Expression, purification, and functional characterization of WhiB proteins of *Mycobacterium tuberculosis*

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Abstract

Mycobacterium tuberculosis (*Mtb*) is the causative organism of one of the deadliest infectious diseases worldwide, tuberculosis (TB). It possesses a remarkable feature of entering into a dormant state upon encountering a stressful milieu and emerging to the active state under favorable conditions; furthermore, it senses and adapts to the different environmental conditions experienced during early infection and persistence in the host cell. This capricious nature has made it an uphill battle to target this stubborn bacterium. *M.tb* deploys several sensors and transcription regulators to sense and subvert the host immune response, regulate the expression of some essential genes, eliminate the redox stress, and survive inside the host for decades. Iron-sulphur cluster-containing WhiB transcription factors are one such redox-sensing internal sensor and regulator. The engagement of the whiB family of genes as redox sensors and regulators in so many fundamental metabolic pathways of *M.tb* and not showing homology with human proteins makes them an attractive drug target. The thesis is concerned with the optimization of expression and purification of unstable and insoluble WhiB proteins (WhiB1, WhiB3, and WhiB6) in *E. coli* and comprehending the structural details of these proteins and the interaction of WhiB proteins with their binding partners (promoter DNA of crucial genes of *Mtb*) employing biophysical techniques. Different strategies were used to overcome the instability, insolubility, and aggregation-prone behavior of WhiB proteins. The low temperature, along with the fusion tags, significantly improved WhiB1 and WhiB3 protein solubility. The co-expression of molecular chaperones and osmolytes remarkably enhanced the solubility of WhiB6 in soluble fraction during over-expression.

The biophysical characteristics of the WhiB6 protein and the interaction between WhiB6 and *espA* promoter DNA were comprehensively investigated. Far-UV CD spectroscopy, surface-enhanced Raman spectroscopy (SERS), and steady-state fluorescence spectroscopy gave insight into the conformational changes in the WhiB6 protein due to the interaction. Furthermore, SERS provided information regarding the residues of the WhiB6 protein involved in the interaction. The thermodynamic parameters and the binding affinity of the WhiB6-DNA interaction were obtained from the ITC experiments. These findings will provide a better understanding of the interaction of WhiB6 with its binding partners.

WhiB3 protein regulates the expression of genes involved in virulence lipid anabolism. Biophysical techniques, including far-UV CD spectroscopy, SERS, steady-state fluorescence spectroscopy, and ITC, provided detailed insight into the binding parameters of interaction, the conformation changes in protein during the interaction, and the residues involved in the binding of WhiB3-*pks2*. The information obtained regarding the biophysical characteristics of the protein, the binding affinity, and the thermodynamic parameters of the WhiB3-*pks2* interaction can be advantageous in designing drugs to cure TB. WhiB1 binds to its promoter DNA and represses the expression. The biophysical characterization of the WhiB1 protein and its interaction with whiB1 promoter DNA was investigated employing biophysical techniques. The thermodynamic parameters associated with the interaction were obtained from ITC. The details of conformational changes in WhiB1 upon interaction with the DNA were obtained from far-UV CD and steady-state fluorescence spectroscopy.

In a nutshell, the present study has provided insight into the biophysical characteristics of WhiB proteins and unveiled the binding parameters of the interaction of WhiB proteins with the promoter DNA. These findings will further help in understanding the structural and functional behaviors of WhiB proteins. They will help design new drug molecules or small-molecule that could imitate the specificity and binding affinity of WhiB proteins. This idea could be deployed in the research of finding promising drugs to cure TB.