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Research paper

Interaction analysis of TcrX/Y two component system from *Mycobacterium tuberculosis*

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ABSTRACT

TcrX/Y is one of the twelve two component system (TCS) present in *Mycobacterium tuberculosis*. We have investigated the TcrX/Y interaction by *in silico* studies, pull down assay, radioactive phosphotransfer, surface plasmon resonance as well as crosstalk analysis of TcrY with TcrA – a non-cognate response regulator. Sequence alignment of TcrY with other histidine kinases revealed His256 as the residue responsible for autophosphorylation. The modeled structure of TcrX/Y was docked with each other by GRAMM-X revealing the interaction of TcrY/His256 with TcrX/Asp54. TcrY dimerization via the formation of four helix bundle was also observed by protein–protein docking. Autophosphorylation of TcrY has been observed followed by the phosphate transfer from TcrY to TcrX. The phosphorylation process required divalent metal ions like Mg^{2+} or Ca^{2+} ions as evident from the radioactive phosphorylation studies. Interaction was not observed between TcrY and TcrA suggesting the signal transduction process is specific in TcrX/Y system. TcrY hydrolyzes ATP and the K_m value has been found to be 10 mM which is comparable to that of Hsp104. TcrX/Y interaction has been determined by surface plasmon resonance and dissociation constant (K_D) was evaluated to be 3.6 μM. We conclude from our results that TcrX and TcrY are part of the same signal transduction pathway without their involvement in crosstalk with non-cognate counterpart.

1. Introduction

Two component signal transduction systems process the signals resulting from the stress developed in the complex environment of a bacterium [1]. This system involves the histidine–aspartate phosphorelay method of signal transduction between a histidine protein kinase (HK) and a phospho-aspartyl response regulator (RR) in response to the external stress [2]. The HK reacts to the environmental stimuli by autophosphorylating itself at a histidine residue. The high energy phosphoryl group thus formed is signaled to the conserved aspartate residue of the RR. The phosphorylated RR then undergoing a conformational change results in the altered activities of its associated domains and finally elicits the specific signals. The autophosphorylation of histidine kinases involves an intermolecular phosphorylation reaction requiring formation of a dimer or oligomer [3]. HKs are of two classes namely the orthodox and hybrid kinases. The main representative of the orthodox kinase is EnvZ from *Escherichia coli* consisting of membrane bound N-terminal signal sensing HAMP linker and a C-teminal cytoplasmic catalytic domain. Orthodox kinases can be cytoplasmic like chemotaxis kinase CheA. The hybrid kinases are mostly found in eukaryotes. They are involved in multiple phosphoryl transfer processes. The kinase catalytic region is composed of a dimerization domain and an ATP binding domain. HK cytoplasmic architecture is represented by H, N, G1, F, G2 boxes. H box is a part of the dimerization domain containing the conserved histidine for most HKs. N, G1, F, and G2 boxes are involved in ATP binding [4,5]. The ATP binding core of HKs is structurally similar to the ATPase domain of Hsp90 and DNA gyrase B [6]. RRs consist of two domains; receiver and effector domain. The receiver domain comprises of the conserved aspartate for phosphorylation whereas the effector domain has a transcriptional regulatory function. OmpR being the most common example of a RR acts both as an activator and a repressor [5].

Abbreviation: ATP, Adenosine triphosphate; GST, Glutathione S-transferase; His_6, Hexahistidine; HK, Histidine kinase; IPTG, Isopropyl β-D-1-thiogalactopyranoside; LDH, L-lactate dehydrogenase; NADH, Nicotinamide adenine dinucleotide-reduced; PBS, Phosphate buffered saline; PEP, Phosphoenolpyruvate; PK, pyruvate kinase; PMSF, Phenylmethylsulphonyl fluoride; RR, Response regulator; TCS, Two component system.

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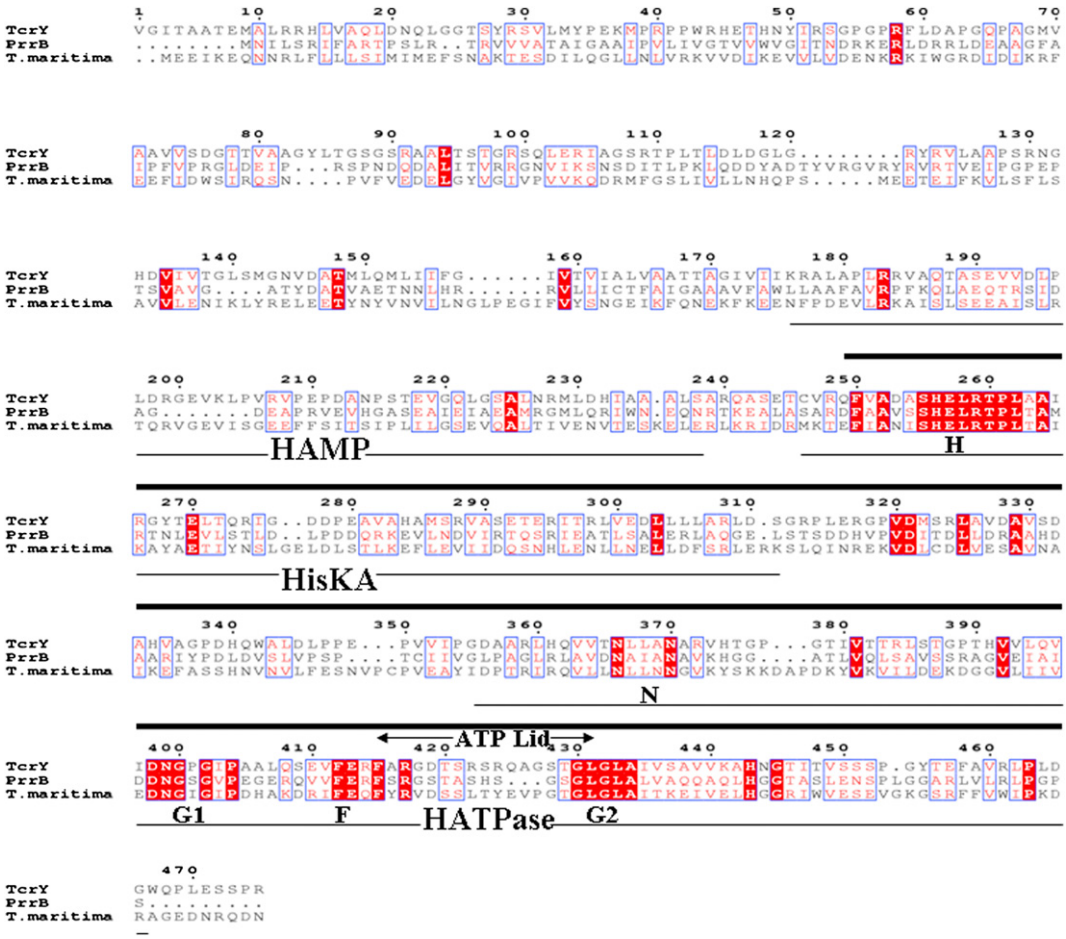

Fig. 1. Amino acid sequence alignment of TcrY with only the HKs having PDB codes from *M. tuberculosis* and *T. maritima*. The conserved boxes are highlighted in red. The domains and boxes are indicated by black lines. ATP lid is shown in solid black. The modeled cytosolic region excluding HAMP linker is indicated in solid black rectangle [For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article].

In *Mycobacterium tuberculosis* H37Rv genome [7], eleven paired two component regulatory system (TCS) genes, two orphan histidine kinase genes, and five orphan response regulator genes have been reported earlier [8]. A new TCS, PdtaR/S has been reported recently [9] making it to twelve known TCS from the organism [10]. Among the TCS, *prrA–prrB* is expressed during growth of *M. tuberculosis* in human; *mtrA* and *senX3/regX3* are required for virulence; PhoP positively regulates the complex lipid biosynthesis in Mycobacteria [11–14]. In *M. tuberculosis*, HK–RR interaction has been observed in PdtaR/S, TrcR/S, SenX3/RegX3, MprA/B, PrrA/B, DevR/S, PhoP/R, Rv0600c/Rv0601c/Rv0602c TCS [9,15–21].

Two adjacent ORFs Rv3765c and Rv3764c encoding RR (TcrX) and HK (TcrY) respectively, is conserved in all species of Mycobacteria except *Mycobacterium leprae* [8]. The gene *tcrX* is significantly induced under iron-limitation [22] and *tcrX* expression has been observed during the post-infection period [23]. In the severe combined immuno deficient (SCID) mouse model, the deletion of *tcrX/Y* shows an increase in virulence, with significantly shorter survival times [24] implying that TcrX/Y interaction may be involved in regulating the genes required for suppressing the intracellular growth of *M. tuberculosis* under physiological conditions.

The present study focuses on the ATPase, autokinase activity of TcrY, phosphorylation of TcrX by Phospho-TcrY and crosstalk analysis of TcrY with non-cognate response regulator TcrA encoded by Rv0602c. Surface Plasmon Resonance has been used to study the dissociation constant (K_D) of TcrX/Y interaction. This is the first report of K_D evaluation in a TCS interaction from *M. tuberculosis* by surface plasmon resonance.

2. Materials and methods

2.1. Material

Phosphoenolpyruvate, L-lactate dehydrogenase, pyruvate kinase, Nicotinamide Adenine Dinucleotide-reduced, Adenosine triphosphate, Isopropyl β-D-1-thiogalactopyranoside, Phenylmethylsulphonyl fluoride, leupeptin, pepstatin, aprotinin, reduced glutathione were purchased from Sigma–Aldrich. Thrombin protease was purchased from GE Healthcare. Glutaraldehyde was purchased from Merck. [γ-^{32}P]ATP (>3500 Ci/mmol) was purchased from BRIT, India.

2.2. In silico studies

The primary sequences of the TcrX/Y were obtained from TB consortium and were analyzed for domain boundaries by SMART (http://www.doe-mbi.ucla.edu/TB/). For defining the boundaries of conserved boxes, sequences of the TcrX/Y were aligned only with the