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Thesis title: Effects of external additives on the fibrillation of the $A\beta_{25–35}$ peptide and of graphene oxide on human serum albumin fibrillation

Abstract: The self-assembly of proteins and peptides is thought to be responsible for commonly occurring neurological disorders such as Alzheimer's disease (AD). The amyloid β-peptide (Aβ), a 40/42 residue long peptide, is reported to be the chief constituent of senile plaques in the AD-affected brain. Several fragments of the Aβ peptides such as $A\beta_{25-35}$, $A\beta_{17-40/42}$, $A\beta_{31-35}$, $A\beta_{11-40/42}$, $A\beta_{1-38}$, $A\beta_{22-35}$, $A\beta_{1-16}$, $A\beta_{12-28}$ etc. are being used for *in vitro* and *in vivo* studies. The amyloid β-(25–35) peptide ($A\beta_{25-35}$) represents the biologically active and toxic domain of the full length Aβ peptide. This fragment has been used for the studies reported in this thesis, as it fibrillates rapidly and also shows toxicity similar to the full length peptide.

The focus of this thesis was to investigate the amyloid fibril formation by the $A\beta_{25-35}$ peptide and its modulation by several external factors *in vitro*. Several spectroscopic and microscopic techniques have been applied to monitor the fibrillation process. Thioflavin T and Congo red, the fluorescent marker dyes were used to confirm amyloid fibril formation. UV-Vis based studies were performed to observe the turbidity of the fibrillar solutions and examine the decrease in soluble protein content. The molecular weight of the protein aggregates were obtained from MALDI-TOF spectrometry and DLS studies provided the size of the protein fibrils. Morphological evolution of amyloid fibrillation and its modulation were further determined by using different microscopic studies.

Amyloid fibrillation of the $A\beta_{25-35}$ peptide was carried out at pH 7.4 and 37 °C. Preformed fibrils of the $A\beta_{25-35}$ peptide were found to disintegrate in the presence of micelles of surfactants with longer hydrophobic tails. Inhibition of amyloid fibrillation of the $A\beta_{25-35}$ peptide was achieved by adhesion of the peptide on a graphene oxide (GO) surface with the aid of hydrophilic and hydrophobic interactions and by oxidizing Met-35 amino acid using H_2O_2 in another study. Elevation of homocysteine levels in the human body shows toxicity by its conversion to homocysteine thiolactone (HCTL) which reacts with the ϵ-amino group of Lys residues of proteins. Treating the peptides with HCTL, an amine oxide osmolyte, glycerol, model phospholipid membranes and silver nanoparticles were found to accelerate the $A\beta_{25-35}$ peptide fibrillation through amino acid modifications and electrostatic interactions among the peptide units and additives. Preformed fibrils were also found to undergo enhanced fibrillation in presence of non-ionic surfactants through possible H-bonding interactions. The studies carried out indicate that the possibilities of hydrophobic interactions constitute a major determining factor in preformed fibril-surfactant interactions. Electrostatic interactions appear to play an important role in the modulation of the amyloid fibrillation process of the monomeric $A\beta_{25-35}$ peptide.

The inhibitory activity of GO was further studied on the *in vitro* fibrillation of the most abundant serum protein, human serum albumin (HSA). HSA undergoes fibrillation in presence of external conditions and serves as a good model protein in protein aggregation studies. HSA fibrils were obtained in a water-ethanol mixed solvent system at 37 °C. Significant restoration of the α-helical content of the HSA was found to occur in presence of higher concentrations of GO in the system. The studies will provide potential information regarding the understanding of the mechanism of amyloid fibrillation process and its inhibition strategies in general.

Keywords: $A\beta_{25-35}$ peptide, Human serum albumin, Amyloid fibrils, Micelles, Homocysteine thiolactone, Phospholipids, Graphene oxide, Silver nanoparticles