CHAPTER 5

BIOCHEMISTRY

Doctoral Theses

027. KULSHRESTHA (Abhishek)
Identification of Immunodominant Epitopes of Mycobacterium Tuberculosis.
Supervisor : Prof. Vijay K Chaudhary
Th 14177

Abstract

M13-g3p whole genome gene fragment phage display library of M. tuberculosis can be used for identification of epitopes using monoclonal antibodies and hyperimmunized polyclonal sera against Mtb antigens, however, it fails to select epitopes recognised by antibodies in patients' sera due to low specific antibody titres, therefore use of high density display systems like lambda display can be advantageous. Recombinant antigens of M. tuberculosis with serodiagnostic potential can be expressed in soluble form under conditions of regulated and slow growth and purified to homogeneity using a 3-step purification protocol. The gene fragment phage display approach can be successfully used for identifying immunodominant regions of individual antigens, using affinity purified patients' sera. The immunodominant fragments selected by phage display approach show better reactivities in ELISA as compared to intact protein using TB patients' sera. A combination of intact proteins and their fragments can dramatically improve diagnosis of Tuberculosis particularly in case of extra-pulmonary infections.

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028. KOUL (Sunaina)

Cloning, Expression and Characterization of Calmodulin Like Protein from Mycobacteria.

Supervisors : Dr. Hemlatha Reddy and Prof. Anil K Tyagi

Th 14277

Abstract

Includes the cloning of calmodulin like protein (CAMLP) from Mycobacterium tuberculosis $H_{37}Rv$, its overexpression in E. coli and purification of this protein to homogeneity. The study characterizes purified calmodulin like protein to ascertain that the protein has some properties akin to eukaryotic calmodulin. In eukaryotic cells, the calcium ion is well known to be involved in the regulation of many cellular processes. Calcium participates in the intracellular signaling system by acting as a diffusable secondary messenger in response to the initial stimuli. Various external signals cause transient increase in calcium levels in the cell. But this free calcium is only briefly available to act as a cellular signal, however various calcium-binding proteins immediately sequester this calcium. The short pulses of Ca^{2+} exert specific changes in cellular function and the information encoded in transient Ca^{2+} signals is deciphered by various intracellular calcium binding proteins, calmodulin being one of the important and intensively studied. Calmodulin is present in all eukaryotic cells and participates in signaling pathways that regulate crucial processes in the cell. Calmodulin was considered to be absent in prokaryotes intially, however reports of regulatory role of Ca^{2+} along with sporadic reports of calmodulin like proteins in prokaryotes indicate the role of Ca^{2+}/ calmodulin complex in prokaryotic cell. Calmodulin like protein has been identified in many prokaryotes like E. coli, Bacillus subtills, B. cereus, Streptomyuces erythraeus, Myxococcus xanthus, Bordetella pertussis, B. parapertussis, B. brochiseptica, Cyanobacteria and in many lower eukaryotes (Onek and Smith, 1992). Calmodulin like protein (CAMLP) is also present in many species of mycobacteria like M.tuberculosis, M.bovis BCG, M.phlei, M.smegmatis and M.leprae (Falah et al., 1988; Sarma et al. 1998; Reddy et al., 2003; Dhople 1999). It has been demonstrated by labelled precursors incorporation that calmodulin affect processes vital for growth of M. tuberculosis $H_{37}Rv$ i.e. synthesis of lipids, DNA and probably carbohydrates derived from glycine Trifluoperazine, a calmodulin antagonist, inhibits the growth of mycobacteria by affecting the synthesis of lipids, DNA and proteins (Ratnakar and Murthy, 1993), indicating that it

plays a crucial role in the survival of the organism. It has also been shown that TFP, a calmodulin antagonist, completely inhibits growth of M. tuberculosis as well as M. tuberculosis resistant to isoniazid (Ratnakar and Murthy, 1992). Similar role of calmodulin like protein has been demonstrated in M. leprae (Dhople, 1999). This study was conducted to clone, purify and characterize calmodulin like protein from M. tuberculosis H_{37}Rv.

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