REGULAR ARTICLE

Special Issue on THEORETICAL CHEMISTRY/CHEMICAL DYNAMICS

In silico studies of the early stages of aggregation of Aβ42 peptides†

PRABIR KHATUA and SANJOY BANDYOPADHYAY∗

Molecular Modeling Laboratory, Department of Chemistry, Indian Institute of Technology, Kharagpur, West Bengal 721 302, India
E-mail: sanjoy@chem.iitkgp.ernet.in

MS received 26 December 2016; revised 3 February 2017; accepted 8 February 2017

Abstract. Accumulation of amyloid beta (Aβ) peptide in the brain is responsible for debilitating neurodegenerative disease, namely, Alzheimer’s disease. We have carried out atomistic molecular dynamics simulation to study the early stages of the aggregation process of five full-length Aβ42 peptide monomers with varying secondary structural contents in aqueous solution. Attempts have been made to study the conformational modifications of the Aβ peptide monomers and their dynamical features during the oligomer formation. In particular, specific molecular interactions that drive the association process leading to the formation of the stable oligomer have been identified. The calculations revealed that the helix–helix linkage plays an important role for bringing the unstructured regions of the monomers closer for self-assembly. Importantly, it is demonstrated that the contribution originating from the nonpolar interactions between the peptides and the corresponding nonpolar solvation more than compensates the weakening effect of unfavorable inter-peptide electrostatic interactions, thereby stabilizing the nucleated oligomer.

Keywords. Molecular simulation; intrinsically disordered protein; amyloid β.

1. Introduction

Protein aggregation is closely linked with several neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease, Type II diabetes, Huntington’s disease, etc. In general, such diseases are believed to be caused by the accumulation of β-sheet-rich structures termed as amyloid fibrils in the brain. Alzheimer’s disease (AD) associated with the deposition of plaques or fibrils composed of 39 to 43 residue peptide named amyloid β-peptide (Aβ) in the brain is the most common form of dementia affecting millions of people worldwide. Aβ peptide is processed from sequential cleavage of the transmembrane amyloid precursor protein (APP) by β- and γ-secretases. The predominant components of fibrillar deposits in the brain of AD patients include Aβ peptides containing 40 and 42 amino acid residues (Aβ40 and Aβ42). Though Aβ42 peptide differs from Aβ40 by only two C-terminal residues, the former has a higher aggregation propensity as compared to the later.

Aβ42 monomer contains two hydrophobic patches, denoted as hp1 (Leu-17 to Ala-21) and hp2 (Ala-30 to Met-35) connected by a hydrophilic patch, denoted as turn (Glu-22 to Gly-29), the C-terminal segment, denoted as C-term (Val-36 to Ala-42), and the disordered N-terminal segment (N-term) containing 16 residues (Asp-1 to Lys-16). Aβ fibril has U-shaped conformation with the β strands formed by the hp1, hp2 and C-term segments. Importantly, the hydrophobic residues in hp1 and hp2 are believed to be primarily responsible for the hydrophobic collapse that occurs during fibrillation. In addition, the salt bridge formed between Asp-23 and Lys-28 in the hydrophilic turn region also plays a crucial role in bringing the hp1 and hp2 segments closer to form the β-hairpin structure, the basic unit of the fibril. The amino acid sequence of the Aβ42 peptide (in one letter code) is D(1)AEFRHDSGYEVHHQKLVFFAEDVGSN-KGAII GLMVGGVVIA(42), with D(1) and A(42) being the N- and C-terminal residues, respectively.

A large body of literature exists on studying the self-assembly of Aβ from experiments as well as from theoretical and simulation studies. It is believed that amyloid formation follows a nucleation-dependent polymerization mechanism. According to this mechanism, fibril formation occurs in two phases, namely, the nucleation/lag phase and the elongation/growth phase. In nucleation phase, soluble monomers undergo conformational modifications and self-associate to form oligomeric nuclei. This process needs to overcome a high energy barrier and thus occurs slowly. In other

For correspondence
†Dedicated to the memory of late Professor Charusita Chakravarty.
words, it is the rate limiting step of the amyloid formation. Nucleation phase is followed by thermodynamically favorable elongation/growth phase. Elongation phase, a faster process as compared to the nucleation phase is associated with the assembly of aggregates and fibril elongation. Neurotoxicity generated from the soluble oligomers and mature fibrils are thought to be the pathological reason for neuronal death of AD affected patients. Recently, it has been shown from several studies that soluble oligomers are more neurotoxic than the insoluble fibrils. Therefore, it is essential to characterize the soluble Aβ oligomers and the mechanism of their formation from monomers in addition to studying the insoluble mature fibrils.

Establishing molecular details of the Aβ aggregation and tracking the intermediate oligomeric structures along the aggregation pathways are two major challenging tasks in studying formation of amyloids. Structural informations of soluble Aβ oligomers have been obtained from solid-state NMR studies. In these studies, small amounts of detergents or fatty acids were used to stabilize the soluble oligomers. For example, Yu et al. used sodium dodecyl sulphate (SDS) to make the soluble Aβ oligomers long-lived and showed that such oligomers contain both parallel and anti-parallel β-sheet structures. X-ray crystallographic studies have also been performed to unravel the structures of soluble oligomers. However, such experimental techniques are often unable to precisely characterize the oligomers as they are highly prone to aggregation and often exist in diverse morphologies due to their inherent fluid-like character. For example, x-ray crystallographic studies often yield data corresponding to the dominant conformational state. Ion-mobility mass spectrometry (IM-MS) is another important technique which can provide more accurate information as it is based on separating the oligomers according to their mass to charge ratio. Besides, IM-MS measures the collision cross-section which can be directly compared with the available experimental and theoretical findings. Recently, Kloniecki et al. detected two families of low-molecular-mass Aβ oligomers, namely, compact and extended from IM-MS measurements.

Computer simulation can provide microscopic understanding of the interactions that drive the formation of Aβ oligomers and the dynamics associated with the early events of fibril formation. Over the years, a number of simulation studies have been attempted to explore different aspects of this problem. In an important study, Urbanc et al. performed discrete molecular dynamics (DMD) simulations based on coarse-grained models to study in detail the dimer formations involving Aβ oligomers. The thermodynamic stability of the dimer conformations as predicted by the DMD simulations were then examined using all-atom MD simulations. Based on the findings, they concluded that Aβ oligomerization is not accompanied by the formation of thermodynamically stable planar β-strand dimers. A recent replica exchange molecular dynamics (REMD) study of Aβ dimer showed that the β-hairpin peptide monomers formed by the central hydrophobic core region (hp1 and hp2) prefer to arrange themselves in anti-parallel orientations in the dimeric form.

Most of the simulation studies reported in the literature are focused on probing the formation of lower order Aβ oligomers, especially dimers. However, the lower order oligomers may not necessarily represent the characteristics of higher order Aβ aggregates which eventually lead to the formation of protofibrils and fibrils. Thus, it is essential to study higher order Aβ oligomers for complete understanding of the driving forces behind Aβ aggregation. In this study, we have performed MD simulation to characterize the early stages of formation of pentameric Aβ aggregate starting from structurally different full-length Aβ peptides in aqueous solution. Our primary objective has been to probe the conformational modifications of the Aβ peptide monomers and their dynamical features during the early stages of the association process. Attempts have been made to identify specific molecular interactions that drive the self-assembly of the Aβ peptide monomers leading to the formation of stable oligomers. In Section 2, we discuss the procedure followed to set up the system and the simulation protocols employed. The results obtained from the calculations are presented and discussed in Section 3. Finally, the important findings of the study and the conclusions reached therefrom are highlighted in Section 4.

2. System setup and simulation methods

We have used the NAMD code to carry out MD simulation of an aqueous solution containing five full-length Aβ oligomers (henceforth will be simply denoted as Aβ) peptide monomers (designated as A1 to A5) with varying secondary structural contents to study the early stages of the aggregation process. The initial configuration of the monomer A1 was taken from model 5 of the solution state NMR structure (PDB ID: 1Z0Q). We carried out 5 ns MD simulations at high temperatures of 450 K and 500 K with the A1 monomer to generate unstructured initial configurations corresponding to A2 and A3, respectively. The initial configurations of the Aβ monomers A4 and A5 were obtained from replica exchange molecular dynamics (REMD) simulations of the NMR structure with 64 replicas in the temperature range of 330 K to 600 K. Each replica was simulated for 10 ns with an
Aggregation of Aβ42 peptides

exchange time of 0.25 ps among the replicas and more than 10% acceptance ratio. The last Aβ conformations as obtained from the trajectories at 530 K and 594 K were taken as the initial configurations of A4 and A5, respectively. It may be noted that the reason for considering Aβ monomers with different initial structures is due to the fact that Aβ being an intrinsically disordered protein (IDP) is highly flexible and exists in different conformational states in its monomeric form in aqueous environment. The terminal residues (Asp-1 and Ala-42) of each of the peptide monomers were taken as standard ammonium and carboxylate forms, respectively. After adding the hydrogen atoms, centers-of-mass of the peptide monomers were placed at least 20 Å away from each other in a cubic cell of dimension 85 Å containing appropriate number of equilibrated water molecules. To avoid unfavorable protein-water contacts, water molecules which were found within 2 Å of the peptide monomers were removed. 15 Na+ ions were then added to the system for charge neutralization.

The simulation system was first minimized using the conjugate-gradient energy minimization method as implemented in NAMD.32 The minimized initial configuration of the system is shown in Figure 1. For easy reference, the individual Aβ monomers are marked (A1 to A5) in the figure. The temperature of the system was then gradually increased to 310 K within a short MD run of 100 picoseconds (ps) under isothermal-isobaric ensemble (NPT) conditions at a constant pressure of 1 atm. The system was then equilibrated for 10 nanoseconds (ns) at 310 K under NPT ensemble conditions. The system temperature was controlled by using the Langevin dynamics method with a friction constant 1 ps\(^{-1}\), whereas the pressure was controlled by the Nosé-Hoover Langevin piston method.33 During this period, the simulation cell volume was allowed to fluctuate isotropically to attain the appropriate density. At this stage, the cell volume attained steady value with cell dimension 82.72 Å. The cell volume was then kept fixed and the simulation conditions were changed from constant pressure and temperature (NPT) to that of constant volume and temperature (NVT). The NVT equilibration run was then continued further at 310 K for another 5 ns, followed by a long NVT production run of 250 ns duration.

The simulation was carried out with an integration time step of 1 femtosecond (fs), and the trajectory was stored with a time resolution of 400 fs for subsequent analyses. The minimum image convention36 was employed to calculate the short-range Lennard-Jones interactions using a spherical cutoff distance of 12 Å with a switch distance of 10 Å. The long-range electrostatic interactions were calculated by using the particle-mesh Ewald (PME) method.37 All bonds involving the hydrogen atoms were constrained using the SHAKE algorithm.38 We have employed the all-atom CHARMM22 force field and potential parameters for the peptide,39,40 while the mTIP3P41 model (modified version of TIP3P42) that is consistent with the chosen protein force field was employed for water.

3. Results and Discussion

3.1 Structural features

A few snapshots of the pentameric aggregate at different time intervals as obtained from our simulation are displayed in Figure 2. For easy reference, the initial configuration of the system containing the five Aβ peptide monomers is included in the figure. Conformational rearrangement of the monomers during their approach to form the oligomer is evident from the figure. It is noticed that such conformational rearrangement of the peptide monomers within the time scale of the simulation is associated with breaking and formation of secondary structural elements (helices, \(\beta\)-strands, turns/coils, etc.). Interestingly, an increase in helical content with time has been observed for a few monomers. This is particularly apparent for A2. Additionally, a closer look at the simulated trajectory reveals that the peptide monomers orient themselves in such a manner so that the helical segments of the adjacent monomers tend to associate with each other during the aggregation process. This is an important observation as close proximity of the helical segments of the monomers is likely to drive the oligomer formation by assisting the nucleation of the unstructured segments of the peptides adjacent to the helices. Our findings are consistent with experimental results where formation of helical intermediates that play important roles in the aggregation process has been suggested.43,44
Figure 2. Snapshots of a few representative configurations of the simulated Aβ pentameric aggregate at different time intervals. For clarity the initial configuration of the system is also included in the figure. The peptide coloring scheme is the same as that in Figure 1.

Figure 3. Time evolutions of RMSD corresponding to all the non-hydrogen atoms of the five Aβ peptide monomers.

To investigate the flexibility of the Aβ monomers and their conformational modifications during the nucleation process, we have monitored the root mean square deviations (RMSD) of the non-hydrogen atoms of each monomer independently. The results are depicted in Figure 3. It is clear from the figure that the peptide monomers deviate significantly from their initial structures. Besides, we observe several jumps in the RMSD values, which suggests sharp conformational transitions of the peptides within the simulation time scale. We believe that such conformational changes of the monomers are key steps for their structural adaptations in the nucleated form. Interestingly, it may be noted that the RMSD of each of the monomers attains a steady limit within ~150 ns. This is an important finding as it provides an estimate of the time scale of structural adaptation necessary for the Aβ peptides to form aggregated pentamer.

To quantify the aggregation of the Aβ monomers, we have monitored the time evolution of the average center-of-mass distance between the closest pairs of the monomers ($R_{CM}$) during the simulation, as shown in Figure 4(a). As a reference, the initial value of $R_{CM}$ (26.88 Å) is marked in the figure (as straight line). Note the rapid decrease in the pairwise distance between the monomers within first 40 ns before attaining a steady mean value with minor fluctuations. This indicates formation of an aggregated pentameric form of the Aβ peptide. The average $R_{CM}$ value of 16.01 (± 0.32) Å as obtained over the last 100 ns of the simulated trajectory has been found to be ~40% lower than the corresponding initial value. The approach between the individual monomers during the aggregation process
should lead to the formation of pairwise inter-peptide residue contacts. The time evolution of the number of such contact pairs found between the Aβ monomers (NC) is shown in Figure 4(b). We have considered two amino acid residues of two monomers to form a contact pair, if the distance between their Cα atoms is within 7 Å. An increase in the number of pairwise contacts with decreasing inter-peptide distance during the aggregation of the Aβ peptides is clearly evident from the data. In addition, it is important to probe the extent of structural adaptations undergone by the Aβ peptides while forming the aggregated oligomer. Radius of gyration (RG) is an important physical parameter which can provide vital information on the size and shape fluctuations of the Aβ peptides during the nucleation process. We have shown the time evolution of RG averaged over the Aβ monomers present in the pentameric system in Figure 4(c). As a reference, the initial average RG value of 13.58 Å is marked in the figure by a straight line. It can be seen that the RG value decreases sharply within first 10 ns and then tend to fluctuate around a mean value of 11.87 (± 0.09) Å over last 100 ns of the trajectory. A decrease in RG by ~13% with respect to the initial value indicates that the Aβ monomers undergo noticeable conformational transformations from expanded to compact forms during the association process. The time evolution of the peptide end-to-end distance (RL) averaged over the Aβ monomers is shown in Figure 4(d). RL is defined as the average distance between the Cα atoms of the two terminal residues of each of the peptide monomers. Again, the initial value of RL (34.33 Å) is marked in the figure (as straight line) for comparison. Transformation of the Aβ monomers into compact forms during their self-assembly is once again evident from decreased RL values with time. The calculated average RL value of 29.04 (± 0.52) Å has been found to be ~15% lower than the initial value. Comparison of the results shown in Figure 4 clearly demonstrates that the nucleation of the Aβ monomers is associated with increased compactness and reduced distance between the end residues of the individual peptide monomers.

3.2 Secondary structure content

A closer examination of Figure 2 reveals change in secondary structural contents of the monomers during the aggregation process. In the previous section, we have discussed the possible role played by the helical segment of the peptide during aggregation. Here, we discuss the secondary structural contents of the peptide monomers in a more quantitative manner. For that, we have computed the residue-specific secondary structural contents of the Aβ monomers using the STRIDE algorithm as implemented in the VMD tool. The time evolutions of the secondary structures of the five Aβ monomers are displayed in Figure 5. The average secondary structural contents (in percentage) of the individual peptide monomers as obtained from the simulated trajectory along with the corresponding initial values are listed in Table 1. It is evident from the figure that in most cases, except A1, the residues present at the two ends of the peptide exhibit propensity to retain random coil-like conformations. Interestingly, we notice that the N-terminal end of A1 undergoes quasi-periodic transitions between coil and helical forms with noticeable lifetimes via momentary formation of β-strand like conformations. Residues Tyr-10 to Lys-16 in the N-term segment of A2 also exhibit such transitions within ~75 ns, before attaining a stable helical form for the rest of the trajectory. On the other hand, non-terminal residues present in the N-term and the two central hydrophobic segments hp1 and hp2 (Tyr-10 to Phe-20 and Ala-30 to Met-35) in general exhibit preference to either remain in helical form or transform from random coil to helical form. This is true for all the monomers.
Figure 5. Time evolutions of residue-wise secondary structures of the five Aβ peptide monomers as obtained from the simulation. The secondary structural elements, such as turn/coil (T/C), β-strand (B), and helix (H) are drawn in green, yellow, and magenta colors, respectively.

Table 1. The average secondary structural content (in percentage) of the five Aβ peptide monomers.

<table>
<thead>
<tr>
<th>monomer</th>
<th>helix</th>
<th>β-strand</th>
<th>turn/coil</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>59.52</td>
<td>0.00</td>
<td>40.48</td>
</tr>
<tr>
<td></td>
<td>(50.00)</td>
<td>(0.00)</td>
<td>(50.00)</td>
</tr>
<tr>
<td>A2</td>
<td>43.78</td>
<td>1.45</td>
<td>54.77</td>
</tr>
<tr>
<td></td>
<td>(19.05)</td>
<td>(0.00)</td>
<td>(80.95)</td>
</tr>
<tr>
<td>A3</td>
<td>20.71</td>
<td>4.44</td>
<td>74.85</td>
</tr>
<tr>
<td></td>
<td>(16.67)</td>
<td>(0.00)</td>
<td>(83.33)</td>
</tr>
<tr>
<td>A4</td>
<td>56.69</td>
<td>7.96</td>
<td>35.34</td>
</tr>
<tr>
<td></td>
<td>(64.29)</td>
<td>(9.52)</td>
<td>(26.19)</td>
</tr>
<tr>
<td>A5</td>
<td>40.23</td>
<td>0.00</td>
<td>59.77</td>
</tr>
<tr>
<td></td>
<td>(38.10)</td>
<td>(0.00)</td>
<td>(61.90)</td>
</tr>
</tbody>
</table>

The values in the parentheses are the corresponding initial values.

Figure 6. Average residue-wise secondary structural propensity (in %) of the Aβ peptide monomers.

(Asp-1 to Gly-9 in N-term and Gly-38 to Ala-42 in C-term) and in the central turn region (Glu-22 to Gly-29) of the peptide exhibit propensity to be in random coil-like conformations. The random conformation of the N-term segment of the peptide as observed in this study is consistent with its experimentally known intrinsically disordered structure.5 Importantly, in consistent with Figure 5 one should note significantly higher propensity of the residues Tyr-10 to Phe-20 and Ala-30 to Met-35 forming the central hydrophobic core (hp1 and hp2) to be in helical form.

3.3 Binding free energy

In this section, we calculate the binding free energy (∆G_{bind}) of the aggregated Aβ pentamer using the Molecular Mechanics Generalised Born Surface Area (MMGBSA) method, as implemented in AMBER14. According to the MMGBSA method,
ΔG_{bind} to form a complex can be written in general as
\[ \Delta G_{bind} = \langle \Delta G_{MM} \rangle + \langle \Delta G_{sol} \rangle - T \langle \Delta S \rangle \]  
(1)
where, ΔG_{MM} is the molecular mechanics contribution originating from the interactions between the ligand and the receptor, and ΔG_{sol} is the solvation free energy. 

\[ T \Delta S \] corresponds to the entropic contribution, which can be calculated either from the normal mode analysis or using quasi-harmonic technique. However, we have not included the entropy contribution arising from changes in the degrees of freedom (translational, rotational, and vibrational) in our calculation due to its high computational demand. Thus, the binding free energy reported in our calculation is indeed the sum of ΔG_{MM} and ΔG_{sol}, i.e.,
\[ \Delta G_{bind} = \langle \Delta G_{MM} \rangle + \langle \Delta G_{sol} \rangle \]  
(2)
ΔG_{MM} is the sum of the contributions originating from the inter-molecular electrostatic and van der Waals (vdw) interaction terms (ΔG_{elec} and ΔG_{vdw}), and can be written as
\[ \Delta G_{MM} = \Delta G_{elec} + \Delta G_{vdw} \]  
(3)
We have calculated ΔG_{elec} and ΔG_{vdw} using the SANDER module of AMBER. The total solvation free energy (ΔG_{sol}) has also been divided into electrostatic and nonpolar components (ΔG_{GB} and ΔG_{nps}) as
\[ \Delta G_{sol} = \Delta G_{GB} + \Delta G_{nps} \]  
(4)
The electrostatic solvation free energy, ΔG_{GB}, has been calculated using the generalised Born (GB) method. On the other hand, the nonpolar contribution to the solvation free energy (ΔG_{nps}) has been calculated as a function of the solvent accessible surface area (SASA), given as
\[ \Delta G_{nps} = \gamma \times SASA + b \]  
(5)
where, the constants γ and b were set to 0.005 kcal mol^{-1} Å^{-2} and 0.0, respectively. Following the discussion, we can rewrite equation 2 for the total binding free energy as
\[ \Delta G_{bind} = \langle \Delta G_{nonpolar} \rangle + \langle \Delta G_{GB,elec} \rangle \]  
(6)
Here ΔG_{nonpolar} and ΔG_{GB,elec} correspond to the contributions originating from non-polar and electrostatic interactions, respectively, which are given by
\[ \Delta G_{nonpolar} = \Delta G_{vdw} + \Delta G_{nps} \]  
(7)
and
\[ \Delta G_{GB,elec} = \Delta G_{GB} + \Delta G_{elec} \]  
(8)
The average binding free energy (ΔG_{bind}) and the corresponding components for the whole peptide monomer in the nucleated pentameric form as obtained from the last 100 ns of the simulation are listed in Table 2. At first, negative ΔG_{bind} value of −50 kcal mol^{-1} for the whole peptide suggests that the nucleation of the Aβ monomers to form the pentameric aggregate is thermodynamically favorable. However, a closer look at the contributions originating from different interactions in ΔG_{bind} reveal interesting behavior. It can be seen that while the nonpolar contribution (ΔG_{nonpolar}) to the binding free energy is favorable, opposite is the case for the electrostatic component (ΔG_{GB,elec}). Further, we notice that though both van der Waals component involving the peptide monomers (ΔG_{vdw}) and their nonpolar solvation (ΔG_{nps}) make favorable contributions, but the former contributes ∼85% of ΔG_{nonpolar}. Interestingly, the contributions originating from electrostatic interactions provide a contrasting picture. It is found that while the polar solvation term (ΔG_{GB}) of the peptides favors the nucleation process, but the unfavorable inter-peptide electrostatic interaction more than compensates the effect of solvation. Such counter-play between the two components weakens the overall electrostatic contribution, thereby resulting in a destabilizing influence on the nucleation process of the Aβ peptide monomers.

We have also calculated the free energy contributions of different secondary structural segments of the Aβ peptide (averaged over the last 100 ns of the trajectory) toward forming the aggregate, which are listed in Table 2. It is apparent that except C-term, other segments of the peptide contribute favorably to the binding free energy (ΔG_{bind}). In particular, the core hydrophobic segments hp1 and hp2 contribute maximum toward negative ΔG_{bind} value. This suggests that the favorable binding between the Aβ peptide monomers during the aggregation process proceeds via preferential association of the hydrophobic segments, hp1 and hp2. On the other hand, the positive value of ΔG_{bind} for the C-term segment suggests that it is unlikely to remain bound in the aggregated state. Importantly, the calculation reveals that the unfavorable contribution of ΔG_{GB,elec} primarily originate from repulsive electrostatic interactions (ΔG_{elec}) involving the two terminal segments of the peptide (N-term and C-term) which are not adequately compensated by the polar component of the solvation free energy, ΔG_{GB}.

It is clear from the above discussion that except the C-term segment, association between the other segments of the Aβ peptide monomers during the early stages of the aggregation process is thermodynamically favorable. Now, it would be interesting to probe the residue-wise contributions to the binding free energy of the peptides. For that, we have calculated the
Table 2. Binding free energies and the corresponding components (in kcal mol⁻¹) for different segments of the Aβ peptide in the nucleated pentameric form as obtained using the MMGBSA method.

<table>
<thead>
<tr>
<th>segment</th>
<th>ΔG_{vdw}</th>
<th>ΔG_{elec}</th>
<th>ΔG_{GB}</th>
<th>ΔG_{aps}</th>
<th>ΔG_{nonpolar}</th>
<th>ΔG_{GB,elec}</th>
<th>ΔG_{bind}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole</td>
<td>-133.92</td>
<td>296.08</td>
<td>-189.70</td>
<td>-22.52</td>
<td>-156.44</td>
<td>106.38</td>
<td>-50.06</td>
</tr>
<tr>
<td>N-term</td>
<td>-47.09</td>
<td>48.62</td>
<td>-0.58</td>
<td>-9.30</td>
<td>-56.39</td>
<td>48.04</td>
<td>-8.35</td>
</tr>
<tr>
<td>hp1</td>
<td>-21.64</td>
<td>-12.76</td>
<td>20.84</td>
<td>-3.71</td>
<td>-25.35</td>
<td>8.08</td>
<td>-17.27</td>
</tr>
<tr>
<td>Turn</td>
<td>-12.85</td>
<td>78.47</td>
<td>-70.06</td>
<td>-2.47</td>
<td>-15.32</td>
<td>8.41</td>
<td>-6.91</td>
</tr>
<tr>
<td>hp2</td>
<td>-36.27</td>
<td>-6.27</td>
<td>16.31</td>
<td>-5.78</td>
<td>-42.05</td>
<td>10.04</td>
<td>-32.01</td>
</tr>
<tr>
<td>C-term</td>
<td>-16.07</td>
<td>188.03</td>
<td>-156.21</td>
<td>-4.92</td>
<td>-20.99</td>
<td>31.82</td>
<td>10.83</td>
</tr>
</tbody>
</table>

The corresponding values averaged over the whole peptide monomers are also listed.

\( \Delta G_{\text{bind}} \) value for each of the amino acid residues averaged over all the peptide monomers and over the last 100 ns of the trajectory. The results are displayed in Figure 7. We have also decomposed the total binding free energy into the contributions originating from the backbones and the side chains of the residues, as shown in the figure. It is found that the contribution from the residue backbones is almost zero. This indicates that the interactions between the residue side chains play dominant role in controlling the formation and stability of the aggregated form. Importantly, we notice that besides the two terminal residues, most of the other residues that contribute unfavorably to \( \Delta G_{\text{bind}} \), namely, Glu-3, Asp-7, Glu-11, Lys-16, Glu-22, Asp-23, contain long side chains that are charged. On the other hand, most of the other residues that make favorable contribution to \( \Delta G_{\text{bind}} \) are either non-polar or polar uncharged residues. This provides microscopic justification of our earlier finding between the contrasting contributions of electrostatic and non-polar interactions toward the overall binding free energy of the aggregate. Note that most of the residues with unfavorable free energy contributions are negatively charged. This is an important observation, which suggests that the side chains of these residues are required to orient in such a manner so that the overall electrostatic repulsion between them minimizes, thereby resulting in formation of stable Aβ peptide aggregates. We are currently exploring this aspect in greater detail in our laboratory.

4. Conclusions

In this article, we have carried out atomistic MD simulation to study the early stages of the aggregation process of Aβ peptides in aqueous environment. Efforts have been made to obtain a microscopic understanding of the conformational modifications of the peptide monomers that occur during the nucleation process and the thermodynamic origin behind that.

The calculations revealed breaking and formation of secondary structural elements with a relative increase in helical content for most of the peptide monomers within the time scale of the simulation. Importantly, it is found that the peptide monomers tend to orient themselves in such a manner so that the helical segments of the adjacent monomers associate with each other during the aggregation process. Propensity to form helical structures by Aβ peptides is consistent with experimental observations at an early aggregation stage.\textsuperscript{33,34} It is further demonstrated that the structural adaptations of the Aβ monomers during nucleation is associated with their increased compactness.

The binding free energy of the nucleated Aβ pentamer as obtained from our calculations revealed that the early stages of the aggregation process involving Aβ peptides are thermodynamically favorable. Importantly, it is observed that the contribution originating from nonpolar van der Waals interactions between the peptides and the corresponding nonpolar solvation more
than compensates the weakening effect of unfavorable inter-peptide electrostatic interactions, thereby stabilizing the nucleated oligomer. It is further shown that the favorable binding between the \( \alpha \beta \) peptide monomers during the aggregation process proceeds via preferential association of the hydrophobic segments, \( hp1 \) and \( hp2 \).

We believe that the long side chains of the charged peptide residues need to orient appropriately during their approach toward each other so that the overall electrostatic repulsion between them is minimized to form thermodynamically stable \( \alpha \beta \) aggregates.

Acknowledgements

This study was supported by grants from the Department of Science and Technology (DST), Government of India, under the DST-FIST programme and the DST-IYC award. PK thanks University Grants Commission (UGC), Government of India for providing a scholarship. We thank Dr. Neelanjana Sengupta for many useful discussions.

References


2. Chiti F and Dobson C M 2006 Protein Misfolding, Functional Amyloid, and Human Disease Annu. Rev. Biochem. 75 333


4. Riek R, Güntert P, Döbeli H, Wipf B and Wüthrich K 2001 NMR Studies in Aqueous Solution Fail to Identify Significant Conformational Differences Between the Monomeric Forms of Two Alzheimer Peptides with Widely Different Plaque-Competence, \( \alpha \beta \) (1–40) ox and \( \alpha \beta \) (1–42) ox Eur. J. Biochem. 268 5930


15. Wasling P, Daborg J, Riebe I, Andersson M, Portelius E, Blennow K, Hanse E and Zetterberg H 2009 Synthetic Retrosynthesis and Amyloid-\( \beta \) in Alzheimer’s Disease J. Alzheimer’s Dis. 16 1

16. Rangachari B D, Reed D K, Sonoda L K, Bridges A W, Conboy E, Hartigan D and Rosenberry T L 2007 Amyloid-\( \beta \) (1–42) Rapidly Forms Protofibrils and Oligomers by Distinct Pathways in Low Concentrations of Sodium Dodecylsulfate Biochemistry 46 12451


34. Sugita Y and Okamoto Y 1999 Replica-Exchange Molecular Dynamics Method for Protein Folding Chem. Phys. Lett. 314 141
45. Frishman D and Argos P 1995 Knowledge-Based Protein Secondary Structure Assignment Proteins 23 566

56. Onufriev A, Bashford D and Case D A 2004 Exploring protein native states and large-scale conformational changes with a modified generalized Born model Proteins 55 383
