SYNOPSIS

Cells are continuously challenged by conditions which cause stress. In order to adapt to environmental challenges and ensure survival, cells respond by a rapid and transient reprogramming of cellular activities called stress response. In response to such critical stress situations, cells employ a variety of mechanisms, and this involves increasing the abundance of an important group of biomolecules in the cells, namely the heat shock proteins (HSPs) or stress proteins. Heat shock proteins were first identified in response to heat stress but were later found to be constitutively present and up-regulated under various stress conditions. HSPs function as molecular chaperones and are involved in a plethora of functions including facilitating the synthesis and folding of proteins throughout the cell.

Heat shock proteins are classified, based on their molecular weights, into six major families *i.e.* Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and small heat shock proteins (sHsps). Chapter I describes role of HSPs in stress response with particular emphasis on sHsps. The sHsps form a ubiquitous family of molecular chaperones, of which the monomer size typically ranges between 12 and 43 kDa. They are characterized by a conserved stretch of 80-100 amino acid residues, called the α-crystallin domain, a more variable N-terminal sequence, and in most cases, a short and variable C-terminal extension. Notably, many of these sHsps are shown to exhibit chaperone-like activity and enhance stress tolerance. The human genome codes for ten sHsps, namely, HspB1 (Hsp27), HspB2, HspB3, HspB4 (αA-crystallin), HspB5 (αB-crystallin), HspB6 (Hsp20), HspB7 (cvHsp), HspB8 (Hsp22), HspB9 and HspB10 (ODF1).

Chapter I provide a contextual review of small heat shock proteins (sHsps). Three sHsps have been crystallized namely Hsp16.5 of *Methanococcus janaschii* (thermophillic methanogenic archae), Hsp16.9 of wheat and Tsp36 of *Taenia saginata* (tapeworm); these provide insights into the structural and functional organization of sHsps. However, no small heat shock proteins of mammalian origin have been crystallized so far. sHsps exhibit ATP-independent chaperone-like activity. Moreover, role of sHsps in cytoskeletal rearrangements, their crosstalk with extracellular environment, interaction with apoptotic cascade and metal

ions have been discussed. sHsps provide protective role in several pathological conditions. Chapter I also describes the role of sHsps in pathological conditions. Several mutations in sHsps have been linked to cataract, myopathies and neuropathies. The work reported in this thesis is related to the structure and function of human small heat shock protein HspB3. This chapter reviews the literature available on HspB3 and provides the scope of this work.

Chapter II describes the cloning, over-expression and purification of the recombinant human HspB3. HspB3 was purified to homogeneity and characterized for its structural and functional properties by various biophysical methods. Circular dichroism (CD) studies revealed that HspB3 has significant β-sheet content, similar to other well characterized members of the sHsp family. Quaternary structure study shows that HspB3 predominantly exists as a trimer. Our studies show that HspB3 efficiently prevents the thermal aggregation of alcohol dehydrogenase (ADH), moderately prevents the thermal aggregation of citrate synthase (CS) and fails to prevent the DTT-induced aggregation of insulin. Thus our study shows that HspB3 exhibits target protein-dependent chaperone-like activity.

HspB3 is a unique member of the small heat shock protein family, it has a very small C-terminal extension (PVGTK). The C-terminal extension of sHsps, have been predicted to play a role in solubilization of target-chaperone complex. In order to investigate the role of C-terminal extension in sHsps, we made a chimeric protein containing the N-terminal domain and the α-crystallin domain of HspB3 fused to the C-terminal extension of HspB5 (αB-crystallin). Chapter II describes our studies on the structure and chaperone properties of the chimeric protein. Our study indicates that the C-terminal extension modulates the oligomerization status and chaperone activity of the protein. The chimeric protein shows a major dimeric population with a significant population of higher oligomeric species. The chapter further shows that the chimeric protein exhibits protection against insulin aggregation in addition to that of ADH and CS. Our results suggest that the C-terminal extension in sHsps may play a role in imparting wide range chaperone-like activity.

Several human sHsps have been associated with neurodegenerative disorders such as Alzheimer's and Parkinson disease. Cu^{2+} has been reported to accumulate in extracellular

spaces of the patient suffering from neurodegenerative disorders. Cu^{2+} deposition could cause neurodegeneration *via* oxidative damage. Cu^{2+} is known to accelerate amyloid fibrillation of proteins such as α-synuclein. Chapter III describes our investigation on the interaction of HspB3 with Cu^{2+}. We found that HspB3 binds to Cu^{2+} with picomolar affinity, sequesters Cu^{2+} and prevents Cu^{2+}-ascorbate-mediated ROS generation. This binding also confers cytoprotection from Cu^{2+}-ascorbate-mediated oxidative damage. Further, our study shows that Cu^{2+} binding also prevents Cu^{2+}-mediated α-synuclein fibrillation, by extracting the Cu^{2+}-bound to α-synuclein. Our study shows that another sHsp, Hsp27, which is associated with neurodegenerative disorders, also exhibits Cu^{2+}-binding property, prevents ROS generation, confers cytoprotection and inhibits α-synuclein fibrillation.

R7S mutation in HspB3 has been correlated with axonal motor neuropathy. Chapter IV describes the cloning, bacterial expression and purification of R7S HspB3 and the effect of R7S mutation on the properties of HspB3. We find that the mutation leads to a small change in the secondary structure and a significant loss in the tertiary structure of the mutant protein. The R7S mutant of HspB3 also shows slight reduction in the chaperone-like activity. Our immunolocalization study on COS-1cells transiently transfected with wild type and mutant HspB3 shows that under normal, unstressed conditions both predominantly localize to the cytoplasm. Further, our study shows that under heat shock conditions wild type HspB3 translocates into the nucleus, whereas R7S mutant fails to do so and remains in the cytoplasm.

Chapter V summarizes our findings and conclusions. The thesis reports the cloning, over expression, purification, structural and functional characterization of the human small heat shock protein HspB3. It also sheds light on the role of C-terminal extension in sHsps. This study reports on the Cu^{2+}-binding property of HspB3 and its role in preventing Cu^{2+}-mediated oxidative damage and amyloidogenesis. Work reported in the thesis characterizes the axonal motor neuropathy-causing R7S mutant of HspB3, and its mis-localization under heat shock conditions. Further studies may help in elucidating the molecular basis of this disorder.