Abstract

Tuberculosis (TB) is one of the major global public health problems, which is caused by contagious bacterium *Mycobacterium tuberculosis*. TB remains one of the top 10 causes of death worldwide in 2015. Novel biomarkers are required for rapid and specific detection of tuberculosis since 47 million lives are saved through TB diagnosis and treatment from 2010 to 2015. MTC28 (Rv0040c), a 28 kDa *Mtb* complex specific antigen, is a potential diagnostic marker against TB. The crystal structure of MTC28 at 2.8Å and 2.15Å resolutions are solved and it shares a 'mog1p' fold consisting of seven antiparallel β strands stacked between three α helices. The probable epitope site of this antigen is mapped by both computationally and experimentally. Five probable epitopes are located by molecular dynamics and simulation studies. Simultaneously, the protein is digested with trypsin and the resulting fragments are purified by HPLC. Such 10 purified peptide fragments are screened against sera from patients infected with pulmonary tuberculosis (PTB). Two out of these ten fragments namely $_{127}$ALDITLPMPPR$_{137}$ and $_{138}$WTQVPDPNVPDAFVVIADR$_{156}$ are found to be major immunogenic epitopes that are localized on the outer surface of the protein molecule and are part of a single continuous epitope. The experimentally mapped epitope is matched with two of the *in silico* predicted epitopes. Mutagenesis and antibody inhibition studies are in accordance with the results obtained from epitope mapping. MTC28 is also shown to be involved in biofilm formation in *Mycobacterium smegmatis* mc^{2}155. Increased surface hydrophobicity and decreased surface charge density are observed when MTC28 is added externally to the culture medium. This alteration of surface properties leads to cell aggregation and biofilm formation. The structural analysis of MTC28 shows that surface of the protein contains a positively charged 'V' shaped cleft, contains an α1β1 region, which might binds to the negatively charged cell surface. Mutagenesis studies confirm that the α1β1 region of the MTC28 is responsible for the biofilm forming activity. MTC28 is found to be localized on the cell wall as evident from FACS and confocal microscopy results. MTC28 along with its signal peptide is cloned and expressed in *Mycobacterium smegmatis,* which eventually secretes the protein in the culture filtrate as confirmed by western blotting and peptide mass fingerprinting. The bacterial cells (secreting MTC28) show increased biofilm formation with increased surface roughness with respect to the wild type as evident from the atomic force micrographs. Finally it is shown that MTC28 stimulates the biofilm formation ability of the cells and ultimately results in their antibiotic/drug resistance.

Keywords: *Mycobacterium tuberculosis*, *Mycobacterium smegmatis*, 28kDa antigen MTC28, X-ray crystallography, Crystal structure, Epitope mapping, Biofilm.