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Misfolding and Oligomerization of Amyloid β(1−40)

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Introduction

Protein misfolding and self-assembly have been identified as important factors in the development of several neurodegenerative diseases. The hallmark of many of these diseases is the deposition of amyloid fibrils and plaques within the extracellular matrix with an organized core structure consisting of β-sheets running perpendicular to the fibril axis. The histologic sign of Alzheimer's disease is the presence of senile plaques formed from Amyloidβ (Aβ) and neurofibrillary tangles formed from hyperphosphorylated tau protein. Despite a more thorough understanding of fibril structure, Aβ(1-40) belongs to an inherently disordered class of polypeptides making its structural determination in both monomeric and oligomeric forms difficult. Previous studies have been dependent on maintaining peptide solubility through the use of organic solvents or detergents which would potentially have significant effects on the overall solution structure of the peptide. Recently, a solution NMR structure of Aβ(1-40) in 50 mM NaCl solution has been determined (PDB id.: 2LFM) [1]. The molecular mechanism of dimerization, subsequent oligomerization and the conformation of the multimeric peptide chains are still not known. Therefore, herein we describe the structural properties of the dimer of Aβ(1-40) using 300 ns replica exchange molecular dynamics (REMD) simulation in TIP3P water as solvent using the CHARMM36 force field as implemented in the GROMACS version 4.6.4 package [2]. The dimer structure was created by randomly docking the NMR-derived solution structure with itself and relaxed with a 1.4 μs MD simulation. The REMD simulations were performed with 70 replicas of the initial structure. The exchange probability was 0.2. The exchange temperatures were obtained from a web-based temperature predictor [3] with the lower and upper limits of 290 K and 470 K, respectively.

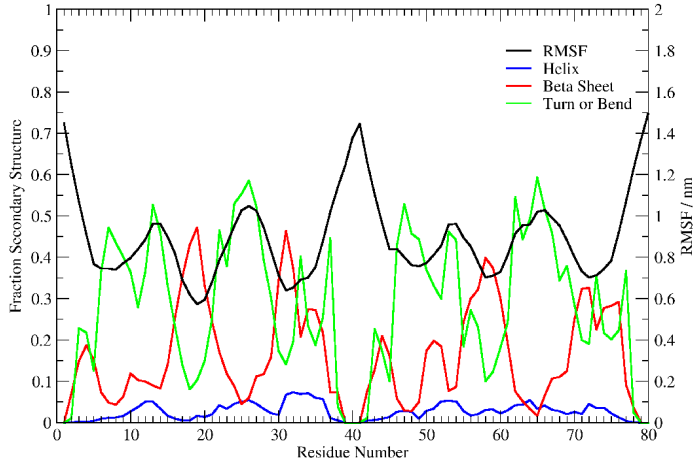

Fig. 1. Backbone RMSF (nm) for each monomer overlaid with its own average structure. The RMSF data compared to a plot of the fraction of sampled secondary structures as a function of residue number. Monomer A constitutes residues 1 through 40 and monomer B constitutes residues 41 through 80.

Results and Discussion

The backbone conformational entropy for the dimer system was calculated for all replica exchange temperatures. Two important aspects of the dimer system are evident from examination of the evolution of entropy during simulations. First, a relatively long, approximately 100000 ps, period of simulation time is required for the system to reach equilibrium. Second, the structures sampled at 310K, the physiological temperature, have a lower value of backbone entropy compared to any other replica temperature during the REMD simulations. This may indicate a slightly more stable system of sampled structures than what occurs at non-physiological temperatures. This temperature effect on the backbone entropy of the system is not unexpected and is confirmed by circular dichroism studies performed as a function of temperature [4].

The sampled secondary structure by the dimer was calculated by the DSSP method [5]. The fraction of sampled secondary structure as a function of residue number in the replica trajectory at 310 K is plotted in Figure 1. The most frequently sampled secondary structure is β-turn/β-bend followed by β-sheet. β-Turn/β-bend regions are prominent between residues 1 to 16 and 21 to 29 with a mixed β-sheet/β-turn/β-bend region between residues 32 to 38 of monomer A and residues 1 to 15 and 21 and 30 with a mixed β-sheet/β-turn/β-bend region between residues 30 and 38 of monomer B. β-sheet regions are prominent between residues 16 to 21 and 29 to 32 of monomer A and between residues 15 to 21 and 30 to 33 of monomer B. There is minimal sampling of helical structures in either monomer. The *C*-terminus of both monomers is largely unstructured with a more prominent turn structure present from residues 36 to 38 of both monomers. The root mean square fluctuation (RMSF) of the backbone atoms shows that the monomers have similar and significant flexibility. The most flexible regions have a larger proportion of β-turn/β-bend secondary structure associated with them.

An average residue-residue distance matrix analysis for the dimer system (Figure 2) shows that many of the intra- and inter-monomer contacts occur at distances of 1.0 nm or greater indicating that the dimer system is loosely packed. Intra-monomer contacts occur between residues 4 to 19 and 15 and 36 of both monomers. There are anti-parallel contacts that occur within the dimer system between residues 16 to 20 of monomer A with residues 17 to 20 and 31 to 36 of monomer B as well as residues 31 to 36 of monomer A with residues 17 to 20 and 30 to 36 of monomer B. The contact map is therefore consistent with a loosely packed antiparallel system that appears to be driven by collapse around the central hydrophobic core of residues 17 to 21 with the *C*-terminal hydrophobic core of residues 30 to 40.

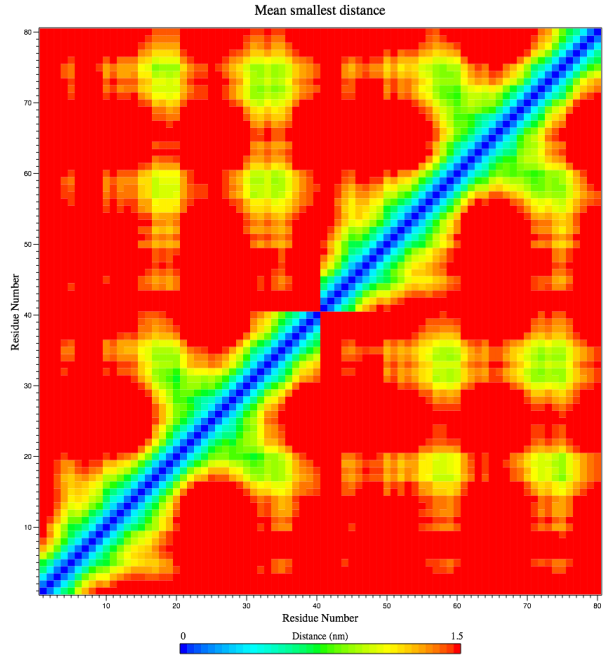

Fig. 2. Distance matrix of the residues at 310 K showing that the system samples an antiparallel global configuration.

The sampled configurations of the dimer and both monomers were de-convoluted using dihedral principle component analysis (dPCA) and the lowest energy configuration identified (Figure 3). Examination of the energy surface shows that the minima are relatively shallow with only 1.86 kJ/mol separating the lowest energy from the highest energy configurations. The majority of the sampled configurations are located within a broad based and shallow region with a total of six identified low energy conformations. The lowest energy configuration (Figure 3) consists of a

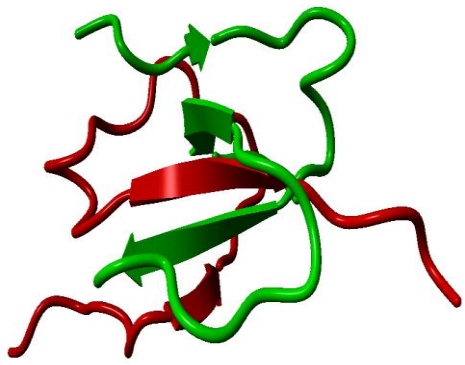

Fig. 3. Lowest energy configuration of Aβ(1-40) dimer. Monomer A is shown in red while Monomer B is shown in Green.

five strand anti-parallel β-sheet from 9 to 11 and 30 to 34 in monomer A and residue 5 and residues 17 to 20, 33 to 36 of monomer B. The remaining five structures from the broad based shallow region show the presence of β-sheet and anti-parallel β-sheet conformations however, the length and location tend to be variable and the majority of the monomers secondary structure is random coil with an overall loose packing of the dimer. This finding explains why the RMSF for the dimer system and individual monomers is high and the contact map demonstrates interaction distances around between 0.7 and 1.0 nm.

Dimers of Aβ(1-40) have a different conformation compared to that of the monomers [1]. The presence of stable β-sheet conformations indicates a misfolded state for the peptide. Furthermore, such conformations are characteristic of the dimers. This finding is in agreement with the dynamic force spectroscopy study (DFS) which predicted the presence of stable dimers in solution with a life-time of 0.1 s at pH 7.0 [6]. The DFS analysis of Aβ(1-40) dimers also showed that various pathways of dimer dissociation occur indicating that the dimers are built with different conformations. The present REMD simulations not only showed relatively high conformation heterogeneity for the dimer but also indicated that the dimers are stable and only a rare and short-time dissociation event occurred at some of the replica trajectories.

Although Aβ(1-40) belongs to the class of natively disordered proteins, neither its monomeric or dimeric forms are entirely in a random coil conformation. Previous MD simulations of structures of monomeric Aβ(1-40) and Aβ(1-42) have demonstrated that a wide variety of conformations occurred during simulations [7]. These simulations demonstrated that the peptides sample a large population of turn type structures and that the driving force of secondary structure formation and fluctuation appears to be the collapse around the central and *C*-terminal hydrophobic cores with resulting "zipping" and "unzipping" of the hydrogen bonds between highly flexible areas with a propensity for β-sheet formation. It is clear that a similar mechanism is occurring in dimeric Aβ(1-40). The two peptides tend to collapse on each other in their most energetically favorable conformation with the driving force of collapse and secondary structure formation being antiparallel contact between the two hydrophobic regions. The fact that these interactions do not result in a stable antiparallel β-sheet dimer but rather a large ensemble of sampled conformations may be one reason that the dimer remains soluble in water.

Acknowledgments

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