LETTER TO THE EDITOR

*Mycobacterium tuberculosis* H37 Rv1222: structural insight in transcription inhibition

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Introduction

Eradiation of multidrug resistant (MDR) *Mycobacterium tuberculosis* is a world-wide focus. A recent study by Fakhar et al is successful to elicit the structural information of a potent drug target of *M. tuberculosis* involved in the peptidoglycan biosynthesis (Fakhar et al., 2016). The computational virtual screening and molecular docking study suggested that bioactive compounds can be a target inhibitor molecule against the MDR pathogen (Skariyachan et al., 2015). Location of specific amino acids in some of the protein is a useful link to predict their function in order to identify the molecular targets for the development of new drugs (Saidijam & Patching, 2015). RNA polymerase is a proven target for anti-tuberculosis drug development. RNA polymerase (RNAP) together with the various sigma factors plays the major role in controlling transcription and thus the gene expression. Sigma factors are further controlled by anti-sigma factors inhibiting promoter recognition process and thereby interfere in transcription initiation (Rodrigue, Provvedi, Jacques, Gaudreau, & Manganelli, 2006; Betts, Lukey, Robb, McAdam, & Duncan, 2002). These anti-sigma factors are regulated by several downstream protein factors called anti-anti-sigma factors (Betts et al., 2002). Hence, the entire biochemical cascade is orchestrated to maintain proper homoeostasis within the host. The complex transcriptional regulation of Mtb-SigE is well regulated by a ZAS family protein, Rv1222, located immediately downstream to the former (Dona et al., 2008). It has been proposed that the interaction of Rv1222 with Mtb-SigE takes place under reducing conditions (Barik, Sureka, Mukherjee, Basu, & Kundu, 2010). However, recent studies have denied the fact of anti-sigma properties of Rv1222. On the contrary, it prompts that Rv1222 is a potent transcription inhibitor that can bind with the RNAP core rather than the sigma factor to paralyse the entire gene regulation process. It has stated that Rv1222 anchors RNAP onto DNA to inhibit transcription (Rudra, Prajapati, Banerjee, Sengupta, & Mukhopadhyay, 2015). This study is first to delineate the structural prediction of Rv1222 and to study its nature of transcription inhibition under multiple mutually exclusive circumstances. Rv1222 is allowed to interact with Mtb-SigE through molecular docking studies and found to behave differentially, like, (1) when only Rv1222 is allowed to interact with the Mtb-SigE and β subunit of Mtb-RNAP complex (Rv1222-SigE::β) (2) if Rv1222 and Mtb-SigE complex binds with the β subunit of Mtb-RNAP (Rv1222::SigE-β) (3) Rv1222 docked with Mtb-SigE and Phsp20 promoter binding complex Rv1222-SigE::Phsp20 (4) the Mtb-SigE and Rv1222 complex interacts with its cognate promoter Phsp20 (Rv1222::SigE-Phsp20). It is well noted from all these interactions that Rv1222 binds efficiently with sigma factor, rather than binding to core RNAP and inhibit transcription. Rv1222 can interact with promoter DNA but this interaction is much weaker than that of sigma factor. Thus, it provides us a great means to analyse the inhibition of gene expression of Rv1222 through RNAP or promoter-mediated pathway. Strenuous molecular simulations have given enough vividness on the validity of the structure of each of the macromolecules.

Materials and methods

Structure prediction

The sequence of Rv1222 is retrieved from protein sequence repository Uniprot (www.uniprot.org). Highly homologous sequences from the available crystal structures in the protein databank (www.pdb.org) are identified using BLAST option of NCBI (National Library of Medicine, USA). The crystal structure of *Rhodobacter sphaeroides* SigE in complex with the anti-sigma ChrR

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Molecular dynamics simulation

Rv1222 is subjected to energy minimisation with GROMACS 4.5.5 software (Hess, Kutzner, van der Spoel, & Lindahl, 2008) as described in Gupta et al. (2015). Molecular dynamics (MD) simulation of various time scales is done using GROMOS96 53a6 force field (Oostenbrink, Villa, Mark, & Van Gunsteren, 2004). Each of the minimised system is equilibrated for a short MD run (150 ps) with position restraints until and unless the potential energy converged and reached the temperature 200 K. This step is vital for the solvent to get well accustomed to the protein, thereby permitting the solvent to move freely, while keeping the non-hydrogen atoms of the proteins more or less affirm in their positions. This is done to assure the solvent configuration that matches the protein. Subsequently, unrestrained MD simulation was turned on with isothermal–isobaric ensemble at 300 K (tau_t 0.1) and 1 atm (tau_p 1.0). The weak coupling method of Berendsen thermostat and barostat is adopted so as to reach the equilibrium efficiently at early stage of simulation. All bonds are constrained with Linear Constraint Solver (LINCS) at relative tolerance of 10^{-4} nm, allowing 2 fs time step for the integration of equation of motion. The stability of the simulated macromolecules is further analysed in terms of differences in their trajectories, root mean square deviation (RMSD), root mean square fluctuations (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA) and the energies of the system using the various GROMACS utilities that comes under the software. The simulation of the docked structure is obtained using standard multiple domain protein simulation protocol from GROMACS.

Molecular docking

All the protein–protein docking experiments are performed using cluspro server (www.cluspro.bu.edu). It is a fully automated web-based program in which the PDB structures are uploaded independently. It uses global rigid body docking method PIPER, which is based on Fast Fourier Transform (FFT) correlation approach. The energy function is given as $E = E_{\text{attr}} + w1E_{\text{rep}} + w2E_{\text{elec}} + w3E_{\text{pair}}$ where, $E_{\text{attr}}$ and $E_{\text{rep}}$ signify the attractive and repulsive contribution to the van der Waals interaction energy $E_{\text{dW}}$, $E_{\text{elec}}$ is electrostatic energy and $E_{\text{pair}}$ represents desolvation contributions. The coefficients $w1$, $w2$ and $w3$ specify the weights of the corresponding terms that are optimised variously in balanced, electrostatic favoured, hydrophobic favoured and van der Waals-electrostatics docking models. The small molecule is treated as ligand and is moved around the protein receptor on a 3D grid with spacing 1.0 Å, using 70,000 rotations at each grid point. The best 1000 translation combinations with lowest energy pose have been chosen for clustering with a 9 Å α-carbon RMSD radius. The clusters are ranked by highest population size and lowest energy structure. The ranked complex undergoes minimisation using charm3 energy to restrict the potential clashes. Cluspro outputs the centre of 10 largest clusters. All docked poses are visualised and analysed with Discovery Studio version 4 from Accelrys (San Diego, CA, USA) and VMD. Accessible surface area is predicted using surface racer version 5 (Tsodikov, Record, & Sergeeva, 2002). DNA docking studies are executed using the methods described in Gupta et al. (2015).

Results

Structure prediction of Rv1222

The present homology model of Rv1222 is a tetra-helical protein consisting of 113 amino acid residues (Figure 1(a)). The template affinity of Rv1222 is portrayed from BLAST analysis against PDB (Figure 1(b)). The structure shows 40% identity with the template. RMSD calculation is 2.6 Å predicting the stability between the model and the template when superimposed upon one another. The crude model is further energy minimised at 4540 steps using conjugate gradient algorithm in the presence of 10,762 water molecules, 34 Na+ and 31 Cl- counter ions. Minimisation is done with 0.003 Fmax (maximum level of tolerance) until and unless the structure reached the final derivative of 1.0 KJ/mol. The φ and ψ torsion angle of the predicted structure is studied.

Figure 1. Tertiary structure of Rv1222. (a) Homology model of Rv1222 showing the helical complex. (b) Phylogenetic relationship between Rv1222 with its template; the crystal structure of Rhodobacter sphaeroides Mtb-SigE in complex with the anti-sigma ChrR (PDB ID: 2Q1Z) highlighted with box.
through Ramachandran plot which showed 93.7% of the residues in the most favoured region, 5.4% in the additional allowed region, but none of them are found in the disallowed region which depicts a good model (Supplementary Figure S1). The compatibility of the 3D structure of Rv1222 is determined with its own amino acid sequence by analysing through verify 3D method. The verify 3D value was found to be 84.07%, indicating a reasonable model. The reliability of the built model is further established through ERRAT scores which analyses the non-bonded interactions between different kinds of atoms to be 96.104%, showing reliability of the structure. ProSA Z-score is −2.88 (Supplementary Figure S1) which typically belongs to the range of score of several experimentally generated (NMR/X-ray) protein structures of similar size (113 amino acids).

**Dynamic propensities of Rv1222, Mtb-SigE and β subunit of Mtb-RNAP**

The dynamic properties as well as the stability of Rv1222, Mtb-SigE and β subunit of Mtb-RNAP are explored for 30 ns using explicit solvent molecular dynamics simulation. The RMSDs of all the three simulations at various time steps with respect to the initial structures are calculated in order to measure the average distance between the α-carbon atoms. RMSD of Rv1222 fluctuates from 0.5 to 1 Å up to 15 ns after which the system gets converged up till 30 ns. The RMSD of Mtb-SigE with respect to the arrangement of α-carbon shows relatively less deviation when compared to the starting coordinates. At the end of 30 ns simulation, the average change in RMSD is 0.5 to 0.6 Å. β subunit of Mtb-RNAP shows an average change in RMSD which is 0.5 Å after 30 ns simulation. These results have indicated that the protein structures have undergone significant changes at various time intervals to reach their stable structural conformation (Supplementary Figure S2). The stability of the structures is further evaluated by calculating the total energy variation during simulation (Supplementary Figure S3).

**Molecular docking studies**

Each of the docking studies are initiated using the refined structures with stable conformational changes at the end of 30 ns simulation studies which further justifies the validity of the predicted interactions.

**Rv1222 non specifically interacts with various sigma factor**

The stable structure of Mtb-SigE is docked with Rv1222 (Figure 2(a) and (b)). The predicted interacting residues are discussed in Table 1. The interaction between Rv1222 and Mtb-SigE is further scrutinised through prolong MD simulation. These have aided to get a clear insight about the nature of binding as well as the changes in the structural conformation. During the time scale of 60 ns, the RMSD of the complex have fluctuated from 0.5 to 1 Å since first 10 ns, then the structure got stabilised to 0.8–1 Å (Supplementary Figure S4 (a)). The flexibility of the complex is assessed through RMSF calculation revealing high fluctuation at N-terminal of Rv1222 and C-terminal of SigE (Supplementary Figure S4 (b)). The complex seems to be less tightly packed for the first 10 ns as evident from its Rg score, after which the structure remained relatively compact until the convergence (Supplementary Figure S4 (c)). The buried surface area of the complex is assessed by SASA analysis through various time-scale evolutions. Supplementary Figure S4 (d) depicts the diminishing nature of accessible surface area by the progress of simulation till its convergence. On the other hand, when Rv1222 is separately allowed to interact with various X-ray crystallographic structures of *E. coli* and *T. thermophilus* RNAP (PDB id: 2CW0, 4LJZ), the same recognition pattern is seen as described in Figure 3. These have thrown enough light about the non-specific nature of anti-Rv1222. In contrast to Rudra et al., 2015, which showed the interaction of Rv1222 with the core RNAP rather than the sigma factor, the present study shows that Rv1222 has more affinity for sigma factor than that of the core RNAP subunits. Though some of the remote docking poses are found with the β and β′ subunits of RNAP but their energy profile is unstable and the interactions are unfavourable showing several steric hindrances (data not shown).

**Rv1222 and β subunit of Mtb-RNAP recognises common site of Mtb-SigE**

Rv1222 typically binds with domain 4 of Mtb-SigE (Barik et al., 2010), which in turn is the β flap-tip interacting region (Geszvain, Gruber, Mooney, Gross, & Landick, 2004; Nickels et al., 2005). The interacting residues reveals the fact that both Rv1222 as well as β subunit of Mtb-RNAP recognises a common patch of Mtb-SigE. The intermolecular interactions of Mtb-SigE and β flap-tip region of Mtb-RNAP (Gupta et al., 2015) have showed remarkable similarity in the binding affinity with that of Mtb-SigE and Rv1222. This indicates that both Rv1222 and β subunit of Mtb-RNAP competes upon one another for interacting with Mtb-SigE. Perhaps, the two repertoire works in a mutually exclusive manner upon one another Mtb-SigE interacts with Rv1222 to turn down the transcription process, whereas Mtb-SigE binds with β subunit of Mtb-RNAP to form the holoenzyme and brings about the process of transcription initiation.
Figure 2. Protein interaction studies. (a) Mtb-SigE (pink) and Rv1222 (green) dimer complex. (b) Close up view of dimer showing hydrophobic interactions (blue dashed line) and hydrogen bonding (yellow dashed line). Gln237, Ala239, Ser242, Arg247 and Arg248 of Mtb-sigE interact with Arg47, Ala 49, Arg51, Asp52, Leu66 and Ile69 of Rv1222. (c) Docked structure of Rv1222-Mtb-SigE (sigma-anti-sigma dimer) with $\beta$ of Mtb-RNAP (complex 1). $\beta$ flap-tip region of Mtb-RNAP in cyan, Rv1222 in green and Mtb-SigE in pink. (d) Mtb-SigE and $\beta$ subunit of Mtb-RNAP with Rv1222 (complex 2). Colour demarcation same as (c).

Table 1. Intermolecular interactions found in the docked complex of Rv1222::Mtb-SigE proteins and Rv1222::SigE-Psp20.

| Complex name       | Residue and atom name | Bond type | Bond distance in Å |
|--------------------|-----------------------|-----------|--------------------|
| Rv1222::MtSigE     | ARG248:NH1 - ASP52:OD2 | Electrostatic | 4.54               |
|                    | ASP52:H – SER242:O    | Hydrogen  | 2.21               |
|                    | GLN237:HE22 – HIS54:ND1 | Hydrogen  | 2.40               |
|                    | SER242:HG – ALA49:O   | Hydrogen  | 1.50               |
|                    | ARG51 – PRO244        | Hydrophobic | 5.14              |
|                    | CYST2 – LEU286        | Hydrophobic | 5.29              |
|                    | ALA239 – LEU66        | Hydrophobic | 4.82              |
|                    | ALA239 – ILE69        | Hydrophobic | 4.75              |
| Rv1222::SigE-Psp20 | GUA32:H22 – GLU75:OE1 | Hydrogen  | 2.66459            |
|                    | CVT35:H41 – SER82:O   | Hydrogen  | 2.84506            |
|                    | SER60:HN – ADE55:O2P  | Hydrogen  | 2.39931            |
|                    | SER60:HG – CYT56:O2P  | Hydrogen  | 2.18264            |
|                    | ARG71:HN – THY36:O2P  | Hydrogen  | 2.75255            |
|                    | GLY76:HN – THY34:O3'  | Hydrogen  | 1.97157            |
|                    | LYS79:HZ3 – THY36:O4' | Hydrogen  | 2.06318            |
|                    | SER81:HG – ADE58:N7   | Hydrogen  | 2.09424            |
|                    | SER82:HG – ADE59:N7   | Hydrogen  | 2.89833            |
|                    | SER85:HG – THY34:O2P  | Hydrogen  | 1.95233            |
|                    | SER86:HN – THY34:O1P  | Hydrogen  | 2.7104             |
|                    | GLY98:HN – THY33:O3'  | Hydrogen  | 2.86997            |

Note: The molecules of Rv1222 are marked in bold.
Rv1222 has no effect on RNAP holoenzyme

To investigate further, we have tried to dock Rv1222::Mtbd SigE complex with β of Mtb-RNAP (complex 1, Rv1222::SigE-β) and Mtb-SigE::β subunit of Mtb-RNAP with Rv1222 (complex 2, SigE::β-Rv1222) (Figure 3). The interaction studies from complex 1 depicts that in presence of the Rv1222 and Mtbd-SigE dimer, β flap-tip region fails to communicate with Mtbd-SigE except a faint electrostatic interaction between Arg226 of Mtbd-SigE and Glu405 of β subunit of Mtbd-RNAP. The interacting site from complex 1 shows that instead of Mtbd-SigE, β subunit of Mtbd-RNAP is found to interact with Rv1222 (Table 2). Structural investigations from the X-ray structures of RNAP from PDB (PDB id: 4LJZ and 2CWO) and further multiple sequence alignment (MSA) with β subunit of RNAP unveils that this region is mainly occupied by β’ subunit of RNAP and is not a freely assessable region as it lies deep into the core region. Hence, this interaction does not play any significant role in the ternary complex formation. The extended study on complex 2 elicits that Rv1222 and Mtbd-SigE dimer fails to form in presence of β subunit of Mtb-RNAP. In other words, Rv1222 will not have any role in impairment on preformed holoenzyme. Instead, Rv1222 is found to form non-specific interaction with β subunit of Mtb-RNAP similar to that found in case of complex 1 (Table 2). Comparative SASA unmasks the reality that Rv1222::Mtbd-SigE dimer is much strong rather than the Rv1222 and β subunit of Mtb–RNAP interactions (Supplementary Figure S5 (a) and (b)) seen in the ternary complex 1 and complex 2. Thus in all possible cases, Rv1222 shows pseudo affinity when challenged with holoenzyme. This in silico study is deviating from the recent findings of Rudra et al. (2015), showing Rv1222 binds to the RNAP holoenzyme rather than the sigma factor.

Figure 3. Rv1222 non-specifically interacts various sigma factor. (a) Rv1222 recognises domain 4 of different sigma factors. (b) Interaction of Rv1222 with domain 4 of sigma factor in T. thermophilus. (c) Rv1222 binds with sigma factor domain 4 in E. coli.

Rv1222 has no effect on RNAP holoenzyme

To investigate further, we have tried to dock Rv1222::Mtbd-SigE complex with β of Mtb-RNAP (complex 1, Rv1222::SigE-β) (Figure 3(c)) and Mtbd-SigE::β subunit of Mtb-RNAP with Rv1222 (complex 2, SigE::β-Rv1222) (Figure 3(d)). The interaction studies from complex 1 depicts that in presence of the Rv1222 and Mtbd-SigE dimer, β flap-tip region fails to communicate with Mtbd-SigE except a faint electrostatic interaction between Arg226 of Mtbd-SigE and Glu405 of β subunit of Mtbd-RNAP. The interacting site from complex 1 shows that instead of Mtbd-SigE, β subunit of Mtbd-RNAP is found to interact with Rv1222 (Table 2). Structural investigations from the X-ray structures of RNAP from PDB (PDB id: 4LJZ and 2CWO) and further multiple sequence alignment (MSA) with β subunit of RNAP unveils that this region is mainly occupied by β’ subunit of RNAP and is not a freely assessable region as it lies deep into the core region. Hence, this interaction does not play any significant role in the ternary complex formation. The extended study on complex 2 elicits that Rv1222 and Mtbd-SigE dimer fails to form in presence of β subunit of Mtb-RNAP. In other words, Rv1222 will not have any role in impairment on preformed holoenzyme. Instead, Rv1222 is found to form non-specific interaction with β subunit of Mtb-RNAP similar to that found in case of complex 1 (Table 2). Comparative SASA unmasks the reality that Rv1222::Mtbd-SigE dimer is much strong rather than the Rv1222 and β subunit of Mtb–RNAP interactions (Supplementary Figure S5 (a) and (b)) seen in the ternary complex 1 and complex 2. Thus in all possible cases, Rv1222 shows pseudo affinity when challenged with holoenzyme. This in silico study is deviating from the recent findings of Rudra et al. (2015), showing Rv1222 binds to the RNAP holoenzyme rather than the sigma factor.

Table 2. Intermolecular interactions found in the docked complex 1 and 2. The molecules of Rv1222 are marked in bold.

| Complex name | Residue and atom name | Bond type | Bond distance in Å |
|--------------|------------------------|-----------|-------------------|
| Rv1222::SigE-β | LYS430:NZ – ASP128:OD1 | Salt bridge | 2.73 |
|              | ARG108:NH2 – ASP428:OD2 | Electrostatic | 5.27 |
|              | ARG137:NH1 – ASP435:OD2 | Electrostatic | 4.40 |
|              | TYR444:N – GLU112:O | Hydrogen | 1.51 |
|              | PRO445:CD – PRO111:O | Hydrogen | 3.27 |
|              | PRO445:CD – ASP128:OD2 | Hydrogen | 3.47 |
|              | ALA130:CA – THR342:OG1 | Hydrogen | 3.39 |
|              | LYS430 – PRO110 | Hydrophobic | 5.21 |
|              | PRO445 – PRO111 | Hydrophobic | 4.59 |
|              | ALA132 – VAL446 | Hydrophobic | 3.28 |
|              | TYR444 – PRO114 | Hydrophobic | 4.05 |
|              | PHE134 – PRO443 | Hydrophobic | 3.34 |
| SigE::β-Rv1222 | ARG437:NH2 – ASP136:OD1 | Salt bridge | 3.94 |
|              | ARG108:NE – TYR444:OH | Hydrogen | 2.73 |
|              | TYR444: OH – PRO111:O | Hydrogen | 3.27 |
|              | ARG137:NH1 – PHE442 | Electrostatic | 4.91 |
|              | ASP128:OD1 – TYR444 | Electrostatic | 4.05 |
|              | GLU440:OE2 – PHE134 | Electrostatic | 3.68 |
|              | ARG137:CD – PHE442 | Electrostatic | 3.68 |
|              | ALA132 – VAL446 | Hydrophobic | 5.35 |
|              | PHE134 – PRO443 | Hydrophobic | 3.88 |
|              | PHE442 – ARG137 | Hydrophobic | 3.84 |

Note: The molecules of Rv1222 are marked in bold.
**Rv1222 interacts with promoters non selectively**

Recent report from Rudra et al. (2015) significantly introduce the promoter-binding nature of Rv1222, which have depicted that the promoter interaction of Rv1222 is mere electrostatic and the C-terminal tail of the protein plays major role in it. The present study analyse the nature of Rv1222 in promoter reorganisation in two ways: (1) by docking it with the Phsp20::Mtb-SigE and also (2) finding interaction of Rv1222::MtbsigE dimer complex with Phsp20. Thus, it is helpful to notice the behaviour of Rv1222 before and after promoter recognition by sigma factor. It can be predicted from this analysis that if Phsp20 and Mtb-SigE complex formation have already been completed then Rv1222 can only bind to Phsp20 non-specifically, but not to the promoter binding region (Figure 4(a)). On the other hand, if the dimer complex of Rv1222 and Mtb-SigE is allowed to bind Phsp20, it is interestingly found that Rv1222 interacts with Phsp20, thereby blocking the sigma factor promoter recognition (Figure 4(b)). The interacting residues of Rv1222::SigE-Phsp20 are discussed in table 1. Even though Rv1222::Phsp20 is non-specific in nature, but SASA studies clearly depict the fact that Rv1222 has got more affinity towards promoter DNA rather than non-promoter DNA. In comparison between the strength of Rv1222 interaction with Mtb-SigE and Phsp20, it can be easily predicted that the latter is much weak than the former due to the fact that average buried surface area for Rv1222::MtbsigE complex is much higher than those of Rv1222::Phsp20 interaction (Supplementary Figure S6).

**Discussions**

Rv1222 is found to be a transcription inhibitor. It shows a tetra-helical structure. Independent MD simulation study of the three macromolecules Mtb-SigE, β subunit of Mtb-RNAP and Rv1222, showed the stability and compactness of the structure in terms of RMSD and the total energy of interaction. Upon trajectory analysis, it can be elicited that Rv1222 is mostly fluctuated with that of the initial structures than that of β subunit of Mtb-RNAP and Mtb-SigE. Thus, Rv1222 is likely to be more flexible in comparison to other two macromolecules. The mode of action of Rv1222 is predicted predominantly upon its interaction with Mtb-SigE, Mtb-RNAP core and putative promoter element (Phsp20). Rv1222 strongly binds with MtbsigE forming a dimer that is competent enough to cease the transcription machinery. Prolonged MD simulation process with Rv1222 and Mtb-SigE complex shows that the interaction is quite stable and have converged after few time frames. Unfavourable and dissatisfied bonding is absent in between the binding patch of the complex. Gln237, Ala239, Ser242, Arg247 and Arg248 of MtbsigE interact with Arg47, Ala 49, Arg51, Asp52, leu66 and Ile69 of Rv1222. When Rv1222 and MtbsigE complex is further allowed to interact with β subunit of Mtb-RNAP, it is found that the conservative β flap-tip binding of MtbsigE completely diminishes and on its place Rv1222 is found to show certain non-specific interactions with β subunit of Mtb-RNAP. On the other hand, if Rv1222 is independently allowed to bind with MtbsigE and β subunit of Mtb-RNAP, the complex from our previous study (Gupta et al., 2015), it is seen that Rv1222::MtbsigE dimer formation is hindered and Rv1222 is showing pseudo affinity for β subunit of Mtb-RNAP. In both the cases, we have tried to compare the nature of interaction between Rv1222::MtbsigE and Rv1222-β subunit of Mtb-RNAP by SASA analysis and have found that the binding affinity of the former is much higher than the latter. Rv1222 is a potent transcription inhibitor that impaired the formation of RNAP holoenzyme by binding itself to the sigma factor. The core RNAP is thus prevented from binding to the sigma factor. On the other hand, Rv1222 fails to disturb the sigma factor once the holoenzyme formation is completed. In such a situation, failure to get access to sigma factor, Rv1222 is compelled to bind with β subunit of Mtb-RNAP. The present study indicates that Rv1222 can non-specifically bind to various sigma factors rather than to only MtbsigE. In all cases, we find domain 4 of sigma factor predominantly plays an important role in Rv1222 interactions. Thus, the strong affinity of Rv1222 towards sigma factor is preferable rather than for the core RNAP. No significant interactions are observed for core RNAP. This is contradictory to Rudra.
et al. (2015); which predicts Rv1222 to bind to the RNAP core rather than the sigma factor. The efficiency of Rv1222 as transcriptional inhibitor is predicted in terms of promoter interactions. When Rv1222::Mtb-SigE complex is allowed to dock with P_hsp20, it is found that Rv1222 directly interacts with it and preventing the interaction of Mtb-SigE with the P_hsp20. Thus, finally lead to inhibit the formation of initiation complex and transcription machinery remains impaired. Similarly, in presence of Mtb-SigE::P_hsp20 complex (Gupta et al., 2015), Rv1222 is found to bind arbitrarily on non consensus of the promoter site. Our study elaborated the fact that Rv1222 can bind to both RNAP holoenzyme as well as the promoter element to block the transcription process, but the binding efficiency is strong as well as energetically stable for the sigma factor than that of promoter DNA.

Conclusions

In silico understanding of the structure of Rv1222 and its characteristic attributes towards RNAP and cognate promoter element have well fulfilled the unknown thirst in its transcription inhibition process. DNA binding of Rv1222 is enhanced if the sigma and Rv1222 complex have already been formed and then interfering the progress of transcription by blocking promoter from binding to sigma protein. Rv1222 is more potent as an anti-sigma that arrests the gene expression at the level of RNAP holoenzyme formation preventing promoter recognition than that of directly binding to DNA and hindering transcription.

Supplementary material

The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2016.1189357

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Disclosure statement

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