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Molecular Modeling of the Core of Aβ Amyloid Fibrils

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ABSTRACT **Amyloid fibrils, a key pathological feature of Alzheimer's disease (AD) and other amyloidosis implicated in neurodegeneration, have a characteristic cross-β structure. Here we present a structural model for the core of amyloid fibrils formed by the Aβ peptide using computational approaches and experimental data. Aβ(15–36) was threaded against the parallel β-helical proteins. Our multi-layer model was constructed using the top scoring template 1lxa, a left-handed parallel β-helical protein. This six-rung helical model has in-register repeats of the Aβ(15–36) sequence. Each rung has three β-strands separated by two turns. The model was tested using molecular dynamics simulations in explicit water, and is in good agreement with a number of experimental observations. In addition, a model based on right-handed helical proteins is also described. The core structural model described here might serve as the building block of the Aβ(1–40) amyloid fibril as well as some other amyloid fibrils. Proteins 2004;57:357–364.**

Key words: Alzheimer's disease; parallel β-helix; protein threading; molecular dynamics simulation; protein structure prediction

INTRODUCTION

The amyloidoses are disorders characterized by the extracellular accumulation of fibrillar protein deposits, called amyloid fibrils.[1] The most studied amyloidosis is Alzheimer's disease (AD). One of the key pathological features in the AD brain is the presence of amyloid plaques which are rich in amyloid-β (Aβ) peptides, 40–42 amino acid fragments derived from the amyloid precursor protein (APP).[2] Aggregates structurally related to amyloid fibrils are also involved in a number of other neurodegenerative diseases, including Huntington's disease, prion diseases, and Parkinson's disease. Although amyloid precursor proteins do not share any homology with respect to amino acid sequence or native structural fold, amyloid fibrils share a number of structural features. Therefore, knowledge of the three-dimensional structure of the amyloid fibrils has great bearing on our understanding of how the fibrils are formed and on the rational design of therapeutics.

Amyloid fibrils are extremely stable and are very resistant to degradation.[3] Electron microscopy reveals that amyloid fibrils are straight and unbranched and are made up of 2–6 bundled and twisted protofilaments, yielding a total fibril diameter of 60–120 Å.[4] X-ray diffraction from amyloid fibrils shows that they share a common cross-β structural motif, in which the β-strands run perpendicular to the long axis of the fibrils while the hydrogen bonds between β-strands are parallel to the axis.[5] Obtaining a higher resolution structural model is crucial to the development of new reagents that inhibit the fibril formation. However, high-resolution structural studies of amyloid fibrils using X-ray crystallography and solution NMR have not been possible, due to the insolubility and noncrystalline nature of the fibrils.

Several other approaches have yielded considerable information that places structural constraints on Aβ fibril models. Using hydrogen-deuterium exchange (HX) technique, Kheterpal et al. showed that only about half of the 39 backbone amide protons are protected from exchange in the fibril form of Aβ(1–40)[6,7] suggesting that a significant number of residues are not involved in β-sheet formation. Limited proteolysis,[8] solid state NMR,[9] and electron paramagnetic resonance spectroscopy (EPR)[10] studies revealed that the N-terminal 13–16 residues of the full-length Aβ are not directly involved in making up the H-bonded fibril core. Based on such experimental observations, several models for full-length Aβ fibrils have been proposed.[9,11–15] While these models differ in many structural details, they generally fall into two main categories: antiparallel and parallel β-pleated sheet arrangements. Compelling evidence from the solid state NMR and liquid suspension EPR studies on full-length Aβ fibrils suggests that the peptides in the fibril core are in an in-register, parallel arrangement.[9,16] At the same time, there is no consensus on a unique structural model for the fibril-folding motif, and in fact a number of models involving parallel β-sheet have been proposed.[9,14,16] One as yet unresolved issue is the number and the location(s) of turns in the peptide when it packs into the amyloid core structure. Recently, proline scanning mutagenesis was used to search for regions involved in turns and disordered structure in Aβ

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peptides.[17–19] The data show that the N-terminal 10–15 residues of Aβ(1–40) are in flexible conformations in the amyloid fibril, which is consistent with the data from limited proteolysis, solid state NMR and EPR studies.[8–10] In addition, the proline scanning data show that the C-terminal residues 37–40 are not involved in H-bonded core structure in the fibril.[17] This is consistent with EPR studies showing enhanced mobility of residues 38–40 in Aβ(1–40) fibrils.[10] Besides indicating that it is the 15–36 sequence of Aβ that is involved in ordered, proline-sensitive structure within the amyloid core, these data also suggest the existence of two turn regions within the 15–36 segment, one at residues 22–23 and one at residues 29–30.[17] The accumulated physical data on Aβ(1–40) amyloid fibrils thus suggests a folding model resembling those of the parallel β-helical proteins.

Here, we describe a structural model for the Aβ amyloid fibril core structure using computational methods in combination of experimental data as constraints. The model was constructed based on the threading of Aβ(15–36) against the representative parallel β-helical proteins using PROSPECT II.[20,21] The model, derived without further input from experimental data, agrees in considerable detail with the proline scanning data. The model was then subjected to molecular dynamics simulation in explicit solvent. Our simulation results indicate that the model has good conformational stability.

MATERIALS AND METHODS

Prediction of Intrinsic Disordered Region

Predictions of intrinsic disorder within Aβ(1–40) monomer were made using the neural network predictor PONDR VL-XT (Molecular Kinetics). The predictor outputs a value for each amino acid. If a residue value exceeds a threshold (0.5), the residue is considered disordered. The accuracy of prediction is about 75%-83%[22].

Threading Analysis

Protein threading was carried out on Aβ(15–36) sequences using PROSPECT II[21] against a preselected structural template database. PROSPECT II employs a knowledge-based energy function and it guarantees to find the globally optimal sequence–structure alignment under the given energy function.[20] Our structural templates include all the left-handed and right-handed parallel β-helical proteins defined by SCOP[23] in the FSSP database[24] that do not share significant sequence similarity (< 25% sequence identity). This set consists of 21 β-helical and four non-β-helical all-β proteins, identified by their PDB codes (with the fifth letter indicating the chain name, if any): 1fwya, 1qrea, 1lxa, 3tdt, 1kqaa, 2xat, 1m8nb (left-handed parallel β-helical proteins), 1hf2a, 1rmg, 1bhe, 1czfa, 1h80a, 1dbga, 1tyv, 1qcxa, 1air, 1qjva, 1daba, 1ee6a, 1kq5a, and 1ezga (right-handed parallel β-helical proteins), and 2paba, 1neu, 1amx, 1c3gu (non-β-helical all-β proteins).

Model Construction

Using the highest-scoring alignments from the threading analysis, and the strong evidence from solid state NMR and EPR studies that Aβ monomers are in an in-register,[9,10] parallel β-sheet organization in Aβ fibrils, 6-mer (Aβ 15–36) models were generated using Modeller.[25] To do this, six successive rungs of β-helices of template structures were aligned to six Aβ(15–36) sequences. The alignment files for Modeller were generated as follows: one copy of Aβ(15–36) was aligned with corresponding residues of template structure as shown in the threading alignments (Fig. 2); five other Aβ(15–36) peptides were placed in successive rungs of the template, one peptide per rung, in an in-register fashion; Gly3 segments were added to connect the N- and C-termini of adjacent Aβ molecules. After the model was constructed using Modeller, the Gly3 connectors were removed. The stereochemical quality of the models was evaluated with Procheck.[26] A left-handed parallel β-helix model was constructed based on the highest scoring template, 1lxa. In addition, a 6-mer right-handed model was generated based on threading result against 1bhe.

Molecular Dynamics Simulations

Molecular dynamics simulations were performed using GROMACS (version 3.1).[27] The simulations employed the GROMACS force field under periodic boundary conditions. The Particle Mesh Ewald (PME) method[28] and a distance cutoff of 10 Å were used for long-range electrostatic interactions and van der Waals interactions, respectively. The time step was 2 femtoseconds (fs). All bonds including hydrogen atoms were constrained using the Lincs algorithm.[29] The protein was first solvated in a cubic box ($60 \times 60 \times 60$ Å) with 7126 SPC water molecules.[30] The simulations were carried out under NPT conditions (constant number of particle, pressure and temperature) at 300 K. A constant pressure of 1 bar in all three directions was used with a coupling constant of $\tau p = 1.0$ picoseconds (ps). The system was energy minimized, followed by equilibration for 30 ps. The simulation was carried out for 2 nanoseconds (ns).

Analysis of the simulation was performed using a set of programs within GROMACS. DSSP program was employed for the secondary structure analysis.[31] Structures were visualized using Rasmol.[32]

RESULTS

A prerequisite for initiating fold identification through threading analysis is to localize the segment(s) of the sequence involved in ordered structure. As reviewed in the introduction, proline scanning mutagenesis [17] as well as other experimental approaches indicate that the first 10–15 amino acids and the C-terminal three amino acids of Aβ(1–40) are relatively disordered in the structure of the amyloid fibril.[8–10] We also used the computer program PONDR,[33] to predict disordered regions in Aβ(1–40). The result is shown in Figure 1. The first seven amino acids of Aβ(1–40) are predicted to be disordered using the default threshold of 0.5. Interestingly, the least disordered segment contains residues 13–33 (Fig. 1), which is in good agreement with the experimental data for the Aβ fibrils.[17] Since the N-terminus and C-terminus are indicated to be

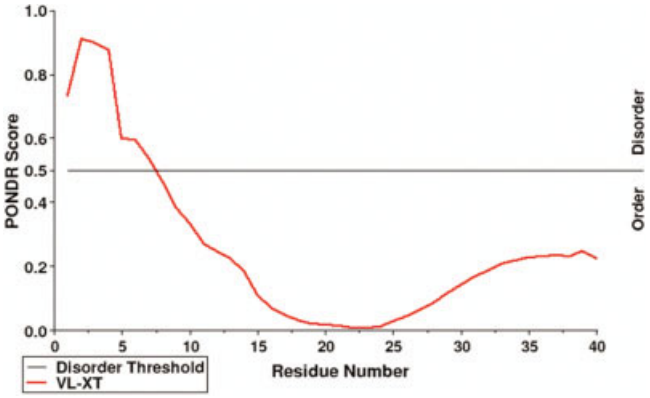

Fig. 1. Prediction of disordered regions in Aβ(1–40) using PONDR predictor, VLXT. The default threshold for disordered region is 0.5.

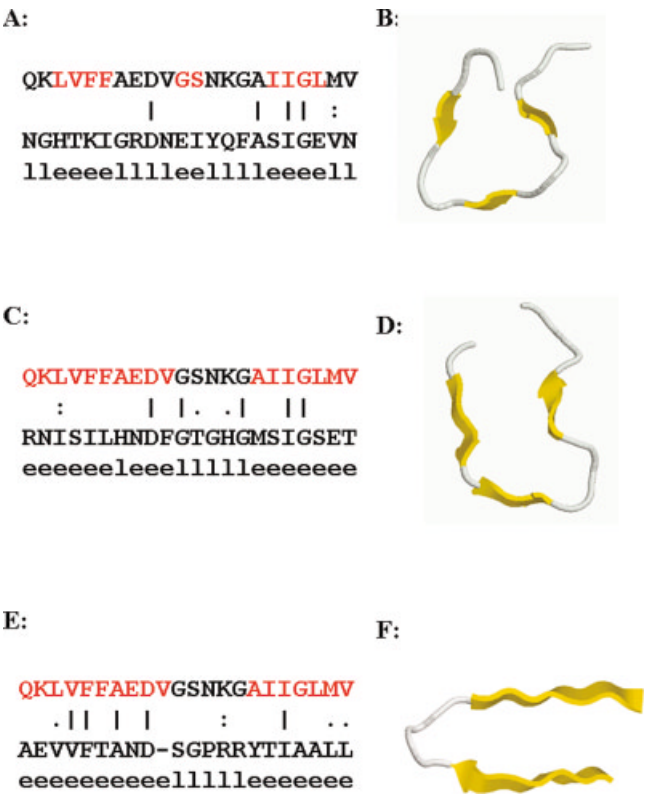

Fig. 2. Threading results of Aβ(15–36). The secondary structure assignment of residues on templates is listed under the template sequence. DSSP program was used for the secondary structure assignment,[31] e: strand; l: coil. **A:** alignment with 1lxa. **B:** template structure for the aligned 1lxa. **C:** alignment with 1bhe. **D:** template structure for the aligned 1bhe. **E:** alignment with 2paba. **F:** template structure for the aligned 2paba.

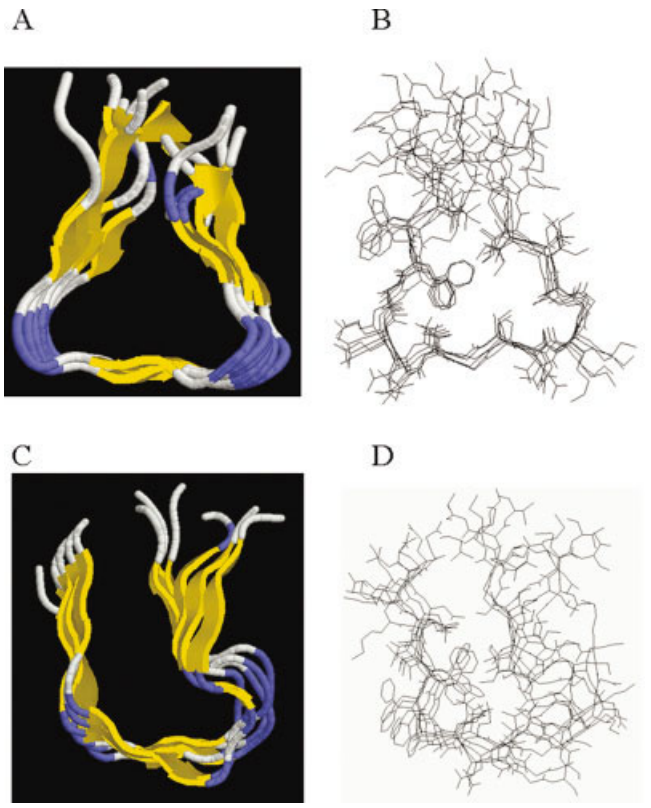

Fig. 3. Structural models for left-handed (**A** and **B**) and right-handed (**C** and **D**) in ribbon (A, C) and in wireframe (B, D) representations. Different color represents different secondary structure, yellow: strand; blue: turn; white: coil.

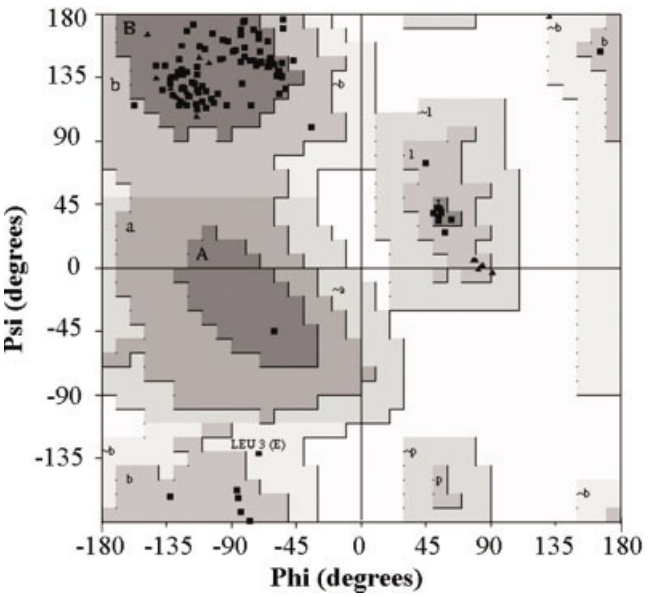

Fig. 4. Ramanchandran plot for the constructed left-handed model, calculated using PROCHECK.[26] None of the residues are in the disallowed regions.

disordered by experimental studies, they are not taken into account in our model.

The parallel β-helix folding motif, an attractive model for the amyloid fibril core structure based on current experimental data, has been proposed for various amyloid fibrils.[15,34,35] The pattern from the proline mutagenesis data is also consistent with a parallel β-helical motif.[17] Here the Aβ(15–36) peptide was threaded against the β-helical proteins using PROSPECT II. The top two hits were the helical domains of 1lxa and 1h80a. 1lxa is a left-handed parallel β-helix protein while 1h80a belongs to

the right-handed category. Figure 2 shows the alignment with 1lxa and the template structure for the aligned part. The alignment between Aβ(15–36) and 1lxa reveals that three pairs of Aβ residues (F19–F20, G25–S26, and I31–I32) are aligned to the three β-sheets of the helical protein and two four-residue regions (residues 21–24, AEDV and residues 27–30, NKGA) of Aβ peptide to the turns [Fig. 2(A)]. The threading result against 1lxa fits well with data from proline scanning mutagenesis in which two pairs of residues (residues 22–23 and residues 29–30) highly resistant to proline substitution exist in the likely β-sheet regions between residues 15 and 36.[17] In contrast, the template structure based on the alignment between Aβ (15–36) and 1h80a, does not fit any experimental observations except that there are two β-strands for sequences VFFA and AIIGLM (data not shown). Figure 2(C, D) show the alignments with 1bhe, another right-handed parallel β-helical protein, and the aligned template structure. The alignment with 1bhe is in agreement with most of the solid state NMR data with two β-strand conformations flanking a non-β-strand segment of residues 25–29. Aβ(15–36) was also threaded against several non-β-helical all-β proteins including the one used by Li et al. for their anti-parallel model construction, 2pab.[11] Results showed that the threading scores against non-β-helical all-β protein templates were much lower than that against β-helical proteins. The alignment with 2paba and the aligned template structure are shown in Figure 2(E,F). The alignment with 2paba is very similar to the model proposed by Petkova et al, [9] in which a bend with residues 25–29 separates two strands.

Based on the threading results, and the requirements for parallel, in-register β-sheet, a structural model of a 6-mer Aβ(15–36) was generated using 1lxa as template as described in Materials and Methods [Fig. 3(A)]. The stereochemical quality of the model was evaluated with Procheck[26] and the Ramachandran plot shows that about 86% of the residues are in the most favored regions, and none of the residues are in disallowed regions (Fig. 4). In this 6-mer Aβ (15–36) model, there are two turns in each rung, residues 21–24 and residues 27–30, and three sheets with the central sheet formed by G25–S26 of each monomer. Several inward-pointing hydrophobic residues are clustered together and form a hydrophobic core (V18, F20, V24, 30A, and 32I) while the hydrophilic residues at the turn regions are pointing outward (E22, D23, N27, and K28) as shown in Figure 3(B). The side-chain orientations for residues 18–32 are consistent in each rung, while the orientations of the flanking residues (residues 15–17 and 33–36) vary a little bit in each rung.

Molecular dynamics simulations were performed for this structural model in explicit water using the PME method to accommodate long-range electrostatic forces and periodic boundary conditions to prevent solvent evaporation. After a short period of equilibration, the structure remains quite stable during the rest of simulation [Fig. 5(A)]. Figure 5(A) shows the structural deviations compared to the starting structure during the course of simulation. It maintained at ~3 Å for most of the simulation. Interestingly, the model acquired more backbone hydrogen bonds [Fig. 6(A)] and more β structure [Fig. 7(A)] over the course of the simulation (2 ns). Figure 7(A) shows that not only is most of the β structure found in the starting model preserved, but also more β structure is formed during the simulation. In particular, in two of the central rungs of the model, the four-residue turns that were built into the starting model contracted, by the end of the 2-ns simulation, to two-residue turns whose positions (residue pairs 22–23 and 29–30) [Figs. 7(A), 8] match those suggested by the proline scanning results described above. Throughout the second half of the simulation, the total number of hydrogen bonds in the six-stranded model remains around 75.

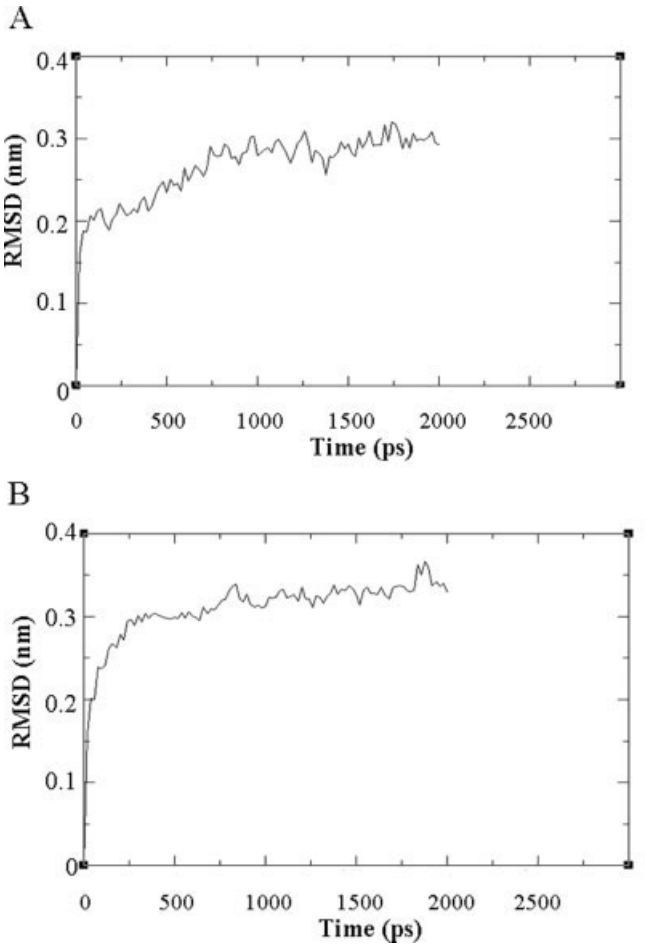

Fig. 5. Root mean square deviation (RMSD) of the backbone atoms relative to the starting structure during 2-ns simulation. **A:** left-handed model. **B:** right-handed model.

Our left-handed helix model is consistent with many experimental observations including the cross-β diffraction pattern. One exception is the model's inability to account for the 10 Å equatorial reflections in X-ray diffraction data on Aβ fibrils.[5] One possible explanation is that the 10 Å diffractions might reflect the inter-protofilament interactions or the packing among protofilaments. The model described above has two hydrophobic faces that can pack together and form the fibrils [Fig. 9(A)].

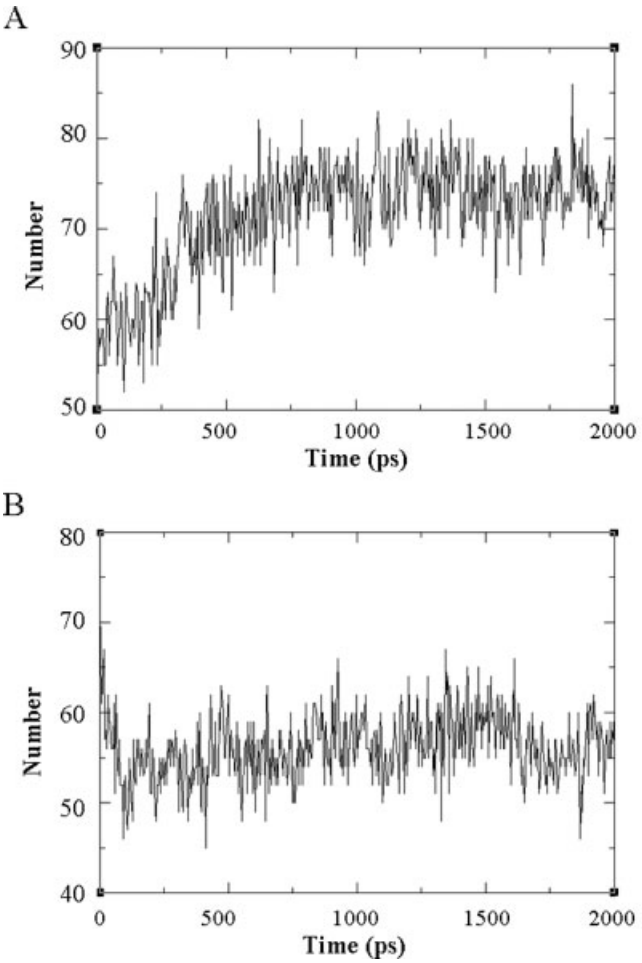

Fig. 6. Analysis of hydrogen bonds among backbone atoms as a function of the simulation time. **A:** left-handed model. **B:** right-handed model.

Another possibility, however, is that the core structure might adopt a slightly different motif resembling some of the right-handed β-helical features, such that the 10 Å spacing might come from two essentially parallel strands within each rung of the helix (Fig. 9B, C). As discussed earlier, the threading result on 1h80a does not yield a model that fits most of the experimental data on amyloid fibrils from full-length versions of Aβ. On the other hand, the structure based on the alignment with 1bhe [Fig. 2(C, D), another high scoring fold in the original threading exercise, is in agreement with most of the solid state NMR data. For example, residues 25–29 are in a non-β-strand segment while the majority of the residues flanking 25–29 are in β-strand conformations.[9] We therefore constructed a right-handed model based on the alignment of Aβ with 1bhe (Fig. 3C, D). The stereochemical quality of this right-handed model is as good as that of the left-handed model. The structure was subjected to molecular dynamics simulations using the same conditions as that used for the left-handed model. The structural deviations compared to the starting structure during the course of simulation are about 3 Å [Fig. 5(B)]. One intriguing feature of the simulation on the right-handed model is that the total number of backbone hydrogen bonds dropped from ~70 at the beginning of the simulation to ~55 in a very short time and remains at this number for the rest of the simulation [Fig. 6(B)]. The locations of some of the β-structure vary over the course of the simulation [Fig. 7(B)]. These results are in direct contrast to those from the simulation on the left-handed model, in which the total number of backbone hydrogen bonds increased from about 58 to 75. The average number of backbone hydrogen bonds for each Aβ fragment at the end of the simulation is therefore different for the two models, with 11.6 (58/5) for the right-handed model and 15 (75/5) for the left-handed model (Fig. 6).

These numbers for H-bonds per strand are low estimates for the amyloid fibril, since the more-disordered strands on the edges of the six-stranded models bring down the averages compared to what one would find in fibrils, which have many more internal strands compared to edge strands. The highest total number of H-bonds per structure during the simulation of the left-handed model is 85 [Fig. 6(A)], which gives an average of 17 H-bonds per strand. The average H-bonds per strand in the central strands of the model would be expected to be somewhat higher. Thus, the left-handed β-helix model after simulation is in good agreement with the estimate of 20–21 protected backbone amide hydrogens in Aβ amyloid fibrils determined by hydrogen exchange-mass spectrometry.[6,7]

DISCUSSION

It is generally accepted that amyloid fibrils are predominantly cross-β structures with β-strands arranged perpendicular to, and their backbone hydrogen bonds parallel to, the fibril axis. Although anti-parallel β-sheet appears to dominate amyloid fibrils made of short Aβ peptides,[12,36,37] evidence from solid-state NMR and EPR studies indicates that the β-strands are in-register and parallel in fibrils composed of longer or full-length Aβ.[9,10,38] The published models for full-length Aβ amyloid fibrils have different β-strand arrangements.[9,13–15,39] The linear parallel model of Aβ(16–35) or Aβ(10–35), with polar residues in the middle of the peptide, appears unfavorable based on simulation experiments by Ma and Nussinov.[40] Therefore, other types of conformations must be considered, such as turn(s) in the middle of the peptide. Solid state NMR reveals a possible non-β-strand region between residues 25 and 29.[9] Kinetics analysis of fibril formation of proline replacements in Aβ(1–42) at positions 19–26 suggests a possible turn region at positions 22 and 23.[19] Thermodynamic analysis of the stabilities of fibrils derived from proline mutants of Aβ(1–40) implicates two turn regions at residues 22–23 and 29–30 within a β-sheet-rich core region involving residues 15–36.[17] The latter data points to a well-known folding motif, the parallel β-helical fold, as a possible model for Aβ fibrils.[34,41] Parallel β-helical-type models have been proposed for other types of amyloid fibrils including polyglutamine,[42] mammalian prions,[35] and insulin amyloid fibrils.[43] The parallel β-helical fold is very stable and natural β-helical domains may well be poised to oligomerize were it not for the intervention of

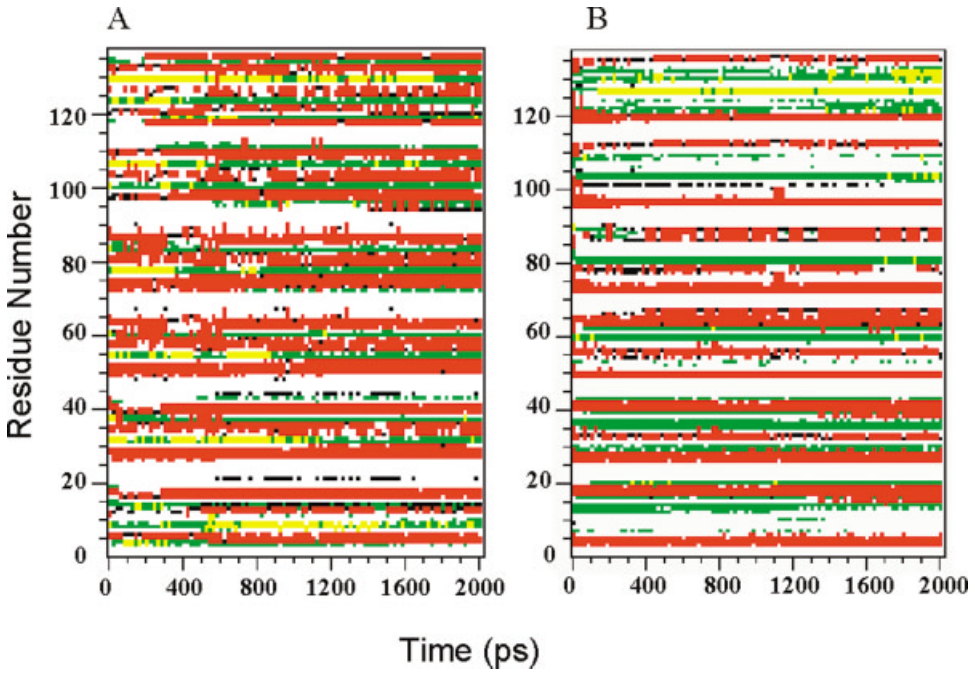

Fig. 7. Secondary structure analysis at each position in each of the six strands during the 2-ns simulation. Residue number of each successive rung of β-helix: 1–22, 23–44, 45–66, 67–88, 89–110, 111–132. Different color represents a different type of secondary structure: white, coil; red, β-sheet; black, β-bridge; green, bend; yellow, turn. **A:** Left-handed model. **B:** Right-handed model.

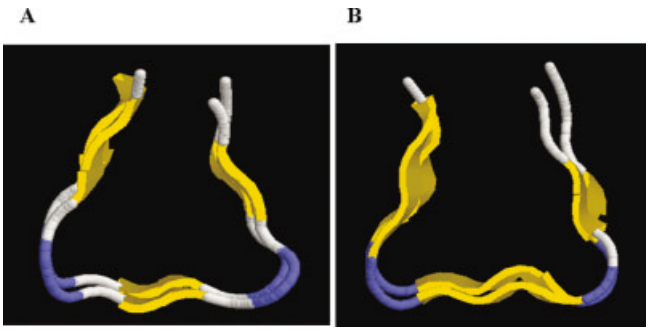

Fig. 8. Comparison of the central two strands of the 6-mer model before and after 2-ns simulation. yellow: strand; blue: turn; white: coil.

structural elements introduced specifically to prevent the edge-to-edge aggregation.[44,45]

Based on recent experimental data, our model does not include the C-terminal residues 37–40 of Aβ(1–40). This data includes the observation of a flexible C-terminus in Aβ fibrils,[10] the finding that proline substitution (which is incompatible with β-sheet stability) in residues 37–39 of Aβ does not significantly destabilize amyloid fibrils,[17] and the observation of little protective H-bonded structure in the C-terminal 35–40 fragment of Aβ (M. Chen, I. Kheterpal, R. Wetzel, and K. Cook, unpublished data). These observations are somewhat surprising, given previous biophysical data suggesting a strong role for the C-terminus of Aβ in amyloid fibril formation[46] and genetic data suggesting the importance of C-terminal residues in disease risk in Alzheimer's disease.[47] These data are not necessarily in conflict, however. First, the C-terminus may play a stabilizing role without being involved in H-bonded structure. Second, the C-terminus appears to play a stronger role in fibril formation kinetics and a lesser role in stabilizing the final fibril product.[17,46] Third, the C-terminus may play a more important role in the 1–42 peptide than in the 1–40 peptide. Strictly speaking, the model presented here is of the Aβ(1–40) protofilament, and further work—both in vitro and in silico, will be needed to investigate how sequence changes alter structure.

The model shown in Figure 3(A) is in good agreement with much of the experimental data on Aβ amyloid fibrils and it is quite stable structurally since it acquires more β-sheet structure during simulation, and has a well-packed hydrophobic core. The degree of stability of Aβ amyloid fibrils may well depend on the overall structural fold. Thus, the model based on right-handed β-helical proteins, although also a parallel β-sheet arrangement, does not show the same degree of stability as the left-handed model does. During the simulation, it maintains fewer backbone hydrogen bonds than does the left-handed model. In the left-handed parallel model, not only hydrogen bondings, but also stabilizing hydrophobic interactions, are maximized. Compared to other parallel Aβ models proposed recently,[9,14] our model agrees with other models in some aspects, and differs mainly in the number of turns in the fibril structure. Rather than having no turn or one turn, our model has two turn regions. It is of interest that the alignment between Aβ(15–36) and 2paba indicates a similar model to the one proposed by Petkova et al.[9] However, the threading score between Aβ(15–36) and

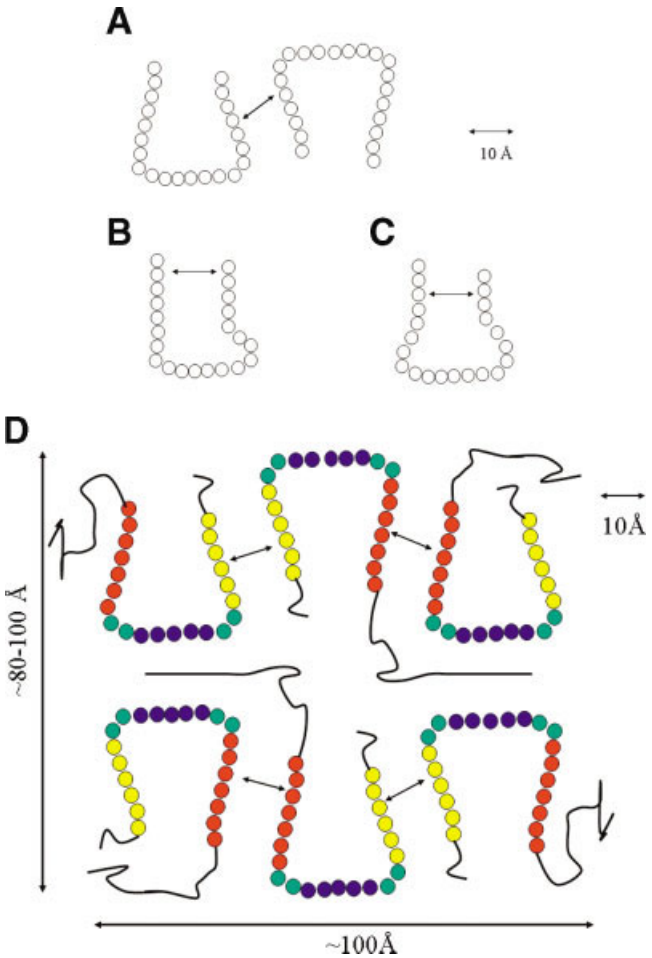

Fig. 9. Schematic representations of the possible sources of the 10 Å equatorial spacing in fibril diffraction patterns. **A:** Packing between protofilament; **B:** β-sheet packing within protofilaments; **C:** β-sheet packing within protofilaments (hybrid model between left-handed and right-handed parallel β-helices; **D:** a schematic model for the protofilaments packing. Red: first strand, residues 15–21; Blue: second strand, residues 24–28; Yellow: third strand, residues 31–36.

2paba is worse than that between Aβ(15–36) and 1lxa. The differences can be resolved in future studies. Table I summarizes the agreements and contradictions of our model and some experimental results.

This model may also provide some insights into the hierarchical structure of amyloid fibrils. Amyloid fibrils are unbranched, 60–120 Å in diameter and composed of four to six 30–50 Å-wide protofilaments.[4] Our model has a diameter of ~35 Å, which could serve as one protofilament of the amyloid fibrils. A schematic model for the β-strand arrangements and protofilaments packing is shown in Figure 9(D). This 6-protofilament Aβ fibril structure is consistent with much of the experimental data in addition to the size of protofilament. Our core structural model has two strands with exposed hydrophobic surface, and such protofilament packing would minimize the overall exposed hydrophobic surface. The distance between adjacent β-strands from neighboring protofilaments could account for the 10-Å diffraction pattern. Size calculation of this

TABLE I. β-Helical Model and Experimental Data

Our model	Experimental data	References
Data support β-helical model		
Cross-β structure	X-ray diffraction	48
Parallel β sheet	Solid-state NMR, EPR	9,16
N-terminal and C-terminal not in core	Limited proteolysis, solid state NMR, EPR	8,9,16
Two turns regions	Proline mutagenesis	17,19
Protofilament size: 30–35 Ã	AEM	49
Data contradict β-helical model		
One turn between 25–29	Solid state NMR	9

fibril model suggests a diameter of about 100 Å [Fig. 9(D)]. The arrangement of the protofilaments requires the N-terminal sequences of two of the protofilament subunits to be embedded near the center of the fibril. This may partially explain why only about 80% of the N-terminus of Aβ peptides is exposed and accessible to enzyme digestion.[8] Our results do not rule out a hybrid model between the left- and the right-handed models if the 10 Å reflection is also from a single protofilament [Fig. 9(C)].

CONCLUSION

We present a core structural model that adopts a β-helical motif, and a fibril model for amyloid β fibrils. Hydrogen-bonding and hydrophobic interactions may play crucial roles in the formation of protofilaments and in the packing in the protofilaments. The proposed model is compact and is consistent with a number of experimental observations. The model may provide insights into structural principles of fibril assembly and should facilitate the design of further experiments to test and refine the model.

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