Abstract

Elucidating the structure-function relationship of proteins provides new perspective about the mechanisms of critical cellular processes and contributes in the understanding of novel drug development against pathogenic bacteria. Presuming the distinctive nature of each protein, investigation of the structure-function relationship becomes essential for the detailed apprehension of cellular processes. Our study is motivated towards the structural and functional characterization of proteins involved in oxidative stress response system and chromosome segregation system. Proteins from *Staphylococcus aureus* and *Mycobacterium tuberculosis* have been taken into account. Both the bacteria being intracellular pathogens, their survival and pathogenesis depend severely on cell cycle progression in a host cell and defense mechanisms to fight against host immune response. Oxidative stress response proteins are members of bacterial defense system, whereas chromosome segregation proteins are indispensable for their growth and survival. Among the various oxidative stress response proteins present in *S. aureus*, we have focused on the proteins involved in repairing oxidative damage on other proteins and maintenance of the redox homeostasis in cell. On the other hand, chromosome segregation is a crucial process to achieve proper cell division and maintain chromosomal inheritance. Little disturbance in the mechanism may cripple the daughter cells and play havoc on the growth and survival of the pathogen. Essentiality of both the processes attracts the attention for the characterization of the proteins involved from a structural and functional aspect.

Part A of the thesis describes structural and functional characterization of oxidative stress response proteins from *S. aureus*. Being an intracellular pathogen, *S. aureus* has to encounter a vast array of reactive oxygen species (ROS) produced by the oxidative defence mechanism of the host. In response, the pathogen implements different strategies to maintain the redox homeostasis of cytoplasm. A series of disulfide exchange reactions mediated by thioredoxin (Trx) and thioredoxin reductase (TR) results in repairing the oxidatively damaged proteins. Here we have characterized an active thioredoxin system of the *S.aureus*. Crystal structure of staphylococcal thioredoxin2 (SaTrx2) in its reduced form reveals that it contains the conserved redox active WCXXC motif and a thioredoxin fold. Staphylococcal thioredoxin reductase2 (SaTR2) is a flavoprotein and consists of two Rossmann folds as the binding sites for FAD and NADPH. Crystal structure of the holoenzyme shows that the protein consists of two domains and the catalytic site is formed by an intramolecular disulfide bond formed between two sequentially distal Cys residues (C248 & C265). Biophysical and biochemical studies unveil that SaTrx2 and SaTR2 can interact in solution and in the course of sustaining the redox equilibrium the latter reduces the former. A mechanism of the redox reaction has been proposed in light of formation of the mixed disulfide intermediate.

Part B of the thesis provides a molecular insight regarding the chromosome segregation process of *M. tuberculosis*. Both ParABS system and SMC-ScpAB complex are crucial for chromosome segregation and cell cycle progression in bacteria. Following origin segregation by the ParABS module, SMC-ScpAB complex takes over the charge of bulk chromosome segregation. Very little is known about the collaboration of ParABS and SMC-ScpAB to accomplish the segregation process in cell. We have characterized an ScpA (Rv1709) from *M. tuberculosis* and proposed its role as a potential ParB, which may function as a linker between the segregation processes carried out by ParABS module and SMC-ScpAB complex. Our results show that ATPase activity of Rv1708, a ParA homolog, is accelerated in the presence of Rv1709 and *parS* sequence. We have also reported that Rv1709 physically interacts with Rv1708 and *parS*, which is a fundamental feature of ParB family of proteins. The N-terminal domain and the central helix of Rv1709 actively participate in the binding events. Self-association of Rv1709 indicates its property to form a large nucleoprotein segrosome complex which is an essential step for faithful chromosome segregation. We have identified Rv1708 and Rv1709 as a novel ParA-ParB pair involved in mycobacterial chromosome segregation process.

Keywords: oxidative stress response, chromosome segregation, thioredoxin, thioredoxin reductase, ParA, ParB, SMC, ScpAB.