

# Structure of a Complex of *E. coli* DNA Topoisomerase III with a Single-Stranded DNA Oligonucleotide

A. Changela, A. Mondragón

*Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL, U.S.A.*

## Introduction

A variety of cellular processes, including DNA replication, transcription, and chromosome condensation, require the presence of enzymes that regulate the ensuing topological changes occurring in DNA. DNA topoisomerases carry out this function in all organisms by catalyzing the transient cleavage of single-stranded (ssDNA) or double-stranded DNA (dsDNA), the passage of DNA through the resulting break, and the rejoining of the broken phosphodiester backbone.<sup>1</sup> Although all known DNA topoisomerases function through the formation of a covalent phosphotyrosine intermediate, several different families of enzymes have evolved that differ in sequence, structure, and mechanism. Type IA DNA topoisomerases change the topology of DNA by forming a transient 5' phosphotyrosine linkage to allow another strand to pass through the break.

The structures of two type IA topoisomerases, *E. coli* DNA topoisomerase I<sup>2</sup> and III<sup>3</sup>, reveal that the main body of the enzyme forms a toroidal molecule comprising four domains that come together to enclose a central hole large enough to accommodate DNA. Based on the structures of *E. coli* DNA topoisomerase I and III, a basic catalytic mechanism has been postulated that explains how type IA topoisomerases can perform complex topological rearrangements of DNA including the relaxation of negatively supercoiled DNA and the catenation or decatenation of DNA molecules. Type IA topoisomerases function by recognizing and binding a ssDNA region within a duplex. The active site of the enzyme is located at the interface of two domains such that the catalytic tyrosine is inaccessible in the absence of substrate.

In order to gain insight into the interactions of a type IA topoisomerase with its ssDNA substrate, we have solved the 2.05 Å resolution structure of an inactive mutant of *E. coli* DNA topoisomerase III in a noncovalent complex with an 8-base ssDNA molecule. This structure is representative of a stage of the type IA topoisomerase catalytic cycle during which the enzyme interacts with ssDNA either prior to cleavage or after religation.

## Methods and Materials

The complex was crystallized by the hanging drop method. Crystals appeared in 2-3 days and grew as thin plates to maximum dimensions of ~0.05 x 0.2 x 1 mm<sup>3</sup> within 2 weeks. The crystals belong to space group C2 with cell dimensions of a=122.0 Å, b=60.8 Å, c=125.4 Å, and  $\beta=90.7^\circ$  and contain one complex per asymmetric unit. Prior to data collection, the crystals were quickly transferred in a single step to mother liquor supplemented with 25% glycerol and then flash cooled in liquid nitrogen. Macromolecular crystal annealing was performed to decrease crystal mosaicity.<sup>4</sup> Crystals diffracted to 2.0 Å at 100K at the APS

although they still displayed a large mosaicity (>1.5°). Diffraction data were collected in 0.5° oscillation steps due to the large mosaicity, processed with the program XDS<sup>5</sup> and scaled with SCALA.<sup>6</sup> The structure of the complex was solved by molecular replacement with AMORE<sup>6</sup> using diffraction data from a single crystal. All refinement was done in CNS.<sup>7</sup> The structure was refined to an R factor of 23.0% and an R<sub>free</sub> of 25.9% with 90.5% of all residues in the most favored regions of the Ramachandran plot and no residues in the disallowed regions.

## Results

The structure reveals that the enzyme undergoes a conformational change that allows the oligonucleotide to bind within a groove leading to the active site and realigns several conserved residues in a catalytically competent conformation. The positioning of the DNA within the active site provides insight into the role of several highly conserved residues and helps to establish a possible mechanism for cleavage and religation. The findings reinforce several aspects of the type IA topoisomerase mechanism while suggesting functional implications for type II topoisomerases and also other proteins that perform DNA rearrangements

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