ABSTRACT

Neurodegenerative (ND) diseases are a group of progressive neurological disorders in which neurons in the brain and peripheral nervous system become dysfunctional with time and ultimately die, causing severe health issues. ND diseases affect millions of people and are the leading cause of weakness in humans; Amyotrophic Lateral Sclerosis (ALS) is one such ND disease. Aggregation of Superoxide dismutase 1 (SOD1) is associated with the pathogenesis of ALS. However, the aggregation mechanism of SOD1 is not fully understood, which is one reason we are still unable to find a cure for ALS. Targeting the steps forming toxic amyloid fibrils with small molecules has emerged as a successful strategy to treat ND diseases. This thesis entitled **"Insights into the mechanism of aggregation of Superoxide Dismutase 1 (SOD1) in the presence and absence of small molecules"** is aimed to understand the different steps involved in the formation of SOD1 aggregates and to find the small molecule inhibitor to prevent, delay or reverse this aggregation process.

The thesis has been comprised of eight chapters. In **Chapter 1** (Introduction), the phenomenon of protein folding, misfolding, protein aggregation, factors responsible for protein aggregation, and diseases related to protein aggregation was discussed in detail. Various kinetics theories were examined to understand the mechanism of protein aggregation, and strategies were mentioned for finding the inhibitors for its collection. A brief introduction of the neurodegenerative disease of interest, ALS, and the associated protein SOD1 is also provided. Further, the literature studies on the current state of understanding of SOD1 aggregation mechanism, aggregation inhibition strategies, and the potential of computer simulations in studying these complex processes were elaborated. At the end of this chapter, the origin of the scientific problem, objectives designed for this thesis, and outline of the research work performed were discussed.

Chapter 2 (Materials and methodologies) lists the details of chemicals procured for protein aggregation-related experiments. The principles of using computational and experimental techniques have been discussed, along with the details of various performed analyses.

Chapters 3-7 present all the work done in this thesis. In **Chapter 3** (Mechanism of Aggregation of Superoxide Dismutase 1 (SOD1)), the aggregation mechanism of Superoxide dismutase 1 (SOD1) was explored employing ThT aggregation kinetics and size exclusion chromatography (SEC) experiments. Fitting the kinetics data to different models suggested that SOD1 follows a fragmentation-dependent mechanism. SEC experiments revealed that initial dimer to monomer conversion is fast compared to the total aggregation time, and aggregation proceeds expeditiously after the formation of a non-native dimer.

Chapter 4 (Computational screening criteria for selecting the aggregation inhibitors of SOD1 linked to ALS) was focused on understanding the effect of metal ions and intramolecular disulfide bonds on the structure and dynamics of SOD1 monomer through molecular dynamics simulations. Further, this chapter developed a computational strategy to predict the aggregation inhibitor of SOD1. The results revealed that removing metal ions destabilizes the protein structure incrementally and that eliminating disulfide bonds causes even more structural disruption. The study found that targeting different stages of amyloid fibril formation using polyphenols, as determined by MD simulations and experimental techniques, requires attention not only to the binding energy of protein-inhibitor interaction but also to the stabilization of the SOD1 interface, formation of native and non-native dimers, elongation, and defibrillation of fibrils in the presence of inhibitors.

Chapter 5 (Myricetin acts as an aggregation inhibitor of SOD1) proposed myricetin as an excellent aggregation inhibitor of DTT-EDTA-induced SOD1 aggregation. We utilized the screening criterion proposed in Chapter 4, and myricetin was screened from a list of

polyphenolic compounds. Our computational results suggested that myricetin binds strongly with apo disulfide reduced SOD1, stabilizes the protein interface, and binds moderately with native SOD1 dimer, Non-native SOD1 trimer, and corkscrew fibril. ThT aggregation kinetics, transmission electron microscopy, dynamic light scattering, and atomic force microscopy demonstrated that myricetin breaks preformed fibrils into shorter fibrils and forms less number of fibrils. Our seeding experiment and MD simulations show that myricetin inhibits fibril elongation, while our size exclusion chromatography experiment shed light on the depolymerization of preformed fibrils. The quenching mechanism and dissociation constant were predicted by fluorescence spectroscopy.

Chapter 6 (Dissecting the mechanism of oligomer interaction with two potent polyphenols) explored the mechanism of action of quercetin and curcumin on corkscrew SOD1 fibril, which are made up of eight chains (A-H), with each chain containing 11 amino acid residues from positions 28 to 38 of the full length SOD1 sequence. Molecular dynamics simulations were used to investigate the effects of these compounds on the fibrils. Quercetin was found to bind to a critical residue involved in the toxic amyloid formation, Trp 32, in chains D, E, and F, and also caused the peptide chains G-H to break away from the rest of the oligomer. In contrast, curcumin is bound to the hydrophobic amino acids of chains B-H, stabilizing the fibril rather than destabilizing it. MM/PBSA analysis was carried out to predict the binding affinity of quercetin and curcumin with corkscrew SOD1 fibril. The simulation results are in accordance with previous experimental results which showed that only quercetin disrupts the pre-formed fibril, not curcumin.

Chapter 7 (Computational alanine scanning mutagenesis of the zinc-binding loop and electrostatic loops of SOD1) cantered to understand the role of each amino acid of zinc binding and electrostatic loops through virtual alanine scanning mutagenesis. It was observed that H71, R79, D125, and S134 are four critical residues that affect the protein structure and

function significantly in fully metallated and disulfide bind intact dimer. Analysis revealed that observed mutations caused disordering in the loops due to the loss of native contacts and disruption of extended H-bond networks.

In brief, **Chapter 8** (Conclusion and future perspectives) provided an overview of the research. Essentially, our discoveries have been critical in comprehending the process of SOD1 aggregation and how employing small molecules to target various stages of the aggregation can be utilized to develop treatments for ALS associated with SOD1 aggregation.