ADENYLYL CYCLASE IN *MYCOBACTERIUM TUBERCULOSIS* H37Rv - A POSSIBLE VIRULENCE FACTOR.

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ABSTRACT

Eukaryotic-like adenylyl cyclase has been identified in *Mycobacterium tuberculosis* H37Rv. The *M. tuberculosis* adenylyl cyclase gene (*cya*) was cloned by complementation of a cAMP dependent expression of maltose operon in a *cya* deletion *E. coli* strain. The nucleotide sequence analysis of the *cya* gene revealed an open reading frame that coded for 443 amino acids. A comparison of *M. tuberculosis* adenylyl cyclase with the protein sequences in the data bank revealed its homology to the eukaryotic Type I and the calmodulin responsive adenylyl cyclases with an average of about 40% identical and 60% similar amino acids in the catalytic domain. The occurrence of eukaryotic-like adenylyl cyclase in *M. tuberculosis* suggests a role for this important enzyme in cell signaling and perhaps in the pathogenesis of *M. tuberculosis*. We suggest that the human pathogen *M. tuberculosis* might have acquired the eukaryotic *cya* by a recombination event.

KEY WORDS : cAMP, signal transduction

INTRODUCTION

Mycobacterium tuberculosis and *Mycobacterium leprae*, the most prevalent pathogenic species of mycobacteria, cause devastating human diseases tuberculosis and leprosy, respectively. Tuberculosis is the leading cause of death from a single infectious agent and it is believed that about one third of the human population is infected with tubercle bacilli (1). The immunological and biochemical events in the clinical manifestation of tuberculosis remain at large elusive. A number of laboratories are currently engaged in understanding the molecular mechanisms in the pathogenesis of tuberculosis. A comparison of differentially expressed genes (*dev*) in a virulent species *M. tuberculosis* H37Rv and its avirulent

derivative *M. tuberculosis* H37Ra is beginning to provide valuable information for further exploring the role of these *dev* gene products in the pathogenicity of tuberculosis (2). Lipoarabinomannan was postulated to be a virulence factor involved in the persistence of *M. tuberculosis* within the host macrophages (3).

In the bacterial infections caused by *Bordetella pertussis* and *Bacillus anthracis*, a secreted form of adenylyl cyclase toxin has been shown to play a critical role in the pathogenicity of the respective disease. For example, in whooping cough caused by *Bordetella pertussis* the secreted adenylyl cyclase is activated by the host calmodulin thereby increasing the cellular concentration of cAMP which in turn triggers a number of events in the signal transduction and compromises the host immune response, enabling the bacterium to become established (4). Similar events have been shown to occur in the disease anthrax caused by *Bacillus anthracis* (5). In our search for the molecular

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events leading to the pathogenicity of tuberculosis, we cloned and sequenced the gene for adenylyl cyclase from *M. tuberculosis* H37Rv.

Cloning of the *cya* gene:

M. tuberculosis chromosomal DNA was partially digested with Sau3AI restriction endonuclease and the fragments in the 2-5 kbp range were purified from an agarose gel. A library of clones was constructed by ligation of the fragments into the BamHI site of pBR322 vector. A strain of *E. coli* TP610 deleted for adenylyl cyclase gene was electroporated with the ligation products and screened for the cAMP-dependent maltose fermentation on MacConkey agarose plates. Recombinant plasmids were purified from eight red colonies considered to be putative *cya* positives. The insert DNA fragment in each plasmid was sequenced with the pBR322 primers flanking the BamHI site. All the eight clones had sequences that begin within 200 bp of a location in the chromosome but had varying lengths from 1.0 to 3.4 kbp. The cAMP synthesis by the *in vitro* adenylyl cyclase assay in the extracts of TP610 harboring one or the other of eight clones was surprisingly barely above the detection level but apparently sufficient enough to turn "on" the maltose operon. Restoration of catabolic defect with all these clones was dependent on cAMP receptor protein since a *crp* deletion strain failed to ferment maltose and lactose in a cAMP dependent manner (Table 1). This observation is reassuring that the clones we obtained so far indeed code for a portion of adenylyl cyclase, as a fusion protein with 97 amino acids of tetracycline resistance protein, capable of synthesizing cAMP. When one of the open reading frames (orf - 200 aa) beginning at the 5' end of the 3.4 kbp clone Mtb *cya* 1 was submitted to Protein Identification Resource, this orf exhibited astonishingly high degree of similarity (40% identical amino acids) with the eukaryotic adenylyl and guanylyl cyclases but none whatsoever with any of the known prokaryotic adenylyl cyclases.

Isolation of full length *cya* gene from *M. tuberculosis* H37Rv:

Since there were no apparent translation initiation signals corresponding to this orf and also the size of the protein is too small compared to the other known adenylyl cyclases, we concluded that the full length *cya* gene was not obtained under the transcription control of the constitutive *tet* promoter in pBR322. This observation is in accordance with our previous finding that the uncontrolled expression of adenylyl cyclase with concomitant overproduction of cAMP is lethal to *E. coli* cells (6). We therefore designed the following strategy to obtain the portion of the *cya* gene coding for the amino terminus of Mtb adenylyl cyclase.

From the nucleotide sequence of the Mtb *cya*1, we have identified a unique BamHI restriction endonuclease sequence about 770 bp from the 5' end of the Mtb *cya* 1. A 22 mer oligonucleotide was synthesized from this region, 5' end labeled with $\gamma^{32}\text{P}$ -ATP and used as a probe in Southern hybridization of the chromosomal DNA completely digested with BamHI. A 2.6 kbp fragment was identified on the Southern blot and the fragment was cloned into the BamHI site of the pRE1 vector (6). About 10,000 recombinants in C600 lambda lysogen were screened with the oligonucleotide probe and positive colonies were purified and rescreened. Plasmids were isolated from pure colonies and sequenced.

Complete coding sequence of Mtb *cya* gene:

The complete coding sequence of *M. tuberculosis cya* gene was assembled from the overlapping sequences of the clones obtained in pBR322 and pRE1 vectors. A putative open reading frame of 443 amino acids with GTG as an initiation codon and terminating with TGA was identified. Comparison of the full length Mtb adenylyl cyclase amino acid sequence with the protein sequences in the data bank revealed its close similarity again to eukaryotic Type I and calmodulin sensitive adenylyl cyclases and guanylyl cyclases.

Table 1 : cAMP dependent fermentation of sugars of the catabolic operon.

Plasmid	<i>cya</i>	Maltose <i>cya / crp</i>	<i>cya</i>	Lactose <i>cya / crp</i>
Mtb 1	+	-	++	-
Mtb 2	+	-	++	-
Mtb 3	+	-	++	-
Mtb 4	+	-	++	-
Mtb 5	+	-	++	-
Mtb 6	+	-	++	-
Mtb 7	+	-	++	-
Mtb 8	+	-	++	-
pBR322	-	-	-	-
pDIA100 (<i>E. coli</i> AC)	+++	-	+++	-

+, ++, +++ indicates strength of fermentation; — indicates no fermentation.

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