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Rv1027c–Rv1028c encode functional KdpDE two – Component system in *Mycobacterium tuberculosis*

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ABSTRACT

In *Mycobacterium tuberculosis Rv1027c–Rv1028c* genes are predicted to encode KdpDE two component system, which is highly conserved across all bacterial species. Here, we show that the system is functionally active and KdpD sensor kinase undergoes autophosphorylation and transfers phosphoryl group to KdpE, response regulator protein. We identified His^{642} and Asp^{52} as conserved phosphorylation sites in KdpD and KdpE respectively and by SPR analysis confirmed the physical interaction between them. KdpD was purified with prebound divalent ions and their importance in phosphorylation was established using protein refolding and ion chelation approaches. Genetically a single transcript encoded both KdpD and KdpE proteins. Overall, we report that *M. tuberculosis* KdpDE system operates like a canonical two component system.

1. Introduction

Mycobacterium tuberculosis, the causative pathogen of the disease tuberculosis, affects almost one third of the world's population. Adaptation to the ever-changing environment within the host, orchestrated by intricate signalling network is one of the strategies utilized by the bacillus for successful survival and infection. One of the major signalling systems in *M. tuberculosis* is two component system (TCS) which contains a sensor kinase protein capable of detecting a signal, paired with a response regulator protein which brings about adaptive response mostly by transcription modulation. These proteins communicate through a well characterized phosphorylation and phosphoryl group transfer process [1]. *M. tuberculosis* has eleven paired two component systems of which two systems viz. KdpDE and NarSL, named based on their homology with the corresponding *Escherichia coli* two component systems, are still not characterized [2] and it is not known if they encode functional signaling proteins.

The yet uncharacterized KdpDE two component system is highly conserved and has been reported to be present in over 1000 bacterial species [3] and responds to a wide variety of stimulus including K^+ ions limitation [4,5], osmotic stress [4,6], turgor pressure [7,8], ATP concentration [9], population density [9], growth temperature [9,10], etc. The system has been shown to regulate virulence or stress resistance in many human pathogens [11–14]. In *M. tuberculosis*, the response regulator protein KdpE was found essential for optimal growth on defined media [15], whereas KdpD is required for *in vivo* survival [16] and a *kdpDE* mutant strain showed hypervirulent phenotype in SCID mouse [17]. Further, *kdpE* is differentially regulated during growth in human macrophages [18] and it is upregulated after 96 h of starvation in an *in vitro* nutrient model [19], hinting towards a possible role in persistence of the tubercle bacilli.

In the present manuscript, we report biochemical characterization of the KdpDE system of *M. tuberculosis* and establish that it is a bonafide and functional two component system.

2. Materials and methods

All cloning steps were carried out in *E. coli* DH10β cells (Invitrogen), while Origami B (Novagen) was used for protein expression. Ampicillin 100 μg/ml was used when required.

2.1. Sequence analysis and structure prediction

Sequences for *kdpD* and *kdpE* genes and proteins were retrieved from Tuberculist server [20] and analyzed on SMART server to identify the domain architecture. Homology modeling of cytosolic domain of KdpD and full length KdpE proteins was performed using Phyre^2 server [21] and the modeled structure was visualized using PyMOL software.

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2.2. Recombinant plasmid construction

Full length *kdpE* gene (681 bp) encoding 226 amino acid protein and the cytosolic domain of *kdpD*, coding for catalytic region (from 1802 bp to 2583 bp) corresponding to a protein of 261 amino acids (position 602–860 aa) were amplified from *M. tuberculosis* H37Rv genome using gene specific primers containing *Nco*I/*Hin*dIII or *Nco*I/*Kpn*I restriction sites respectively. Both the genes were cloned in pPROEx-HTa vector (primer details in Supplementary Table 1). H642Q mutation in the KdpD and D52N or D8N mutations in KdpE expression plasmids were introduced using QuickChange Site-Directed Mutagenesis protocol. The mutants were confirmed by DNA sequencing.

2.3. Protein expression and purification

For protein expression, each clone was grown at 37 °C,180 rpm, in 2× YT broth till OD_{600} of 0.8–0.9 followed by 1 mM IPTG addition. The culture was incubated for 15–20 h at 12–15 °C. The proteins were purified under native conditions by Ni^{+2}-NTA chromatography as per manufacturer's protocol (Qiagen Inc). The purified proteins were dialyzed against storage buffer (50 mM Tris–HCl, pH 8.0, 50 mM NaCl, 1 mM DTT, and 50% glycerol) and stored at −20 °C.

2.4. KdpD autophosphorylation

KdpD (150 pmol) was incubated in the autophosphorylation buffer (50 mM Tris–HCl, pH 8.0, 50 mM KCl, 10 mM $MgCl_2$) containing 50 μM ATP and 1 μCi of [γ-^{32}P] ATP for indicated time periods at 30 °C. Reaction was terminated by adding 1× SDS–PAGE gel loading buffer and kept on ice. For analysis, the reaction mixture was resolved on 15% SDS–PAGE at 150 V, the gel was washed with water and exposed to an image film (Fujifilm, Japan) followed by phosphoimaging (Typhoon, GE Healthcare, USA). For quantitative measurements, densitometry was performed using ImageJ software.

2.5. Transphosphorylation of KdpE by KdpD

KdpD (150 pmol) was autophosphorylated for 60 min as described above in 10 μl reaction volume, then KdpE (150 pmol) was added to the reaction mixture in total volume of 20 μl and incubated at 30 °C for the indicated time periods. Analysis was done as described above.

2.6. Autophosphorylation of KdpE with acetyl phosphate

Radiolabeled acetyl phosphate was generated as described before [22] and KdpD phosphorylation was performed as described previously [23].

2.7. Surface plasmon resonance analysis

KdpE protein (~500 ng) was immobilized on the CM5 chip as per manufacturer's protocol and its interaction with KdpD protein, in phosphorylated, non-phosphorylated and the mutant form was evaluated using Biacore instrument (GE Healthcare).

3. Results

3.1. KdpDE locus organization

Loci organization of *kdpDE* genes in five mycobacterial genomes was analyzed and compared with organization in *E. coli*. Except for *M. bovis* and *M. tuberculosis*, where the *kdpDE* genes were divergent to the *kdpFABC* operon, in other bacteria the *kdp* genes were organized in a unidirectional arrangement where *kdpABC*/*FABC* and KdpDE genes are adjacent to each other and perhaps form a part of an operon (Supplementary Fig. 1). We also confirmed the presence of homologues of these proteins across various mycobacterial species, except for *M. leprae* and *M. ulcerans* where they were missing (Supplementary Table 2).

3.2. Structural motifs prediction

KdpD and KdpE protein sequences were analyzed using SMART server. Domain organization of KdpD sensor kinase, which is a large protein of 860 aa, revealed presence of an large N-terminal domain, three transmembrane domains followed by catalytic histidine kinase domain and ATPase domain which are conserved across many mycobacterial species. Similarly, KdpE response regulator protein sequence was analyzed and a transcriptional regulatory domain of 76 amino acids in C-terminal end was predicted along with a conserved N-terminal receiver domain (Fig. 1 and Supplementary Fig. 2).

3.3. Functional characterization of KdpDE two component system

Known biochemical properties of the typical sensor kinases (SK) and response regulator (RR) proteins were utilized to validate the functionality of the KdpDE system [24]. Towards this, both KdpE and KdpD were overexpressed as 6×-his tagged fusion proteins in *E. coli*. Since full length KdpD protein is predicted to contain 3 transmembrane domains (Fig. 1A), we used only the C-terminal catalytic domain. It has been shown previously that the C-terminal domain of SKs is active and can be used for functional characterization of two component system proteins [25]. Both KdpD and KdpE proteins were obtained in soluble form and the overexpressed proteins were purified using Ni^{+2}-NTA affinity chromatography and were confirmed to be >90% pure by SDS–PAGE (Fig. 1B).

KdpD protein autophosphorylation analysis was performed using γ^{32}P-labeled ATP, wherein the labeled γ-phosphate from ATP is transferred to a conserved histidine residue of the sensor kinase protein. The occurrence of phosphorylation is detected by the presence of radiolabeled protein on the autoradiogram. In the time course experiment, KdpD incorporated maximal γ^{32}phosphoryl moiety in 4 h and remained stable for 24 h with 50% incorporation taking place within 1 h of reaction initiation (Fig. 2A). The reduction in the signal at 24 h was further examined by incubating the KdpD protein in the assay buffer without ATP for 24 h followed by autophosphorylation with labeled ATP. This revealed that KdpD did retain its activity even after prolonged incubation in the reactions conditions and can undergo phosphorylation similar to fresh protein (Supplementary Fig. 3) indicating that loss of signal at long time points is perhaps due to auto-dephosphorylation. We confirmed that the bands on autoradiograms were not due to physical binding of ATP, by heating the sample in SDS–PAGE sample buffer before loading. We further confirmed this by using α^{32}P-ATP in autophosphorylation assays which failed to show any radiolabel incorporation in KdpD (not shown). Interestingly, we detected presence of labeled dimeric species on longer incubations suggesting presence of stable oligomeric species (Fig. 2A) which was further confirmed by glutaraldehyde cross-linking, where stable dimers of the KdpD SK were detected (Supplementary Fig. 4).

The phosphorylated KdpD was used to assess the functionality of RR, KdpE through a His-to-Asp phosphotransfer reaction. The reaction involves direct transfer of phosphoryl moiety from His of the active SK to a conserved Asp residue on the RR protein [1]. To confirm this, 150 pmol of KdpD was autophosphorylated with radiolabeled ATP for 60 min followed by mixing with equimolar amount of KdpE, for different durations before analysis. In this

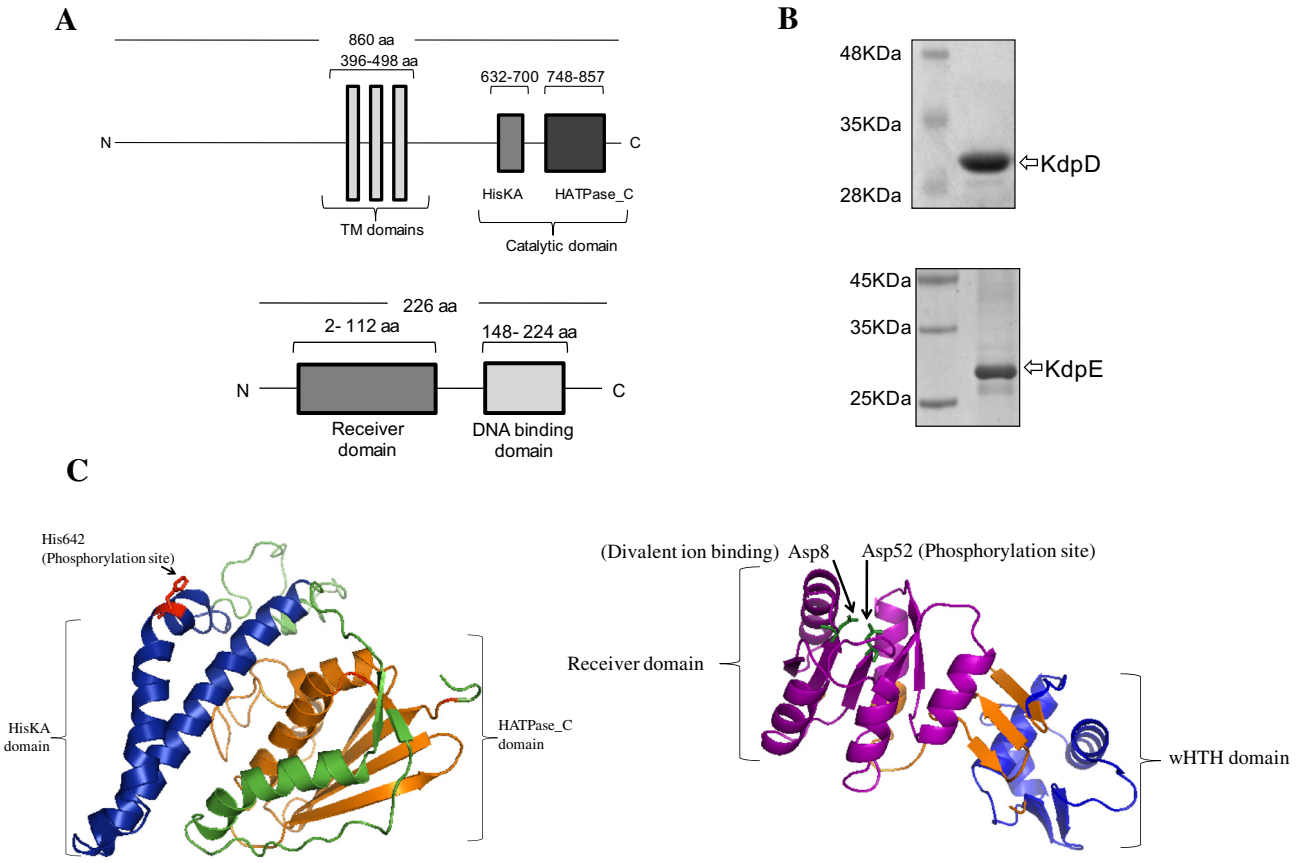

Fig. 1. Features and purification of KdpD and KdpE proteins. (A) Domain organization of KdpE and KdpD proteins of *M. tuberculosis* H37Rv. KdpD (top panel) has three transmembrane domains (TM); a histidine kinase domain (HisKA); and a histidine kinase-type ATPase catalytic domain (HATPase_C). KdpE (Bottom panel) has a receiver domain (Rec) and a winged helix-turn-helix DNA binding domain (wHTH). (B) SDS–PAGE analysis of purified cytosolic fragment of KdpD (top panel) and purified full length KdpE (bottom panel) proteins. The proteins were overexpressed and purified as described in Section 2. (C) 3-D structure model of KdpD (cytosolic fragment) and KdpE. The locations of the residues critical for phosphorylation activities are marked. Left, modeled structure of KdpD (cytosolic fragment from 602 to 860 aa). Blue portion represents His KA domain containing the conserved His^{642} and orange represents the HATPase_C domain. Right, modeled structure of KdpE. Purple portion represents receiver domain and blue represents winged helix-turn-helix DNA binding domain. Two conserved Aspartate residues Asp^{52} and Asp^{8} critical for phosphorylation are marked. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reaction, the transfer of γ^{32} labeled phosphoryl group from KdpD to KdpE is detected by the appearance of a band corresponding to the position of KdpE and also a decrease in signal of the KdpD (Fig. 2B). We observed robust phosphorylation of the KdpE protein almost immediately which peaked at 2 min (Fig. 2B), validating that KdpE is active and serves as a cognate RR of KdpD and can potentially regulate gene expression through a phosphorylation switch event (Fig. 2B). Surprisingly, the phosphorylated KdpE was quite stable in 20 min experimental window, which is unlike what is reported for many other SK–RR pairs [26]. To confirm that KdpE is not binding to ATP or accepting phosphoryl group directly from ATP, KdpE protein was incubated with ATP in the reaction buffer without KdpD as described above. As expected, KdpE failed to retain any radiolabel when analyzed (not shown). Overall, we demonstrated that both SK and RR Kdp proteins are active and encode a 'bonafide' two component system (TCS).

3.4. Residues involved in phosphorylation of KdpDE TCS

Sequence comparison allowed us to identify His^{642} and Asp^{52} as putative phosphorylation sites in KdpD and KdpE respectively (Supplementary Fig. 1). To confirm this, H642Q substitution in KdpD and D52N in KdpE were made by site directed mutagenesis and the mutated proteins were tested for phosphorylation. Both the mutated proteins were defective in their phosphorylation capacities, confirming that the predicted sites are indeed the sites of phosphorylation (Fig. 3A, right panel).

To address possibility of loss of protein activity simply due to loss of protein structure, we analyzed the chemical nature of phosphoryl bond on the SK and RR proteins. Phosphoacyl bond, formed during RR phosphorylation on an aspartate residue and the phosphoramidate bond, formed by histidine phosphorylation on SK have different chemistries from the phosphoester bonds [1,27]. In our experiments we found that while KdpD∼P species was less stable in the presence of alkali, KdpE∼P was labile in acidic condition (Fig. 3B), confirming that the phosphorylation had indeed occurred on His and Asp residues of KdpD and KdpE respectively.

3.5. Divalent ions in KdpDE activity

To confirm that KdpDE system also uses divalent metal ions for their catalytic activities [1], we initially tested for autophosphorylation of KdpD in absence of Mg^{+2} ions and surprisingly observed distinct phosphorylation (Fig. 3C, left panel). The activity was detectable even with 25 mM EDTA, suggesting that either divalent ions were not necessary for KdpD activity or the purified protein contains prebound divalent ions. To address this, we denatured KdpD and then renatured it on Ni^{+2}-NTA matrix in the absence of

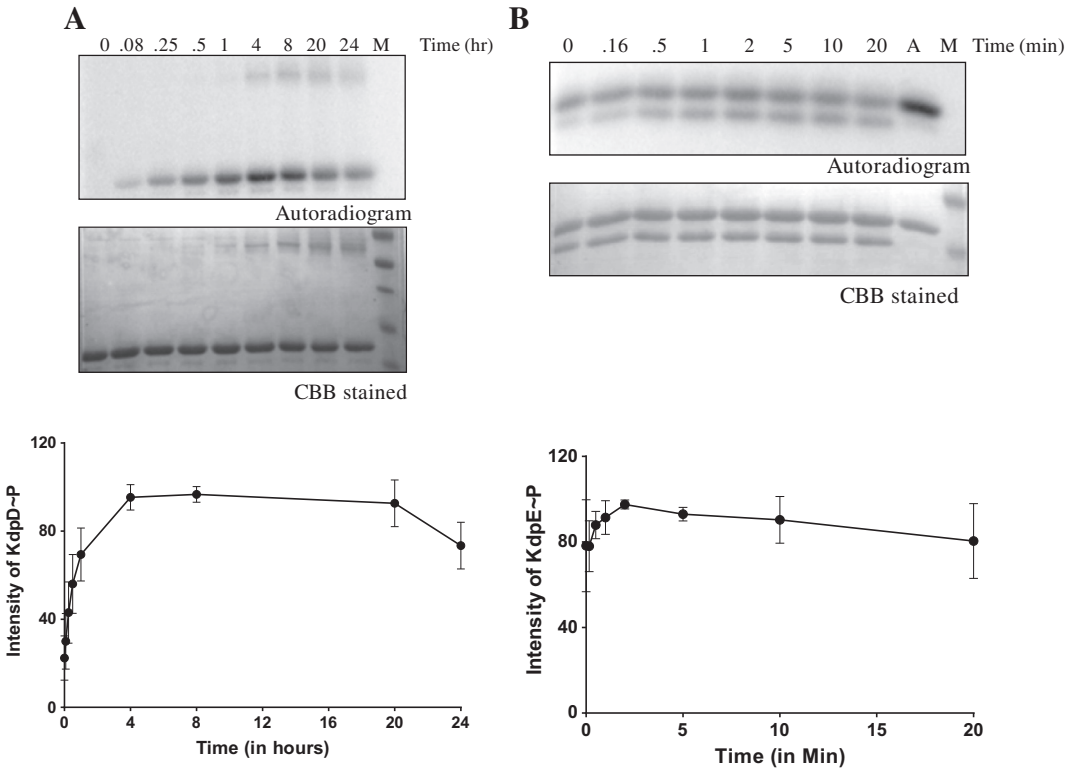

Fig. 2. Phosphorylation analysis of the KdpD and KdpE proteins. Reactions were set up as described in Section 2. (A) KdpD autophosphorylation time course. (B) Time course of phosphotransfer of phosphoryl group from KdpD~P to KdpE. (M represents Marker and A represents autophosphorylation control). Top panel shows the autoradiogram and the corresponding Coomassie Brilliant Blue (CBB) stained gel. Lower panel shows densitometric plot of phosphorylated protein from the autoradiogram as a function of time.

any divalent ion, using an on-column refolding approach [28]. The refolded protein was inactive in the autophosphorylation reaction in the absence of Mg^{+2} ions, but regained activity when Mg^{+2} ions were added (Fig. 3C, left panel). This confirmed that Mg^{+2} ions were critical for activity of KdpD and the protein was purified from *E. coli* with pre bound divalent ions. Similarly, the ionic requirement was also tested for phosphotransfer reaction and no transfer to RR was detected in presence of EDTA, thereby confirming the essentiality of divalent ions in phosphotransfer reaction (Fig. 3C, right panel).

To evaluate if other divalent ions can substitute for Mg^{+2}, Mn^{+2} and Ca^{+2} ions were tested. While Ca^{+2} was able to weakly catalyze both the reactions, Mn^{+2} could only support weak autophosphorylation of KdpD (Fig. 3D). This suggested that Mg^{+2} is the preferred ion in KdpDE phosphorelay similar to many other two component systems.

3.6. Structure prediction

Using the sequence based structure homology modeling, we generated a 3D protein structure model for both KdpD and KdpE proteins, which allowed us to predict Asp^{8} as the critical ion binding aspartate residue in the KdpE (Fig. 1C). The prediction was confirmed by generating $KdpE^{D8N}$ mutant which was found be defective in phosphorylation (Fig. 3A, left panel).

3.7. KdpD and KdpE protein interaction

Occurrence of phosphotransfer reaction mandates physical interaction between the SK and RR proteins, which we examined using surface plasmon resonance. We evaluated KdpD–KdpE interaction using a CM5 chip in which KdpE protein was immobilized and KdpD protein was used as an analyte. Supplementary Fig. 5A shows that the KdpD and KdpE proteins interact in basal conditions and the interaction is weaker when KdpD is phosphorylated (Supplementary Fig. 5B), indicating that phosphorylation induces dissociation of KdpD from KdpE to initiate the effector activities of the latter. We observed very weak interaction between the phosphorylation defective $KdpD^{H642Q}$ and KdpE (Supplementary Fig. 5C), suggesting that either His residue is critical for basal state interaction, or the mutant KdpD is structurally disturbed hence fails to interact with response regulator. To ascertain that the interaction of two component proteins detected in our setup is relevant, we confirmed the interaction for another two component system, TcrX–TcrY as reported previously [29].

3.8. Phosphorylation of KdpE with acetyl phosphate

We tested if KdpE can accept phosphate from acetyl phosphate and unlike KdpE protein of *E. coli* and *Clostridium acetobutylicum* [30], KdpE of *M. tuberculosis* failed to undergo phosphorylation through acetyl phosphate, suggesting towards differences in activities between the two proteins despite of having high sequence homology (Supplementary Figs. 2 and 6).

3.9. Co-Expression analysis and regulation of KdpD and KdpE genes

As shown in Supplementary Fig. 1, *KdpDE* gene organization suggests existence of co-transcription, which we evaluated by using a combination of intergenic primers. Primer P1 which mapped inside *kdpE* gene and primers P2/P3 which mapped inside in *kdpD* gene were used in pairs in a RT–PCR reaction using RNA isolated from *M. bovis* BCG grown in conditions of K^{+} limitation. As represented in the Fig. 4, we detected presence of single transcript, using both P1/P2 and P1/P3 primer pairs and confirmed

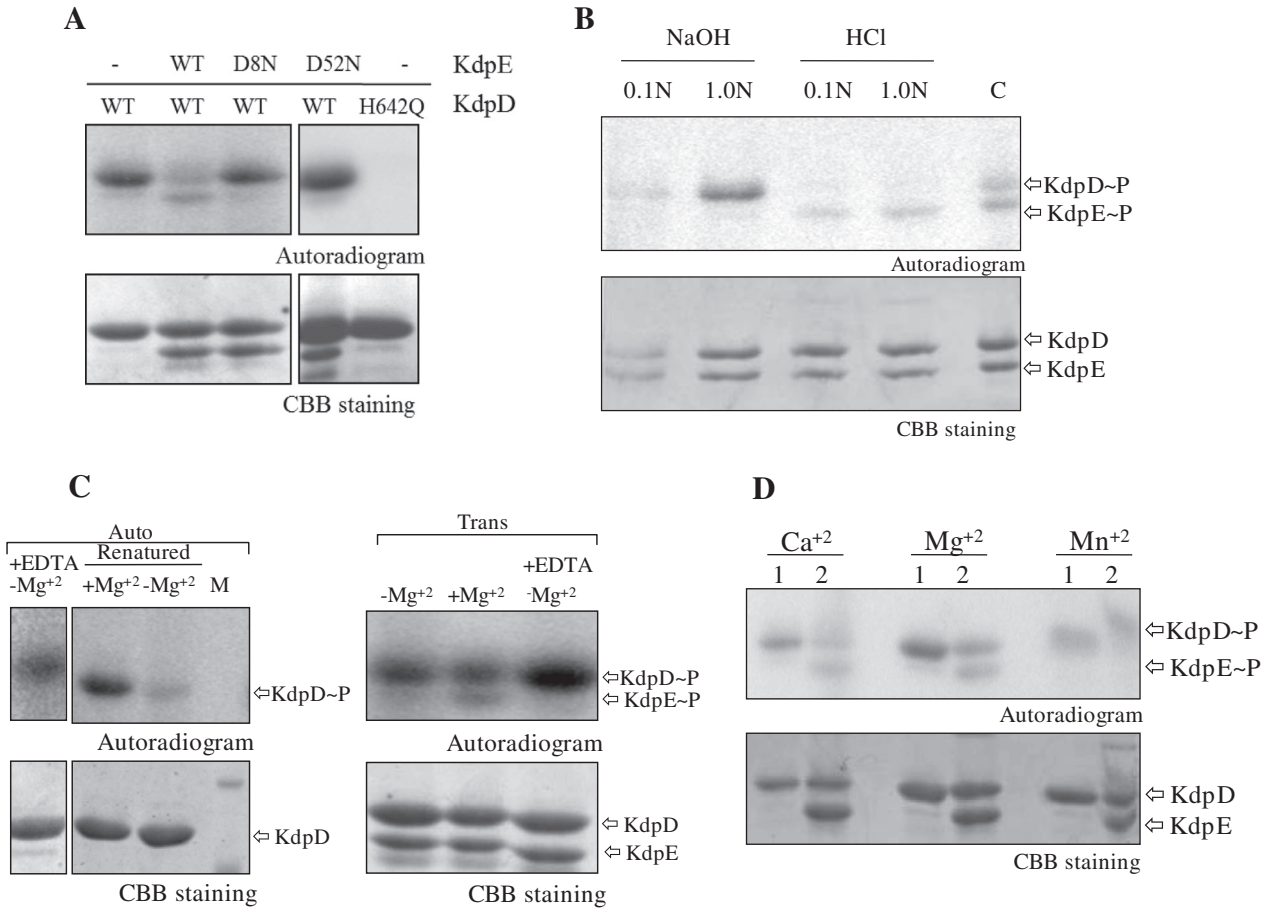

Fig. 3. Characterization of KdpD and KdpE phosphorylation. (A) Residues involved in phosphorylation reactions. Mutant proteins were generated as described and tested in the reaction similar to the wild type proteins. (B) Effect of acid and alkali on phosphorylated KdpD and KdpE. Phosphorylated proteins were treated with HCl (acid) or NaOH (alkali) for 60 min at indicated concentrations at 37 °C. C, control untreated samples (C) Essentiality of divalent cations in phosphorylation reactions. Autophosphorylation (left), transphosphorylation (right) reactions were set up with or without divalent ions and analyzed. (D) Effect of various divalent ions in auto and trans phosphorylation reactions. Phosphorylation reactions were performed in the presence of Ca^{2+}, Mg^{2+} or Mn^{2+} ions at 10 mM concentration. All reactions were set up as described in materials and methods. Top panel shows the autoradiogram and lower panel shows the corresponding CBB stained gel.

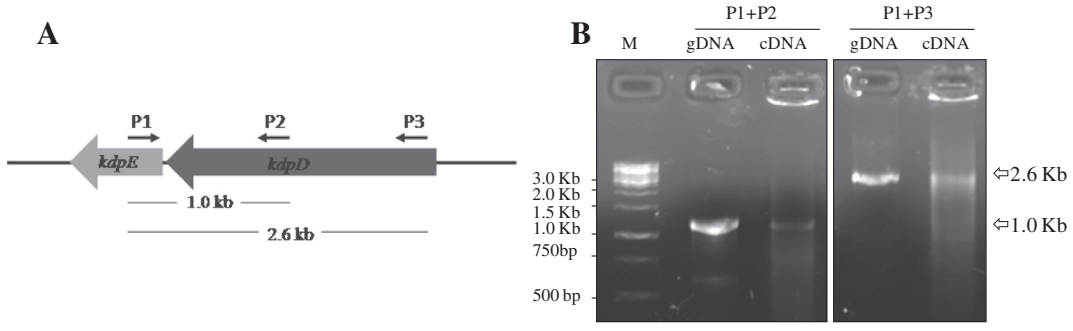

Fig. 4. Co-transcription of *kdpD* and *kdpE* genes. (A) Location of primer pairs used to study co-transcription is indicated. (B) PCR analysis for co-transcription using cDNA prepared from *M. bovis* BCG showing the presence of common transcript for *kdpDE*. Genomic DNA from *M. bovis* BCG was used as a positive control.

that the genes are expressed as an operon (Fig. 4) and perhaps have a common upstream promoter region. We also tested for autoregulatory nature of the *kdpDE* operon by examining the binding of KdpE with the intergenic region (between *kdpDE* and *kdpABC* operons). We failed to detect any binding KdpE protein under the tested conditions indicating that perhaps the system is not autoregulated or some other accessory factor is needed for the regulatory function which is not included in our experiments (not shown).

4. Discussion

KdpDE two component system is classically associated with responses to stimuli like low levels of potassium ion, osmotic imbalance, acid and nutrient stresses. The adaptive responses by this two component system are mediated through regulation of the *kdpFABC* operon expression (which encodes for K^+ transporter proteins) amongst many other target genes [9]. Since it is of advantage to couple the structural genes tightly with the signal transduction

systems, in most bacteria the regulatory and structural *kdp* genes are generally present as a single operon. However, it is not the case for *kdpDE* system of *M. tuberculosis* where the operon is divergent with regulatory and structural genes arranged in opposite orientations. This arrangement proposes possibility of independent regulation of *kdpDE* and *kdpABC* genes, which was strengthened by absence of direct binding of KdpE to the common promoter region.

At the biochemical level, the KdpDE two component system of *M. tuberculosis* is not well studied, though one report showed interaction of the C-terminal domain of KdpD and RR KdpE using yeast two hybrid assay [31] and another reported the interaction of the SK with LRP protein [32]. Besides these associations, there are no evidences for the functionality of this system and it is still a putative two component system. The present study establishes that the system is a bonafide two component system of *M. tuberculosis*, and KdpD undergoes autophosphorylation at His^{642} residue in presence of Mg^{+2} ions. The phosphoryl group is then transferred to a conserved Asp^{52} residue on the KdpE RR protein.

The acid – base stability analysis confirmed the nature of chemical bonds present in the KdpD and KdpE proteins. It is known that phosphoamidate bond formed by phosphorylation of histidine residue is sensitive to low pH and phosphoacyl bond formed by phosphorylation of aspartate residue, is sensitive to acidic and alkaline pH [27]. The bond properties complemented the mutagenesis studies where the conserved residues were identified. The system functionality necessitated intermolecular interaction of SK and RR, which was validated with SPR analysis. The KdpD and KdpE interacted in the basal conditions and the interaction affinity was reduced when SK was phosphorylated, allowing us to speculate possible dissociation of SK and RR protein during phosphotransfer, which allows RR to exert their regulatory effect. On the similar line, the phosphorylation defective SK ($KdpD^{H642Q}$) had least affinity with RR suggesting that perhaps this mutant SK, fails to interact with the RR. Overall, our work is amongst very few reports which demonstrate that phosphorylation status of SK alters the interaction kinetics with the RR proteins.

Further, the 3D-structure modeling validated the role of Asp^{8} in KdpE, which recruits the divalent ion in the phosphorylation reaction. The genetic characterization revealed that both the genes of the system, *kdpD* and *kdpE* are part of the single operon and are encoded as a single transcript, a feature which is well conserved across most two component systems. However, no direct binding of RR KdpE on its own promoter region, which is also the putative promoter region of *kdpFABC* operon was detected by us. This was surprising since in other bacterial species the *kdpDE* two component systems are tightly autoregulated and are direct regulators of functional *kdpABC* operon as well [33]. The finding suggested towards a more complex mode of regulation of *kdp* genes in *M. tuberculosis* which needs further investigation.

Overall, using the biochemical approaches we have established that putative *Rv1027c–Rv1028c* operon of *M. tuberculosis* encodes a functional and typical KdpDE two component signal transduction system. Both the genes of the system are co-transcribed and the encoded proteins show phosphorylation dependent interaction.

Author Contributions

R.A., designed and performed the experiments and wrote the paper; and D.K.S., designed the experiments, analyzed the data and wrote the paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.03.066.

References

[1] A.M. Stock, V.L. Robinson, P.N. Goudreau, Two-component signal transduction, Annu. Rev. Biochem. 69 (2000) 183–215.
[2] D.J. Bretl, C. Demetriadou, T.C. Zahrt, Adaptation to environmental stimuli within the host: two-component signal transduction systems of *Mycobacterium tuberculosis*, Microbiol. Mol. Biol. Rev. 75 (2011) 566–582.
[3] R. Heermann, K. Jung, K^+ supply, osmotic stress, and the KdpD/KdpE two-component system, in: R. Gross, D. Baler (Eds.), Two Component Systems in Bacteria, Caister Academic Press, Norfolk, UK, 2012.
[4] A. Sugiura, K. Hirokawa, K. Nakashima, T. Mizuno, Signal-sensing mechanisms of the putative osmosensor KdpD in *Escherichia coli*, Mol. Microbiol. 14 (1994) 929–938.
[5] A.J. Roe, D. McLaggan, C.P. O'Byrne, I.R. Booth, Rapid inactivation of the *Escherichia coli* Kdp K^+ uptake system by high potassium concentrations, Mol. Microbiol. 35 (2000) 1235–1243.
[6] H. Asha, J. Gowrishankar, Regulation of kdp operon expression in *Escherichia coli*: evidence against turgor as signal for transcriptional control, J. Bacteriol. 175 (1993) 4528–4537.
[7] L.A. Laimins, D.B. Rhoads, W. Epstein, Osmotic control of kdp operon expression in *Escherichia coli*, Proc. Natl. Acad. Sci. USA 78 (1981) 464–468.
[8] R. Malli, W. Epstein, Expression of the Kdp ATPase is consistent with regulation by turgor pressure, J. Bacteriol. 180 (1998) 5102–5108.
[9] Z.N. Freeman, S. Dorus, N.R. Waterfield, The KdpD/KdpE two-component system: integrating $K(^+)$ homeostasis and virulence, PLoS Pathog. 9 (2013) e1003201.
[10] D.T. Hughes, M.B. Clarke, K. Yamamoto, D.A. Rasko, V. Sperandio, The QseC adrenergic signaling cascade in enterohemorrhagic *E. coli* (EHEC), PLoS Pathog. 5 (2009) e1000553.
[11] T. Xue, Y. You, D. Hong, H. Sun, B. Sun, The *Staphylococcus aureus* KdpDE two-component system couples extracellular K^+ sensing and Agr signaling to infection programming, Infect. Immun. 79 (2011) 2154–2167.
[12] L. Zhao, T. Xue, F. Shang, H. Sun, B. Sun, *Staphylococcus aureus* AI-2 quorum sensing associates with the KdpDE two-component system to regulate capsular polysaccharide synthesis and virulence, Infect. Immun. 78 (2010) 3506–3515.
[13] R.A. Alegado, C.Y. Chin, D.M. Monack, M.W. Tan, The two-component sensor kinase KdpD is required for *Salmonella typhimurium* colonization of *Caenorhabditis elegans* and survival in macrophages, Cell. Microbiol. 13 (2011) 1618–1637.
[14] J.L. O'Loughlin, J.L. Spinner, S.A. Minnich, S.D. Kobayashi, Yersinia pestis two-component gene regulatory systems promote survival in human neutrophils, Infect. Immun. 78 (2010) 773–782.
[15] C.M. Sassetti, D.H. Boyd, E.J. Rubin, Genes required for mycobacterial growth defined by high density mutagenesis, Mol. Microbiol. 48 (2003) 77–84.
[16] C.M. Sassetti, E.J. Rubin, Genetic requirements for mycobacterial survival during infection, Proc. Natl. Acad. Sci. USA 100 (2003) 12989–12994.
[17] T. Parish, D.A. Smith, S. Kendall, N. Casali, G.J. Bancroft, N.G. Stoker, Deletion of two-component regulatory systems increases the virulence of *Mycobacterium tuberculosis*, Infect. Immun. 71 (2003) 1134–1140.
[18] S.E. Haydel, J.E. Clark-Curtiss, Global expression analysis of two-component system regulator genes during *Mycobacterium tuberculosis* growth in human macrophages, FEMS Microbiol. Lett. 236 (2004) 341–347.
[19] J.C. Betts, P.T. Lukey, L.C. Robb, R.A. McAdam, K. Duncan, Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling, Mol. Microbiol. 43 (2002) 717–731.
[20] A. Kapopoulou, J.M. Lew, S.T. Cole, The MycoBrowser portal: a comprehensive and manually annotated resource for mycobacterial genomes, Tuberculosis (Edinb) 91 (2011) 8–13.
[21] L.A. Kelley, M.J. Sternberg, Protein structure prediction on the Web: a case study using the Phyre server, Nat. Protoc. 4 (2009) 363–371.
[22] V. Molle, M.J. Buttner, Different alleles of the response regulator gene bldM arrest *Streptomyces coelicolor* development at distinct stages, Mol. Microbiol. 36 (2000) 1265–1278.
[23] S. Chauhan, J.S. Tyagi, Cooperative binding of phosphorylated DevR to upstream sites is necessary and sufficient for activation of the Rv3134c-devRS operon in *Mycobacterium tuberculosis*: implication in the induction of DevR target genes, J. Bacteriol. 190 (2008) 4301–4312.
[24] A.H. West, A.M. Stock, Histidine kinases and response regulator proteins in two-component signaling systems, Trends Biochem. Sci. 26 (2001) 369–376.
[25] M.T. Laub, E.G. Biondi, J.M. Skerker, Phosphotransfer profiling: systematic mapping of two-component signal transduction pathways and phosphorelays, Methods Enzymol. 423 (2007) 531–548.
[26] S.A. Thomas, J.A. Brewster, R.B. Bourret, Two variable active site residues modulate response regulator phosphoryl group stability, Mol. Microbiol. 69 (2008) 453–465.
[27] S. Klumpp, J. Krieglstein, Phosphorylation and dephosphorylation of histidine residues in proteins, Eur. J. Biochem. 269 (2002) 1067–1071.

[28] D.K. Saini, N. Pant, T.K. Das, J.S. Tyagi, Cloning, overexpression, purification, and matrix-assisted refolding of DevS (Rv 3132c) histidine protein kinase of *Mycobacterium tuberculosis*, Protein Expr. Purif. 25 (2002) 203–208.

[29] M. Bhattacharya, A. Biswas, A.K. Das, Interaction analysis of TcrX/Y two component system from *Mycobacterium tuberculosis*, Biochimie 92 (2009) 263–272.

[30] M. Behrens, P. Durre, KdpE of *Clostridium acetobutylicum* is a highly specific response regulator controlling only the expression of the kdp operon, J. Mol. Microbiol. Biotechnol. 2 (2000) 45–52.

[31] H.N. Lee, K.E. Jung, I.J. Ko, H.S. Baik, J.I. Oh, Protein–protein interactions between histidine kinases and response regulators of *Mycobacterium tuberculosis* H37Rv, J. Microbiol. 50 (2012) 270–277.

[32] A.J. Steyn, J. Joseph, B.R. Bloom, Interaction of the sensor module of *Mycobacterium tuberculosis* H37Rv KdpD with members of the Lpr family, Mol. Microbiol. 47 (2003) 1075–1089.

[33] J.W. Polarek, G. Williams, W. Epstein, The products of the kdpDE operon are required for expression of the Kdp ATPase of *Escherichia coli*, J. Bacteriol. 174 (1992) 2145–2151.