Computational Biology and Chemistry 59 (2015) 37–47

Contents lists available at ScienceDirect

Computational Biology and Chemistry

journal homepage: www.elsevier.com/locate/compbiolchem

Research article

Identification of chebulinic acid as potent natural inhibitor of *M. tuberculosis* DNA gyrase and molecular insights into its binding mode of action

Kunal Patel[a], Chetna Tyagi[b], Sukriti Goyal[b], Salma Jamal[b], Divya Wahi[c], Ritu Jain[c], Navneeta Bharadvaja[a], Abhinav Grover[c,*]

[a] Department of Biotechnology, Delhi Technological University, Delhi 110042, India
[b] Department of Bioscience and Biotechnology, Banasthali University, Tonk, Rajasthan 304022, India
[c] School of Biotechnology, Jawaharlal Nehru University, New Delhi 110067, India

ARTICLE INFO

Article history:
Received 14 November 2014
Received in revised form 2 September 2015
Accepted 8 September 2015
Available online 11 September 2015

Keywords:
Chebulinic acid
DNA gyrase
Multiple drug resistance
Tuberculosis
Virtual screening
Molecular dynamics simulations

ABSTRACT

Drug resistant tuberculosis has threatened all the advances that have been made in TB control at the global stage in the last few decades. DNA gyrase enzymes are an excellent target for antibacterial drug discovery as they are involved in essential functions like DNA replication. Here we report, a successful application of high throughput virtual screening (HTVS) to identify an inhibitor of *Mycobacterium* DNA gyrase targeting the wild type and the most prevalent three double mutants of quinolone resistant DNA gyrase namely A90V + D94G, A74S + D94G and A90V + S91P. HTVS of 179.299 compounds gave five compounds with significant binding affinity. Extra presicion (XP) docking and MD simulations gave a clear view of their interaction pattern. Among them, chebulinic acid (CA), a phytocompound obtained from *Terminalia chebula* was the most potent inhibitor with significantly high XP docking score, −14.63, −16.46, −15.94 and −15.11 against wild type and three variants respectively. Simulation studies for a period of 16 ns indicated stable DNA gyrA–CA complex formation. This stable binding would result in inhibition of the enzyme by two mechanisms. Firstly, binding of CA causes displacement of catalytic Tyr129 away from its target DNA-phosphate molecule from 1.6 Å to 3.8–7.3 Å and secondly, by causing steric hindrance to the binding of DNA strand at DNA binding site of enzyme. The combined effect would result in loss of cleavage and religation activity of enzyme leading to bactericidal effect on tuberculosis. This phytocompound displays desirable quality for carrying forward as a lead compound for anti-tuberculosis drug development. The results presented here are solely based on computations and need to be validated experimentally in order to assert the proposed mechanism of action.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Mycobacterium tuberculosis causes one of the most severely affecting human diseases called tuberculosis. With present scenario of time-taking, inappropriate use of the first line of tuberculosis therapeutics led to emergence of multiple-drug resistant *M. tuberculosis* (MDR-TB) and has threatened the TB control programme at global stage (World Health Organization, 2013a). According to WHO report of 2012, 750,000 cases of MDR-TB cases of MDR-TB have been reported and near about 170,000 deaths have been attributed to MDR-TB. Along with this, 92 countries have reported cases of extensively-drug-resistant (XDR) TB with half of the cases coming from China, India, and the Russian Federation (World Health Organization, 2013a,b). In case of MDR-TB, WHO advocates fluoroquinolone drugs like ofloxacin when predisposition to the first line drug's report are not available in the continuation period (18 months) before changing the course of treatment or if resistance to at least two drugs rifampicin and isoniazid is proven (Walwaikar et al., 2003). However, in light of emergence drug resistance cases, key for controlling both wild type and MDR-TB is the search of a common inhibitor of tuberculosis DNA gyrase and its drug resistant mutant gyrase.

Type II topoisomerases are crucial enzymes involved in controlling, modifying DNA's topology and regulating supercoiling

Abbreviations: HTVS, high throughput virtual screening; CA, chebulinic acid; XP, extra precision; MDR-TB, multiple-drug resistant tuberculosis; XDR-TB, extensively-drug resistant tuberculosis; QRDR, quinolone resistance determining region; CTD, C-terminal domain; PSVS, protein structure validation suite; MD, molecular dynamics.

* Corresponding author. Fax: +91 1126742558.
E-mail addresses: abhinavgr@gmail.com, agrover@mail.jnu.ac.in (A. Grover).

http://dx.doi.org/10.1016/j.compbiolchem.2015.09.006
1476-9271/© 2015 Elsevier Ltd. All rights reserved.

of DNA (Champoux, 2001). DNA gyrA aids in relaxing of DNA at the replication forks by inducing a nick and DNA topoisomerase IV mediates the process of unlinking of interlocked daughter chromosomes (Levine et al., 1998). DNA gyrA is a part of type II topoisomerase and performs both the functions of supercoiling DNA and inducing breaks in DNA, relieving the tension in DNA strands supercoil and after DNA replication unlinking of daughter DNA efficiently (Aubry et al., 2006a; Cole et al., 1998). Both type II topoisomerases comprises two subunits of gyrA and gyrB and together they form the catalytically active heterotetrameric enzyme (i.e. A2B2). Subunit A consists of two domains, the N-terminal breakage-reunion domain also known as G segment at the DNA gate. It functions by binding the DNA segment, cleaving and resealing it. The second domain is C-terminal domain (CTD). Subunit B is also comprised of two domains, ATPase domain and the toprim domain (Schoeffler and Berger, 2008). Fluoroquinolone shows its powerful bactericidal activity by meddling with the enzymatic reaction process of bacterial type II topoisomerases. The drug binds to the enzyme–DNA complex with high affinity and stabilizes the covalent bond formed between catalytic 129 tyrosine residue of enzyme and DNA phosphate. Stabilization of this ternary complex hinders with normal function of the enzyme and halts the DNA replication process which ultimately causes bacterial cell death (Oates et al., 1991). However, resistance was reported against fluoroquinolone-based drugs. A study conducted by Shi et al. in 2003 on 109 clinical isolates showed that 87 samples of them were fluoroquinolone resistant. Sequencing of quinolone binding pocket (QBP) of DNA revealed many drug resistant mutations, however, we would focus on more frequently occurring mutations, specifically at 74, 90, 91, and 94 codon position. At 94th codon position four different type of point mutations are observed (Asp94 to Gly/Ala/Tyr/Asn) (Shi et al., 2006). This study mostly confirmed the findings of other researchers (Alangaden et al., 1995; Cheng et al., 2004; Xu et al., 1996). However, two unique observations were made for the first time, one observation was that 49 of the 87 ofloxacin-resistant isolates (56%) had double point mutations and the second was presence of a unique Ala74-Ser mutation in 20% (10/49) of the isolates (Shi et al., 2006). The high rate at which these double point mutations were observed in gyrA and emergence of Ala74-Ser mutation showed rise in development of fluoroquinolone resistant strains (Cheng et al., 2004; Xu et al., 1996). A real threat of rise in extensively drug-resistant (XDR) strains has been posed by the findings of this study (An et al., 2009; Sun et al., 2008; van Doorn et al., 2008). This QRDR segment is located in the breakage-reunion domain of gyrA subunit (QRDR-A) and with low frequency it also has been seen in the toprim domain of gyrB (QRDR-B) (Aubry et al., 2006b; Veziris et al., 2007).

Despite of having a number of potent drugs for tuberculosis, hunt for new potential drug candidates are continuously on the rise owing to increasing incidence of drug resistance. Screening of potential drug compounds using computational techniques possesses great potential to expedite the process of drug discovery. (Sun, 2008; Goyal et al., 2014; Dhiman et al., 2013; Dhanjal et al., 2014). Considering the need for drugs effective against both wild type and fluoroquinolone resistant mutants caused by mutations in A90V + S91P, A90V + D94G, and A74S + D94G (Piton et al., 2010), natural compounds were docked against *M. tuberculosis* DNA gyrase. A phytocompound library of 169,109 natural compounds taken from zinc database along with 10,000 compounds from Myria-screening databases and 190 self-created compounds was screened using parallel high throughput virtual screening (Dhanjal et al., 2014; Goyal et al., 2013). The study identified chebulinic acid (CA), a natural phytocompound obtained from *Terminalia chebula* as the most potent drug candidate to treat both TB and fluoroquinolone resistant mutant gyrase by inhibiting DNA gyrA. *T. chebula* is one of the three constituent of triphala (Lu et al., 2012) and is being used since ages in the Indian Ayurvedic discipline for curing many ailments related to the gastrointestinal and cardio-vascular systems (Singh et al., 2008; Pawar et al., 2009; Baliga, 2010). Recently, in vivo studies in mice has demonstrated that triphala constituents can inhibit the growth of stomach cancer, murine thymic lymphoma and human pancreatic cancer, it also has antibacterial properties but its effect particularly on TB have not been studied much (Baliga, 2010; Deep et al., 2005; Shi et al., 2008; Malekzadeh et al., 2001). The study attempts to find a natural compound which has bactericidal properties against wild type *M. tuberculosis* DNA gyrA and which can also be effective against multi-drug resistant (MDR)-TB.

2. Materials and methods

2.1. Preparation of wild type and mutated DNA gyrA structures

The crystal structure of *M. tuberculosis* DNA gyrA, consisting of two domains i.e. the N-terminal breakage-reunion domain and a C-terminal domain (CTD) having PDB accession code—3IFZ and *Streptococcus pneumoniae* topoisomerase IV catalytic core with PDB accession code—3FOF was retrieved. Crystal water molecules and all non-bonded heteroatoms including the docked ligand were removed from the protein structures using Accelerys Viewerlite 5.0 (Accelrys, 2015). For the sake of reducing the computational time, only A chain of DNA gyrase subunit A was chosen for docking and molecular dynamics studies. Using tfmodeller, DNA was docked with *M. tuberculosis* DNA gyrase subunit A taking *S. pneumoniae* topoisomerase IV as a reference structure already docked to DNA (Laponogov et al., 2009). Tfmodeller screens a library of known experimental protein structures in complex with DNA to drive docking of the query protein with the DNA molecule. The model was validated using protein structure validation suite (PSVS) (Bhattacharya et al., 2007) which comprises a number of software. PROCHECK (3.5.4) (Laskowski et al., 1993) and Molprobity (Davis et al., 2007) provide a Ramchandran plot analysis.

The DNA-protein docked structure was used for analyzing the impact of inhibitors obtained after screening. As crystal structures of mutant DNA gyrA were not available, the three double mutants were created by incorporating point mutations in the primary structure. The first double mutant of DNA gyrA chain A was obtained by mutating Ala90 to Val90 and Asp94 to Gly94. The second double mutant of DNA gyrA chain A was obtained by introducing mutation at 74th and 94th positions corresponding to Ala to Ser and Asp to Gly point mutations, respectively. Similarly, in the third double mutant, Ala90 was mutated to Val and Ser91 to Pro through Schrodinger's protein preparation wizard (Wizard, 2009). The wild type and three mutant DNA gyrA structures were stabilized through several structural modifications and energy minimization (Madhavi Sastry et al., 2013; Schrödinger, 2013). In this process hydrogen atoms were added, bond lengths were augmented, disulphide bonds were generated, capping of terminal residues were done and selenomethionines were converted into methionines. Molecular dynamic simulations were then performed for a period of 12 ns to stabilize the mutant structure forms to be used for high throughput virtual screening and evaluation of interaction patterns.

2.2. Binding cavity prediction

In search for a single drug that can inhibit both wild type and mutant type, choice of ligand binding cavity which can produce the desired inhibitory effect is of fundamental importance. Binding cavity was predicted using Q-SiteFinder server (Laurie and Jackson, 2005). Q-SiteFinder uses energetic criteria to identify binding

Download English Version:

https://daneshyari.com/en/article/14970

Download Persian Version:

https://daneshyari.com/article/14970

Daneshyari.com