Impact of Mutations on the Conformational Transition from α-Helix to β-Sheet Structures in Arctic-Type Aβ40: Insights from Molecular Dynamics Simulations

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ABSTRACT: The amyloid-β (Aβ) protein aggregation into toxic oligomers and fibrils has been recognized as a key player in the pathogenesis of Alzheimer’s disease. Recent experiments reported that a double alanine mutation (L17A/F19A) in the central hydrophobic core (CHC) region of [G22]Aβ40 (familial Arctic mutation) diminished the self-assembly propensity of [G22]Aβ40. However, the molecular mechanism behind the decreased aggregation tendency of [A17/A19/G22]Aβ40 has not well understood. Herein, we carried out molecular dynamics simulations to elucidate the structure and dynamics of [G22]Aβ40 and [A17/A19/G22]Aβ40. The results for the secondary structure analysis reveal a significantly increased amount of the helical content in the CHC and C-terminal region of [A17/A19/G22]Aβ40 as compared to [G22]Aβ40. The bending free-energy analysis of D23–K28 salt bridge suggests that the double alanine mutation in the CHC region of [G22]Aβ40 has the potential to reduce the fibril formation rate by 0.57 times of [G22]Aβ40. Unlike [G22]Aβ40, [A17/A19/G22]Aβ40 largely sampled helical conformation, as determined by the minimum energy conformations extracted from the free-energy landscape. The present study provided atomic level details into the experimentally observed diminished aggregation tendency of [A17/A19/G22]Aβ40 as compared to [G22]Aβ40.

1. INTRODUCTION

Alzheimer’s disease (AD) is a complex and progressive neurodegenerative disease that is affecting the elderly population.1 The 2019 World Alzheimer’s report highlighted that globally more than 50 million people have dementia, and these figures are going to be three times of the present value by 2050.2 The accumulation of misfolded amyloid-β (Aβ) peptide aggregates (oligomers, protofibrils, and fibrils) in patient’s brain is the key pathological hallmark of AD.3 Although there are different isoforms of Aβ peptide, Aβ40 and Aβ42 are the most abundant isoforms.4 Aβ exists largely as a random coil in solution, however, the conformational transition to β-sheet (aggregation-prone conformation) results in the formation of toxic aggregates.5 Recently, soluble Aβ oligomers were identified as the most toxic and harmful species and are the main culprit for neuronal damage in comparison to mature fibrils.6

Significant advancement has been made to understand the pathological mechanism of AD by examining rare genetic cases that are linked with the early commencement of the disease. Till date, more than 20 single residue mutations are reported in the Aβ peptide sequence.7 Among the reported mutations, six single-point mutations occurring near the α-secretase cleavage site have been extensively evaluated by computational and experimental methods. The Flemish (A21G),7 Arctic (E22G),8 Italian (E22K),9 Dutch (E22Q),10 Osaka or Japanese (E22A),10 and Iowa (D23N)13 variants have an alteration in the single amino acid residue at 21–23 region of Aβ. The fibrillogenic properties of Aβ peptide alter drastically in the above-listed mutants. Out of the above-listed single-point mutations, Arctic-type (E22G) mutation can be examined to obtain key insights into Aβ aggregation processes as Arctic-type Aβ easily form soluble Aβ protofibrils in vitro and has a purely cognitive phenotype typical of AD.14,15 E22G mutation in Aβ peptide lead to enhanced Aβ aggregation with an increased amount of soluble oligomeric species of Aβ fibrillation.16,17 A recent study demonstrated the effect of E22G substitution in Aβ40 and described the role of α/β discordant segment (15–23) in the aggregation of Aβ40 and Arctic Aβ40 ([G22]Aβ40).18 The double alanine mutation (L17A/F19A) in the central hydrophobic core (CHC) region of [G22]Aβ40 resulted in the considerable loss in the aggregation propensity and neurotoxicity of [G22]Aβ40.18,19
However, mechanistic details about the modulation in the aggregation propensity of [G22]Aβ40 upon double alanine mutation in the CHC region of [G22]Aβ40 remain unclear. Extensive studies, both experimental and computational, investigated the structural properties of the monomeric and oligomeric Aβ alone as well as in the presence of various aggregation inhibitors.20 However, investigation of the Aβ aggregation mechanism by experimental techniques is highly challenging due to the intrinsic disorder, conformational heterogeneity, and high aggregation tendency of Aβ. Molecular dynamics (MD) simulation is a useful method for investigating Aβ dynamics as well as the conformational behavior of Aβ variants at the atomic level.21 Herein, we have investigated the effect of double alanine mutation in the CHC region of [G22]Aβ40 on the structure and dynamics of [G22]Aβ40. The conformational clustering, secondary structure analysis, and free-energy landscape (FEL) provided insights into the structural changes, in experiments with experimental results, which highlight reduced [G22]Aβ40 aggregation propensity upon double alanine mutation in the CHC region of [G22]Aβ40.

2. MODEL AND SIMULATION DETAILS

2.1. Starting Structures for [G22]Aβ40 and [A17/A19/G22]Aβ40. In this work, explicit-solvent MD simulations of a total 2 μs simulation length have been performed. The systems were labelled as [G22]Aβ40 and [A17/A19/G22]Aβ40 (Figure 1). The atomic structure of wild-type (wt) Aβ40 was retrieved from PDB: 1BA422 and residues were mutated in PyMol23 to generate the structures of [G22]Aβ40 and [A17/A19/G22]Aβ40. The PDB, 1BA4, has been previously employed to elucidate the structural and dynamical changes in the Aβ40 monomer and mutants as well as its interaction with inhibitors.24,25

2.2. MD Simulations. The MD simulations were performed with the GROMACS 5.026 package using OPLS-AA27 force field as conformations sampled by OPLS for Aβ40 and Aβ12 matches with NMR data.28 Carballo-Pacheco and Strodel compared various force fields (AMBER99SB, AMBER99SB*ILDN, AMBER99SBILDN-NMR, CHARMM22*, and OPLS) for their ability to reproduce the NMR experimental observables for Aβ12 peptide.29 Except AMBER99SBILDN-NMR, all other force fields reproduced the local NMR experimental observables. Moreover, numerous studies highlighted that the OPLS force field is the most balanced for examining the secondary structural changes occurring in the Aβ fragments in an aqueous medium.30 Additionally, the OPLS force field has been employed to investigate the structure and dynamics of Aβ and its mutant systems in a number of recent studies.31,32,33 In the present study, MD simulations were carried out using the OPLS-AA force field and TIP3P water model.34 The OPLS and TIP3P were previously utilized to generate Aβ ensemble in a number of reported studies.35,36 The Na+ ions were added to achieve overall charge neutrality in each system in an octahedron box. We used the LINCS algorithm to constrain the bond lengths. The temperature and pressure were kept constant by employing Berendsen’s coupling algorithm.37 For the short-range nonbonded interactions, the cut-off was kept 1.0 nm, and the long-range electrostatic interactions were evaluated by employing the particle–mesh Ewald summation scheme.38 The simulations were run for 500 ns for each system, and sampling was carried out at 10 ps time interval. Furthermore, to evaluate the reliability and reproducibility of the MD simulations, repeat simulations of 500 ns with different initial velocities were carried out for each system. The trajectories were examined by utilizing GROMACS tools as used previously in our earlier study.39 The bending free-energy was evaluated as reported in the previous study.39

2.3. Evaluation of 3JNH–HN Coupling Constants. The MD simulation data were validated by comparing 3JNH–HN coupling constants of the [G22]Aβ40 residues with the NMR data. The Karplus equation along with the parameters reported by Vogeli et al.37 was employed to evaluate the 3JNH–HN coupling constants from dihedral angles ϕ and ψ.

2.4. FEL. The dihedral principal component analysis (dPCA)38 and the first two principal components (PC1 and PC2) were used to build the FEL at 300 K. The gmx covar and gmx anaeg tools were used to generate and analyze the covariance matrix of Cα atomic coordinates of [G22]Aβ40 and [A17/A19/G22]Aβ40. The probability density of the distribution of PC1 and PC2 was plotted and evaluated as a function of free energy (kcal/mol) for [G22]Aβ40 and [A17/A19/G22]Aβ40 at 300 K. The gmx sham tool was used to convert the conformational distribution into a three-dimensional plot (FEL).

3. RESULTS AND DISCUSSION

3.1. Validation of MD Simulations. The NMR chemical shift values for the amide protons and Hα atoms of [G22]Aβ40 residues were evaluated using SHIFTX2 program39 and compared with experimental values reported by Usachev et al. in 2014.40 The calculated Hα atoms chemical shift values of [G22]Aβ40 residues from the MD structural ensemble (δsim) show a decent correlation (R = 0.94) with experimental NMR chemical shift (δexp) values (Figure S1a, Supporting Information). The chemical shift values for the amide protons of [G22]Aβ40 residues display good correlation (R = 0.95) between δsim and δexp values (Figure S1b, Supporting Information), which highlight that conformations sampled during MD simulations were almost similar to the experimental data.

In comparison to chemical shifts, the J-coupling (3JNH–HN) constants display subtle variations on conformational fluctuations.41 The average values of the 3JNH–HN coupling constant of [G22]Aβ40 residues were evaluated and compared with NMR

Figure 1. Starting structures of [G22]Aβ40 and [A17/A19/G22]Aβ40 are shown in (panels a,b), respectively.
data reported by Rodziewicz-Motowidlo and co-workers in 2008 (Figure S2, Supporting Information). The average value of computational \( J_{\text{H1-40}} \) was noted to be 7.50 Hz as compared to 7.41 Hz from the experimental data, which indicate the consistency of structural ensembles with the experiment.

### 3.2. Structural Parameters of [G22]\\( \alpha \beta_{40} \) and [A17/A19/G22]\\( \alpha \beta_{40} \)

A quantitative assessment of the conformational behavior and stability of the structural ensemble was achieved by monitoring conformational cluster population, root-mean-square deviation (RMSD), and \( R_g \) during simulation. In addition, to assess the qualitative features, representative conformations of the five most-populated clusters were examined. Applying Daura and co-workers algorithm, the conformational clusters were examined for both systems. The thermodynamic stability of the clusters is directly related to population of clusters. In addition, the number of clusters during simulation were evaluated for the structural ensembles of [G22]\\( \alpha \beta_{40} \) and [A17/A19/G22]\\( \alpha \beta_{40} \) (Figure S3, Supporting Information). Both systems have attained equilibrium and saturated to distinct populations in the number of clusters. Clustering analysis over the entire trajectory identified 92 clusters for [G22]\\( \alpha \beta_{40} \) and a significantly lower number (78) of clusters were observed for [A17/A19/G22]\\( \alpha \beta_{40} \). The relative population distribution of clusters of both systems is listed in Table 1. The most-populated cluster sampled 19.9% of the total conformations for [A17/A19/G22]\\( \alpha \beta_{40} \) as compared to 16.9% for [G22]\\( \alpha \beta_{40} \) (Table 1, Figure 2). The population distribution of the most-populated clusters highlighted the greater thermodynamic stability of the structural ensemble of [A17/A19/G22]\\( \alpha \beta_{40} \) than [G22]\\( \alpha \beta_{40} \). The higher conformational heterogeneity observed in the conformational ensemble of [G22]\\( \alpha \beta_{40} \) is consistent with the results reported by Lu et al., which highlighted that [G22]\\( \alpha \beta_{40} \) samples different aggregates such as oligomers, single fibrils, clusters, fibril bundles, and aggresomes.

The representative [G22]\\( \alpha \beta_{40} \) conformations in the five most-populated clusters adopt predominantly coil conformations, and clusters \( c_1-c_5 \) highlight occurrence of \( \beta \)-hairpin like conformation with two distinct \( \beta \)-strands near the C-terminal region of [G22]\\( \alpha \beta_{40} \) (Figure 2). The observation of \( \beta \)-strands near the C-terminal region of [G22]\\( \alpha \beta_{40} \) is consistent with Lin and Pandey, which reported that small \( \beta \)-hairpins and \( \alpha \)-helices were observed in the conformational ensemble of [G22]\\( \alpha \beta_{40} \) generated by MD simulations.21c Panda et al. reported that the probability of formation of \( \beta \) sheets in the region 21–42 of [A17]\\( \alpha \beta_{40} \) peptide was increased upon E22G mutation, which is consistent with the occurrence of \( \beta \) hairpin structures near the C-terminal region of [G22]\\( \alpha \beta_{40} \) in the present study. The results of the present study are consistent with Mudedla et al., which revealed that helical peptides converted to \( \beta \)-sheet structures through coil-like conformations as intermediates upon E22G mutation in \( \alpha \beta \) dimer (residues 16–24). The five most-populated clusters of [A17/A19/G22]\\( \alpha \beta_{40} \) adopt significantly different conformations as compared to [G22]\\( \alpha \beta_{40} \) with a visibly high helix content and a significantly lower population of clusters sampling \( \beta \)-sheet conformation (Figure 2). The preservation of native random coil/helix conformation and a loss of \( \beta \)-sheet conformation indicate the prevention of conformational transition in [A17/A19/G22]\\( \alpha \beta_{40} \).

The RMSD, \( R_g \) and root-mean-square fluctuation (RMSF) were calculated to assess the stability of [G22]\\( \alpha \beta_{40} \) and [A17/A19/G22]\\( \alpha \beta_{40} \) structural ensemble. For both systems, RMSD fluctuates initially up to \( \sim 100 \) ns and attains equilibrium during the rest of the simulation (Figure 3a). For [G22]\\( \alpha \beta_{40} \), MD trajectory displays a significantly higher average value of RMSD (\( \sim 1.12 \) nm) in comparison to \( \sim 0.88 \) nm for [A17/A19/G22]\\( \alpha \beta_{40} \), which indicate enhanced stability of the structural ensemble of [A17/A19/G22]\\( \alpha \beta_{40} \). For [G22]\\( \alpha \beta_{40} \), \( R_g \) displays higher fluctuation for initial 200 ns and thereafter system attain equilibrium till the end of simulation with an average value of \( \sim 1.10 \) nm (Figure 3b). For [A17/A19/G22]\\( \alpha \beta_{40} \), a lower average value of \( R_g \) (\( \sim 1.03 \) nm) was observed and \( R_g \) oscillates near its average value during the simulation (Figure 3b). The \( \text{C} \alpha \) RMSF of [G22]\\( \alpha \beta_{40} \) and [A17/A19/G22]\\( \alpha \beta_{40} \) were evaluated (Figure 3c). As depicted in Figure 3c, conformational fluctuations observed in [A17/A19/G22]\\( \alpha \beta_{40} \) residues are significantly lower in comparison to [G22]\\( \alpha \beta_{40} \), which, in turn, depict greater structural stability of [A17/A19/G22]\\( \alpha \beta_{40} \). The observed lower values of RMSD, \( R_g \) and RMSF for [A17/A19/G22]\\( \alpha \beta_{40} \) as compared to [G22]\\( \alpha \beta_{40} \) indicated higher stability of the structural ensemble of [A17/A19/G22]\\( \alpha \beta_{40} \) than Arctic variant.

The RMSD and \( R_g \) distributions were noted to be identical for simulations with different initial velocity for both systems (Figures S4 and S5, Supporting Information). For both systems, the \( \text{C} \alpha \) RMSF displays identical fluctuations with

### Table 1. Number of Conformational Clusters and Sampling of the Most-Populated Clusters of [G22]\\( \alpha \beta_{40} \) and [A17/A19/G22]\\( \alpha \beta_{40} \) during MD Simulation

| model system                 | number of clusters | \( c_1 \) | \( c_2 \) | \( c_3 \) | \( c_4 \) | \( c_5 \) |
|------------------------------|--------------------|----------|----------|----------|----------|----------|
| [G22]\\( \alpha \beta_{40} \) | 92                 | 16.9     | 15.1     | 13.0     | 8.6      | 7.5      |
| [A17/A19/G22]\\( \alpha \beta_{40} \) | 78                 | 19.9     | 16.9     | 12.6     | 11.5     | 6.8      |
different initial velocity simulations (Figures S4 and S5, Supporting Information). Overall, these results depict the reliability and reproducibility of the MD simulation data.

3.3. Effect of Double Alanine Mutation in the CHC Region of [G22]Aβ40 on the Secondary Structure.

For [G22]Aβ40, the secondary structure composition was noted to be 7, 14, 21, 31, and 17% for helix, β-sheet, turn, coil, and bend, respectively (Table 2). The lower sampling of helical conformation (7%) in [G22]Aβ40 is consistent with the results reported by Rodziewicz-Motowido et al., which highlighted that E22G mutation lead to shortening of the α-helical fragment.14 The lower sampling of helical and concomitant higher sampling of β-sheet conformation in [G22]Aβ40 is in accordance with the CD data.18 For [G22]Aβ40, a significant β-sheet structure was observed at the C-terminal region that is consistent with earlier reports as region 30−40 of Aβ40 displayed higher propensity to form β-sheet and considered to be critical in Aβ40 aggregation.45 The observation of turn conformation in the decapeptide region Ala21−Ala30 of [G22]Aβ40 after ~190 ns indicates a significant reduction in the helical propensity which highlighted reduced conformational stability of the decapeptide region (Figure 4, upper panel). Lazo et al. (2009) highlighted that the decapeptide region of Aβ40 (Ala21−Ala30) comprises a nucleus for the Aβ40 folding.40 NMR analysis on the Aβ(21−30) decapeptide region highlighted that the turn formation within the Val24−Lys28 region results in the Aβ monomer folding, which, in turn, leads to subsequent self-assembly process.46 Thus, the enhanced aggregation rate of [G22]Aβ40 is associated with the formation of the β-sheet structure at the C-terminal region as well as a significantly higher propensity to adopt turn conformation in the decapeptide region Ala21−Ala30 of [G22]Aβ40.

For [A17/A19/G22]Aβ40, the secondary structure content was noted to be 13, 7, 17, 27, and 26% for helix, β-sheet, turn,
concomitant increase in the helical content in [A17/A19/G22]β40 (lower panel). Thus, a decrease in the secondary structure analysis in both wt Aβ40 and [A17/A19/G22]β40 got significantly reduced in [A17/A19/G22]β40 (Figure 4, lower panel). Thus, a decrease in the β-sheet structure and a concomitant increase in the helical content in [A17/A19/G22]β40 confirm that double mutation by alanine in the C-terminal region and turn conformation abundant in the Ala21–Ala30 region of [G22]β40 got significantly reduced in [A17/A19/G22]β40 (Figure 4, lower panel).

Thus, secondary structure analysis in both studies highlighted the lower aggregation tendency in [A17/A19/G22]β40 which, in turn, highlighted the lower tendency of aggregation.

The GxxxG (Gly33–Gly37) repeat motifs with-in peptides have been found to be responsible for the formation of ridges on the amyloid surface, which lead to enhanced packing of the sheets.46 The glycines in the G33–G37 region displayed a higher propensity to adopt β-sheet conformation in [G22]β40 as compared to [A17/A19/G22]β40 (Figure 5b). Thus, a significant decrease in the β-sheet content of the GxxxG motif prevented close packing of the sheets, which, in turn, led to reduced aggregation tendency in [A17/A19/G22]β40.

The turn conformation at Ser8 and Gly9 residues of the Aβ40 permit N-terminal region to make contacts with the CHC region and promotes Aβ self-assembly.49 For [G22]β40, the Ser8 and Gly9 residues sampled 91 and 98% turn conformation as compared to [A17/A19/G22]β40 (Figure 5b). Thus, a significant decrease in the β-sheet content of the GxxxG motif prevented close packing of the sheets, which, in turn, led to reduced aggregation tendency in [A17/A19/G22]β40 and hence the lower aggregation tendency.

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3.5. Tertiary Interactions and Solvent Accessible Surface Area of [G22]β40 and [A17/A19/G22]β40. To elucidate the tertiary interactions, a contact map between side chain of residues was plotted for both systems, which displays...
probabilities of contact formation for all possible residue pairs (Figure 6). In [G22]Aβ40, the contacts between CHC residues and mid-domain/C-terminal residues were observed (Figure 6a), which is consistent with earlier results.50 A lower tendency for the contacts between CHC and C-terminal regions was observed in [A17/A19/G22]Aβ40 (Figure 6b), which, in turn, led to a lower propensity of the formation of aggregation-prone conformation. These results are consistent with the results reported by Coskuner et al.,51 which highlighted that the interactions between CHC and C-terminal regions become less abundant upon E22Δ mutation in the wt Aβ40 monomer peptide and resulted in the lower aggregation propensity of E22Δ mutant.

The surface exposure of hydrophobic residues is considered to be important for recognition and assembly of fibrils.52 The per-residue solvent accessible surface area (SASA) for [G22]Aβ40 and [A17/A19/G22]Aβ40 was evaluated (Figure S6, Supporting Information). SASA analysis depicts reduced values for most of the residues in [A17/A19/G22]Aβ40 as compared to [G22]Aβ40. The change in the area for hydrophobic and hydrophilic residues in SASA analysis was utilized to monitor the stability of Aβ40 monomer.53 The lower SASA values in the CHC region of [A17/A19/G22]Aβ40 highlighted low propensities of the residues for self-assembly. Thus, double alanine mutation in the CHC region of [G22]Aβ40 prevented [G22]Aβ40 aggregation by reducing the CHC residues area exposed to the solvent.

### 3.6. Impact of Double Alanine Mutation in the CHC Region of [G22]Aβ40 on the Rate of Fibril Formation.

The D23−K28 salt bridge probability was evaluated in [G22]Aβ40 and [A17/A19/G22]Aβ40 as it plays a critical role in the Aβ fibrils stability (Figure 7a).25a,54 Previous studies revealed that the salt bridge interactions between Asp23 and Lys28 lead to U-shaped conformation, which is an important motif in amyloid formation and hence enhance the structural rigidity of Aβ42 peptide.52,55 The occurrence of salt bridge among Asp23 and Lys28 residues in the bend region of Aβ peptide stabilizes the β-sheet structure. It has been known that the salt bridge was considered to be formed when the distance between Cγ atom of Asp23 and Nζ atom of Lys28 remained within 0.46 nm.25a The population distribution of the D23(Cγ atom)−K28(Nζ atom) distance was evaluated (Figure 7a).

The average D23−K28 distance increases significantly from ∼0.35 nm in [G22]Aβ40 to ∼0.47 nm in [A17/A19/G22]Aβ40 that highlights D23−K28 salt bridge destabilization in [A17/A19/G22]Aβ40, which, in turn, highlights reduced aggregation tendency in [A17/A19/G22]Aβ40.

Sciarretta et al. investigated the fibril formation rate of Aβ40 and Aβ40-lactam(D23−K28).56 In Aβ40-lactam(D23−K28), the fibrillogenesis rate was increased to 1000 fold, and the lag phase period got suppressed. The lactam linkage in Aβ40-lactam(D23−K28) resulted in the formation of a bend-like structure in the Aβ40 fibril formation process. Thus, to study the rate and kinetics of Aβ40 fibril formation it is important to investigate the probability of bend formation between D23−K28 residues. To examine the fibril formation rate of [G22]Aβ40 and [A17/A19/G22]Aβ40, the bend formation probability between D23−K28 residues needs critical inves-
tigation. Thus, binding free energy and the rate of fibril formation were evaluated for both systems. The probability distribution of the Ca distance between D23–K28 residues has been calculated for both systems (Figure 7b). The probability distribution \( P(R_{23}^{G22} < R_{23}^{G22}) \) and \( P(R_{23}^{G22} > R_{23}^{G22}) \) are reported in Table 3.

| system            | [G22]Aβ40 | [A17/A19/G22]Aβ40 |
|-------------------|-----------|-------------------|
| \( R_{23}^{G22} \) (nm) | 0.29 ± 0.66 | 0.40 ± 0.78       |
| \( P(R_{23}^{G22} > R_{23}^{G22}) \) (%) | 42 ± 1.24 | 58 ± 1.01         |
| \( P(R_{23}^{G22} < R_{23}^{G22}) \) (%) | 51 ± 0.16 | 49 ± 0.97         |

As \( \Delta \Delta G_{\text{bond}} \) is related to the fibril formation, the double alanine mutation in the CHC region of [G22]Aβ40 has the potential to slow down the fibril formation rate by 0.57 times of [G22]Aβ40. In another mutation study, Truong et al. performed replica-exchange MD simulations with the OPLS force field and implicit solvent and reported that aggregation rates were reduced by 0.59 and 0.64 times for Aβ40 and Aβ42, respectively, upon D7H mutation.

3.7. Study of Conformational Substates by FEL. The FEL depicting the conformational preferences of [G22]Aβ40 and [A17/A19/G22]Aβ40 was investigated based on first two PCs (Figure 8). As shown in Figure 8a, [G22]Aβ40 has more complex FEL depicting four minimum free-energy basins. In [G22]Aβ40, the lowest energy conformation corresponding to basin i displays 8, 15, 29, 30, and 18% content for helix, \( \beta \)-sheet, turn, coil, and bend conformation, respectively (Table 4). The conformation corresponding to basin i possesses a higher propensity for \( \beta \)-sheet, coil conformation, and a lower propensity for helical conformation. Interestingly, a \( \beta \)-sheet conformation at the C-terminal region was observed in the minimum energy conformations corresponding to basin i, ii, and iii for [G22]Aβ40 (Table 4) that matches with overall secondary structure analysis. FEL analysis highlights higher sampling aggregation-prone conformations in the minimum free-energy basins in [G22]Aβ40. For [G22]Aβ40, the FEL was noted to be rugged with a number of minimum free-energy basins separated by low-energy barriers, which, in turn, promoted the conformational transitions between various conformations. These results are consistent with that of Xu et al., which highlighted that E22G mutation in Aβ12 accelerated fibril formation by altering the microstate dynamics, as revealed by dihedral dynamics analysis.

The FEL of [A17/A19/G22]Aβ40 displays two minimum free-energy basins and the conformation extracted from the minimum free-energy basin i sample higher helix (19%), coil (33%) conformations, and a lower turn (21%) conformation (Figure 8b, Table 4). Most importantly, no aggregation-prone \( \beta \)-sheet conformation at the C-terminal region was observed in the conformations corresponding to minimum free-energy basins i and ii of [A17/A19/G22]Aβ40. FEL analysis highlighted that tendency for oligomerization and fibrillation was significantly higher for [G22]Aβ40 in comparison to [A17/A19/G22]Aβ40 as the aggregation-prone conformation was observed at the C-terminal region of [G22]Aβ40. The double alanine mutation in the CHC region of [G22]Aβ40 resulted in a loss in the \( \beta \)-sheet content at the C-terminal region, which, in turn, lowered the self-aggregation tendency in [A17/A19/G22]Aβ40.

4. CONCLUSIONS

In this study, the conformational fluctuations and structural changes in Arctic-type Aβ40 upon double alanine mutation (L17A/F19A) in the CHC region were explored by using MD simulations. MD simulations suggested that the lower aggregation tendency of [A17/A19/G22]Aβ40 has been related to an augmentation in the helical conformation and a simultaneous decline in the \( \beta \)-sheet content, which is consistent with CD studies. The representative conformations from clustering analysis and FEL highlighted the existence of \( \beta \)-sheet conformations in [G22]Aβ40, whereas conformations obtained from the lowest energy basins of [A17/A19/G22]Aβ40 adopt native conformations (helical or random coil). Moreover, a reduction in the intrapeptide side chain contacts among CHC and mid-domain or C-termini in [A17/A19/G22]Aβ40 highlighted lower propensity of oligomerization and formation of aggregation-prone conformation. The higher aggregation propensity of [G22]Aβ40 was associated with the formation of a stable D23–K28 salt bridge, which becomes considerably less stable in [A17/A19/G22]Aβ40. The bending free-energy analysis suggested that the double alanine mutation (L17A/F19A) in the CHC region has the potential to decrease the fibril formation rate of [A17/A19/G22]Aβ40 as compared to [G22]Aβ40 which is in accordance with the experimental results. The present study unraveled mechanistic

Figure 8. FEL of [G22]Aβ40 (panel a) and [A17/A19/G22]Aβ40 (panel b) along PC1 and PC2 obtained from dPCA. The conformations extracted from the minimum free-energy basins are displayed underneath FEL.
details about the conformational changes in Arctic-type Aβ40 upon double alanine mutation in the CHC region. The findings in the present study uncovered the structural basis of the experimentally observed lower aggregation propensity of [G22]Aβ40 upon double alanine mutation in the CHC region of [G22]Aβ40.

■ ASSOCIATED CONTENT

1 Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02983.

Correlation between simulated and experimental $^1$H NMR chemical shifts for $\Delta H$ atoms and amide protons of [G22]Aβ40 residues; comparison of simulated $^1$HHN−$^1$Hα coupling constants of [G22]Aβ40 residues with experimental measurements; evolution of clusters for [G22]Aβ40 and [A17/A19/G22]Aβ40 RMSD, $R_g$ and RMSF of [G22]Aβ40 and [A17/A19/G22]Aβ40 for simulations with different initial velocities; SASA of each residue in [G22]Aβ40 and [A17/A19/G22]Aβ40; and secondary structure component statistics of [G22]Aβ40 and [A17/A19/G22]Aβ40 for simulations with different initial velocities (PDF)

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Table 4. Helix, β-Sheet, Turn, Coil, and Bend Content of the Conformations Extracted from the Minimum Free-Energy Basins of [G22]Aβ40 and [A17/A19/G22]Aβ40

| model system | conformation | helix$^a$ | β-sheet$^b$ | turn | coil | bend |
|--------------|--------------|-----------|-------------|------|------|------|
| [G22]Aβ40   | i            | 8         | 15          | 29   | 30   | 18   |
|              | ii           | 7         | 14          | 28   | 30   | 21   |
|              | iii          | 8         | 15          | 27   | 31   | 19   |
|              | iv           | 7         | 0           | 32   | 41   | 20   |
| [A17/A19/G22]Aβ40 | i | 19        | 0           | 21   | 33   | 27   |
|              | ii           | 16        | 0           | 19   | 35   | 30   |

$^a$Helix comprises $3_{10}$ α- and π-helix. $^b$β-sheet comprises β-strand and β-bridge.
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