Summary
Recent years have seen increased incidence of tuberculosis and leprosy in both the developing and the developed countries. Information available from the complete genome sequence of *Mycobacterium tuberculosis* and *Mycobacterium leprae* has the potential of providing the information that would generate knowledge that will enable to elucidate the unusual biology of its etiological agents.

Serine hydroxymethyltransferase (SHMT), L-serine:tetrahydrofolate 5, 10-hydroxymethyltransferase, a member of the α-class of the pyridoxyl-5′-phosphate (PLP)-dependent enzyme, is a catabolic protein involved in the reversible interconversion of serine to glycine. The SHMT reaction plays a major role in cell physiology as it is considered to be a key enzyme in the pathway for interconversion of folate coenzymes which provides almost exclusively one carbon fragments for the biosynthesis of a variety of end products such as DNA, RNA, ubiquinone, methionine, etc. Increased activity coupled with enhanced DNA synthesis in neoplastic tissues has suggested SHMT as a possible target for cancer chemotherapy. The importance of SHMT as a drug target in malaria has also been predicted and being evaluated.

Genome analysis revealed that there are two putative genes namely GlyA and GlyA2, for *Mycobacterium tuberculosis* and one GlyA for *Mycobacterium leprae*, which encode for the enzyme serine hydroxymethyltransferase. All the three genes were cloned and their respective protein products were over-expressed and purified to homogeneity. SHM1 and SHM2 had around 66% homology and mlSHMT had 89% homology with SHM1. The recombinant proteins exist as homodimers of molecular mass about 90kDa under physiological conditions but differ much with respect to their structural properties like, SHM2 has more compact conformation and higher thermal stability than SHM1 and SHM1 has higher thermal stability than mlSHMT.

The most interesting structural observation was that SHM1 and mlSHMT contains one mole of PLP per mole of enzyme dimer. This is the first report of such a unique stoichiometry of PLP and enzyme dimer for SHMT. This was confirmed by taking visible CD spectra of the enzymes which gives a positive peak at around 425nm for bound PLP and it shows lower chiral values for SHM1 and mlSHMT which are about 8 times less than that for SHM2, which confirms that SHM1 and mlSHMT has lower content of bound PLP. Sequence alignment of all these three SHMTs with other SHMTs shows that they
have all the residues conserved which are required for binding of PLP but the octapeptide sequence near the active-site lysyl residue (K229 as in eSHMT) 223-VTTTTHK(Pyr)T-230 in *E. coli*, 221-VSTTVHK(Pyr)T-228 in SHM1 and mISHMT and 225-VTSTTHK(Pyr)T-232 in SHM2, which clearly indicates a non-homologous mutation in threonine-225 in SHM1 and mISHMT corresponding to threonine-227 in *E. coli*. These observations led to the conclusion that may be this octapeptide sequence is responsible for binding of the cofactor PLP and any alteration may lead to change in its stoichiometry.

Functionally, all the mycobacterial SHMTs showed catalysis of reversible interconversion of serine and glycine and aldol cleavage of a 3-hydroxyamino acid. However, unlike SHMT from other sources all the three do not undergo half-transamination reaction with D-alanine resulting in formation of apoenzyme but L-cysteine removed the prosthetic group, PLP, from both the recombinant enzymes leaving the respective inactive apoenzyme. In terms of kinetic properties it was observed that all the three SHMTs has $K_m$ value for L-Serine comparable to that reported for SHMTs from other sources i.e. binding affinity to substrates is same for all the enzymes with respect to SHMTs from other organisms. However, a significantly lower catalytic efficiency ($k_{cat}/K_m$) for the reaction was observed which shows that mycobacterial enzymes are less active as compared to other organisms.

pH denaturation showed that all the three enzymes are resistant to alkaline denaturation and had pH of 6.12, 6.05 and 6.16 for SHM1, SHM2 and mISHMT, respectively. SHM1 and mISHMT are more resistant to alkaline denaturation and maintains it oligomeric structure whereas the native SHM2 dimer dissociates into monomer at pH 9. Urea- and guanidinium chloride-induced denaturation profiles show a two-step unfolding with the first step being dissociation of dimer into apomonomer at low denaturant concentrations followed by unfolding of the stabilized monomer at higher denaturant concentrations. All the denaturation profiles show that as the bounded PLP starts coming the enzyme also loose activity, thus cofactor is responsible for the activity of the enzyme.

Thus the mycobacterial SHMTs reported in this thesis although are from same genus but have many different properties among themselves and that from other
organisms. The unusual behavior of SHM1 and that of mISHMT can be exploited for various other purposes of study.

To summarize the work in this thesis, a specific amino acid sequence determines folding, function and degradation of any protein. Homologous proteins from different species or may be from same species bearing same function can differ in their structural and functional properties. The amount of residual structures in the presence of different denaturants is relevant to study the confirmation of any protein in its folding pathway.