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Effects of the Arctic (E22→G) Mutation on Amyloid β-Protein Folding: Discrete Molecular Dynamics Study

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Abstract: The 40–42 residue amyloid β-protein (Aβ) plays a central role in the pathogenesis of Alzheimer’s disease (AD). Of the two main alloforms, Aβ40 and Aβ42, the longer Aβ42 is linked particularly strongly to AD. Despite the relatively small two amino acid length difference in primary structure, in vitro studies demonstrate that Aβ40 and Aβ42 oligomerize through distinct pathways. Recently, a discrete molecular dynamics (DMD) approach combined with a four-bead protein model recapitulated the differences in Aβ40 and Aβ42 oligomerization and led to structural predictions amenable to in vitro testing. Here, the same DMD approach is applied to elucidate folding of Aβ40, Aβ42, and two mutants, [G22]Aβ40 and [G22]Aβ42, which cause a familial (“Arctic”) form of AD. The implicit solvent in the DMD approach is modeled by amino acid-specific hydrophobic and electrostatic interactions. The strengths of these effective interactions are chosen to best fit the temperature dependence of the average β-strand content in Aβ42 monomer, as determined using circular dichroism (CD) spectroscopy. In agreement with these CD data, we show that at physiological temperatures, the average β-strand content in both alloforms increases with temperature. Our results predict that the average β-strand propensity should decrease in both alloforms at temperatures higher than ~370 K. At physiological temperatures, both Aβ40 and Aβ42 adopt a collapsed-coil conformation with several short β-strands and a small (<1%) amount of α-helical structure. At slightly above physiological temperature, folded Aβ42 monomers display larger amounts of β-strand than do Aβ40 monomers. At increased temperatures, more extended conformations with a higher amount of β-strand (≥30%) structure are observed. In both alloforms, a β-hairpin at A21-A30 is a central folding region. We observe three additional folded regions: structure 1, a β-hairpin at V36-A42 that exists in Aβ42 but not in Aβ40; structure 2, a β-hairpin at R5-H13 in Aβ42 but not in Aβ40; and structure 3, a β-strand A2-F4 in Aβ40 but not in Aβ42. At physiological temperatures, the Arctic mutation, E22G, disrupts contacts in the A21-A30 region of both [G22]Aβ peptides, resulting in a less stable main folding region relative to the wild type peptides. The Arctic mutation induces a significant structural change at the N-terminus of [G22]Aβ40 by preventing the formation of structure 3 observed in Aβ40 but not Aβ42, thereby reducing the structural differences between [G22]Aβ40 and [G22]Aβ42 at the N-terminus. [G22]Aβ40 is characterized by a significantly increased amount of average β-strand relative to the other three peptides due to an induced β-hairpin structure at R5-H13, similar to structure 2. Consequently, the N-terminal folded structure of the Arctic mutants closely resembles the N-terminal structure of Aβ42, suggesting that both Arctic Aβ peptides might assemble into structures similar to toxic Aβ42 oligomers.

1. Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that is characterized pathologically by extensive neuronal loss and the accumulation of extracellular senile plaques and intracellular neurofibrillary tangles. Senile plaques contain fibrillar aggregates of the amyloid β-protein (Aβ). Aβ is produced through cleavage of the amyloid precursor protein (APP) and is normally present in the body predominantly in two alloforms, Aβ40 and Aβ42, that differ structurally by the absence or presence of two C-terminal amino acids, respectively.1,2 An important hypothesis of disease causation, strongly supported by genetic and experimental evidence, posits that Aβ oligomers, rather than fibrils, are the proximate neurotoxic agents in AD.3 In particular, Aβ42 oligomers appear to be the most toxic Aβ assemblies.4 The linkage of Aβ oligomerization to AD makes imperative the detailed elucidation of the oligomerization process. Unfortunately, the Aβ system is remarkably complex.

in its conformational and assembly dynamics. This has complicated the application of classical structure determination methods such as X-ray crystallography and solution state NMR to the oligomerization question. One approach that has provided information on the initial self-association of Aβ has been in situ chemical cross-linking (for a review, see ref 6). This approach allowed Bitan et al. to determine quantitatively the Aβ oligomer size distribution, which demonstrated that Aβ40 and Aβ42 exhibit different oligomerization pathways. Aβ42 assembled into pentamer/hexamer units (paramuclics) and multiples of paramuclics, while Aβ40 only formed dimers through tetramers in equilibrium with monomers. However, the resolution of the method was insufficient to reveal the interatomic interactions controlling the oligomerization processes.

In vitro studies showed that Aβ40 and Aβ42 monomers adopted a predominantly α-helical structure in a membrane-mimicking environment,9,10 while a collapsed coil structure was reported for Aβ(10–35) in an aqueous solution.10 Aβ folded structure clearly depends on the solvent. Earlier studies of Aβ40 using a mixture of trifluoroethanol and water demonstrated a substantial amount of α-helical structure.11,12 Initial studies of temperature dependence of the secondary structure of Aβ40 in aqueous solution demonstrated that β-strand propensity increased with temperature.13 Using CD spectroscopy on both Aβ40 and Aβ42 monomers in aqueous solution, Lim et al. recently demonstrated14 that the average β-strand structure increased with temperature, in agreement with Gursky and Aleshkov,15 with Aβ42 monomers having a slightly higher amount of average β-strand structure than Aβ40 monomers, suggesting that the two alloforms are characterized by differences in folded structures.

Knowledge-based therapeutic drug design requires the definition of target structures at atomic resolution. In silico approaches provide a powerful means to achieve this goal. Several in silico studies addressed folding of Aβ42,15,16 Aβ39,17 Aβ(10–35),18,19 Aβ(25–35),20 Aβ(1–28),21 and Aβ(21–30) decapetide.22–27 The latter was hypothesized to nucleate monomer folding.28,29 Replica-exchange all-atom molecular dynamics (MD) study of Aβ42 monomer in implicit water by Baumketner et al. showed three distinct families of folded structures, all dominated by turns and loops with a small amount of α-helical structure at the C-terminus.10 Using similar all-atom MD approach, Anand et al. found that at room temperature, the Aβ(1–39) monomer did not have a unique structure but rather three distinct families of mostly collapsed coil-like structures existed.17 Using all-atom MD in explicit water, Massi et al. demonstrated that although Aβ(10–35) is somewhat disordered in water, the central hydrophobic cluster, L17–A21, and the turn region, V24–N27, were particularly stable.18,19 Wei et al. used replica-exchange MD in pure water and HFIP/water cosolvent to demonstrate that Aβ(25–35) preferentially populated an α-helical structure in apolar organic solvent, while in water, a collapsed coil, and to a lesser extent β-hairpin conformations, were observed. Dong et al. explored the energy landscape of Aβ(1–28) monomers and concluded that the monomer was predominantly in a collapsed coil conformation with a non-negligible β-strand structure at the N-terminus.

An ab initio DMD approach using a four-bead protein model with backbone hydrogen bonding in implicit solvent recently demonstrated that despite relatively small differences in the primary structure, Aβ40 and Aβ42 not only followed different oligomerization pathways but also folded differently, with Aβ42 displaying a turn structure centered at G37-G38 that was not present in Aβ40 monomer.30 This structural difference between Aβ40 and Aβ42 was corroborated by several independent in vitro studies.28,31,32 In addition, a combined MD/NMR study confirmed that Aβ42 monomer was more structured at the C-terminus than Aβ40.33 Using the same DMD approach as the initial study by Urbanc et al., Lam et al. showed that only selected regions of Aβ42 had a well-defined folded structure and that the average amount of β-strand increased with temperature,34 consistent with in vitro findings.13 Because Aβ40 and Aβ42 were shown to oligomerize through distinct pathways in vitro and in silico,30 the present study is based on a hypothesis that different oligomerization pathways leading to distinct effects in vitro are a consequence of folding differences between Aβ40 and Aβ42. We employ the DMD approach with

implicit solvent parameters mimicking the in vitro aqueous solution to elucidate all structural differences between Aβ40 and Aβ42 monomers at different temperatures. Because the Arctic mutation, E22, is associated with the familiar form of AD with distinct pathology relative to the sporadic AD, we examine also the effects of the Arctic mutation on folded structures of both isoforms and discuss the implications of our findings for understanding Aβ isoform-specific folding and its relationship to AD.

2. Methods

2.1. Discrete Molecular Dynamics. Zhou et al. proposed the idea of applying discrete molecular dynamics (DMD) combined with a simplified protein model to study protein folding. Since then, many groups have implemented this approach to investigate protein folding mechanisms. In DMD, all interparticle interactions are modeled by square-well and step-like potentials. Particles move with constant speeds along straight lines. When two particles reach a distance at which the potential is discontinuous, a collision occurs. The pair of particles with the shortest collision time is chosen as the next collision event and the new positions and velocities of the two particles involved are calculated based on conservation laws for the linear momentum, angular momentum, and total energy. The advantage of DMD is that the numerical integration of Newton’s second law equations is avoided, resulting in a substantial decrease in computational burden. This makes the DMD approach much faster than all-atom MD with continuous interparticle potentials.

2.2. Four-Bead Protein Model and Interactions. We use a four-bead protein model, in which up to four beads are used to represent an amino acid. Three beads are used to model the backbone groups N, Cα, and C′. The fourth bead represents the side chain centered at the Cβ group. Only glycine lacks the Cβ bead and is thus modeled by three beads only. Adjacent beads are connected to each other through covalent or peptide bonds, which are modeled as square well potentials with infinite walls but of finite width corresponding to ~2% variability in covalent/peptide bond lengths. In addition to covalent and peptide bonds, constraints are implemented to ensure the proper geometry of the protein backbone. These constraints are modeled in the same ways as the bonds. All lengths of bonds and constraints are based on statistical properties derived from the protein database of known protein structures.

The backbone hydrogen bond was introduced into the four bead model to account for the α-helical and β-strand secondary structure. The bond is introduced between the Nβ bead of amino acid i and C′β bead of amino acid j. For a hydrogen bond between Nβ and C′β to form, these two beads need to be at a distance <4.2 Å. In addition, auxiliary bonds between the two amino acids involved are introduced to account for the particular backbone geometry allowing hydrogen bond formation. The absolute value of the potential energy associated with formation of a single hydrogen bond, $E_{HB}$, represents a unit of energy in our approach. The simulation temperature $T_{sim}$ is expressed in units of $E_{HB}/k_B$, where $k_B$ is the Boltzmann constant.

The model implements amino acid-specific interactions between two side chain beads due to effective hydrophobicity and charge. The side chain bead of each amino acid is characterized by an effective hydrophobicity following the Kyte and Doolittle scale. Because the solvent is not explicitly present in the model, effective attractive interactions between two hydrophilic, and repulsive interactions between two hydrophobic, side chain beads are introduced. The strength of the effective hydrophobic interactions as given by the absolute value of the potential energy between two side chains $E_{HB}$ (relative to the energy unit $E_{HB}$) is the first interaction parameter. A double square-well potential is applied to model the effective electrostatic interactions between two charged side chain beads. The maximal absolute value of the potential energy between two charged side chains $E_{CH}$ (relative to the energy unit $E_{HB}$) is the second interaction parameter. Both interaction parameters $E_{HB}$ and $E_{CH}$ strongly depend on and need to be adjusted to the particular solvent.

2.3. Secondary Structure Analysis. The secondary structure propensities of each amino acid were calculated using the STRIDE program within the Visual Molecular Dynamics (VMD) software package. The secondary structure propensities included α-helical, β-strand, turn, and random coil per amino acid. We calculated the average β-strand and α-helix propensities, (β) and (α), by averaging the β-strand/α-helix propensity over all amino acids at a given temperature and interaction parameters ($E_{HB}$, $E_{CH}$).

2.4. Intramolecular Contact Map. We determined the average intramolecular contact frequency for each temperature and interaction parameter set ($E_{HB}$, $E_{CH}$). Two amino acids, i and j, were considered to be in contact when the distance between them $d_{ij}$ ≤7.5 Å. The contact was counted with variable $C_i$ that was defined as the average number of contact pairs between amino acids i and j from different trajectories. Because each amino acid had up to four beads, the maximum number of contacts $C_i$ (between any two amino acids) was 16. We normalized the contact maps to the same maximum value.

3. Results

The DMD approach employed here has been described in detail by Urbanc et al. In earlier studies, DMD combined with a four-bead amino acid model, and considering backbone hydrogen bonding only, resulted in β-hairpin monomer and planar dimer conformations. Introducing amino acid-specific interactions due to hydrophobicity into the four-bead model enabled the successful in silico reproduction of experimentally observed oligomerization differences between Aβ40 and Aβ42 and yielded new structural predictions amenable to in vitro testing. This same study indicated that alloform-specific differences already existed at the stage of monomer folding. In particular, the turn structure centered at G37-G38 was present in a folded Aβ42 monomer but not in a folded Aβ40 monomer and was associated with the first contacts that formed during monomer folding. Yun et al. using the same DMD approach, showed that electrostatic interactions promote formation of larger oligomers in both Aβ40 and Aβ42 while preserving the
Figure 1. (A) CD data obtained for Aβ40 (black squares) and Aβ42 (red squares) by Lim et al.14 (B) Average β-strand propensity, ⟨β⟩, of Aβ40 (black squares) and Aβ42 (red squares). For all the parameter sets (E\text{int}, E\text{CH}), we map the CD data in panel A using the functions defined in the dashed-line box above the plot into the data enclosed in the dashed box in panel B. T\text{sim} and T\text{real} are related by a conversion factor (T* = T\text{real}/T\text{sim}) that maps all the simulation temperatures onto real temperatures. A similar relationship exists for the CD intensity conversion factor α. ⟨β⟩₀ and β₀ are the average β-strand propensity and the CD intensity at 5 °C, respectively.

Differences between the two alloforms at both the folding and oligomerization stages of assembly. Lam et al. applied this approach in studies of the temperature dependence of Aβ42 folding, showing that a collapsed coil conformation at low temperatures converts to a more extended, β-strand-rich conformation at higher temperatures.34 Interestingly, while several regions of Aβ42 consistently exhibit a temperature-dependent fold, significant variability of folded structures was found at each temperature. The coexistence of a multitude of monomer conformations is characteristic of naturally unfolded proteins. Here we explore more deeply the differences between the full-length Aβ40 and Aβ42 peptides. We first describe a technique that allows us to determine the implicit solvent parameters that best match experimental conditions. We then use these parameters to explore not only the folding of wild type Aβ40 and Aβ42 but also of two clinically relevant mutant alloforms, Arctic [G22]Aβ40 and [G22]Aβ42.

The primary structure of Aβ42 differs from Aβ40 by two additional amino acids of Aβ42, I and A, at the C-terminus. The sequence of Aβ42 is DAEFRHSDGYEVHHQK\textsuperscript{16}LVFGK\textsuperscript{18}DVGSNKG\textsuperscript{20}IILMV\textsuperscript{26}GGVV\textsuperscript{30}IA. We refer to the segments (L17−A21) and (I31−V36) as the central hydrophobic cluster (CHC) and the mid-hydrophobic region (MHR), respectively. We define the C-terminal region (CTR) as the segment V39-V40 in Aβ40 or V39-V42 in Aβ42.

The energy unit is set to the absolute value of the hydrogen bond potential energy, E_{HB} = 1.0 and the simulation temperature T\text{sim} is expressed in units of E_{HB}/k_B. We explore folding at four different strengths of hydrophobic interactions, E_{CH} = 0, 0.15, 0.3, 0.4, and three different strengths of ELs, E_{CH} = 0, 0.15, 0.3. For each set of these effective interaction parameters (E_{CH}, E_{CH}), we first perform DMD simulations of a monomer at a high temperature (T_{sim} = 4) to obtain 100 distinct random coil-like initial conformations to be used in production runs. For given interaction parameters (E_{CH}, E_{CH}), we simulate 100 trajectories spanning a temperature range of [0.10, 0.18].

3.1. Interaction Parameters for Aqueous Aβ Folding. In an aqueous environment, hydrophobic and hydrophilic effects play key roles in protein folding and assembly. In the DMD approach, we model hydrophobic effects by a single-well attractive/repulsive potential between two side-chain beads. The strength of the effective hydrophobic interactions, E\text{HB}, is by definition equal to the absolute value of the potential energy between two I residues at a distance of <7.5 Å. Similarly, the strength of effective electrostatic interactions, E\text{CH}, is defined as the absolute value of the potential energy between two oppositely charged side-chain atoms at a distance of <6 Å.

We examined the temperature dependence of Aβ40 and Aβ42 folding using 12 different sets of interaction parameters (E_{CH}, E_{CH}). Our goal was to select interaction parameters that would best fit the folded structure of Aβ in aqueous solution. As an input, we used the in vitro data by of Lim et al.,14 who applied CD spectroscopy to characterize the secondary structure of Aβ40 and Aβ42 monomers in a broad temperature range (Fig. 1A). The resulting CD intensities for Aβ40 and Aβ42 increased linearly with temperature T\text{real} and above T\text{real} = 298 K, the CD intensity for Aβ42 surpassed the Aβ40 intensity, indicating a higher ⟨β⟩ in Aβ42 for T_{real} > T_{real}'.

At given interaction parameters (E_{CH}, E_{CH}), we calculated the average β-strand propensity, ⟨β⟩, for each Aβ40 and Aβ42, which could be compared to the CD intensities. Our in silico results for ⟨β⟩ for both Aβ40 and Aβ42 were in agreement with the CD data obtained by Lim et al. (Fig. 1B). At low temperatures, ⟨β⟩ in both peptides increased with temperature linearly, and above the temperature T_{sim}', Aβ42 had more β-strand structure than Aβ40.

Assuming that ⟨β⟩ was proportional to the CD intensity, we fitted the calculated temperature dependence of ⟨β⟩ for Aβ42 monomer to the temperature dependence of the CD intensity. For each set of interaction parameters (E_{CH}, E_{CH}), we first determined the simulation temperature T_{sim}', above which ⟨β⟩ of Aβ42 was larger than ⟨β⟩ of Aβ40. We matched T_{sim}' to T_{real}' to obtain the temperature scaling factor T* (eq 1),

\[
T^* = \frac{T_{\text{real}'} \cdot T_{\text{sim}'} - 1}{T_{\text{real}'}}
\]

and used T* to scale the simulation temperature T_{sim} to the real temperature T_{real}.

CD spectroscopy provides a rapid method for the determination of the population average secondary structure frequency.
distribution. In a previous paper, Greenfield suggested that a linear relationship exists between CD intensity and the sum of the secondary structure elements contributing to it.\textsuperscript{50} To relate the average \( \beta \)-strand propensity \( \langle \beta \rangle \) derived from our simulations to CD intensities \( I_{\text{CD}} \) determined experimentally, we used the data of Lim \textit{et al.} \textsuperscript{14} These data were obtained at 222 nm, not at \( \sim 218 \) nm, where the minimum in \( \beta \)-strand ellipticity occurs. Nevertheless, because the \( \beta \)-strand is the dominant secondary structure element in our A\( \beta \) monomer conformations and because \( \theta_{218} \approx \theta_{222} \), \( \langle \beta \rangle \) will be proportional to CD intensity at 222 nm. We thus relate the CD intensity data, \( I_{\text{CD}} \) - \( I_0 \) (the \( y \)-axis of Fig. 2C in ref 14), to the calculated \( \langle \beta \rangle \) using the following equation:

\[
\langle \beta \rangle - \langle \beta_0 \rangle = \alpha (I_{\text{CD}} - I_0)
\]

(2)

where \( \langle \beta_0 \rangle \) was \( \langle \beta \rangle \) at 5 °C (278 K) and \( I_0 \) was the corresponding CD intensity at 5 °C. The scaling factor \( \alpha \) was obtained by fitting \( I_{\text{CD}} \) to \( \langle \beta \rangle \).

For all sets of interaction parameters \( (E_{\text{HP}}, E_{\text{CH}}) \), the physiological temperature was found to be within the simulation temperature range \( T_{\text{sim}} \in [0.11, 0.12] \) (Fig. 2).

We fitted \( \langle \beta \rangle \) of the folded A\( \beta \)42 monomer to the CD intensities for 12 different sets of interaction parameters \( (E_{\text{HP}}, E_{\text{CH}}) \). Once the two fitting parameters, \( T^* \) and \( \alpha \), were obtained we used them to calculate \( \langle \beta \rangle \) versus temperature not only for A\( \beta \)42 but also for A\( \beta \)40. For each interaction parameter set \( (E_{\text{HP}}, E_{\text{CH}}) \), we quantified the quality of the fit, including temperature dependencies of \( \langle \beta \rangle \) for both A\( \beta \)40 and A\( \beta \)42, by calculating \( \sigma \):

\[
\sigma = \sqrt{\frac{1}{(N-1) \cdot \langle \beta \rangle_{\text{max}}^2} \sum (\langle \beta \rangle_{\exp} - \langle \beta \rangle_{\text{sim}})^2}
\]

(3)

Table 1. Calculation of \( \sigma \) To Determine the Quality of the Fitting\textsuperscript{a}

<table>
<thead>
<tr>
<th>( E_{\text{HP}} )</th>
<th>( E_{\text{CH}} = 0.00 )</th>
<th>( E_{\text{CH}} = 0.15 )</th>
<th>( E_{\text{CH}} = 0.30 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>0.0459</td>
<td>0.0898</td>
<td>0.0734</td>
</tr>
<tr>
<td>0.20</td>
<td>0.0387</td>
<td>0.0490</td>
<td>0.0682</td>
</tr>
<tr>
<td>0.30</td>
<td>0.0461</td>
<td>0.0690</td>
<td>0.0478</td>
</tr>
<tr>
<td>0.40</td>
<td>0.0596</td>
<td>0.0440</td>
<td>0.0243</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The parameter set \( (E_{\text{HP}}, E_{\text{CH}}) \) that best fit the CD data was \( (0.40, 0.30) \) (in italics).

where \( N \) is the number of experimental temperatures at which the CD intensity was measured, \( \langle \beta \rangle_{\text{max}} \) is the highest value of \( \langle \beta \rangle \) in the simulation data set, \( \langle \beta \rangle_{\exp} \) is the experimental value of \( \langle \beta \rangle \) obtained from fitting the CD intensities to \( \langle \beta \rangle \) (eq 2), and \( \langle \beta \rangle_{\text{sim}} \) corresponds to the value of \( \langle \beta \rangle \) obtained by simulations and interpolated to exactly match the temperatures at which the CD intensities were measured. \( \sigma \) values for each interaction parameter set \( (E_{\text{HP}}, E_{\text{CH}}) \) are listed in Table 1.

Examining the effect of varying the strengths of the two effective interaction parameters, we observed that \( \langle \beta \rangle \) decreased with increasing \( E_{\text{HP}} \) but showed only minor changes with increasing \( E_{\text{CH}} \). For \( E_{\text{HP}} \in [0.1, 0.2] \), \( \langle \beta \rangle \) did not change significantly (Fig. 2G-L). Increasing \( E_{\text{CH}} \) from 0 to 0.15 at \( E_{\text{HP}} = 0.2 \) did not change \( \langle \beta \rangle \) in either of the two peptides. However, at \( E_{\text{CH}} = 0.3 \) (Fig. 2I), \( \langle \beta \rangle \) amounts in both peptides were higher than the amounts observed at \( E_{\text{CH}} \in [0, 0.15] \) but they followed the experimentally observed tendencies: \( \langle \beta \rangle_{42} > \langle \beta \rangle_{40} \). \( \langle \beta \rangle \) strongly decreased when \( E_{\text{HP}} \) changed from 0.2 to 0.3 (Fig. 2D-L). Variations in \( E_{\text{CH}} \) did not significantly affect \( \langle \beta \rangle \) at \( E_{\text{HP}} = 0.3 \). When \( E_{\text{HP}} \) increased from 0.3 to 0.4 (Fig. 2A-F), we observed a further decrease of \( \langle \beta \rangle \) at all temperatures in both peptides while preserving the experimentally observed differ-

ences in $\langle \beta \rangle$ between the two alloforms. At $E_{HP} = 0.4$ (Fig. 2A-C), increase in $E_{CH}$ from 0 to 0.3 resulted in a decrease of $\langle \beta \rangle$ at all temperatures in both peptides.

In the following, we used the interaction parameters that best matched the experimental data, $E_{HP} = 0.4$ and $E_{CH} = 0.3$, to characterize the structural differences in $\beta$-40 and $\beta$-42 monomer folding at different temperatures. The physiological temperature $T_{\text{real}} = 310$ K was found to correspond to the simulation temperature $T_{\text{sim}} = 0.124$.

3.2. $\beta$-40 and $\beta$-42 Folded Structures Differ. Fig. 3 shows typical folded structures of both peptides at four selected temperatures. We define “typical” as a conformation that possesses the average amount of $\beta$-strand structure that strongly increases with temperature. At a fixed temperature, a large variability in the conformational space of folded $\beta$ monomers has been observed in our own previous study and by others. With increasing temperatures, a larger number of $\beta$-strand-rich conformations were observed. At high temperatures, thermal fluctuations destroy any secondary structure and result in random-coil conformations. Similar conformational transitions in a mixture of monomers and dimers in aqueous solution were reported by Gursky and Aleshkov. To gain more detailed structural information, we calculated amino acid-specific $\beta$-strand propensities (Fig. 4) and constructed intramolecular contact maps. These maps show the pairwise amino acid interaction frequencies, thereby allowing identification of peptide regions contributing significantly to folding (Fig. 5).

Fig. 4 shows that all $\beta$-strand propensities were below 40% at $T_{\text{sim}} = 0.1$, increased with temperature, reached the highest values of up to 80% at $T_{\text{sim}} = 0.14$, and decreased at yet higher temperatures. At the physiological temperature $T_{\text{sim}} = 0.12$, the regions with the highest $\beta$-strand propensities were A2-F4 ($\beta$-40

Figure 3. Typical conformations of $\beta$-40 (top) and $\beta$-42 (bottom) at different temperatures.

Figure 4. Amino acid-specific $\beta$-strand propensities for $\beta$-40 (black) and $\beta$-42 (red) at four different temperatures using the parameter set (0.40, 0.30). Differences in the termini between $\beta$ peptides are highlighted by the dashed-line boxes. The solid-line boxes show the turn/loop centered at G25-S26 and G37-G38 observed experimentally.28,31,32
These two segments formed a \( \beta \text{-hairpin} \) centered at S8-Y10 in A\( ^{\beta42} \). The hairpin occurs significantly less frequently in A\( ^{\beta40} \), in which the entire segment R5-Q15 shows a significantly smaller \( \beta \)-strand propensity (\( \leq 20\% \)) at all temperatures than does the equivalent segment in A\( ^{\beta42} \) (Fig. 4). Lim et al. reported temperature-induced changes at the N-terminal region of A\( ^{\beta40} \) and A\( ^{\beta42} \) that may be important for their amyloidogenic properties.\(^{14} \) However, the N-terminal (A2-Q15) structural differences between A\( ^{\beta40} \) and A\( ^{\beta42} \) were not observed so far.

We next calculated the average \( \alpha \)-helix propensity, \( \langle \alpha \rangle \), at each simulation temperature \( T_{\text{sim}} \). At the physiological temperature, \( T_{\text{sim}} = 0.12 \), \( \langle \alpha \rangle \) was 0.1\% for A\( ^{\beta40} \) and 0\% for A\( ^{\beta42} \). At lower temperatures, \( \langle \alpha \rangle \) was 0\% and 3\%, respectively. At \( T_{\text{sim}} = 0.1 \), \( \langle \alpha \rangle = 0.3\% \) in A\( ^{\beta40} \) and \( \langle \alpha \rangle = 0.1\% \) in A\( ^{\beta42} \). At temperatures above the physiological temperature, \( \langle \alpha \rangle \) was 0\% in both A\( ^{\beta40} \) and A\( ^{\beta42} \) (data not shown).

### 3.3. Effect of the Arctic (E\(^{22} \rightarrow G \)) Mutation on A\( ^{\beta} \) Folding

We explored here the effects of the clinically relevant Arctic mutation\(^{51} \) on A\( ^{\beta} \) folding. In \textit{in vitro} studies have determined that [G22]A\( ^{\beta40} \) has a higher propensity to form protofibrils than does A\( ^{\beta4} \) but that the overall rate of fibril formation remains constant.\(^{51} \) A recent study by Grant et al.\(^{29} \) showed that the Arctic mutation significantly destabilized the turn structure in the central folding region A21-A30.

We used the optimal interaction parameters \( E_{\text{HP}} = 0.4 \) and \( E_{\text{CH}} = 0.3 \) in our simulations. Fig. 6A shows \( \langle \beta \rangle \) over a wide temperature range for A\( ^{\beta40} \), A\( ^{\beta42} \), [G22]A\( ^{\beta40} \), and [G22]A\( ^{\beta42} \). The temperature dependence of \( \langle \beta \rangle \) for Arctic peptides followed the same trend as A\( ^{\beta4} \). At low temperatures, \( \langle \beta \rangle \) increased gradually until it reached a maximum at \( T_{\text{sim}} = 0.14 \). For \( T_{\text{sim}} > 0.14 \), \( \langle \beta \rangle \) decreased.

As described above, \( \langle \beta \rangle \) in A\( ^{\beta4} \) increased with temperature in both all- \( \beta \)-strands and at \( T_{\text{sim}} > 0.11 \), the average \( \beta \)-strand propensity of A\( ^{\beta42} \), \( \langle \beta \rangle_{12} \), surpassed the average \( \beta \)-strand propensity of A\( ^{\beta40} \), \( \langle \beta \rangle_{10} \). In contrast, in the Arctic mutants in the same temperature range, \( \langle \beta \rangle_{[G22]A^{\beta40}} > \langle \beta \rangle_{[G22]A^{\beta42}} \) (Fig. 6A). As shown in Fig. 6A, at \( T_{\text{sim}} \in [0.11, 0.15] \), \( \langle \beta \rangle \) of [G22]A\( ^{\beta40} \) (blue line with squares) was larger than that of A\( ^{\beta40} \) (black line with circles) by 3–5\%, while \( \langle \beta \rangle \) in [G22]A\( ^{\beta42} \) was 2–3\% larger than in A\( ^{\beta42} \).

Important structural differences between [G22]A\( ^{\beta40} \) and [G22]A\( ^{\beta42} \) were observed in the calculated \( \beta \)-strand propensities per amino acid (Fig. 6B). At a physiological temperature, \( T_{\text{sim}} = 0.12 \), both all- \( \beta \)-strands showed similar \( \beta \)-strand propensities in the segment F21-V24 (50\%), whereas the \( \beta \)-strand propensity of the segment N27-G29 was significantly larger in [G22]A\( ^{\beta40} \) (50\%) than in [G22]A\( ^{\beta42} \) (35\%). Overall, the \( \beta \)-strand propensities in the central folding region of A\( ^{\beta40} \), A\( ^{\beta42} \), and [G22]A\( ^{\beta42} \) were comparable. In the intramolecular contact map

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corresponding to the Arctic mutants, decreased numbers of contacts were observed in the central folding region of both [G22]Aβ40 and [G22]Aβ42 relative to Aβ40 and Aβ42 (Fig. 7), suggesting that the Arctic mutation destabilizes the central folding region as also observed in vitro for the decapeptide Aβ (21–30) by Grant et al.29

At the N-terminus, the β-strand propensity of the segment R5-Q15 (Fig. 6B, top and bottom) was similar in [G22]Aβ40, [G22]Aβ42, and Aβ42, while in Aβ40, no significant β-strand propensity was associated with this region. The β-strand propensity of the segment A2-F4 was small (10%) and similar in both Arctic peptides, similar to Aβ42 but in contrast to that same region in Aβ40, where the β-strand propensity was 40–50%. The segments R5-D7 and Y10-V12 in [G22]Aβ40 had β-strand propensities >50%, compared to only slightly reduced propensity (≈47%) in [G22]Aβ42. In both Arctic peptides, as well as in Aβ42, these two segments form a β-hairpin centered at S8-Y10.

In [G22]Aβ42, the β-strand propensity of the CTR was 20% higher than that in Aβ42, however, the intramolecular contact map shows a slightly decreased number of contacts in this region, suggesting that the Arctic mutation induced a confor-

Figure 6. (A) The average β-strand propensity of [G22]Aβ (solid lines with empty squares; blue for [G22]Aβ40 and green for [G22]Aβ42) and Aβ (solid lines with filled circles; black for Aβ40 and red for Aβ42) at different temperatures. (B) Amino acid-specific β-strand propensity at T_{sim} = 0.120 for Aβ (top) and [G22]Aβ (bottom) peptides with parameters E_{HP} = 0.40 and E_{CH} = 0.30. The boxes indicate the structural differences between wild type and Arctic peptides. The yellow X indicates the substantial differences between the N-termini of the two peptides.

Figure 7. Intramolecular contact maps for Aβ (top) and [G22]Aβ (bottom) peptides with parameters E_{HP} = 0.40 and E_{CH} = 0.30 at T_{sim} = 0.120. The black boxes highlight differences in contact frequency between segments of wild type (top) and Arctic mutant (bottom) peptides.

In these segments was lower than suggested by the folding region, NTR). The hydrogen bond formation propensity important segments: F20-I31 (central folding region TR1), V36-V39 (TR2), and R5:S8, R5:V12, D7:Y10, S8:E11, and H13:K16 were present with the highest propensities at 11% and 14%, respectively, while R5:S8 had 1% propensity in Aβ42, the hydrogen bond F20:D23 had low hydrogen bond propensities of 4%, while E22:G29 and V24:N27 had propensities of 8% and 13%, respectively. In [G22]Aβ42 only the hydrogen bonds R5:S8, D7:Y10, and S8:E11 were present, with 15%, 20%, and 13% propensities, respectively. Here, R5:S8 was absent and H13:K16 remained with propensity of 11%. These results indicate that the Arctic mutation increased the propensity for backbone hydrogen bond formation with respect to wild type peptides. However, the pattern of the backbone hydrogen bonds in [G22]Aβ40 and [G22]Aβ42 was consistent with a β-hairpin structure at R5:Q15 similar to the one in Aβ42.

### 4. Conclusions

In this paper we examined folding of full-length Aβ40 and Aβ42, and their Arctic mutants, using DMD combined with a four-bead protein model and implicit solvent interactions. The temperature-induced conformational transitions obtained in silico were consistent with in vitro experiments that showed conformational transitions from a collapsed coil at low temperatures to a β-sheet-rich extended conformations at higher temperatures. Consistent with the CD measurements by Lim et al., we observed a faster increase of the average amount of β-strand in Aβ42 relative to Aβ40. Our model predicted the central folding region centered at G25-S26 in both Aβ40 and Aβ42, and the C-terminal folded structure centered at G37-G38 in only Aβ42, in agreement with in vitro findings of several groups.

Existing experimental and all-atom MD studies on the fragment Aβ(10–35) are consistent with our observation of the collapsed coil monomer structure dominated by loops, bends, and turns at low temperatures. Our results demonstrate that small changes in the primary structure can have significant impact on folding, suggesting that full-length Aβ40 and Aβ42 and their mutants need to be examined to gain insights into pathological differences between the alloforms. The present study extends our understanding of how the additional amino acids I41 and A42 at the C-termini of Aβ40 and Aβ42 and their mutants need to be examined to gain insights into pathological differences between the alloforms. The present study extends our understanding of how the additional amino acids I41 and A42 at the C-termini of Aβ40 and Aβ42 and their mutants need to be examined to gain insights into pathological differences between the alloforms. 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in the redox peptides. As the structure at the N-terminal region was suggested to impact the amyloidogenic properties of Aβ, the structural difference between the two alloforms reported here might provide a new clue to understanding oligomerization differences between Aβ40 and Aβ42.

Examining folding of the two Arctic mutants, [G22]Aβ40 and [G22]Aβ42, we showed that the presence of Gly²² disrupts contacts close to position 22, and importantly, also at the N-terminus of Aβ40, resulting in a [G22]Aβ40 conformer that is structurally similar to Aβ42 in this region. The average amount of β-strand formed at a physiological temperature in [G22]Aβ40 is higher than in [G22]Aβ42. Our observation that the substitution E22G increases the propensity for β-strand formation is not surprising. This substitution not only reduces the overall negative charge of the Arctic peptides but also, through the G²² substituent, increases the local backbone flexibility needed for a collective hydrogen bond ordering into a β-strand. In our study, the Arctic mutation did not significantly alter the structure of Aβ42. Instead, the major effect appeared to be on the secondary structure of Aβ40, which was more “Aβ42-like”. The increased level of regular secondary structure in Aβ40 is likely to affect its oligomerization pathway, as observed in vitro and in vivo. Several studies have reported that the Arctic mutation significantly increases the protofibril formation rate relative to the wild type. Our simulation result for [G22]Aβ40 shows an increase in the average β-strand propensity when compared to the wild type, which is consistent with these experimental findings. Dahlgren et al. developed two aggregation protocols for the production of stable oligomeric or fibrillar preparations of Aβ42 and its Dutch (E²²→Q) and Arctic mutants. In terms of neurotoxicity, the wild type and the mutants were not significantly different, but they observed extensive protofibril and fibril formation by the mutant peptides. Experimental studies by Murakami et al. demonstrated that the mutations at positions 22 and 23 played a significant role in Aβ assembly. Specifically, the Arctic mutant showed a 50% increase in the average β-strand content in Aβ oligomers. Whalen et al. found that Arctic Aβ had an increased rate of assembly into oligomers and that these oligomers were more toxic to neurons in culture than were wild type oligomers. These experimental findings on Arctic peptides are consistent with the increased β-strand propensity in folded Arctic monomers relative to their wild type counterparts. Take together with other data extant, our results suggest that small changes in the primary structure of Aβ not only may affect peptide monomer folding itself but also the rate of formation, structure, and neurotoxic properties of higher order assemblies.

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