CHAPTER V
CONCLUDING REMARKS
The biological function of a protein depends on its three-dimensional structure, which is determined by its amino acid sequence and cellular environment surrounding the polypeptide chain. A large number of debilitating human diseases are associated with protein misfolding and aggregation (amyloid fibril formation), collectively called protein conformational disorders. A group of proteins unrelated in size and sequence forms a highly ordered structure called amyloid fibrils. Deposition of such fibrils in cells or tissues causes several diseases. Importantly, a large number of such aggregates are present in the brain and play a major role in neurodegeneration. Amyloid fibrils formed by protein in vitro are morphologically indistinguishable from those extracted from patients. Therefore, investigating the mechanism and factors that influence amyloid fibril formation in vitro are of great importance in understanding amyloidosis.

Heat shock proteins (Hsps) are important in protein-aggregation-related disorders since they are known to prevent aggregation of proteins in their non-native conformation. Interestingly, small heat shock proteins have been observed in intra- or extra-cellular plaques, characteristically seen in several neurodegenerative diseases such as Alzheimer's, Parkinson's and poly Q diseases. The work reported in this thesis is an attempt to understand the mechanism of fibril formation of human α-synuclein and the effect of the small heat shock protein, αB-crystallin, and its phosphorylation-mimicking mutant on fibril formation of α-synuclein.

α-Synuclein is a presynaptic protein with unknown function. Aggregation of α-synuclein into ordered filament-like structure is a major cause for Parkinson's disease and multiple system atrophy. Factors that favor amyloid fibril formation have been the subject of intense research in the recent past. α-Synuclein interacts with membranes; such membrane interactions are shown to promote as well as inhibit the fibril formation. The role of membrane interaction, thus, became controversial. We have investigated the molecular mechanism of fibril formation of α-synuclein in the presence of SDS. SDS mimics lipid molecules in terms of its amphiphilic nature. We
find that the fibril growth of α-synuclein does not proceed rapidly despite providing preformed fibril (seed) for nucleation, unlike other amyloidogenic proteins such as Aβ and β2-microglobulin. Addition of SDS promotes fibril formation. We have investigated the dependence of this process on the concentration of SDS and, interestingly, found that α-synuclein-SDS form two types of ensembles: 1) the fibrillogenic ensembles characterized by partial helical conformation with highly exposed hydrophobic surfaces that are highly competent for fibrillogenesis; 2) non-fibrillogenic ensembles characterized by fully folded α-synuclein with less accessible hydrophobic surfaces that are less or non-fibrillogenic. Our study also shows that higher concentrations of SDS inhibit fibril formation of α-synuclein. Such dual behavior, perhaps, explains the controversial reports of the role of membrane in fibril formation. However, despite our extensive studies using circular dichroism, steady state and time resolved fluorescence and isothermal titration calorimetry, the exact nature of the ensembles remains unclear.

Phosphorylation, which is one of the important post-translational modifications of small heat shock proteins that modulates its chaperone activity, may play a role in alleviating the pathogenic effect of protein conformational diseases. Our present study contributes towards the understanding of the effect of phosphorylation on the molecular chaperone-like activity of αB-crystallin in preventing the ordered amyloid fibril formation of human α-synuclein as well as amorphous aggregation. We find that phosphorylation increases the rate of subunit exchange of the homo-oligomers of αB-crystallin as well as hetero-oligomers of phosphorylated and unphosphorylated αB-crystallin. Since αB-crystallin, in a given tissue, is present both in the phosphorylated and the unphosphorylated forms, properties of the mixed of the hetero-oligomers is of importance.

Elevated levels of Cu\(^{2+}\) have been reported in amyloid plaques of Parkinson’s, Alzheimer’s and prion diseases. Interestingly, αB-crystallin was found to co-exist with amyloid plaques. However, the significance of such co-existence is not understood. We set out to investigate the effect of αB-crystallin on the Cu\(^{2+}\)-induced
fibril formation, generation of reactive oxygen species and cytotoxicity. Our study demonstrates that Cu$^{2+}$ accelerates α-synuclein fibril formation as well as aggregation of Aβ peptide. αB- and 3DαB-crystallin prevents the Cu$^{2+}$-mediated fibril formation of α-synuclein and aggregation of Aβ peptide. Our study also shows the selective Cu$^{2+}$-binding to αB- and 3DαB-crystallin with picomolar affinity. We found that Cu$^{2+}$-binding influences the structure and stability of αB- and 3DαB-crystallin to a significant extent. Interestingly, we find that the small heat shock protein, αB-crystallin and its phosphorylation-mimic mutant 3DαB-crystallin, silence the Cu$^{2+}$-mediated oxidative stress, inhibiting the generation of ROS and thereby conferring cytoprotection. Thus, αB-crystallin and possibly other small heat shock proteins appear to have multiple roles in preventing or slowing the disease process: they prevent formation of toxic protein aggregates and they also provide cytoprotection by checking the oxidative damage. Strong metal-ion-binding and consequent cytoprotection is a novel finding that needs further investigation.