thermodynamic energies are subsequently evaluated. Additional simulations with different simulation protocols (starting conformation, heating, and equilibration) were also performed.

MD simulations were performed using the NAMD package (23) and the Charmm 27 force field (24), with constant pressure ensembles (NPT) at 1 atm and temperature of 330 K. The short range van der Waals interactions were calculated using the switching function, with a twin range cutoff of 10.0 and 12.0 Å. The long range electrostatic interactions were calculated with the Particle Mesh Ewald method with a cutoff of 12.0 Å (25). The average effective energies are calculated from 500 snapshots obtained in the last 5 ns of the MD simulations, using the Generalized Born with the Molecular Volume method (26).

Comparison of the “Closed” and “Open” Amide Groups and Experimental H/D Exchange Protection Ratios—For hydrogen exchange, the amide groups need to break their hydrogen bonds with other residues and be accessible to water molecules. In Scheme 1, NHc and NHo represent the amide groups in closed and open conformations; ND indicates the deuterated amide group, and Kc and Kr are the rate constants of the amides opening and closing, respectively, and Kr is an intrinsic exchange rate constant (27).

![Polymorphic Triple β-Sheet Fibrillar Structure](image)

In this work, we calculate two amide hydrogen bonding properties as follows: 1) NHsol, which measures the average number of hydrogen bonds between NH and water oxygen within 3.0 Å distance; and 2) NHbp, which measures the average number of hydrogen bonds between NH and C=O backbone oxygen in β-sheets within 2.6 Å distance. It is reasonable to assume that shown in Equation 3,

\[
K_o = C_o \cdot \text{NH}_{\text{sol}} \\
K_r = C_r \cdot \text{NH}_{\text{bp}}
\]  

where \( C_o \) and \( C_r \) are coefficients relating \( K_o \) and \( K_r \) to NHsol and NHbp, respectively. Thus, we have Equation 4,

\[
K_{\text{sol}}/K_{\text{int}} = C \cdot \text{NH}_{\text{sol}}/(\text{NH}_{\text{sol}} + \text{NH}_{\text{bp}})
\]

In the work by Olofsson et al. (28), the solvation protection factor can be related to Equation 4 as shown in Equation 5,

\[
(100 - P_{\text{sol}}) = C \cdot \text{NH}_{\text{sol}}/(\text{NH}_{\text{sol}} + \text{NH}_{\text{bp}})
\]

NHsol and NHbp are calculated for each residue using the Charmm module in the Charmm35 program, averaged over a total of 60 ns of simulations. Experimental H/D exchange protection ratios are taken from the literature (5, 13, 28, 29). We use the residues 10–42 with non-zero protection ratio values. We then compare the linear correlation based on Equation 5. If only one model (Table 2) is used, the values directly obtained from the MD simulations are used to compare with the H/D exchanges. In mixtures of multiple models, we assume that each model contributes equally to the overall solvation factors, and the average values of the models are used for the comparison (Table 3).

RESULTS

Comparison of Simulations and Experimental H/D Exchange Protections for the Aβ42 Pentamer—The H/D exchange solvation protection of the Aβ42 pentamer is totally different from that of the Aβ42 fibril, with the correlation coefficient value (R2) only 0.26 (Fig. 1A). The pentamer protection indicates that there are turns in the His13–Gln15, Gly25–Gly29, and Gly37–Gly38 regions (13). Ahmed et al. (13) suggested that the Aβ42 pentamer is loosely packed in a disk-like cluster. Following this schematic Aβ42 pentamer arrangement, we constructed a disk-like model (disk1) and simulated the Aβ42 pentamer as indi-
cated in Fig. 2A. However, the loosely packed pentamer quickly dissociates into random states, and the calculated solvation protection does not correlate with experimental observations.

The His$^{13}$–Gln$^{15}$, Gly$^{25}$–Gly$^{29}$, and Gly$^{37}$–Gly$^{38}$ turn regions also suggest intra-chain $\beta$-hairpin structure for the pentamer. Yu et al. (30) characterized the partially structured oligomers, which have $\beta$-hairpin structure with a turn region in Gly$^{25}$–Gly$^{29}$. A $\beta$-hairpin structure with a turn region at this location was also observed in the solution structure of the A$\beta$ peptide in complex with an antibody mimetic protein (31). We tested a disk-like arrangement of $\beta$-hairpin structures (disk2, Fig. 2B). disk2 is flexible, with large changes leading to the loop-like structure and with few $\beta$-interactions (Fig. 2B). Its solvation exposure has some correlation with the experimental protection (Table 1, $R^2 = 0.43$).

Because experimentally observed pentamers could be mixtures of conformations in equilibrium, we examine other possible $\beta$-hairpin structures. We constructed four models (Fig. 3), with solvation protection patterns that could be consistent with those observed in the experiments. All have the same structure from Val$^{18}$ to Val$^{36}$, which fits the experimental observation of the Phe$^{19}$–Leu$^{34}$ interaction in the pentamer. Model m1 (Fig. 3, A and E) straightforwardly considers that all have $\beta$-hairpin turns, and oligomers can be formed through the interactions indicated in Fig. 3A. In m2, His$^{13}$–Gln$^{15}$ and Gly$^{37}$–Gly$^{38}$ are bulges in $\beta$-strands, making it possible for the C terminus in one monomer to interact with the N terminus in another. m3 and m4 have similar monomer structures but different dimeric associations (m3, parallel; m4, anti-parallel). In both monomers, the C terminus is $\alpha$-helix. The rationale for the $\alpha$-helical C terminus was as follows: 1) FTIR spectra suggested that there is 27% $\alpha$-helix in the pentamer (13), and 2) the C terminus presented strong H/D exchange protection, which is hardly

![FIGURE 1. A$\beta$42 pentamer and fibril have different structure and different H/D exchange protection patterns. A, correlation of the H/D exchange protections for A$\beta$42 pentamer and fibril is low. B, H/D exchange protections for A$\beta$42 pentamers can be fitted with the mixture of loop-like structure and several structures with $\beta$-hairpins. Because of highly flexible conformations of A$\beta$42 pentamers, amide hydrogen solvation exposures dominate the H/D exchange process. The solvation exposure values in the y axis are best fit values in Table 1. C, H/D exchange protections for A$\beta$42 fibril can be fitted with the mixture of triple $\beta$-sheet fibril seed oligomer and globulomer. Unlike pentamer, amide hydrogen solvation exposures and backbone hydrogen bonding contribute to H/D exchange protection. The values in x axis correspond to the $K_{ex}/K_{int}$ in Equation 4 and are obtained with the mixture of Go3 and Tf2 models.](image1)

![FIGURE 2. Simulations of two disk-like A$\beta$42 pentamer arrangements indicate that the pentamers are highly flexible. A, disk-like A$\beta$42 pentamer arrangement based on the suggestion from Ref. 13. Because of limited interactions among monomers, the arrangement quickly disassociates into monomeric loop structures. B, disk-like A$\beta$42 pentamer arrangement with $\beta$-hairpin structures, which change into loop-like structures with a few $\beta$-interactions.](image2)

| Table 1: Correlation coefficients (R2) of H/D solvation protections for the simulated pentamer models with the experimental H/D exchange of A$\beta$42 pentamer and A$\beta$42 fibril |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Pentamer models | $K_x$ | $K_c$ | $K_{ex}/K_{int}$ | $K_x$ | $K_c$ | $K_{ex}/K_{int}$ |
| disk2           | 0.43 | 0.16 | 0.29            | 0.15 | 0.12 | 0.16            |
| m1              | 0.29 | 0.19 | 0.23            | 0.12 | 0.20 | 0.19            |
| m2              | 0.34 | 0.21 | 0.31            | 0.21 | 0.24 | 0.24            |
| m3              | 0.37 | 0.15 | 0.27            | 0.01 | 0.11 | 0.02            |
| m4              | 0.38 | 0.22 | 0.43            | 0.14 | 0.16 | 0.20            |
| Average mixture | 0.60 | 0.22 | 0.50            | 0.25 | 0.18 | 0.21            |
| Best fit mixture | 0.68 | 0.23 | 0.46            | 0.21 | 0.19 | 0.22            |

$^a$ This is assuming an equal mixture of Disk2, m1, m2, m3, and m4.

$^b$ The mixture concentration is 6 disk2 + 3m3 + 3m2 + m4.
possible at the pentamer level otherwise (a similar consideration was also applied in disk2).

All pentamer models have certain correlations with H/D exchange protection (Table 1). Solvation exposure ($K_o$) correlates with the H/D exchange better than the backbone hydrogen bonding ($K_c$), indicating that solvation factors dominate exchange protection in loosely associated Aβ42 pentamers. Experimentally observed protection can be captured by including multiple models. As can be seen in Table 1, good correlation with H/D exchange ($R^2 = 0.60$) can be achieved with simple assumption that all models (disk2, m1, m2, m3, and m4) have equal contributions. Higher correlation can be achieved by varying the contribution of the models. The best fitted ($R^2 = 0.68$, Table 1 and Fig. 1B) has a large contribution from loop-like structures in disk2 and smaller contributions from m2, m3, and m4. Removing m1 increases the correlation, suggesting that m1 has minimal contribution.

As can be seen in Table 1, mixtures of β-hairpin pentamers have negligible correlations with H/D exchange protection for the fibril, within a range similar to that between experimental H/D exchange protection for the pentamer and fibril ($R^2 = 0.26$). This observation is consistent with the experimental conclusion that the pentamer and fibril have different structural elements.

Comparison of Simulations of the Aβ42 Fibril Seed and Experimental H/D Exchange Protection—Amyloid growth depends on the formation of seed oligomers, which catalyze aggregation and dictate amyloid structure. Established computational and experimental results revealed that Aβ fibril can be characterized by a double sheet U-turn structure, with two double sheet protofilaments (Fig. 4, A and B). The protofilaments may have two distinct interfaces (20), one similar in Aβ40 and Aβ42, and the other is unique to Aβ42. Met35 in one protofilament contacts Gly33 in the Aβ40/42 interface (Fig. 4B) but with Gly37 in the Aβ42 interface (Fig. 4A) (20).

We studied six double β-sheet models, with different combinations of U-turn structures and protofilament interfaces. Four (F1b, F1c, F1d, and F2b) have the Met35–Gly37 Aβ42 interfaces, and two (F1 and F2) have Gly37 in the Aβ40/42 interface. However, all failed to correlate with H/D exchange protection

![FIGURE 3. Intramolecular β-hairpin could contribute to the stabilization of Aβ42 pentamers. All these models have the same structure from Val18 to Val36, which fits experimental observation of Phe19–Leu24 interaction in the pentamer. A and E are secondary structures for model m1, which straightforwardly consider that all turns are turns for β-hairpin; B and F are secondary structures for model m2, which consider the region His13–Gln15 and Gly37–Gly38 as bulge in β-strands, making it possible for the C terminus in one monomer to interact with the N terminus in another monomer; C and G are secondary structures for model m3; and D and H are secondary structures for model m4. In the monomer structure of m3 and m4, the C terminus was constructed as an α-helix. The monomers are parallel in the model m3 and anti-parallel in the model m4.](image-url)
We examined two additional double β-sheet models (F3 and F4) with the protofilaments associated as a mirror image (supplemental Fig. 1). In F4, Met35 is buried between the two β-sheets as proposed based on ssNMR experiments (21). Comparing the simulations of these models with experimental H/D exchange, we again found no correlation (Table 1, model number 7 and 8).

Because the traditional double β-sheet protofilament model cannot explain the experimental H/D exchange, we consider novel motifs. Based on experimental and computational work, we consider a possible turn around Gly9–His14 (13, 32). We tested triple β-sheet protofilament conformations, in which the N-terminal region of Aβ42 folds back to a conventional double β-sheet core (Fig. 4C). Triple β-sheet can be formed from pen-
Polymorphic Triple β-Sheet Fibrillar Structure

A recent NMR study indicated that Aβ40 D23N mutant fibril may have anti-parallel β-sheets (7). We examined two possible anti-parallel conformations. In model AF1 Val¹⁸ aligns with Val¹⁸ in neighboring N-terminal strands, and Met⁵⁵ aligns with Met⁵⁵ in neighboring C-terminal strands (Fig. 6A). In AF2, the N-terminal strands have the same register as in Aβ(16–22) (33) and in the D23N mutant Aβ40 fibril (7). In the C terminus, Met⁵⁵ aligns with Gly³³ in neighboring C-terminal strands (Fig. 6B). We also included a globulomer model (34) GO3 (supplementary Fig. 2).

tamers with turn regions in His¹³–Gln¹⁵ and Gly²⁵–Gly²⁹, as illustrated in Fig. 4C. We investigated five triple β-sheet models with different turn conformations and protofilament interfaces (Fig. 5). In model TF1, the N-terminal turn allows hydrophobic interactions of Phe⁴ and Phe²⁰ (Fig. 5A); in model TF2 Phe³–Phe²⁰ and Tyr¹⁰–Val¹⁸ interactions (Fig. 5B), and in model TF3 Phe³–Phe²⁰ but no Tyr¹⁰–Val¹⁸ (Fig. 5C). The protofilament interfaces in TF1, TF2, and TF3 are the Aβ40/42 interface. Models with the unique Aβ42 interface (TF1b and TF2b) were also tested (Fig. 5, D and E).

FIGURE 5. Structures of triple β-sheet models indicate that Aβ peptide can form fibril with minimum exposure of hydrophobic residues. These models are among those simulated in this work. Met³⁵ residues, large balls with sulfur in yellow; Glu²² residues, ball-and-stick with oxygen in red; and Phe⁶, Tyr¹⁰, His¹⁴, Val¹⁵, Phe²⁰, and Val²⁴ are sticks. A, TF1 model with hydrophobic interactions (HPI) between Phe⁴ and Phe²⁰; B, TF2 model with HPI between Phe³–Val¹⁸ and Tyr¹⁰–Val¹⁸; C, TF3 model with HPI between Phe³–Val¹⁸; D, TF1b model with Met³⁵–Gly³⁷ interaction; E, TF2b model with Met³⁵–Gly³⁷ interaction; and F, TF3E22Q model indicates that buried charges of Glu²² can be neutralized by the E22Q mutation.

FIGURE 6. Polymorphic ensemble of Aβ can include anti-parallel β-strand arrangements. The models shown here were also simulated in this work. Glu²² and Met³⁵ residues are depicted as ball-and-stick (sulfur, yellow; oxygen, red). A, anti-parallel model AF1 with Val¹⁸ aligns with Val¹⁸ in neighboring N-terminal strands, and Met³⁵ with Met³⁵ in C-terminal strands; B, anti-parallel model AF2 with the same register as Aβ(16–22) in the D23N mutant Aβ40 fibril (7); in the C-terminal register, Met³⁵ aligns with Gly³³ of the neighboring C terminus.
Polymorphic Triple β-Sheet Fibrillar Structure

Our simulations revealed that these new motifs correlated with experimental H/D exchange protection (Table 2, model number 9–16). Unlike the highly fluctuating pentamer whose solvation exposure dominates the H/D exchange protection, in these possible fibril seeds both solvation factor ($K_s$) and backbone amide hydrogen bonding factor ($K_m$) contribute to the exchange protection. Triple β-sheet models TF1 and TF2 have the best fit to the experimental protection ratio; however, the other three models (TF3, anti-parallel AF2, and globulomer model GO3) also have certain correlations. Models with the Aβ40/42 interface (TF1, TF2, and TF3) correlate with experiments better than those with the Aβ42 interface (TF1b and TF2b).

| Polymorphic Aβ mixture* | $R^2(K_s)$ | $R^2(K_m/K_m^0)$ |
|------------------------|------------|-----------------|
| GO3+TF1               | 0.66       | 0.72            |
| GO3+TF2               | 0.74       | 0.77            |
| GO3+TF3               | 0.63       | 0.69            |
| GO3+AF2               | 0.62       | 0.60            |
| TF1+TF2               | 0.57       | 0.63            |
| TF1+TF3               | 0.44       | 0.53            |
| TF1+AF2               | 0.54       | 0.59            |
| TF2+TF3               | 0.45       | 0.54            |
| TF2+AF2               | 0.58       | 0.62            |
| TF3+AF2               | 0.49       | 0.55            |
| GO3+TF1+TF2           | 0.72       | 0.77            |
| GO3+TF1+TF3           | 0.62       | 0.70            |
| GO3+TF1+AF2           | 0.66       | 0.68            |
| GO3+TF2+TF3           | 0.66       | 0.74            |
| GO3+TF2+AF2           | 0.73       | 0.72            |
| TF1+TF2+TF3           | 0.50       | 0.58            |
| TF1+TF2+AF2           | 0.60       | 0.66            |
| TF2+TF3+AF2           | 0.55       | 0.62            |

* This is assuming that each model contributes equally to the overall solvation factors, and the average values from mixed models are used in the comparison.

It is possible that multiple triple β-sheet fibrils can coexist in sufficiently high populations to explain the experimental protection. We examined the correlation of the H/D data with an ensemble of five possible models GO3, TF1, TF2, TF3, and AF2. As can be seen in Table 3, if only two polymorphic states are observed experimentally, the mixture of GO3 and TF2 has the highest correlation ($R^2 = 0.77$, Fig. 1C). If we consider three states, the combinations of GO3 + TF1 + TF2 ($R^2 = 0.77$) also show similar correlations. Therefore, it is likely that amyloid states observed under experimental conditions are the mixture of GO3-, TF1-, and TF2-like models. Thus, a novel triple β-sheet model such as TF2 could be a main fibril form observed in H/D exchange protection ratios. The correlations were calculated based on the assumption that each model contributes equally. We also tested variable contributions. However, the best correlations are near the range obtained for equal ones.

### Triple β-Sheet Aβ42 Models Have Minimum Solvent Exposure of Hydrophobic Residues in the Fibril

Structured, all models are stable in the core region (residues 17–42), with the N terminus (residues 1–14) fluctuating. The Generalized Born with the Molecular Volume energy calculations indicate that F1d, TF2b, and AF2 are among the most stable conformers, whereas F3, F4, and TF2 have the highest energies (Table 2). Overall, the three groups of conformers (double β-sheet, triple β-sheet, and anti-parallel; Fig. 7A) have comparable energies, indicating that from the thermodynamic standpoint these polymorphic forms can coexist in the heterogeneous ensemble. The individual energetic contributions differ substantially among the double β-sheet, triple β-sheet, and anti-parallel oligomers. For example, the double β-sheet oligomers have the largest number of hydrogen bonds between β-strands, whereas anti-
Polymorphic Triple β-Sheet Fibrillar Structure

Parallel structures have the least (Fig. 7B). However, anti-parallel structures have favorable electrostatic interactions between the β-strands due to salt bridges between Asp²³–Lys²⁸ and Glu²²–Lys¹⁶ (Fig. 8A). The Asp²³–Lys²⁸ salt bridge is important for the chemical and mechanical stability of the Aβ amyloid (35, 36). In the parallel oligomers (for both double and triple β-sheet), the Asp²³–Lys²⁸ salt bridges can be formed inter- or intramolecularly, and Glu²² and Lys¹⁶ (and other charged residues) have large electrostatic repulsion. As a result, electrostatic attraction within the oligomers increases from double (for example, F1d, 563 kcal/mol), to triple β-sheet (TF2b, −559 kcal/mol), to the anti-parallel structure (AF2, −1997 kcal/mol). Thus, favorable electrostatic interaction could help the formation of anti-parallel structures such as the AF2 model.

The stability of the triple β-sheet may stem from hydrogen bonds and electrostatic interactions between N-terminal residues and the Lys¹⁶–Glù²² region. In addition, in triple β-sheet oligomers, exposed hydrophobic surfaces significantly decrease, which contributes to better solvation. All exposed hydrophobic residues in the double β-sheet and anti-parallel models (Phe⁶, Tyr⁴⁰, Val¹⁸, Phe²⁰, and Val²⁴, see supplemental Fig. 3) are buried in the triple β-sheet TF2 and TF2b models. As can be seen in Fig. 8B, there are less water molecules around hydrophobic residues in these models than in the double β-sheet and anti-parallel models. The interaction of the N terminus and the Lys¹⁶–Glu²² β-sheet is not tight enough to completely prevent some water molecules from diffusing toward the hydrophobic residues. However, the E22Q mutation makes the triple β-sheet TF2 highly compact, with the lowest solvation of hydrophobic residues (Fig. 8B).

**E22Q Dutch Mutation Increases the Stability of the Triple β-Sheet Aβ42 Fibril Model—**We tested the following five E22Q models: two double β-sheet models (F1-E22Q and F1d-E22Q), one anti-parallel model (AF2-E22Q), and two triple β-sheet models (TF2-E22Q and TF3-E22Q). We found that the triple β-sheet TF2-E22Q has the lowest energy (Table 2), implying that the triple β-sheet TF2-E22Q model can be the preferred form for the Dutch mutant.

One of the traditional explanations for the accelerated aggregation and toxicity of the Dutch mutation in Alzheimer disease is that E22Q decreases electrostatic repulsion between parallel β-strands in the Aβ fibril (37). Our study indicated that the E22Q mutation indeed increases electrostatic attraction for all three oligomeric states (double β-sheet, triple β-sheet, and anti-parallel; Fig. 8A); however, the changes in electrostatic attraction were compensated by a decrease in the electrostatic contribution from solvation. Similar results were found for the Aβ(17–42) E22Q mutant (38). Neither the double β-sheet nor the anti-parallel oligomers gain structural stability from increased electrostatic attraction due to the E22Q mutation.

On the contrary, the total number of hydrogen bonds in the F1d model decrease by the E22Q mutation (Fig. 7, B and C), explaining the less favorable energies for the F1d-E22Q model.

Triple β-sheet models provide a new angle to the accelerated aggregation of the E22Q mutant. The highly charged Glu²² patches are buried in the TF2 and TF3; however, the Gln²² mutation would decrease the desolvation energy and stabilize the triple β-sheet. As can be seen from a comparison of Fig. 7, B and C, the total number of hydrogen bonds increases from the WT TF2 (red lines) to the TF2-E22Q (light blue line). The interaction of the N terminus and the β-sheet of the Lys¹⁶–Glù²² region is tighter (Fig. 8C) than in the wild type TF2. The E22Q mutation decreases the solvation of hydrophobic residues, mainly because of the compact fibril form. These results are consistent with the driving forces of amyloid formation being a tight geometric match between β-sheets (39) and the hydrophobic effect (6, 8).

We also tested three E22A mutant models (F1-E22A, TF2-E22A, and TF3-E22A). Unlike the E22Q, triple β-sheet E22A models (TF2-E22A and TF3-E22A) are not necessarily more stable than the double β-sheet F1-E22A model (Table 2). One reason could be that the E22A side chain does not provide steric match between the N terminus and the main β-sheets, in which case E22A may not accelerate aggregation.

**DISCUSSION**

Proteins can fold into functional states or misfold into toxic aggregation-prone forms. Highly polymorphic protein structures pose challenges for computational prediction and experimental identification. The information contained in NMR chemical shifts has already been demonstrated to assist in pro-
tein fold prediction and identification (40). Our work focuses on the misfolded Aβ aggregate conformations. Because the energy landscape is broad and the polymorphic range of pre-existing misfolded aggregates is vast (8), current methods cannot exhaustively search and identify all possible structural entities. Here, we probe the polymorphic range of the oligomers and compare amide solvation obtained from simulations with H/D exchange protection ratio. Our work represents an effective approach to delineate observed and likely aggregation states that could relate to toxic mechanisms in Alzheimer disease. For the Aβ42 pentamer, our simulations confirmed that highly flexible pentamers do not share structural similarities with fibril seed oligomers, except the turn regions. Using amide hydrogen solvation exposure and backbone hydrogen bonding protection factors, we can reproduce experimental H/D exchange protection for both species.

Our analysis revealed coexistence of nano-particle-like globulomer and fibril structures of the triple β-sheet amyloid. The novel triple β-sheet fibril has minimum exposure of hydrophobic residues, which is further stabilized by the E22Q mutation in the familial Dutch in Alzheimer disease.

Even though there are surely entirely different polymorphs, the correlation of experimental and computational approaches can serve as the basis for explaining observations, especially the triple β-sheet Aβ42 fibril structure. 1) The E22Q mutant has lower proteolytic degradation as compared with WT Aβ (41, 42), in agreement with our finding that the mutant is highly stable and is more compact than WT Aβ. 2) The English (H6R) mutation accelerates Aβ fibril formation (43). His6 is in contact with Glu22 in the triple β-sheet structure. The H6R mutation would lead to the formation of a salt bridge between Glu22 and Arg6 and enhance the stability of the triple β-sheet conformer. 3) The N-terminal truncated Aβ(12–42) E22Q aggregates less (44), which could relate to the absence of a triple β-sheet. These experimental observations and the triple β-sheet fibril model highlight recent findings of the N-terminal role in the formation of the tubular Aβ fibril (32, 45). The coexistence of globulomer and fibril also suggests that there are critical intermediates (8, 34) that could lead to both ADDL-like (Amyloid β-Derived Diffusible Ligands) and fibril amyloids. The triple β-sheet Aβ42 structure may also apply to Aβ40. We tested two interfaces between Aβ protofibrils, one similar in Aβ40 and Aβ42 and the other unique to Aβ42. For Aβ42, the interface does not necessarily provide better interaction than the Aβ40/ Aβ42 interface. Under our simulation conditions, although TF2b has lower energy than TF2, TF1b is very close to TF1, and F1b/F1c has higher energy than F1 (Table 2). Fibrils with Aβ40/ Aβ42 interface correlate better with H/D exchange protection.

A recent NMR study revealed that the Phe19–Leu34 packing in the Aβ42 oligomer (13) is consistent with most oligomer structures tested in our study. Our models also include an earlier structure proposed by Petkova et al. (21), in which Met55 is buried in the protofibril between two β-sheets. Even though this structure does not fit the observed H/D exchange protection ratio, based on free energy landscape concepts, it may have higher population under different conditions. Furthermore, heterogeneous β-sheet associations involve parallel and anti-parallel β-strand orientations; according to our results, the anti-parallel AF2 could fit experimental H/D exchange protection. We note that the AF2 model has the same register around Aβ(16–22) as in the D23N mutant Aβ40 fibril (7), and our results suggest that the experimentally observed anti-parallel Aβ40 D23N fibril could also exist for wild type Aβ42.

In conclusion, our results indicate that a combination of H/D exchange NMR and computational screening can help in delineating observed and likely aggregation states. An extension of the approach to analyze Aβ aggregate forms under more in vivo-like conditions may provide further insight into the preferred aggregate states and their mechanisms of toxicity.

H/D exchange provided important information related to protected/dynamic regions in amyloids (29), offering insights into possible kinetic intermediates (18). However, it may also have significant errors because of the difficulties that are involved. This highlights the advantages of integrating computational and experimental approaches. On their own, neither NMR protection nor computational modeling provides robust atomic scale data of preferred conformational species in solution. Our results illustrate that the combination of modeling and H/D exchange NMR protection data can be useful. A similar strategy could be used in prediction of structures of complexes if the separate structures of the components (e.g. single chain proteins, RNA, and DNA) are available. Here we model amyloids; however, in a similar manner structures docked by automated algorithms can be usefully combined with NMR exchange data.

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