## Abstract

Inside the bacterial cell, the genetic material is maintained in a negatively supercoiled state within a compact space. The supercoiling of the genome undergoes topological perturbations during a variety of cellular processes such as replication, transcription, chromosome segregation etc. Throughout these processes, topological homeostasis is maintained by a ubiquitous class of enzymes called topoisomerases. These enzymes solve the problems associated with the DNA topology by cutting and rejoining the DNA. Based on their structure and mechanism of action, these enzymes are broadly classified as type I and type II. The genome of *Mycobacterium tuberculosis* codes for a single type I and a single type II topoisomerase while *Escherichia coli* and many other eubacteria have additional topoisomerases. Recently, the essentiality of the mycobacterial topoI was validated. Genus Mycobacterium includes many species including the well-studied saprophytic *Mycobacterium smegmatis* as well as the pathogenic *Mtb*, the causative agent of the deadly disease tuberculosis (TB), which claims millions of deaths worldwide annually. DNA gyrase has been extensively exploited as a target for development of anti-bacterial compounds. There is a dearth of such compounds that target bacterial topoI. Therefore, as a part of this study, efforts have been made to identify small molecule inhibitors against mycobacterial topoI. Biochemical studies from the laboratory have revealed several unusual characteristics of the topoisomerase I from *Msm* such as site specificity, ability to bind single as well as double stranded DNA with high affinity and absence of Zn2+ fingers required for DNA relaxation activity in other Type I enzymes. Similar to a host of nucleic acid transaction enzymes which depend on divalent metal ions for catalytic activity, type IA and type II topoisomerases also require Mg2+ for their activity. However, the role of the metal ion in the reaction cycle of type IA topoisomerases is as yet not clearly understood. A part of the study is therefore aimed at understanding the role of Mg2+ in the reaction cycle of bacterial topoI.

In **Chapter 1** of the thesis, an introduction has been provided about DNA topology, DNA topoisomerases with respect to their biochemical properties and structure and the role of Mg2+ in the reaction cycle. A brief overview of TB epidemiology and pathogenesis is presented. This is followed by a description of the repertoire of drugs presently available to treat TB. Finally, the emerging anti-TB drugs are discussed.

In **Chapter 2,** the studies are directed to understand the biochemical properties of the single type IA topoisomerase from *Mtb*. Previous studies with the *Msm* topoisomerase I (MstopoI) revealed several of its distinct properties compared to the well-studied *E. coli* topoisomerase I (EctopoI). Also, essentiality of topoisomerase I in both *Msm* and *Mtb* has been validated in the laboratory. In spite of their crucial role in cellular function and survival, so far, no small molecule inhibitors have been identified for this class of enzymes from any bacteria. Now, *Mtb* topoisomerase I (MttopoI) is characterized and its properties have been compared with MstopoI and EctopoI. The enzyme is shown to cleave DNA at preferred sites in a pattern similar to its ortholog from *Msm*. Oligonucleotides containing the specific recognition sequence inhibit the activity of the enzyme in a manner similar to that of MstopoI. Previously in the laboratory, monoclonal antibodies (mAbs) were raised against mycobacterial topoisomerase I. One of the mAbs, 2F3G4, is shown to inhibit the relaxation activity of MttopoI. These studies affirm the characteristics of MttopoI to be like MstopoI and set a stage to target it for the development of specific small molecule inhibitors.

In **Chapter 3,** the inhibition of MttopoI by two related small molecule inhibitors is shown. These molecules were identified as high scoring hits from an *in-silico* screen and subsequently their inhibitory potential was tested. These molecules showed growth inhibition of both *Msm* and *Mtb* cells. The compounds stimulated the DNA cleavage thereby perturbing the cleavage-religation equilibrium. Consequently, these molecules inhibited growth of the cells over expressing TopoI at lower minimum inhibitory concentration (MIC). Docking of the molecules on the MttopoI model suggested that they bind near the metal binding site of the enzyme. The DNA relaxation activity of the metal binding mutants harboring mutation in the DxDxE motif was differentially affected by the molecules, suggesting that the metal coordinating residues contribute to the interaction of the enzyme with the drug. These results highlight the potential of these small molecules, which poison the *Mtb* and *Msm* topoisomerase I, as leads for the development of molecules to combat the mycobacterial infections.

In **Chapter 4**, m-AMSA, a known type II topoisomerase poison is shown to act as a type IA topoisomerase poison. m-AMSA is an established inhibitor of eukaryotic type II topoisomerases. The drug exerts its cidal effect on treated cells by binding to the enzyme-DNA complex, inhibiting the DNA religation step by altering the cleavage-religation equilibrium. Due to this cellular poisoning effect, the molecule and its analogues have been successfully used as chemotherapeutics against different forms of cancer. After virtual screening using a homology model of MttopoI, m-AMSA was identified as a high scoring hit. Subsequent *in vitro* testing demonstrated that m-AMSA can inhibit the DNA relaxation activity of type IA topoisomerase in a whole cell assay against *Msm* and *Mtb*.

In **Chapter 5,** the role of $Mg^{2+}$ in the clamp operation step during the second transesterification is studied. A mAb, 1E4F5, is shown to stimulate religation even in the absence of $Mg^{2+}$ suggesting that the metal ion is dispensable for chemical catalysis. Further characterization showed that the antibody closes the clamp of the enzyme. In this study, $Mg^{2+}$ is shown to be needed primarily for triggering conformational changes in the enzyme that are needed for religation to occur. Using tryptophan induced quenching method; closing of enzyme clamp is demonstrated in presence of the mAb alone as well as $Mg^{2+}$. The biochemical and the biophysical evidence suggest a novel role for $Mg^{2+}$ in the clamp operation mechanism of topoisomerase I.

In **Chapter 6,** the conformational changes that drive strand passage in bacterial topoisomerase are studied. Topoisomerases modulate the topology by concerted DNA binding, cleavage, strand passage and relegation through enzyme-bridged mechanism. During the relaxation cycle, TopoI transits through various conformational states and domain rearrangements. The catalytic activities i.e. DNA cleavage and religation reside in the NTD while the CTD takes part in strand passage between the two catalytic steps. The two domains in the NTD that act as clamps must come together or move apart during the two sequential transesterification steps. Although the CTD is indispensable and shown to be involved in strand passage, the mechanism by which it participates in the relaxation is not understood. Here, it is demonstrated that conformational transitions in the NTD are relayed to the CTD via a transducer region to activate CTD for strand passage activity.