Stability and Structure of Amyloid-Forming Peptides from Computer Simulation

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Proteins and peptides can fold into their unique 3-dimensional (3D) structures to perform their biological functions, or they can misfold to form insoluble amyloid fibrils, which are highly ordered protein aggregates currently known to be associated with more than 20 neurodegenerative diseases, including Alzheimer's, Parkinson's, Huntington's, diabetes type II, and various prion diseases (1-4). Regardless of the differences in size, function, sequence, and native structures of these amyloid-forming proteins/peptides, all amyloid fibrils adopt a common cross-β-sheet structure, in which the β-sheets are parallel to the fibril axis and the β-strands within a sheet are perpendicular to the fibril axis. Moreover, recent experiments found that even those disease-unrelated proteins/peptides also have an intrinsic tendency to form highly ordered amyloid fibrils under appropriate conditions (5). These findings imply that a general principle may govern amyloid fibril formation (6). Most importantly, although fibril formation is linked to neurotoxicity, accumulating evidences point to soluble oligomeric intermediates, rather than insoluble fibrils, as the primary toxic species (7-9), possibly due to their membrane-disruption abilities, while mature fibrils may represent an inert monomer reservoir. Amyloid oligomers are directly linked to infectivity as well arising from the instability of fibrils (10, 11) -- the higher tendency of the fibrils to break into oligomers, the more infection they are. Additionally, these prefibrillar intermediates display various discrete morphologies (micelle-like, annular-like, and linear-like structures) as observed by atomic force microscopy (AFM) and electron microscopy (EM) when exposed to different environmental conditions (12-14), suggesting that fibrillization/oligomerization may proceed through multiple assembly pathways. Despite substantial efforts and progresses have been made, the mechanism of amyloid formation and the origin of its toxicity are still not fully understood, primarily because little was known until recently about the molecular-level structures of fibrils, apart from the existence of the cross-β motif (15).

Obtaining atomic-level structures of various amyloid species is the first and important step towards understanding the principles of amyloid formation. Once the amyloid structures are available, the rational design of therapeutic agents and strategies becomes feasible to fight against neurodegenerative diseases. Many different experimental techniques are used to probe structural information and biological function of amyloidogenesis. Solid-state NMR and x-ray diffraction approaches are good at resolving atomic-level structural information (16-18), but the nature of protein aggregation (noncrystallization and insolubility of fibrils, small size and short-lived of oligomers, involvement of cell membrane) renders these experimental studies extremely challenging (19, 20). AFM and EM techniques can provide morphological data by capturing nanometer images over time (21-23), but detailed structural and kinetic information are not reliable. Surface plasmon resonance (SPR) is able to explore the kinetics of protein

aggregation by measuring the mass amount of aggregates, but it has difficulty to provide structural information.

The difficulties and limitations of these experimental methods have inspired intensive computational studies as complement to experiments. Most computer simulations of amyloid-forming peptides fall into two categories at the atomic and coarse-grained levels with explicit and implicit solvent models. All-atom molecular dynamics (MD) simulations have been applied to study amyloid oligomer stability alone by testing different candidate β-sheet arrangements of preformed oligomers mimicking possible nucleus seeds at the very early stage of fibril formation (24-26). This approach can determine the most stable conformation for minimal nucleus seeds at the lowest free energy state, but can not provide the aggregation scenario of amyloid intermediates/fibril growth since aggregation is an extremely slow process on the timescale of minutes to days, which is typically beyond the timescale of nanoseconds for conventional MD simulations. To overcome computational limitations, alternative computer simulations using low-resolution models (e.g. coarse-grained protein models and implicit solvent models) have been used to directly study the formation of oligomers (small species) and even fibrils (large species) (19, 27). These simulations can qualitatively provide information on the kinetic pathways of protein aggregation, but can not adequately capture different detailed interactions, such as hydrophobic interactions, electrostatic interactions, and hydrogen bonding. Once the amyloid structure and its pathway are determined, various peptide inhibitors can be designed on the basis of complementarity of shape, hydrophobicity, charge, and hydrogen bonding against original oligomers. Then, molecular docking and binding simulations (28) can help to locate possible binding sites and evaluate binding strength for preventing amyloid fibril formation. Each experimental or theoretical method has its strengths and weakness, but a combination of experimental, theoretical, and computational methods can illustrate various aspects of fibril structure, formation, and toxicity at the molecular-level and thus provide an improved understanding of the complicated picture of protein aggregation.

In this work, a systemic analysis of preformed oligomeric structures is performed to examine their sequence and structural characteristics (10, 11, 29-35) using conventional all-atom molecular dynamics and replica exchange molecular dynamics with CHARMM force field. We identify several stable oligomeric structures with different structural morphology, size, and shape, delineate several common features in amyloid organizations and amyloid structures, and illustrate aggregation driving forces that stabilize these oligomeric structures using computational simulations. The structural comparison among different oligomers suggests that the aggregation mechanism leading to distinct morphologies and the aggregation pathways is sequence specific due to differences in side-chain packing arrangements, intermolecular driving forces, sequence composition, and residue positions. Furthermore, we systemically analyze two amyloid peptides which have available crystal structures and other amyloid sequences with proposed structures using computational simulations, we delineate three common features in amyloid organizations and amyloid structures: (i) Sheet-sheet recognition via a steric zipper characterized by complementarity: of shape, hydrophobicity, charge, and of hydrogen bonding; (ii) β-strand–loop-β-strand; (iii) twisted cross β-sheet. The common structural

characteristics of fibril arrangement permit explanation of the observation that most peptide chains appear able to form amyloid fibrils under appropriate conditions, regardless of their sequences (33). A catalogue of these structural motifs is expected to be valuable targets in drug design for prevention and treatment of amyloid-related diseases.

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