Title
Amyloid β-Protein Monomer Folding: Free-Energy Surfaces Reveal Alloform-Specific Differences

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Alloform-specific differences in structural dynamics between amyloid β-protein (Aβ) 40 and Aβ42 appear to underlie the pathogenesis of Alzheimer’s disease. To elucidate these differences, we performed microsecond timescale replica-exchange molecular dynamics simulations to sample the conformational space of the Aβ monomer and constructed its free-energy surface. We find that neither peptide monomer is unstructured, but rather that each may be described as a unique statistical coil in which five relatively independent folding units exist, comprising residues 1–5, 10–13, 17–22, 28–37, and 39–42, which are connected by four turn structures. The free-energy surfaces of both peptides are characterized by two large basins, comprising conformers with either substantial α-helix or β-sheet content. Conformational transitions within and between these basins are rapid. The two additional hydrophobic residues at the Aβ42 C-terminus, Ile41 and Ala42, significantly increase contacts within the C-terminus, and between the C-terminus and the central hydrophobic cluster (Leu17-Ala21). As a result, the β-structure of Aβ42 is more stable than that of Aβ40, and the conformational equilibrium in Aβ42 shifts towards β-structure. These results suggest that drugs stabilizing α-helical Aβ conformers (or destabilizing the β-sheet state) would block formation of neurotoxic oligomers. The atomic-resolution conformer structures determined in our simulations may serve as useful targets for this purpose. The conformers also provide starting points for simulations of Aβ oligomerization—a process postulated to be the key pathogenetic event in Alzheimer’s disease.

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Keywords: Alzheimer’s disease; amyloid β-protein; protein folding; molecular dynamics; free-energy surface

Introduction

Alzheimer’s disease (AD) is the most common cause of late-life dementia.1 The pathognomonic neuropathologic features of AD are extracellular amyloid deposits comprising primarily fibrils of the amyloid β-protein (Aβ) and intracellular neurofibrillary tangles formed by tau protein.2 Compelling evidence supports a seminal role of Aβ in AD. Fibrils originally were thought to be central to AD pathogenesis,3 but recent studies support the hypothesis that the proximate neurotoxic agents in AD are Aβ oligomers.4–6 In fact, recent experiments have shown that some pathways of Aβ oligomerization and fibril formation are independent7,8 and that fibrillization may be protective.9 Aβ is produced naturally and ubiquitously in vivo as an ~4-kDa peptide.10 It exists predominately in two forms, Aβ40 and Aβ42, which contain 40 and 42 amino acids, respectively (Fig. 1). Despite the small structural difference between Aβ40 and Aβ42 (the C-terminal Ile-Ala sequence), the peptides display significantly different behaviors in vitro and in vivo. Aβ42 is the principal component in parenchymal plaques.11–13 An increase in the Aβ42/Aβ40 concentration ratio is associated with familial forms of early-onset AD.14,15 Treatments that reduce Aβ42 levels have been shown to correlate with a decreased risk for AD.16 In addition, Aβ42 displays enhanced neurotoxicity relative to Aβ40.17–19 In vitro studies have shown that Aβ42 displays fibril nucleation and elongation rates that are significantly higher than...
those of Aβ-40, and that Aβ-42 forms larger oligomers than does Aβ-40. These results support the conclusion that development of efficacious therapeutic agents for AD would be facilitated by knowledge in at least two areas: (1) the structural dynamics of Aβ monomer folding and oligomerization, and (2) differences in the dynamics between Aβ-40 and Aβ-42.

Experimental studies of Aβ monomer structure and dynamics are complicated by the lack of existence of a stable fold and the propensity of the peptide to aggregate into amorphous assemblies or multiple fibrillar forms. NMR experiments on Aβ fragments or full-length Aβ-40 and Aβ-42 performed in the absence of solvent additives consistently reveal little regular structure. A small increase in C-terminal rigidity has been observed in Aβ-42 versus Aβ-40. Consistent with these data, studies of region-specific endoprotease sensitivity showed increased resistance of the Aβ-42 C-terminus. These studies have provided relatively coarse insights into local Aβ structure, but they were not capable of elucidating the Aβ conformational ensemble in atomic detail. Substantial helical structure was revealed in Aβ studied in mixtures of fluorinated alcohols or SDS with water. However, the relevance of these systems to understanding extramembranous assembly is unclear.

Molecular dynamics (MD) simulations complement experimental studies through their ability to define the conformational space and dynamics of a macromolecule(s). This approach is being applied actively in the Aβ field (for recent reviews, see Teplow et al. and Urbanc et al.). Recently, we studied Aβ dynamics computationally, integrating these data with experimental results obtained using ion mobility spectroscopy–mass spectrometry. We found that Aβ-42 conformational space is dominated by loops and turns. Comparative studies with Aβ-40 were not performed. Sgourakis et al. performed Aβ simulations using both Aβ-40 and Aβ-42 in an explicit water environment. Structured regions were observed, one of which was a β-hairpin within the C-terminal peptide segment Ile31-Ala42. The simulation employed a virtual cubic space that was designed to contain a collapsed peptide that then was solvated by explicit water molecules. This system size may not accommodate extended conformers and thus not completely sample conformational space—a result that would produce a biased view of Aβ structure and dynamics. Here, using replica-exchange molecular dynamics (REMD) with an all-atom protein model, we sample and compare the conformational spaces and the corresponding free-energy surfaces of Aβ-40 and Aβ-42. We assess the relevance of the data by comparison with experimental information extant. Through comparison of the structural dynamics of Aβ-40 and Aβ-42, we establish their shared and distinct features. Finally, we discuss the implications of these findings for understanding and potentially controlling neurotoxic Aβ assembly.

Results and Discussion

Agreement between simulation and experiment

Before performing extensive analyses of our simulation data, we sought to establish their physical relevance by comparison with experimental data produced using NMR—the experimental method that has provided the highest-resolution structural information on Aβ. To do so, we used chemical shift values predicted from our simulations and determined experimentally. The experimental chemical shift values were predicted with the SHIFTS program, based on the conformational ensemble collected at 278 K. We used experimental values of monomeric Aβ that had been measured at the same temperature and pH. As shown in Fig. 2, the small standard deviations of the chemical shift values indicate the convergence of our simulation. The chemical shift values of the Cα and Hα atoms of both Aβ-40 and Aβ-42 were highly correlated with those observed experimentally. A strong correlation (r = 0.942) was also observed for the Nα atoms of Aβ-40. The chemical shifts of Aβ-42 were less correlated (r = 0.883) than those of Aβ-40, but remained well correlated with experimental values. It is possible that the modestly weaker correlations of Nα atoms are illusory because the SHIFTS program is known not to consider all the factors contributing to chemical shifts in proteins. It is also possible that the decreased correlation of the Aβ-42 experimental data results from intermolecular interactions among monomers in aqueous solution in the NMR experiment (Dr. Michael Zagorski, personal communication). In conclusion, the high correlations observed between our in silico data and those produced experimentally indicate that our simulations reproduce the Aβ structural ensemble well.

Secondary structure of Aβ

We first calculated and compared the secondary structures of Aβ-40 and Aβ-42. The secondary structure was assigned according to criteria defined by STRIDE (Fig. 3) or DSSP (Fig. S1). Both programs yielded qualitatively similar secondary structure assignments (see below). STRIDE defines a higher percentage of “turn” structure. This is
consistent with the fact that distance criteria are used by STRIDE to assign turn structure, whereas DSSP uses H-bonding as a criterion. Turns defined by DSSP thus comprise a subset of all turns defined by STRIDE. Because Aβ is very dynamic conformationally, it does not display high levels of classical α-helix, β-strand, or β-turn. Therefore, to represent both classical and nonclassical turn structures, we discuss here the results obtained using STRIDE. The term “turn” thus refers to short peptide segments in which the peptide chain reverses direction, regardless of the presence or the absence of H-bonds. Residues 6–40, except those at the N- or C-termini, exist as turn structures with ~30–80% probability. Residues more likely to exist as β-strand or α-helix are those at or adjacent to the central hydrophobic cluster (CHC) region (residues 17–21) and C-terminus (residues 30–36). In the CHC, a significant (~30–40%) secondary structure comprising these types was observed. Residues 16–27 and 30–36 of Aβ42 display β-structures with frequencies of

![Fig. 2. Correlation of simulated and experimental chemical shifts. Chemical shift values were determined using the SHIFTS program following REMD (Δδ_{sim}) and were compared with values measured experimentally at 278 K by NMR (Δδ_{exp}). The Pearson correlation coefficient (r) is listed at the bottom right of each panel. Left: Aβ40; right: Aβ42. We divide the 100-ns trajectory into three 33-ns frames and calculate the standard deviations of the chemical shifts from the three samples. Small standard deviations indicate the convergence of our simulation.](image-url)
∼6–8% and ∼8–14%, respectively. These data are consistent with prior experimental studies that have suggested that Aβ belongs to the class of “natively disordered” proteins. However, Aβ is not entirely a “random coil.” Two highly populated (>60%) turn structures, centered at residues 6–9 and 23–27, were observed. The positions of these turns correspond precisely with turn or bend-like structures observed by NMR to occur at Asp7-Glu11 and Phe20-Ser26. We found that residues 14–16 and 31–35 also had high probabilities of existing as turns.

One experimental approach that has been used successfully to identify structured and unstructured regions within Aβ is limited proteolysis. This approach revealed a protease-resistant decapeptide segment, Ala21-Ala30, that was found in NMR studies to form a turn-like structure. This region was postulated to nucleate folding of the Aβ monomer. Interestingly and consistent with this interpretation, pathogenic (linked to familial AD or cerebral amyloid angiopathy) amino acid substitutions within this region alter the stability of this.

Fig. 3. Secondary structure of Aβ. Secondary structure occurrence frequencies for Aβ40 (white) and Aβ42 (black) were determined using the STRIDE program following REMD simulation. Turn, β-strand, α-helix, 3_10-helix, and total secondary structure (3_10-helix + α-helix + β-strand) are shown. The frequencies of other structures were negligible (<0.1%).
Based on these experimental findings, simulations of turn dynamics have been performed on the decapeptide. These studies all revealed that a majority of the peptide conformers possess a turn-like structure that is stabilized by hydrophobic interactions between Val24 and Lys28 and by electrostatic interactions between Glu22 or Asp23 and Lys28. Baumketner et al. have suggested that hydrogen bonds involving the side chain of Asp23 and the amide hydrogen atoms of adjacent residues are important in stabilizing this turn.  

These hydrogen bond interactions also recently were reported by Fawzi et al., although their data suggested a lower occurrence frequency (∼40%) for this type of conformer. A stable turn formed by residues Val24-Asn27 and an intramolecular Asp23-Lys28 salt bridge also were observed in computational studies of Aβ(10–35) structural dynamics. Experiments on the Ala21-Ala30 decapeptide or on Aβ(10–35) cannot reveal effects of adjacent (missing) regions of the peptide that may occur in the biologically and clinically relevant full-length peptide. To determine whether such effects exist, we studied the structure and dynamics of the decapeptide turn element within the native Aβ monomer.

![Fig. 4. A typical central turn structure formed by residues 21–30. Dashed yellow lines indicate hydrogen bonds stabilizing the turn structure. The frequency of each hydrogen bond is shown in Table 1. The turn structure, occurring with a probability of ≈28% in full-length Aβ40 and ≈23% in full-length Aβ42, is similar (RMSD ≈ 1.0 Å) to that found in the most populated conformational cluster (gray) populated by the Aβ(21–30) decapeptide in simulations by Baumketner et al.](image)

<table>
<thead>
<tr>
<th>Hydrogen bond pair</th>
<th>Aβ40 (%)</th>
<th>Aβ42 (%)</th>
</tr>
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<tbody>
<tr>
<td>Asp23 Oγ⋯HN Val24</td>
<td>20.0</td>
<td>18.6</td>
</tr>
<tr>
<td>Asp23 Oγ⋯HN Gly25</td>
<td>27.4</td>
<td>35.9</td>
</tr>
<tr>
<td>Asp23 Oγ⋯HO Ser26</td>
<td>75.2</td>
<td>70.3</td>
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<td>Asp23 Oγ⋯HN Ser26</td>
<td>38.2</td>
<td>46.3</td>
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<tr>
<td>Asp23 Oγ⋯HN Asn27</td>
<td>18.5</td>
<td>20.9</td>
</tr>
<tr>
<td>Asp23 Oγ⋯HN Lys28</td>
<td>16.5</td>
<td>17.8</td>
</tr>
<tr>
<td>Asp23 Oγ⋯HN Gly29</td>
<td>12.3</td>
<td>13.5</td>
</tr>
</tbody>
</table>

The occurrence frequencies are listed for hydrogen bonds in the network involving the Asp23 Oγ atom.
solvent, one using the OPLS-AA force field\textsuperscript{47} and the other using the Gromos force field (Dr. Yundong Wu, personal communication).

Our simulations suggest that A\textsubscript{β}(21–30) adopts similar structures in its isolated decapeptide form and in the context of the full-length A\textsubscript{β} monomer. In practice, this result suggests that the decapeptide is a relevant proxy for the cognate segment within the A\textsubscript{β} monomer. In theory, our observation would be predicted for a structural domain that serves to nucleate protein folding. It should fold in isolation, and this folding should not be affected significantly by adjacent regions. In the full-length peptide, decapeptide folding would facilitate contacts between the CHC and the C-terminal regions of A\textsubscript{β}—contacts that comprise key stabilizing elements of fibril structure. However, this fold forms infrequently, as A\textsubscript{β} exists in many conformations with comparable free energies and does not display a dominant global minimum on its free-energy surface (Fig. 8).

In our simulation, the C-terminus of A\textsubscript{β}-42 is significantly more structured than that of A\textsubscript{β}-40, as reflected by a higher percentage of turn structure within peptide segment 34–41 and of β-structure within segment 30–42. It is noteworthy that residues 30–36 in A\textsubscript{β}-42 have almost twice the β-structure that they do in A\textsubscript{β}-40 and that residues 39 and 40 have no β-structure in A\textsubscript{β}-40, whereas they can exist in β-structure in A\textsubscript{β}-42 with a likelihood of ~5%. The more frequent turn and β-structure at the A\textsubscript{β}-42 C-terminus may be due to increased intramolecular contacts stabilized by the two hydrophobic residues Ile\textsubscript{41} and Ala\textsubscript{42}. In fact, such stabilizing interactions may account for the increased C-terminal rigidity observed in NMR experiments.\textsuperscript{32–34} Higher β-structure frequency is observed also among residues 20–27 of A\textsubscript{β}-42, the basis of which is a β-hairpin centered at residues 28 and 29 (Fig. 8b, cluster 8). Cluster 8 does not exist with A\textsubscript{β}-40. Interestingly, residues 3–6, 11–14, and 17–18 have higher β-content in A\textsubscript{β}-40 than in A\textsubscript{β}-42, and residues 17–28 are more likely to exist in α-helices in A\textsubscript{β}-40. For both peptides, residues 14–28 are more likely to form an α-helical structure than the C-terminus, consistent with a number of experiments in which central region α-helices were more stable than C-terminal helices.\textsuperscript{40}

**Tertiary structure**

We assess tertiary structure by analyzing contacts among amino acids (Fig. 5). Two amino acids were considered in contact if any heavy atom (C, N, O, or S) of one residue was ≤5 Å from any heavy atom of the other residue. Globally, the contact maps of A\textsubscript{β}-40 and A\textsubscript{β}-42 are similar and are characterized by relatively frequent contacts of residues within peptide segments 3–13, 10–20, and 17–36 (Fig. 5, red boxes). These segments correspond to populated turn structures centered at residues 6–9 (T\textsubscript{1}), 14–16 (T\textsubscript{2}), and 23–27 (T\textsubscript{3}), respectively. Weaker but noticeable contacts also were observed between two discontinuous segments, 6–10 and 23–27. We note that the CHC contacts residues 30–36 at the C-terminus with 20–30% probability. However, with the two extra residues Ile\textsubscript{41}–Ala\textsubscript{42} at the C-terminus, residues 30–42 of A\textsubscript{β}-42 are more likely to contact each other, presumably in the context of a turn (Fig. 5b).

Despite the overall global similarity in contacts between A\textsubscript{β}-40 and A\textsubscript{β}-42, differences are discernible (Fig. 6, red boxes). Residues 24–30 of A\textsubscript{β}-40 are more compact than those of A\textsubscript{β}-42, as shown by 2.5–30% more contacts in this region (Fig. 6a). This is consistent with the higher helical content of this region (Fig. 3). The A\textsubscript{β}-40 CHC is more likely (5–17.5%) to contact the N-terminus. In contrast, the A\textsubscript{β}-42 CHC is 2.5–10%
more likely to contact the C-terminus (residues 34–41) (Fig. 6b). Residues 3–8 contact residues 10–14 more frequently, corresponding to higher turn formation of residues 6–9 and higher \( \beta \)-content of residues 2–14. A peculiar residue is Phe19, which is more buried in A\( \beta \)40, as evidenced by its more frequent contacts with residues 13–32 more frequently (Fig. 6b, red box), suggesting that it is more shielded from the solvent. In fact, recent chemical cross-linking experiments suggest that Tyr10 is more difficult to cross-link in A\( \beta \)42 than it is in A\( \beta \)40 (unpublished observation).

Contact maps (Fig. 5) suggest that the N-terminus of A\( \beta \)40 is more compact than that of A\( \beta \)42, whereas the opposite is true of the C-terminus. MD simulations of A\( \beta \) oligomerization performed by Urbanc et al. showed that the N-termini of A\( \beta \)42 monomers comprising oligomers were significantly more extended and unstructured than those in the corresponding A\( \beta \)40 assemblies.\(^{59}\) Our observation here suggests that this feature exists at the monomer level. We postulate that the increased hydrophobicity of the A\( \beta \)42 C-terminus, due to the presence of Ile41 and Ala42, increases the contact frequency between residues in the C-terminus and the CHC. As a result, the N-terminus of A\( \beta \)42 has less contact with the CHC and exists most frequently as an extended or coil structure. This postulation is consistent with results of studies of the assembly state dependence of the intrinsic fluorescence of a Tyr residue substituted for Phe20.\(^{60}\) These studies showed that a significant increase in fluorescence intensity occurred in A\( \beta \)40, but not in A\( \beta \)42, during the initial oligomerization of the peptides. This change would result from a disruption of the N-terminus–CHC interaction in the A\( \beta \)40 monomer and the subsequent formation of the more apolar CHC-C-terminus complex, in which quenching of the fluorescence would be substantially lower. It is intriguing that recent scanning cross-linking studies have suggested that such competition between the N-terminus and the C-terminus for interaction with the CHC is a fundamental feature of A\( \beta \) conformational dynamics.\(^{61}\) In A\( \beta \)42, relative to A\( \beta \)40, the C-terminus “wins” because the presence of Ile41 and Ala42 facilitates formation of a C-terminal bend that creates a larger and more stable hydrophobic region—one that can interact especially strongly with the CHC. The biological consequence of this interaction is the formation of folded A\( \beta \) monomers with the well-known increased propensity to form neurotoxic higher-order assemblies.

**Correlated motion**

Secondary and tertiary structure analyses suggest that A\( \beta \) is relatively disordered and thus populates a large conformational space. However, like proteins in general,\(^{62,63}\) and from the computational and experimental results discussed above, the A\( \beta \) monomer does not appear to be a random coil, if we define “random coil” as an entity that can populate every point in monomer conformational space with equal probability. The implication of this fact is that valuable knowledge may be obtained if nonrandom features of conformational space can be identified. One method to achieve this goal is to identify segments of A\( \beta \) whose motions are correlated. To do so, we quantify the cross-correlations of atomic positional fluctuations.\(^{64}\) This approach has shown that many regions in proteins, especially those involving secondary structure elements, display correlated motions.

We represent each residue with its corresponding \( \text{C}^\alpha \) atom. In Fig. 7, each grid square indicates the correlation \( r_{ij} \) between a residue pair. \( r_{ij} = 1 \) indicates...
that two residues are completely correlated, whereas $r_{ij} = -1$ indicates completely anticorrelated motions. Complete correlation means that two residues always move together with the same phase and period. For simplicity, we arbitrarily define meaningful correlations to be those with $|r_{ij}| \geq 0.5$.

For both Aβ40 and Aβ42, positive correlations are observed only along the diagonal line (i.e., residues have motions that are correlated only with their immediate neighbors). All nonneighbor motions are anticorrelated. Aβ40 and Aβ42 each display four sets of peptide segments whose motions are correlated significantly (Fig. 7, red boxes 1–4). The location of each set is similar, but the sizes of the sets and the magnitudes of the correlations within them vary (Fig. 7, Table 2). In Aβ40, the largest negative correlations exist between residues 1–3 and 6–10 (box 1; $T_1$), between residues 9–11 and 15–18 (box 2; $T_2$), between residues 21–23 and 29–31 (box 3; $T_3$), and between residues 30–35 and 38–40 (box 4; $T_4$). The correlations in boxes 1 and 2 of Aβ42 occur over a similar segment range as do those in Aβ40 and have similar total magnitudes. The largest difference occurs for box 3, in which substantially greater total correlation exists for Aβ42. The segment range in box 4 is translated two residues towards the C-terminus in Aβ42 relative to Aβ40, and the total correlation magnitude is greater. These differences result from the presence of Ile41 and Ala42, which likely stabilize a C-terminal turn centered at Gly38. This postulation is consistent with our secondary structure analyses (Fig. 3), which show that residues adjacent to the Gly37-Gly38 dipeptide in Aβ42 have particularly high probabilities of forming β-structures.

If monomer tertiary structure is maintained during peptide oligomerization and fibril formation, our data allow certain structure–activity predictions. For example, models of Aβ fibril structure produced using NMR data suggest that the fibril is formed by parallel in-register packing of β-strand–turn–β-strand motifs, in which D23-K28 salt bridges stabilize the turn. The formation of the $T_3$ turn identified in our simulation would increase contacts between peptide segments immediately adjacent to it, facilitating fibril formation. In contrast, formation of the $T_4$ turn (centered at Gly38) would disrupt the extended conformation that exists in the C-terminal β-strand element of the fibril. In considering these predictions, however, it is important that Aβ conformational dynamics in the monomer state be distinguished from that in the fibril state. The two are related, but the most stable conformers in one state are not entirely isomorphous with those in the other. Experimental and computational studies have shown that conformational conversions may occur both in incoming monomers attaching to fibril ends and in the monomers comprising the fibril ends themselves.

Our observation that all the negatively correlated peptide segments reside on the opposite sides of turns identified at residues 6–9, 14–16, and 23–27 (Fig. 3) suggests that segmental motion within Aβ is dominated by “zipping” and “unzipping” motions around these turn structures. It is intriguing to consider Aβ monomer folding as a process involving five relatively independent folding units (residues 1–5, 10–13, 17–22, 28–37, and 39–42) that are connected by four turn structures. This simple consideration would have a highly significant

![Fig. 7. Cross-correlation of atomic positional fluctuations. Intramolecular cross-correlation coefficients are shown for (a) Aβ40 and (b) Aβ42. The motion of a residue pair is considered correlated if its corresponding correlation coefficient ($r_{ij}$) has an absolute value of $\geq 0.5$. Red boxes highlight strong anticorrelated motions.](image)

<table>
<thead>
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<th>Table 2. Total cross-correlation magnitude</th>
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<td>3</td>
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To enable a quantitative comparison of segmental cross-correlations, boxes that delineated regions of the highest residue cross-correlations (Fig. 7, boxes 1–4) were created. The total cross-correlation magnitude within each box then was determined according to $r_{\text{total}} = \sum_{i=1}^{2} r_{ij}$. 

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implication: the degrees of freedom† for Aβ folding would decrease from $\sim 2000$ to $\sim 10$, and this would enable ab initio determination of the structures of higher-order assemblies such as Aβ oligomers, now thought to be the proximate neurotoxins in AD.\textsuperscript{10}

**Free-energy surface**

To facilitate our understanding of the conformational equilibria of Aβ, we used principal coordinate analysis\textsuperscript{70} to project its conformations onto a two-dimensional space comprising the first two principal coordinates. By defining a third coordinate as conformational free energy, we constructed the free-energy surface for Aβ (Fig. 8). This method has been shown to effectively preserve the essential features of the conformational space. Surfaces of Aβ\textsubscript{40} and Aβ\textsubscript{42} share two global features: (1) two large basins, one dominated by extended or $\beta$-sheet conformations (“$\beta$-basin”) and the other dominated by $\alpha$-helical structures (“$\alpha$-basin,” except position 8 in Fig. 8b); and (2) a number (\~{}20) of minima that have comparable free energies and are separated by shallow barriers. This latter feature predicts that conformational conversions (movement among these minima) occur frequently.

The first principal coordinate of Aβ\textsubscript{40} dominates the conformational conversion within each basin, and the $\alpha$-helix→$\beta$-sheet conversion occurs along the second principal coordinate (Fig. 8a, white double-headed arrow). In contrast, the $\alpha$-helix→$\beta$-sheet conversion of Aβ\textsubscript{42} occurs along its first

\begin{equation}
\text{df} = 3N - 6,
\end{equation}

where $N$ is the number of elements in the system. For Aβ\textsubscript{42}, $N = 630$, where $N$ is the number of atoms in the peptide. For a system with five folding units, $N = 5$ if the structures of the units are known.

\textsuperscript{†} We calculate degrees of freedom $df = 3N - 6$, where $N$ is the number of elements in the system. For Aβ\textsubscript{42}, $N = 630$, where $N$ is the number of atoms in the peptide. For a system with five folding units, $N = 5$ if the structures of the units are known.
principal coordinate, and conformational conversions within the α- or β-basins occur along the second principal coordinate (Fig. 8b, white double-headed arrow). This suggests that α-helix → β-sheet transitions occur more readily in Aβ42 than in Aβ40. We also see that the local minima in the Aβ40 α-basin have lower free energies, on average, than those in the β-basin (Fig. 8a), whereas for Aβ42, the magnitudes of the free energies of local minima in both basins, on average, are comparable (Fig. 8b). Previous experimental studies have shown that α-helix-containing conformers are obligatory but transitory intermediates in Aβ fibril assembly, both for Aβ40 and for Aβ42. During fibril assembly, in which a statistical coil → α-helix → β-sheet conformational conversion path is observed, the maximum α-helix content of Aβ40 and Aβ42 was 32% and 19%, respectively, and Aβ42 reached its maximal α-helix content significantly sooner than did Aβ40. Consistent with this finding, solvent conditions that facilitate the initial coil → α-helix transition significantly accelerate fibril assembly, whereas increased α-helix stabilization blocks β-sheet formation. The thermodynamics of these systems revealed in the respective energy surfaces provides a mechanistic explanation for this observation. First, the deeper α-helix basin in Aβ40 means that Aβ40 conformers will populate these regions more frequently. Second, the lower barriers for α-helix → β-sheet transitions in the Aβ42 surface mean that the equilibrium amount of α-helix-containing Aβ42 conformers will be lower. The differential stability of α-helix-containing conformers of Aβ40 and Aβ42 explains the well-known difference between the two peptides in assembly rate—a difference that appears to be

Fig. 8. Free-energy surfaces. (a) Aβ40; (b) Aβ42. Both surfaces exhibit two large basins: an α-basin and a β-basin. Red lines have been superimposed on the figures as gross indicators of the interfaces between the basins. White double-headed arrows denote the direction of the conformational transition between the two basins. Representative conformers from a number of low-energy wells are enumerated with black numbers outside of each panel, and the corresponding well within the panel itself is indicated by a white arrow and number. The positions of the amino acids in each conformer are indicated spectrally, from the N-terminus (indigo) to the C-terminus (red), and by small black residue numbers. Topological analyses of the Aβ40 and Aβ42 surfaces allow comparisons to be made of the distributions of conformers and their stabilities. The reader should note, however, that no formal correspondence exists between the principal coordinates in each system.
related directly to the increased neuroticity of the longer Aβ alloform.\textsuperscript{10}

\section*{Summary}

The impetus for our work was the importance of elucidating alloform-specific differences in structural dynamics between Aβ40 and Aβ42—differences that appear to underlie the pathogenesis of AD. REMD simulations at microsecond timescales reproduced aspects of Aβ conformational dynamics that had been revealed previously using NMR, supporting the biological relevance of the simulations and, importantly, providing insights not obtainable experimentally. We find that neither peptide monomer is unstructured, but rather that each may be described as a unique statistical coil in which five relatively independent folding units exist, comprising residues 1–5, 10–13, 17–22, 28–37, and 39–42, which are connected by four turn structures. Incorporating this modular organization into folding algorithms could simplify analyses of Aβ assembly and facilitate \textit{ab initio} studies of Aβ oligomerization. Our determination of the free-energy surfaces of Aβ40 and Aβ42 revealed that both peptides may possess significant amounts of \( \alpha \)-or \( \beta \)-structure and that conformers within and between each structural class are in rapid equilibrium. The two additional hydrophobic residues at the Aβ42 C-terminus, Ile41 and Ala42, significantly increase contacts within the C-terminus and between the C-terminus and the CHC. As a result, the \( \beta \)-structure of Aβ42 is more stable than that of Aβ40, and the conformational equilibrium in Aβ42 shifts towards \( \beta \)-structure. Considered together with our previous studies of the role of \( \alpha \)-helix formation in Aβ assembly,\textsuperscript{71,72} these results suggest that drugs stabilizing \( \alpha \)-helical Aβ conformers (or destabilizing the \( \beta \)-sheet state)\textsuperscript{73} would block formation of neurotoxic oligomers. The atomic-resolution conformer structures determined in our simulations may serve as useful targets for this purpose.

\section*{Materials and Methods}

\subsection*{Replica-exchange MD simulation}

Our first consideration in selecting a method for simulating Aβ monomer conformational dynamics was how to model a highly flexible peptide chain that, in its extended conformation, is \( \sim 156 \) Å in length. A simulation space fully enclosing such a conformer would contain >100,000 water molecules. Simulations of systems of this size over meaningful timescales currently are computationally impractical. Solvating a more compact peptide conformer would produce a much smaller system. For example, a 50- to 60-Å simulation space would contain only \( \sim 5000 \) water molecules. However, natively disordered peptides sample extended states; when this occurs during a simulation, interimage interactions that would affect the results can occur. Even in situations in which a pronounced bias toward compact states exists, interconversion among different states likely would proceed through extended intermediates. For these reasons, we used the generalized Born (GB) implicit solvation model\textsuperscript{74} to mimic the aqueous environment and did not represent water explicitly.

By eliminating the degrees of freedom and the viscosity associated with solvent water, simulations using the GB model can converge rapidly and explore conformational space efficiently. However, as with any simulation system, limitations exist. In the absence of explicit water, the simulated events take place much faster due to the lack of frictional effects. This complicates studies of kinetics. Our primary focus here is the thermodynamics of the systems, not their kinetics; thus, a more rapid attainment of equilibrium is useful. Another potential limitation of the GB model is that it does not allow the simulation of water bridges. However, for a highly flexible molecule such as Aβ, there is no evidence that water bridges contribute significantly to its stability. In practice, the GB approach has been implemented frequently and has yielded reliable results,\textsuperscript{75–78} including results from \textit{ab initio} protein folding experiments in which structures of unprecedented accuracy were produced.\textsuperscript{79,80}

We used the REMD technique to further enhance conformational sampling. The simulations were performed with the Sander module of the Amber simulation package (version 9).\textsuperscript{81} The proteins were modeled by FARM99SB, a recently improved Amber force field.\textsuperscript{82} We implemented an effective 0.2 M salt concentration in the GB solvent model. Nonpolar solvation effects were represented using a surface tension coefficient of 0.005 kcal/mol Å\(^2\). Starting from extended Aβ monomer, 16 replicas that exponentially spanned the temperature range 276–400 K were created. The temperature of the system was regulated using the Berendsen coupling algorithm\textsuperscript{83} with a coupling constant of 1.0 ps. Hydrogen atoms were constrained using SHAKE.\textsuperscript{84} The integration time step was 2 fs. Exchange between replicas was attempted every 2 ps. We used the default values of the Sander module for other relevant parameters.\textsuperscript{85} This system mimics a very dilute aqueous Aβ solution at neutral pH. For each replica, the simulation length was 110 ns, and 110,000 conformations were collected. The first 10 ns was treated as equilibrium, and the last 100 ns was used for data analysis. The total simulation time was 3.52 μs.

\section*{Correlation of computationally and experimentally determined chemical shifts}

Chemical shifts were determined from our simulation data using the SHIFTS program.\textsuperscript{48} Correlation of these data with those obtained by NMR then was determined using the Pearson correlation coefficient (Eq. (1)): \( \rho = \frac{\langle (x_{i} - \langle x \rangle)(y_{i} - \langle y \rangle) \rangle}{\sqrt{\langle (x_{i} - \langle x \rangle)^{2} \rangle \langle (y_{i} - \langle y \rangle)^{2} \rangle}} \) (1)

\section*{Correlated motion}

Isotropically distributed ensemble analysis\textsuperscript{85} was used to characterize the correlated molecular motions of Aβ. We represent each residue with its corresponding C\(^{\alpha} \) atom. For a protein containing \( n \) amino acids, we first constructed an \( n \times n \) matrix \( \mathbf{P} \) with elements \( P_{ij} = \frac{1}{2} \langle \mathbf{r}_{i} \cdot \mathbf{r}_{j} \rangle \), where \( \mathbf{r}_{i} \) (or \( \mathbf{r}_{j} \)), the position vector of residue \( i \) (or \( j \)), originates from the center of mass of the protein
and ends at each Cα atom. Each element $P_{ij}$ of the matrix is the vector product averaged over the entire conformational ensemble. The cross-correlation coefficient $r_{ij} = P_{ij} / (P_{iii}P_{jj})^{1/2}$, with the overall rotational modes eliminated. If $r_{ij} = 1$, the motions of the two residues $i$ and $j$ are highly positively correlated. If $r_{ij} = -1$, the motions are strongly negatively correlated. If $r_{ij} = 0$, no correlation exists.

**Principal coordinate analysis**

Energy surfaces were constructed by principal coordinate analysis. To do so, the collected conformations were clustered with a threshold RMSD distance of 3.0 Å. The cluster centers were used to build a distance matrix $A$ with elements $A_{ij} = -\frac{1}{2}d_{ij}^2$, where $d_{ij}$ is the power distance between conformations $i$ and $j$. The matrix $A$ was “centered” by Eq. (2), where $\langle \ldots \rangle_k$ is the mean over all specific indices $k = i$, $j$ or $i'j'$:

$$A^*_{ij} = A_{ij} - \langle A_{ij} \rangle_i - \langle A_{ij} \rangle_j + \langle A_{ij} \rangle_{ij}$$  

(2)

This centering process guarantees a zero root of matrix $A^*$. Matrix $A^*$ was diagonalized, and the resulting eigenvectors were sorted in descending order according to their corresponding eigenvalues. Finally, we constructed the free-energy surface by projecting all the collected conformations onto the two-dimensional space defined by the first two eigenvectors. This procedure is similar to that described by Yang et al., with the exception that the “minimum energy envelope” was not adopted. The conformations in our analysis were not minimized, and all collected conformations were projected onto the surface to retain the free-energy information.

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**Supplementary Data**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmb.2008.09.039

**References**


