Abstract

In the elderly population around the world, neurodegenerative disorders are a leading cause of disability and mental decline. Alzheimer's Disease (AD) and Parkinson's Disease (PD) are the most frequent neurodegenerative disorders. AD is the most prevalent form of dementia in the world, accounting for 60¯80 percent of all dementia cases and impacting an estimated 24 million individuals worldwide. Utilizing nationally representative data gathered in India between 2017 and 2020, a nationwide research study by J.Lee et. al., 2023 discovered that 7.4% of those 60 years and older were thought to have dementia (8.8 million individuals). According to the Alzheimer's Association, AD primarily affects individuals aged 65 years and older.

AD has no preventive treatment, according to the National Institute on Ageing. There are evidences that early disease detection improves treatment possibilities. The most commonly supported hypothesis for the cause of AD relates to Amyloid-Beta (Aβ) peptide, an intrinsically unstructured protein which is formed after the sequential cleavage of the Amyloid Precursor Protein (APP), a type 1 integral cell surface membrane protein which resembles a signal transduction receptor. Aβ peptide occurs in two isoforms: $A\beta_{1-40}$ and $A\beta_{1-42}$. The $A\beta_{1-42}$ peptide is discovered to be the most neurotoxic of the two alloforms. The $A\beta_{1-42}$ peptide is more hydrophobic and more likely to fibrillate because it has two more amino acids in the C-terminal region. Hence, more research emphasis is given on $A\beta_{1-42}$ peptide. Research suggests that $A\beta_{1-42}$ peptide activates kinase enzymes, protects against oxidative stress, and modulates cholesterol transport. There are presently no effective treatments to completely cure AD. So, there is always a constant search for inhibition strategies to prevent AD.

PD is the second most prevalent neurodegenerative disorder after AD. According to the Parkinson's Foundation, nearly 1 million people are affected by PD. PD is projected to have a prevalence of 0.3% in the general population, 1.0% in those older than 60 years of age, and 3.0% in those aged 80 years or older. The ultimate reason for the onset of PD is yet unknown. In the affected region of the brain, misfolded proteins are associated with PD. These proteinaceous deposits are caused by amyloid fibrils containing 140 amino acid residues of the presynaptic α-Synuclein (αS) protein. It is the primary factor related with the start of PD and a major contributor to the formation of aberrant neuronal protein aggregates known as Lewy Bodies (LBs). It has been observed that αS is an intrinsically disordered protein because it lacks a distinctive secondary

structure conformation. Since the precise mechanism by which αS fibrils are formed is unclear, numerous studies are focusing on the mechanism of αS aggregation. There is currently no disease-modifying treatment for the most difficult disabling condition, PD.

Though the AD and PD has been identified since many decades, it is still incurable due to its complex pathogenesis. Thus, inhibiting the aggregation of the causative proteins of these two diseases to prevent formation of oligomers and fibrils has been considered as a potential goal in their therapies. Medicinal herbs, nutraceuticals, pharmacological techniques, nano-pharmaceuticals, and gene therapy are used in clinical trials for AD and PD. Therefore, it can be said that research is evolving in this field to find out better inhibition strategies or approaches to prevent or cure AD and PD. Hence, in this thesis, different inhibition approaches are investigated with the help of computational approaches to find out strategies to prevent the aggregation of the causative proteins of AD and PD: $A\beta_{1-42}$ peptide and αS protein respectively.

Within the first part of the thesis, three different inhibition approaches were discussed that affects $A\beta_{1-42}$ peptide aggregation. These inhibition approaches were effect of ionic strength, effect of dimerization of peptides and role of a small molecule, Resveratrol (a polyphenol). All these inhibition approaches have highlighted their effect on the aggregation of the $A\beta_{1-42}$ peptide.

For prevention or treatment of AD, ionic strength-dependent studies of amyloid formation have suggested that ions can influence the kinetics and thermodynamics of the aggregation process. However, the details of the effect of ionic strength on the aggregation properties of $A\beta_{1-42}$ peptide have not been studied in the molecular level. Hence, the first inhibitory study in this thesis examined how ionic strength affects $A\beta_{1-42}$ peptide aggregation propensity computationally. This study discussed the stability of the $A\beta_{1-42}$ peptide in different concentrations (0 M, 0.15 M and 0.30 M) of an ionic medium, Sodium Chloride (NaCl). Molecular Dynamics (MD) trajectory analysis demonstrates solution ionic strength inhibits $A\beta_{1-42}$ peptide monomer aggregation. Root Mean Square Deviation (RMSD) and Solvent Accessible Surface Area (SASA) showed that the $A\beta_{1-42}$ peptide monomer rapidly changes shape as solution ionic strength increases. Radius of Gyration (R_g) shows that moderate ionic strength solutions condense the $A\beta_{1-42}$ peptide monomer. The $A\beta_{1-42}$ peptide monomer retains its helical secondary structure in moderate or higher ionic strength solutions. Moderately ionic conditions increase $A\beta_{1-42}$ peptide

monomer diffusion coefficient. In conclusion, this computational analysis indicates that solution ionic strength greatly influences $A\beta_{1-42}$ peptide monomer aggregation, suggesting that limiting this aggregation may prevent AD.

Replacement of serum albumin in plasma has been proposed as a favorable therapy for the cure of AD. It has also been reported that albumin binds with Aβ-peptide impeding its aggregation. Therefore, in this second investigation of $A\beta_{1-42}$ peptide aggregation inhibition, C-terminal (CTerm) region of the Human Albumin (HA) have been used to form Heterodimer with $A\beta_{1-42}$ peptide. Potential of Mean force (PMF) and Binding free energy (BFE) analysis have been studied to understand the association of monomeric units in $A\beta_{1-42}$ peptide-$A\beta_{1-42}$ peptide Homodimer and $A\beta_{1-42}$ peptide-CTerm Heterodimer complexes. It has been found that the $A\beta_{1-42}$ peptide-$A\beta_{1-42}$ peptide homodimer complex has higher dissociation energy than the heterodimer complex. These findings show that CTerm of HA can dimerize and disassemble $A\beta_{1-42}$ peptide.

Recent studies have shown the potential of flavonoids and polyphenolic compounds to play an important role in the cure of AD. Hence, the third inhibitory research examined how Resveratrol (RSV), a polyphenol, attaches to the $A\beta_{1-42}$ peptide monomer and how it affects the aggregation characteristics of $A\beta_{1-42}$ peptide. Secondary structural and conformational investigations demonstrate that RSV attachment to $A\beta_{1-42}$ peptide increases its helical content. RSV binds $A\beta_{1-42}$ peptide well according to BFE. The MM-PBSA/GBSA algorithm showed that the receptor-ligand binding affinity is high, and the $A\beta_{1-42}$ peptide monomer residues Val 37, Gly 28, Leu 35, Ile 32, Val 25, Lys 29, Phe 20, Met 36, Val 40, Ala 31 are responsible for their intermolecular interaction. This work also shows how RSV interacts with residues Asp 23 and Lys 28 to reduce harmful amyloid oligomers and fibrils.

In the second part of the thesis, three different inhibition approaches that affects αS aggregation were discussed. These inhibition approaches are namely macromolecular crowding or influence of crowding agent, effect of dimerization of peptides and role of a small molecule, Oleuropein aglycone (a polyphenol). All these inhibition approaches have highlighted their role in preventing the aggregation of αS protein.

Literature studies have shown that macromolecular crowding is one of the essential cellular environment elements that can influence the aggregation mechanism of

αS. Therefore, the effect of crowding agents have been studied on the αS protein. The structural and conformational changes of αS were studied in the presence and absence of crowding agents. RMSD data reveal that the αS protein is more stable in a crowded environment (10 PEG molecules) than in a less/non crowded environment (5 PEG molecules and control). Secondary structure study suggests that crowding agent PEG has reduced the number of alpha-helices in the N-terminal and NAC domain of the αS protein. This study helps to understand how crowding agent PEG affects αS protein structure and conformation.

Designing of peptides as inhibitor has gained new momentum as inhibition strategy for PD. Hence, an inhibition approach of two peptides K84s and K102s affecting the aggregation of αS were studied. It was found that the binding free energies between the αS protein and the two peptides were found to be indeed high. The (α-Synuclein-K84s) and (α-Synuclein-K102s) complexes have binding free energies of -33.61 kcal/mol and -40.88 kcal/mol respectively. Using PDBsum server and PRED analysis, the residues of αS and the residues of the peptides K84s and K102s which were involved in the protein-protein interaction were identified. It has also been noticed that the αS has retained the helical content to a significant extent when it is in complex form with the K84s and K102s peptides**.**

In the third inhibition study for preventing the αS aggregation, the role of OleA was studied. OleA was found to interact with the N-terminal domain of αS, making this region unavailable for interaction with membranes and lipids for the formation of cellular toxic aggregates. From the binding free energy (BFE) analysis, it is found that the binding affinity between αS and OleA to be indeed high (ΔG_{bind} = -12.56 kcal mol^{-1} from MM-PBSA and ΔG_{bind} = -27.41 kcal mol^{-1} from MM-GBSA).The Per-residue energy decomposition (PRED) analysis identified the residues involved in the interaction between OleA and αS protein.