SUMMARY

Structural Studies on the Chaperonin-10 of Mycobacterium Tuberculosis.

The landmark work of Anfinsen showed for the first time that primary structure dictates the tertiary structure and introduced the concept of self-assembly of proteins. However, the cellular conditions of high protein concentration, ionic strength *etc*. are often debated to be non-ideal for the spontaneous self-assembly of proteins as they favour aggregation over correct folding.

Under these non-ideal cellular conditions, a group of specialized proteins designated as molecular chaperones have been shown to be essential for the correct folding of polypeptides in a number of cellular compartments in eukaryotes as well as in prokaryotes. Chaperonins are an important class of molecular chaperones. Chaperonin-60 or GroEL are large cylindrical protein complexes with an ATPase activity that assist the folding of a large number of cellular proteins. Chaperonin-10 or GroES acts as an allosteric modulator GroEL and assists GroEL in its function of ATP-dependent protein folding cycle.

Chaperonin-60 and chaperonin-10 of *Mycobacterium tuberculosis*, the GroEL and GroES homologues of *E. coli*, have been shown to be important T-cell antigens. In addition, the chaperonin-10 of *M. tuberculosis* (Mt-cpn10) has been postulated to be the major factor responsible for spinal tuberculosis or Pott's disease.

This thesis entails structural studies on Mt-cpn10 both at the primary level as well as tertiary level to understand its interesting immunological and biological properties. reviews the function of chaperonin-10 in the GroEL-GroES protein folding cycle with its interesting pathological and biological properties in details. Sufficient

background on the role of molecular chaperones *in vivo* and a brief discussion on other classes of molecular chaperones has been given.

The GroES-homologue of *M. tuberculosis* shares ~90% sequence identity with the closely-related GroES-homologue of *M. leprae*. Conserved residues in the interface and core regions of *M. tuberculosis* were identified and mapped using the *M. leprae* GroES structure. The irregular β-barrel structure of chaperonin-10 has earlier been classified as GroES fold. Other members of the GroES fold family are the quinone oxidoreductases, glucose dehydrogenases and alcohol dehydrogenases. Structural comparisons of members of this fold along with an extensive analysis of the available sequences for each class were carried out. These indicated several interesting features including the conservation of a glycyl-aspartyl dipeptide across the various protein families. We have proposed these residues to be responsible for the maintenance of the GroES fold. The results of this work have been published (Taneja, B., and Mande, S.C., (1999), *Protein Eng.*, **12**, 815-818). Chapter 2 reports these results in the form of a reproduction of the published work. Subsequent molecular dynamics work on this conserved dipeptide by Ms. Rohini Qamra in the lab has confirmed the role of this dipeptide in the maintenance and integrity of the GroES fold.

Chapter 3 details the journey from the chaperonin-10 clone to the purified protein to the struggle to obtain diffraction quality crystals. Since most of the standard techniques for purification and crysallizations were used, the methodology followed has been given in brief. This chapter contains no separate 'Methods' section, instead the results are reported in a chronological manner.

Chapter 4 is the reproduction of another published work (Taneja, B. and Mande, S.C., (2001), *Protein Eng.*, **14**, *in press*), as in the case of Chapter 2. The high degree of plasticity

in the chaperonin-10 structures has been an enigma. This chapter explores the role of metal ions in modulating the metastable states of Mt-cpn10 using intrinsic Trp fluorescence as the major tool. This result also helped us exploit the role of metal ions in obtaining diffraction quality crystals.

Chapter 5 describes the crystal structure of this important mycobacterial protein. The structure of Mt-cpn10, though at a modest resolution of 3.5 Å reveals several interesting conformational features. The most interesting outcome of the structure is the visualization of its mobile loop, which appears to be in a partially stable conformation. The conformation of the mobile loop, reported for the first time in any stand-alone crystal structure of chaperonin-10, assumes immediate functional relevance since chpaeronin-10s interact with chaperonin-60s through this mobile loop. This work has recently been accepted for publication (Taneja, B. and Mande, S.C., (2001), *Curr. Sci.*, **81**, 87-91). We have also identified binding site for metal ions in the dome loop of Mt-cpn10, which was earlier shown to modulate the different plastic states of Mt-cpn10 in Chapter 4. These results along with other crystallographic details are being readied for communication elsewhere.