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Biophysical and Structural Characterization of Transcription Factor Rv0047c of Mycobacterium Tuberculosis H37Rv

Authors : Md. Samsuddin Ansari, Ashish Arora

Abstract : Every year 10 million people fall ill with one of the oldest diseases known as tuberculosis, caused by Mycobacterium tuberculosis. The success of M. tuberculosis as a pathogen is because of its ability to persist in host tissues. Multidrug resistance (MDR) mycobacteria cases increase every day, which is associated with efflux pumps controlled at the level of transcription. The transcription regulators of MDR transporters in bacteria belong to one of the following four regulatory protein families: AraC, MarR, MerR, and TetR. Phenolic acid decarboxylase repressor (PadR), like a family of transcription regulators, is closely related to the MarR family. Phenolic acid decarboxylase repressor (PadR) was first identified as a transcription factor involved in the regulation of phenolic acid stress response in various microorganisms (including Mycobacterium tuberculosis H37Rv). Recently research has shown that the PadR family transcription factors are global, multifunction transcription regulators. Rv0047c is a PadR subfamily-1 protein. We are exploring the biophysical and structural characterization of Rv0047c. The Rv0047 gene was amplified by PCR using the primers containing EcoRI and HindIII restriction enzyme sites cloned in pET-NH6 vector and overexpressed in DH5α and BL21 (λDE3) cells of E. coli following purification with Ni2+-NTA column and size exclusion chromatography. We did DSC to know the thermal stability; the Tm (transition temperature) of protein is 55.29ºC, and ΔH (enthalpy change) of 6.92 kcal/mol. Circular dichroism to know the secondary structure and conformation and fluorescence spectroscopy for tertiary structure study of protein. To understand the effect of pH on the structure, function, and stability of Rv0047c we employed spectroscopy techniques such as circular dichroism, fluorescence, and absorbance measurements in a wide range of pH (from pH-2.0 to pH-12). At low and high pH, it shows drastic changes in the secondary and tertiary structure of the protein. EMSA studies showed the specific binding of Rv0047c with its own 30-bp promoter region. To determine the effect of complex formation on the secondary structure of Rv0047c, we examined the CD spectra of the complex of Rv0047c with promoter DNA of rv0047. The functional role of Rv0047c was characterized by over-expressing the Rv0047c gene under the control of hsp60 promoter in Mycobacterium tuberculosis H37Rv. We have predicted the three-dimensional structure of Rv0047c using the Swiss Model and Modeller, with validity checked by the Ramachandra plot. We did molecular docking of Rv0047c with dnaA, through PatchDock following refinement through FireDock. Through this, it is possible to easily identify the binding hot-stop of the receptor molecule with that of the ligand, the nature of the interface itself, and the conformational change undergone by the protein pattern. We are using X-crystallography to unravel the structure of Rv0047c. Overall the studies show that Rv0047c may have transcription regulation along with providing an insight into the activity of Rv0047c in the pH range of subcellular environment and helps to understand the protein-protein interaction, a novel target to kill dormant bacteria and potential strategy for tuberculosis control.

Keywords : mycobacterium tuberculosis, phenolic acid decarboxylase repressor, Rv0047c, Circular dichroism, fluorescence spectroscopy, docking, protein-protein interaction
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