Computational study of aggregation mechanism in lysozyme [D67H]

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Protein unfolding or misfolding changes the confirmation of protein and leads to protein aggregation. Amyloid disorders involve atypical deposits of the amyloid protein in body tissue and are characterized by abnormally unfolded forms of the proteins which lead to aggregation either intra or extracellularly. Single point mutations aspartate to histidine (D67H) and isoleucine to threonine (I56T) were found in human lysozyme which is associated with hereditary systemic amyloidosis. Two single-point mutation D67H and I56T have been studied and compared to wild type (WT) structure for structural instability due to mutation which leads to conformational change and formation of amyloid fibrils. Various experiments have been conducted to understand the process behind the fibrils formation; including thermal denaturation, pH change, and hydrostatic pressure. From these studies, it was found that conformational rearrangements or partial unfolding is a key factor for aggregation. Several approaches have been tried to reduce fibril formations, for example, small molecule inhibitors, polyamines, surfactants etc. Still there appears to be no effective treatment for amyloid disorders such as Alzheimer disease, Parkinson disease etc. Therefore, understanding the mechanisms underpinning conformational change of soluble lysozyme to insoluble fibrils is important to prevent aggregation.

D67H fibrils have tendency to refold to the native structure *ex-vivo* under some environmental conditions which indicates D67H variant fold orderly in the cell before formation of fibrils in tissues. So it is essential to study partially folded states or initial change of conformation of D67H variants which leads to aggregation called “seed”. In the present study, we have done MD simulations of D67H variant and wild type (WT) at high temperature(400K) to study partially unfolded intermediate states and opened structures were confirmed with RSMD of backbone atoms, residue wise RMSD of C-α atoms and number of water molecules in hydrophobic regions. Then docking algorithm was applied between the D67H and WT structures simulated at room and high temperature to study putative binding modes and “seed” complexes and we clustered the binding poses based on interactions and docking score. Then MD simulations were done on complexes for stability and to analyse binding interactions. Potential mean force (PMF) was calculated on stable complexes using steered molecular dynamics simulations (SMD) to find their binding strength through binding free energy.

It was found that two loops in β-domain are separating away from each other and hydrophobic region between the loops is exposed to solvent in D67H structure compared to WT. This hydrophobic region is found responsible to interact with another monomer in docking and MD simulations of the docked complexes simulated at high temperature in D67H. There was no stable binding mode found between WT structures at room temperature as well as high temperatures. The pi-pi interactions were found in complex D67H(400K)-D67H(400K) between His67, Tyr54 and Tyr45 which showed higher binding free energy in PMF compare to complex D67H(400K)-D67H(RT). This study opens up the path for developing therapeutic agents which maintain the interactions between two loops. This method can be used to study other protein aggregation problems such as antibody aggregation which is major problem in vaccine development. With this method aggregation prone regions can be identified and mutation of interaction residues can prevent aggregation.