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Crystal structure of the DNA Gyrase GyrA N-terminal domain from *Mycobacterium tuberculosis*

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Introduction

In all cells, the topological problems associated with DNA replication, transcription and repair are managed by a class of enzymes known as DNA topoisomerases¹. Many bacteria retain a unique topoisomerase, DNA gyrase, which maintains genomes in a negatively-supercoiled state using an ATP-dependent DNA duplex strand passage mechanism². A key intermediate in gyrase's catalytic cycle is the formation of an enzyme-mediated, double-stranded break in which a tyrosine resident in each of the two GyrA subunits of the heterotetrameric enzyme becomes covalently attached to the DNA³. This "cleavage complex" is potentially cytotoxic and is stabilized by the action of clinically-used antibiotics such as the fluoroquinolones⁴.

Despite their clinical success, fluoroquinolone efficacy is being eroded by increasing levels of antibiotic resistance. In 2007, there were an estimated 9.2 million new *Mycobacterium tuberculosis* infections, 4.9% of which were caused by multidrug-resistant strains⁵. The challenge of designing new antibiotics to combat rising resistance can be assisted by high-resolution structures of antibiotic targets; to this end, we have solved the crystal structure of the 59kDa *M. tuberculosis* DNA gyrase GyrA N-terminal domain (*MtGyrA59*), the principal drug-binding region of the enzyme. A comparative structural analysis of the *M. tuberculosis* and *E. coli* GyrA N-terminal domains reveals previously unobserved structural flexibility in a key hotspot for fluoroquinolone resistance.

Materials and Methods

Protein Purification

The coding region for residues 34–500 of *M. tuberculosis* GyrA (corresponding to residues 27–525 of the homologous *E. coli* subunit) was amplified from genomic DNA (American Type Culture Collection) and cloned into a derivative of pET28b with an N-terminal, tobacco etch virus (TEV) protease-cleavable hexahistidine tag. Protein was overexpressed in *E. coli* BL21-CodonPlus(DE3)-RIL cells (Stratagene) by inducing log-phase cells with 0.25 mM isopropyl- β -D-thiogalactopyranoside for 14 h at 25°C. Cells were harvested by centrifugation, resuspended in 20 mM HEPES pH 7.5, 800 mM NaCl, 30 mM imidazole, 10% glycerol with protease inhibitors, and frozen dropwise into liquid nitrogen.

For purification, cells were sonicated and centrifuged, and the clarified lysate was passed over a Ni²⁺ affinity column (Amersham Biosciences). His-tagged protein was eluted with 20 mM HEPES pH 7.5, 400 mM NaCl, 500 mM imidazole and 10% glycerol, then concentrated and exchanged into the same buffer containing 30mM imidazole, followed by incubation overnight at 4° C with hexahistidine-tagged TEV protease⁶. This mixture was passed over a Ni²⁺ affinity column, and the flow-through was collected, concentrated and run over an S-200 gel filtration column (Amersham Biosciences) in 50 mM HEPES pH 7.5, 500 mM KCl, 10% glycerol, and 2 mM 2-mercaptoethanol. Peak fractions were pooled and concentrated by ultrafiltration (Millipore Centriprep-10).

Crystallization

Purified *MtGyrA59* at 11 mg/ml was dialyzed overnight at 4° C against 10 mM HEPES pH 7.5, 50mM KCl, and 1mM Tris(2-carboxyethyl)-phosphine. Crystals were grown in hanging drop format by mixing 1 μ l of protein with 1 μ l well of solution containing 26% 2-methyl-2,4-pentanediol, 100 mM Tris pH 9.0, and 1 mM Ciprofloxacin. Crystal reproducibility was extremely erratic, and crystal formation proved not to be drug dependent. For harvesting, crystals were transferred to a cryoprotectant solution containing well solution plus 25% glycerol for 1 minute, followed by looping and flash-freezing in liquid nitrogen.

X-ray diffraction data collection and structure determination

A single native dataset was collected at Beamline 8.3.1 at the Advanced Light Source at Lawrence Berkeley National Laboratory⁷. Data were indexed and reduced as P1 using HKL2000⁸. Phasing by molecular replacement with a poly-serine model of the *E. coli* GyrA NTD (residues 2–523, PDB 1AB4) with PHASER^{9,10} revealed two *MtGyrA59* protomers per asymmetric unit. Density modification and initial building were performed by PHENIX¹¹; subsequent cycles of manual rebuilding and refinement were performed using COOT¹². TLS parameters were analyzed with the TLSMD server and six TLS groups were introduced in the last stages of refinement¹³. The structure consists of residues 2–467(chain A) and 4–467(chain B) of *MtGyrA*. The final model (Figure 1A) was refined to 1.60 Å resolution, with a final $R_{\text{work}}/R_{\text{free}}$ of 17.8/20.1%. Molprobit¹⁴ analysis showed a total of 98.4% of residues in the most favored regions of Ramachandran space, with none in

disallowed regions. (Table I). The atomic structure and coordinate files have been deposited with the PDB, ID code 3ILW.

Results and Discussion

At 1.6 Å, the *MtGyrA59* structure determined here is the highest resolution yet obtained for a type II topoisomerase domain. *MtGyrA59* assembles into a heart-shaped dimer (Figure 1B), with an overall fold that closely resembles the structure of the homologous *E. coli* GyrA NTD (44% sequence identity, 1.27 Å rmsd). One notable difference between the two proteins is in the loop connecting helices $\alpha 3$ and $\alpha 4$ (Gly88-Ala92), a region prone to acquiring quinolone-resistance mutations. The position of this loop in *MtGyrA59* deviates significantly from its location in *E. coli* GyrA NTD⁹, and in the quinolone- and DNA-bound structure of the homologous region of *S. pneumoniae* topoisomerase IV¹⁵, likely due to crystal-packing influences (Figure 1C). Superposition of *MtGyrA59* on the topo IV fluoroquinolone-DNA structure reveals that the position of Asp89 in *MtGyrA59* is likely to clash with moxifloxacin as bound by *S. pneumoniae* topo IV. The novel loop conformation is present in both subunits in the asymmetric unit, suggesting that this region is more conformationally dynamic than previously suspected, at least in *MtGyrA*.

MtGyrA59 and the *E. coli* GyrA NTD also differ significantly in the loop encompassing Ile181 (Ile174 in *E. coli*) (Figure 1D), a region that has been shown to bend DNA in *S. cerevisiae* topoisomerase II¹⁶. Interestingly, conformational diversity has been observed in other regions that interact with DNA. The loop bearing the active site tyrosine differs significantly between *E. coli* GyrA (residues 129–131) and the paralogous subunit of topoisomerase IV^{9,17}. These examples demonstrate that the critical DNA-engaging regions of the GyrA NTD are structurally malleable, a property that may ultimately play an important role in modulating inhibitor potency. Studies of *MtGyrA* and other type II topoisomerase homologs bound to drug and DNA substrates will be needed to address this issue in the future.

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Figure 1.

MtGyr59 structure. A) Representative electron density from a refined $2F_o - F_c$ map contoured at 2σ . B) Cartoon representation (Pymol¹⁸) of the *MtGyr59* dimer. The active site Tyrosine (Tyr129) is highlighted pink. C) Structural alignments of the *MtGyr59* (yellow) and *EcGyrA59* (fuchsia) helix $\alpha 4$ region. Gly88 and Asp82 are shown as space filling spheres to highlight the clashes that would occur if *MtGyr59* region adopted the same structure as seen in *EcGyrA59*⁹. Asp89 of *MtGyrA59* is also shown, together with moxifloxacin superposed from the *S. pneumoniae* topo IV co-crystal structure¹⁵, to highlight how the *MtGyr59* loop would clash with bound drug. D) Overlay of the DNA bending loop region between *MtGyr59* (yellow) and *EcGyrA59* (fuchsia).

Table I**Data Collection and Refinement Statistics**

<i>Data Collection</i>	
Beamline	ALS 8.3.1
Wavelength (Å)	1.1
Space Group	P1
Cell Dimension (Å) <i>a, b, c</i>	61.23, 70.05, 97.81
α, β, γ (°)	109.96, 91.16, 114.16
Resolution (Å)	50.00-1.60
R_{merge} (%) ^a	5.7 (47.8) ^b
I/ σ (I)	20.6 (1.95)
Redundancy	3.6 (2.8)
Completeness (%)	99.0 (43.5)
Unique Reflections ^c	152754
<i>Refinement</i>	
R_{work} (%) ^d	17.8
R_{free} (%) ^e	20.1
No. atoms	
Protein	7265
Water	406
Glycerol Molecules	7
B factor (Å ²)	
Protein	28.18
Water	34.89
<i>RMSD</i>	
Bond lengths (Å)	0.017
Bond Angles (°)	1.562
<i>Ramachandran (%)</i> ^f	
Favored regions	98.4
Allowed regions	1.6
Outliers	0

^a $R_{\text{merge}} = \frac{\sum_{\text{hkl}} \sum_i |I_i(\text{hkl}) - \langle I(\text{hkl}) \rangle|}{\sum_{\text{hkl}} \sum_i I_i(\text{hkl})}$, where $I_i(\text{hkl})$ is the intensity of an observation and $\langle I(\text{hkl}) \rangle$ is the mean value for its unique reflection. Summations cover all reflections.

^b The values in parentheses indicate the highest resolution shell.

^c $N_{\text{Obs}}/N_{\text{Unique}}$.

^d $R_{\text{work}} = \frac{\sum_{\text{hkl}} \|F_{\text{obs}} - k|F_{\text{calc}}|\|}{\sum_{\text{hkl}} |F_{\text{obs}}|}$

R_{free}^e was calculated same way as R_{work} , but with the reflections excluded from refinement. The R_{free} set was chosen using default parameters in PHENIX¹¹.

f_c^f Categories were defined by Molprobit.