

Determination of the structure of a type IV topoisomerase from *Staphylococcus aureus*

Stephen Carr, George Makris, Simon E. V. Phillips and Chris D. Thomas

Background

DNA topoisomerases are ubiquitous enzymes responsible for resolving topological problems arising during DNA transcription, recombination, replication and chromosome partitioning. Topoisomerase IV (topoIV) of *Staphylococcus aureus* is a type II topoisomerase and is composed of two homodimeric subunits: GrlA, which is responsible for DNA binding, strand cleavage and religation, and GrlB, which hydrolyses ATP enabling enzyme turnover. The action of topo IV *in vitro* include a reduction in the superhelical density within the target DNA; it has also been shown to be one of the primary targets of fluoroquinolone antibiotics in *S. aureus*.

A 56 kDa proteolytic fragment of GrlA (GrlA56) has been produced and this displays atypical DNA cleavage activity *in vitro*, as it is active in the absence of the ATP hydrolysing subunit, GrlB. GrlA56 has been crystallised and diffraction data collected at the ESRF (Grenoble, France) to a resolution of 2.8 Å.

Recent findings

Molecular replacement using fragments of GyrA (a homologous protein from *E. coli*) resulted in interpretable electron density maps for approximately 50% of the molecule. Iterative rounds of model building and refinement generated electron density for the missing regions, allowing the entire polypeptide chain to be traced (Fig. 1).

The overall fold of topoIV is similar to that seen in other type II topoisomerase enzymes with a DNA binding groove at the “top” of the enzyme, the active site at the central interface between the two monomers and the major dimerisation interface at the base of the protein. The relative orientation of the domains at the larger interface (boxed in Fig. 1) is significantly different to that seen elsewhere. The structure could therefore represent another crystallographic “snapshot” of an intermediate conformation involved in the catalytic cycle of topoIV, providing yet more information about the order of events within the protein during catalysis.

The DNA-binding/cleavage site (indicated by an arrow in Fig. 1) contains two tyrosine residues (Tyr 119) positioned on either side of a positively-charged DNA-binding groove. These residues undergo a transesterification reaction, attacking the “target” phosphoryl groups forming a covalent link to the DNA. This results in a covalent link between protein and DNA and cleavage of the DNA strand. There are many conserved, basic residues in the vicinity of Tyr119 thought to be involved in stabilisation of the transition state and of the 3' end of the DNA in the complex containing cleaved DNA (Figs. 2a and b).

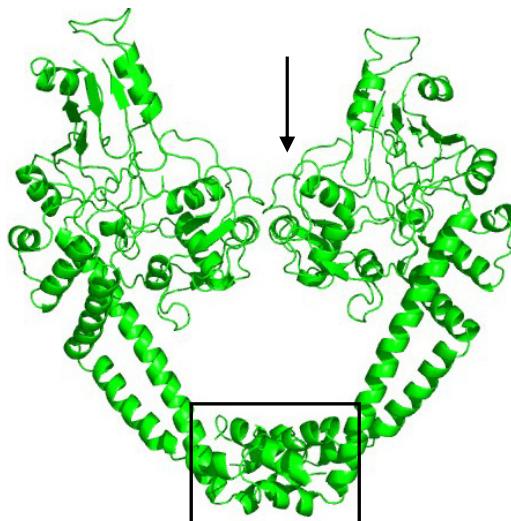


Fig. 1. TopoIV structure with domains in a novel orientation (boxed) and active site (arrow) indicated

This region is also involved in the binding of quinolone antibiotics and naturally occurring resistant phenotypes have altered residues at these positions (Fig. 2a). The various quinolones also display differing propensities for inactivating topoIV from various organisms and it is likely that changes in these residues contribute to this preference. The quinolone interaction region of GrlA contains of a number of different residues to those seen in the homologous proteins from *E. coli*, and these differences could contribute to variation in quinolone activity against topoisomerases from these different sources.

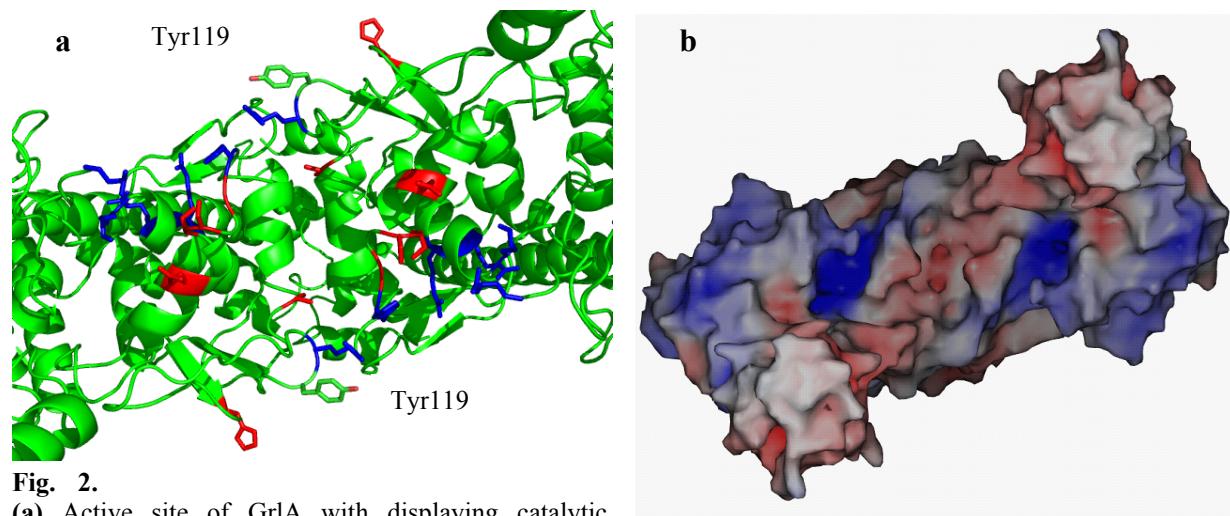


Fig. 2.

(a) Active site of GrlA with displaying catalytic tyrosine residues and conserved basic residues in blue and residues involved in quinolone binding in red. (b) Surface representation of GrlA displaying electrostatic charges highlighting the basic nature of the active site, positive charged regions are coloured blue and negative charges coloured red.

Acknowledgements

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