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Molecular dynamics simulations of Aβ fibril interactions with β-sheet breaker peptides

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ABSTRACT

Accumulation and aggregation of the 42-residue amyloid-β (Aβ) protein fragment, which originates from the cleavage of amyloid precursor protein by β and γ secretase, correlates with the pathology of Alzheimer's disease (AD). Possible therapies for AD include peptides based on the Aβ sequence, and recently identified small molecular weight compounds designed to mimic these, that interfere with the aggregation of Aβ and prevent its toxic effects on neuronal cells in culture. Here, we use molecular dynamics simulations to compare the mode of interaction of an active (LPFFD) and inactive (LHFFD) β-sheet breaker peptide with an Aβ fibril structure from solid-state NMR studies. We found that LHFFD had a weaker interaction with the fibril than the active peptide, LPFFD, from geometric and energetic considerations, as estimated by the MM/PBSA approach. Cluster analysis and computational alanine scanning identified important ligand–fibril contacts, including a possible difference in the effect of histidine on ligand–fibril π-stacking interactions, and the role of the proline residue in establishing contacts that compete with those essential for maintenance of the inter-monomer β-sheet structure of the fibril. Our results show that molecular dynamics simulations can be a useful way to classify the stability of docking sites. These mechanistic insights into the ability of LPFFD to reverse aggregation of toxic Aβ will guide the redesign of lead compounds, and aid in developing realistic therapies for AD and other diseases of protein aggregation.

1. Introduction

The molecular origins of certain neurodegenerative disorders, including Alzheimer's, Huntington's [47] and Parkinson's diseases, have been associated with the aggregation of proteins [36]. In particular, the development of Alzheimer's disease (AD) is accompanied by accumulation of "plaques" in the brain formed primarily by aggregates of a small 42 amino acid fragment, Aβ. Self-aggregation of peptides similar to Aβ also plays a role in renal failure in diabetes [19–21] and aggregation of other proteins is present in many other diseases [34,35]. Thus novel drugs that can reverse protein aggregation are the subject of many recent investigations. The small size and biological significance of Aβ make it an ideal test system for studying both aggregation and methods to inhibit or reverse the process. Compounds that inhibit aggregation of Aβ have been identified, often in a serendipitous fashion, in several groups [2,13,15,16,25,29,42]. However, these compounds are often large molecules with substantial toxicity, and as their mechanism of action is not known, further development to produce useful drugs is difficult.

Structural studies have shown that the Aβ peptide, as a monomer in detergent solution, is helical. However, upon aggregation, the monomers change conformation [5] to form β-strand sheets, stabilized within monomers by antiparallel interactions and between monomers by parallel ones [3,8,26,31,32]. A fibril structure that forms in conditions similar to those used for the inhibitor assay has been determined from extensive solid-state NMR experiments in the Tycko group [30,44,45]. This fibril is stabilized by both

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid-β; MD, molecular dynamics; RMSD, root mean square deviation; REMD, replica exchange molecular dynamics.

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hydrogen bonds associated with the backbone structure, and salt bridges formed between the key charged residues Asp23 and Lys28. While other, asymmetric, fibril forms can be produced under different conditions [51], the symmetric structure used here was chosen as it forms when Aβ is induced to aggregate under similar incubation conditions to those used for the assays of the peptide inhibitors. In our search for new inhibitors, we began with "β-blocker peptides", inhibitory peptides, originally based on the sequence of Aβ, that were empirically optimized by changing discrete positions and even whole side chains [29]. We docked a series of β-blocker peptides with known ability to affect Aβ aggregation to the NMR fibril structure. The results were used to design molecular pharmacophores and identify compounds that were shown experimentally to be good inhibitors of aggregation and prevent the neurotoxic effects of Aβ on cultured neuroblastoma cells [10]. These studies also showed the limitations of using Autodock scores, on an absolute level, to distinguish good inhibitors from inactive ones, when applied to discriminate active peptides from very similar, but inactive ones [10]. The docking methodology postulates that binding stability should equate with activity, and that certain positions on the fibril would be more advantageous for inhibitor binding than others.

In this work, we explored other computational methods that could give complementary information on the stability of the positions predicted by the docking studies. To provide insight into the potential inhibitory mechanism of active compounds, we used molecular dynamics (MD) simulations to probe the stability of the top-ranked poses predicted from docking. Simulations (20 ns in length) were performed of an $A\beta_{9-40}$ 12-mer protofibril derived from solid-state NMR, complexed with a β-sheet breaker peptide, LPFFD and with an inactive control peptide, LHFFD, at three discrete lowest energy docking positions. We use $A\beta_{9-40}$ as a model for $A\beta_{1-40}$ as the first eight N-terminal residues are structurally disordered and not required for fibril growth [7,28,31]. We found that MD simulations could indeed distinguish a useful peptide inhibitor from an inactive one that differed at only one residue position. The inactive LHFFD bound less strongly to the fibril than the active peptide, LPFFD, and formed different interactions. LPFFD interacted significantly with the fibril throughout the simulation, while LHFFD, depending on initial location, detached from the fibril, to rebind at a later time point. Cluster analysis suggested that the difference in bound poses between the active and inactive peptides, especially a key groove-bound orientation of LPFFD, could affect the ability of these compounds to disrupt intermolecular hydrogen bonding between Aβ monomers, which may account for their different activities. These results illustrate the promise of MD simulations in compound selection and design procedures, providing an alternate way to estimate the stability of interactions with the target.

2. Computational methods

2.1. Molecular dynamics simulation of peptide–fibril complexes

A 3D structural model of an $A\beta_{9-40}$ fibril was constructed based on the solid-state NMR structure of Tycko and co-workers [31,32] (Fig. 1). Initial poses of the peptide inhibitor of Aβ aggregation, LPFFD, and its inactive analogue, LHFFD, were obtained from previous computational docking studies using Autodock [27]. The docked structures were then examined by 20 ns molecular dynamics simulations in explicit water. All simulations were performed using *sander* from the Amber 9 modeling suite [9], and the Amber *ff03* forcefield [14]. For each ligand, the fibril complex structure obtained from docking [10] was solvated in a TIP3P waterbox [22] and sodium ions were added to neutralize the total charge of the simulation box.

MD simulations used the particle mesh Ewald [12] method, a time step of 2 fs and the SHAKE algorithm [33], used to constrain bonds involving hydrogen. The simulation temperatures were controlled using the Berendsen thermostat [4] and a heat bath coupling constant of 2 ps. The systems were heated to 310 K and their densities were allowed to equilibrate in an NPT ensemble, with harmonic positional restraints on the fibril atoms, for 200 ps. A weak coupling algorithm was used to maintain a pressure of 1 bar, with a pressure relaxation time of 1 ps. The restraints were removed and a further 200 ps period of equilibration performed. The equilibrated system was subjected to a 20 ns production run, sampling an NVT ensemble.

2.2. Calculation of peptide–fibril binding free energies

The binding energies of the ligands with the fibril, ΔG_{bind}, were calculated the by MM/PBSA method (Eqs. (1) and (2)) [38]:

$$G = \bar{E}_{MM} + \bar{G}_{PBSA} - TS \quad (1)$$

$$\Delta G_{bind} = G_{complex} - (G_{fibril} + G_{ligand}) \quad (2)$$

Here, the free energy of each species is approximated by the average of its molecular mechanics force field energy, $\bar{E}_{MM}$, Poisson–Boltzmann solvation energy, $\bar{G}_{PBSA}$, and the energy contribution of configurational entropy, *TS* (Eq. (1)). Binding free energies can then be estimated by the free energy difference between the bound and unbound states (Eq. (2)). A single trajectory approach was employed, such that the snapshot coordinates for both the bound and unbound states were obtained from a single molecular dynamics simulation. The molecular mechanics contributions were calculated using the Amber *ff03* forcefield [14]. The Poisson–Boltzmann solvation contributions were calculated using the optimized atomic radii generated by Tan et al. [41] and a probe radius of 1.6 Å. The repulsive non-polar contribution to the solvation energy was calculated by a solvent accessible surface area dependent term of coefficient 0.04356 kcal/mol Å^2 and offset −1.008 kcal/mol; while the attractive nonpolar contribution was calculated according to the 6–12 decomposition scheme outlined by Tan et al. [40]. Although the MM/PBSA calculations omitted entropic contributions, the approach is known to usually given reasonable estimates for comparison of similar ligands. Computational alanine scanning was performed on the ligand position identified as being the most strongly bound. In this approach, each fibril residue identified as interacting with LPFFD was mutated independently to an alanine residue. MM/PBSA analysis was then repeated for each mutated complex.

The RMSD of each ligand, relative to the fibril, during each molecular dynamics trajectory, was obtained by fitting 400 equally spaced snapshots from each trajectory by minimizing the RMSD of the fibril atoms, relative to the first snapshot. The fitting was performed using the *ptraj* module present in Amber 9 [9]. Cluster analysis of the ligand poses were performed using *kclust* from the MMTSB Tool Set [18]. The snapshots closest to the centre of each cluster were visualized, and the interactions between the ligands and fibril identified.

3. Results

Molecular dynamics simulations were performed on our previously generated [10] structures of complexes of amyloid fibrils with active and inactive pentapeptides, LPFFD and LHFFD, respectively. These complexes were obtained by using the three lowest energy Autodock positions of the two peptides bound to a $A\beta_{9-40}$ fibril model, based on the solid-state NMR structure of Tycko et al. [31,32], in which 12 $A\beta_{9-40}$ chains each adopt a horseshoe-like