

Amyloid β -Peptide Oligomerization *in Silico*: Dimer and Trimer

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Soluble oligomers of Alzheimer's amyloid beta protein ($A\beta$) may act as effectors of neurotoxicity in early stages of Alzheimer's disease. Detailed information about the structure of $A\beta$ in atomistic level and the dynamics of assembly of monomeric $A\beta$ into oligomeric structures is rather elusive. We have performed replica exchange molecular dynamics (REMD) simulations on the formation of the dimer and trimer of $A\beta_{10-35}$ peptide. We have observed spontaneous formation of several basic structural units that may act as a template or an intermediate for further aggregation of Alzheimer's $A\beta$ protein. Various conformers, including interlocking structures of experimentally known bend double β strands, are identified.

According to the amyloid hypothesis, structurally well organized amyloid fibril is closely related to many neurodegenerative diseases.^{1,2} For therapeutic purposes, the detailed understanding of the structure and function of protein aggregates in such diseases is of primary interest. Despite the limitations of conventional NMR or X-ray in the amyloid fibril structural study,^{3,4} some structural information has recently begun to emerge thanks to various techniques⁵⁻¹⁰ including solid-state NMR.¹¹⁻¹⁵ The Alzheimer's amyloid beta ($A\beta$) peptide with chain length of 40 and 42, denoted as $A\beta_{1-40}$ and $A\beta_{1-42}$ respectively, is in the dominant form when it is released from amyloid β -protein precursor ($A\beta$ PP). The assembly of monomeric $A\beta$ into oligomeric structures is an important step in amyloid formation,^{16,17} and it was recently revealed that soluble oligomers of the $A\beta$ protein can be more neurotoxic than the fibrillar form.¹⁸⁻²⁰

Complimentary to the experimental studies, computer simulations can yield valuable information on structure, stability, and the possible fibril formation mechanism of the $A\beta$ protein. Several computational studies, ranging from the coarse grained model to the all atom model, provide insight into the characteristics of the amyloidogenic protein aggregate.²¹⁻³⁸ It is known that the short fragment cannot represent the full $A\beta$ protein structure because $A\beta$ has different conformations depending on the peptide length and sequence.⁴ Further, it was found that the $A\beta_{10-35}$ has an in-register parallel β strand structure.¹⁴ The solid-state NMR study^{15,39} showed that the full length $A\beta_{1-40}$ and $A\beta_{1-42}$ retain the same structural features as $A\beta_{10-35}$, indicating that $A\beta_{10-35}$ can represent the full length $A\beta$ structural features very well. It was also found that the $A\beta_{1-40}$ has two β strands connected with a turn region around residues 25–29.^{4,40} According to this study, the residues 1–11 are unstructured and 12–24 and 30–40 have the β strand structure. Independent computational study of $A\beta_{16-35}$ and $A\beta_{10-35}$ at high temperature by Ma and Nussinov⁴¹ supports the same feature. It is noted that the two β strand regions have hydrophobic residues (17–21 and 29–35) and the bend region has polar and charged

residues. Recently, Petkova et al.⁴² observed the formation of two morphologically different amyloid fibrils with distinctive growth, i.e., different association of $A\beta_{1-40}$. Some likely interaction patterns between monomeric $A\beta_{1-40}$ have been previously suggested.¹⁵

Considering that $A\beta_{10-35}$ can represent the main structural features of the full $A\beta$ and several aggregation patterns are possible for oligomerization of the $A\beta$ peptide,^{24,29,42} the computational study of $A\beta_{10-35}$ oligomers might capture important structural and mechanistic information on the $A\beta$ aggregation. In the present work, we attempted to address three important issues. First, is it possible to observe the ordering and assembly of the $A\beta_{10-35}$ aggregates by computer simulation with atomistic details? Second and most importantly, if so, what would be the possible structures and association patterns of these oligomers and their relative populations? Finally, how do the simulation results relate to the experimental studies and their implications? We studied the dimer and trimer formation since it is the first stage of oligomerization. It is noted that the full $A\beta_{1-42}$ dimer formation with several conformations was observed by Urbanc et al. from discrete molecular dynamics simulation with the coarse-grained model.²⁴

In an attempt to observe the spontaneous ordering of $A\beta_{10-35}$ oligomers, we have performed the replica exchange molecular dynamics (REMD) simulation^{34,43} for two systems. One is composed of two copies of the $A\beta_{10-35}$ peptide (dimer) and the other is composed of three copies of the $A\beta_{10-35}$ peptide (trimer). Initially, each $A\beta_{10-35}$ is randomly placed inside the simulation box. The initial conformation of each $A\beta_{10-35}$ strand is obtained from the NMR structure (PDB code 1HZ3) of the monomeric peptide, which adopts a collapsed coil structure in water. Each $A\beta_{10-35}$ strand was patched in a manner similar to the preparation of the same model peptide in the experiment.¹⁵ With regard to the simulation box, the whole system is confined within an imaginary sphere such that if the atoms are beyond the given boundary distance from the center of mass of the system, the attracting harmonic force centered at that boundary position will prohibit the molecules from flying apart from each

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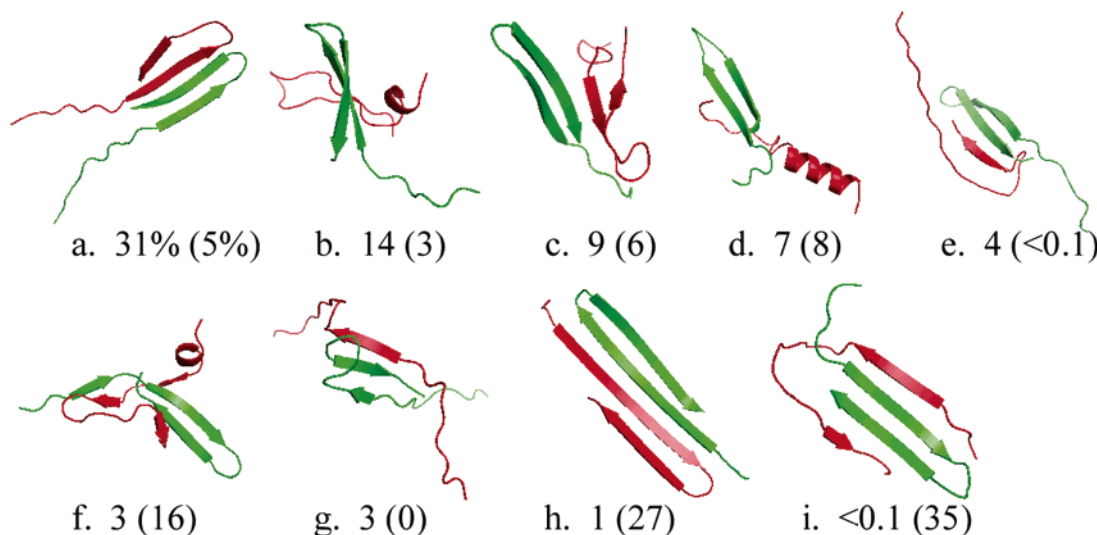


Figure 1. Various dimer conformers from REMD simulations of two copies of $A\beta_{10-35}$. The numbers represent their populations at 300 K, while the values in parentheses are populations at 280 K.

other, mimicking the crowded cellular environment.^{29,44} The radius from the center of mass was maintained as 45 Å for the dimer simulation and 50 Å for the trimer simulation. Initially each $A\beta_{10-35}$ is separated by the distance about 25 Å within the simulation box. Considering that the end-to-end distance of $A\beta_{10-35}$ is about 27 Å, this separation provides sufficient space for the overall tumbling of each $A\beta_{10-35}$ molecule. It was observed that the motion of each unit of $A\beta_{10-35}$ was not hindered inside the spherical simulation box during the initial stage of the simulation. It is noted that the simulation results are independent of the initial placement of $A\beta_{10-35}$.

The all atom force field of Amber96 with generalized Born surface area correction (GBSA) solvation model by Onufriev et al.⁴⁵ (Mode II) was used in this study. The time step was 2 fs with a bond constraint involving only hydrogen atoms using SHAKE. The nonbonded cut off distance was maintained at 15 Å, and the temperature was controlled by Berendsen thermostat with coupling constant of 0.1 ps. The REMD simulation was performed with 32 replicas, and the temperature range was 280.0 ~ 405.0 K. It is noted that 405 K is high enough to make the $A\beta$ oligomer into completely unstructured and dissociated conformation.³⁴ The REMD simulation time of each replica was 146 ns for dimer and 150 ns for trimer. The resulting average exchange acceptance was around 36% for dimer and 34% for trimer throughout the simulation. The replica exchange interval was set to 0.4 ps, and we have performed no replica exchange for the initial 100 ps in order to ensure no structural correlation between the replicas before exchange. For REMD simulation, we have used the molecular dynamics package AMBER8⁴⁶ with potential energy modification in order to account for the abovementioned soft spherical wall boundary condition. A total of 128 CPUs, which corresponds to four CPUs with each replica, were used for REMD simulation. The configuration was stored at every 200 fs for further analysis.

All the results presented in this paper are from the trajectory at two temperatures (280 and 300 K) for the last 4 ns of REMD simulation (20 000 conformations). It was found that essentially no monomeric states exist at 280 K (~0.1%), while monomers and dimers are in equilibrium at 300 K. The conformational distribution of dimer simulation shows 72% of the trajectory exists as a dimer and the remaining 28% exists as a monomer at 300 K. The root-mean-square displacement (RMSD) based clustering analysis⁴⁷ of the last 4 ns trajectory shows that there are nine structurally different dimer conformers. Figure 1 shows

TABLE 1: Average Potential Energies of Various Conformers for the Dimer and the Trimer as Shown in Figures 1 and 2^a

conformer (dimer)	total energy	solvation energy	conformer (trimer)	total energy	solvation energy
a	-1673 (44)	-1114 (71)	a	-2598 (108)	-1534 (93)
b	-1684 (8)	-1145 (41)	b	-2618 (36)	-1451 (119)
c	-1707 (6)	-793 (167)	c	-2632 (34)	-1075 (134)
d	-1679 (34)	-1022 (133)	d	-2649 (4)	-1553 (92)
e	-1684 (33)	-985 (150)	e	-2655 (4)	-1158 (46)
f	-1708 (34)	-775 (102)	f	-2680 (3)	-1175 (164)
g	-1626 (47)	-1170 (93)			
h	-1717 (35)	-732 (29)			
i	-1731 (4)	-813 (55)			

^a The energies are in units of kcal/mol and the values in parentheses represent uncertainty.

these conformers in the order of observed percentage at 300 K. We also indicated the relative population of each conformer at 280 K. It is noted that more “ordered” conformers are dominant at lower temperature.

Some of the assembly patterns of planar β strand dimers are consistent with schematic $A\beta_{1-42}$ dimer peptide models suggested by recent coarse grained discrete molecular dynamics simulations (Figure 3 of ref 24). We also observed some dimer conformations containing helix or random-coil conformations, which might be transient structures during the formation of ordered dimers. To compare the energetics of the conformers, we calculated average potential energies by locally minimizing 10 different conformations for each conformer cluster. It was found that the average potential energies for different conformers are very similar, even though conformers with highly ordered β strands have somewhat lower total potential energies (Table 1). On the other hand, the GBSA solvation energies for different conformer clusters show subtle differences. It was found that the GBSA solvation energy shows opposite behavior compared to the total potential energy, and conformers with high solvation energy (low total potential energy) have relatively low population at high temperature (300 K).

REMD simulations for the trimer formation at 300 K showed that the trimer and the [dimer + monomer] are in equilibrium with [dimer + monomer] as one of the major species. Six structurally distinct trimer conformers are observed from cluster analysis of the last 4 ns trajectory. Figure 2 shows the conformations of the major trimer conformers. We also calcu-

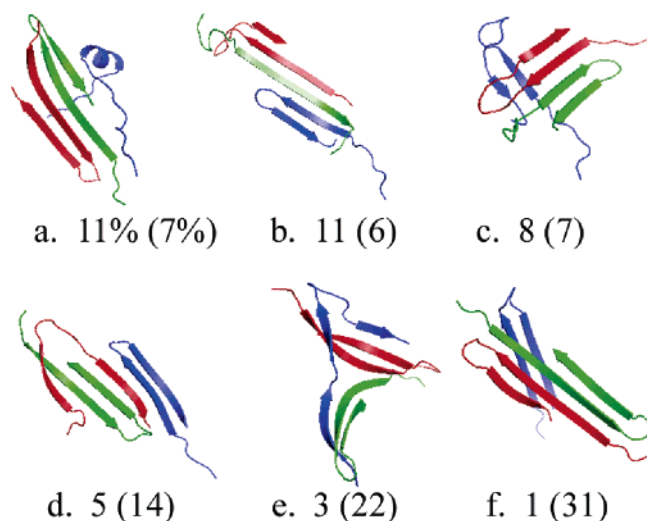


Figure 2. Various trimer conformers from REMD simulations of three copies of $A\beta_{10-35}$. The numbers represent their populations at 300 K, while the values in parentheses are populations at 280 K.

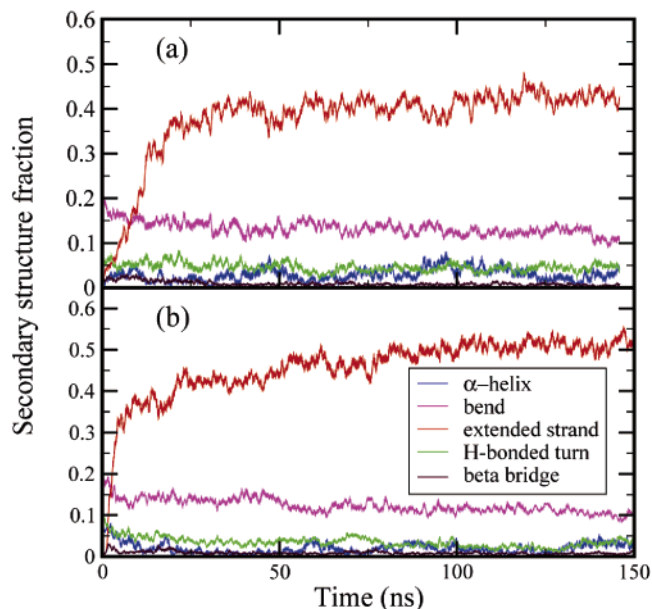


Figure 3. Time profile of secondary structure contents for (a) the dimer and (b) the trimer at 300 K.

lated the average total potential energies and the GBSA solvation energies for each conformer (Table 1). As in the case of dimer, conformers with high solvation energy (or low total potential energy) have rather low population at 300 K.

The results of REMD simulations illustrated the overall structural features associated with the formation of $A\beta$ oligomers. For both the dimer and trimer, each $A\beta_{10-35}$ unit forms two β strands joined by a turn region, as was observed in the experiment,^{4,40} and the assembly of such bend double β strands exhibits several different interlocking patterns. It is interesting to note that partial helical conformations are observed for both the dimer and trimer.^{25,31} Figure 3 shows the time profile of secondary structures for the dimer and trimer at 300 K. In both cases, significant amounts of extended β strand and bend structures are observed along with some α -helix content. It is noted that the presence of α -helical content persists throughout the simulations, even though its percent is rather small (under 5%). Experimentally, the possibility of α -helical conformer as a key intermediate step for fibril formation of $A\beta_{1-40}$ and $A\beta_{1-42}$ has been suggested.^{16,48-50} The computational study of antipar-

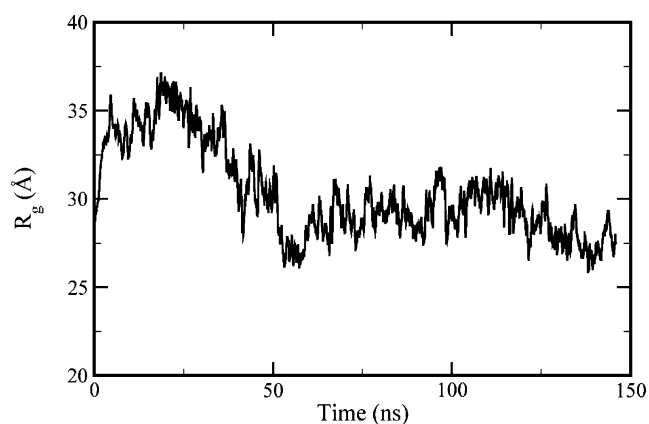


Figure 4. Time profile of the radius of gyration (R_g) for the dimer at 300 K.

allel β -sheet forming $A\beta_{16-22}$ peptide⁵¹ by Klimov and Thirumalai²⁵ indicated that the α -helical structure is an obligatory intermediate. On the contrary, the $A\beta_{16-22}$ simulations by Santini et al.^{31,32} using activation relaxation technique (ART) showed that α -helical intermediate is just one of the intermediates in multiple folding pathways. Our simulation results suggest that, at least for $A\beta_{10-35}$ dimer and trimer, the α -helical conformer might be one of the folding intermediates with several different structures.

Recently, Tarus et al.⁵² studied the dimerization of $A\beta_{10-35}$ by performing umbrella sampling and molecular dynamics simulations of putative stable dimers, generated by a protocol based on coarse grained shape complementarity followed by energy minimization. They showed that the hydrophobic interaction between each monomer acts as a stabilizing force and the dimerization process involves substantial structural reorganization of both the C and N terminus regions. Their conclusions are somewhat related to the recent simulations by Han et al.,⁵³ where it was shown that the strand-loop-strand structure could be a possible intermediate in fibril elongation. Figure 4 shows the radius of gyration (R_g) for the dimer as a function of time at 300 K. The initial increase of R_g followed by the subsequent decrease may represent a metastable dimer formation, possibly stabilized by hydrophobic interactions. It should be noted that the time evolution in REMD simulations may not represent dynamical events in real time, especially considering additional limitation due to the use of implicit solvation model. However, one can deduce the general characteristics of assembly processes from REMD trajectories. The details of the simulation results including atom-by-atom interaction patterns, the temperature dependence behavior, and energetics will be reported elsewhere.

In a physiological condition, not all conformations shown here might be possible. It should be noted that there is an order of difference in the monomer concentration between *in silico* and *in vivo* (or *in vitro*).²⁹ Also, there could exist some force field dependency.⁵⁴ In particular, the force field used in the present study is known to overestimate the β strand propensity.⁵⁵ DSSP analysis⁵⁶ for the trajectories at 300 K shows that the contents of β structures is about 40% for the dimer and 50% for the trimer (Figure 3). The previous experiments estimated the population of β -sheet structures to be about 13% (30% after ~ 30 days).⁵⁷ The relative propensities for different conformers and the time scale for the formation of the oligomers may be dependent on a particular force field used in the simulations. However, it can be argued that the oligomerization process observed in the present study illustrates a generic behavior irrespective of the force fields.

The formation of amyloid fibril is a very slow process (several weeks to months), and one cannot rule out a possibility that the simulation is not fully converged. The time evolutions of the secondary structure contents (Figure 3) and the radius of gyration (Figure 4) seem to indicate that simulation results begin to converge roughly after 60 ns for the dimer and 100 ns for the trimer. We also observed very slow structural changes for some of the trimers, even after 130 ns of REMD simulation. Therefore, the conformations from the current simulation should be understood as “quasi-stable” structures rather than in “final equilibrated form”, even though we have observed no noticeable conformational distribution change for the last 6 ns of simulation time. Some of the conformations observed in the present study may represent transient structures during oligomerization of A β proteins. Experimentally, very short-lived A β oligomer intermediates are hard to detect¹⁶ and it may proceed to further oligomerization or fibril growth before reaching the equilibrium state. In other words, fibril formation is a kinetically controlled process.²⁹

We also note that our simulation may not rigorously represent both A β _{1–40} and A β _{1–42} oligomeric states since there could be subtle structural differences between them due to extra 41–42 residues in the A β _{1–42}.^{16,24} However, despite these limitations, the current study demonstrates that the spontaneous formation of various basic structural units with a structurally relevant A β _{10–35} dimer and trimer can be examined at the atom level simulation. Such simulations are capable of suggesting, at least within the simulation time, possible conformations that may act further as a template or an intermediate for larger oligomer growth. Since monomers are in a steady state with larger oligomers and some oligomers can serve as important intermediates,¹⁶ it would be interesting to observe possible conformation(s) of the larger nucleus (tetramer, pentamer, or hexamer) by simulations, which will be the subject of future studies.

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