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Structural Studies of *E. coli* Topoisomerase III-DNA Complexes Reveal a Novel Type IA Topoisomerase-DNA Conformational Intermediate

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Escherichia coli DNA topoisomerase III belongs to the type IA family of DNA topoisomerases, which transiently cleave single-stranded DNA (ssDNA) via a 5′ phosphotyrosine intermediate. We have solved crystal structures of wild-type *E. coli* topoisomerase III bound to an eight-base ssDNA molecule in three different pH environments. The structures reveal the enzyme in three distinct conformational states while bound to DNA. One conformation resembles the one observed previously with a DNA-bound, catalytically inactive mutant of topoisomerase III where DNA binding realigns catalytic residues to form a functional active site. Another conformation represents a novel intermediate in which DNA is bound along the ssDNA-binding groove but does not enter the active site, which remains in a catalytically inactive, closed state. A third conformation shows an intermediate state where the enzyme is still in a closed state, but the ssDNA is starting to invade the active site. For the first time, the active site region in the presence of both the catalytic tyrosine and ssDNA substrate is revealed for a type IA DNA topoisomerase, although there is no evidence of ssDNA cleavage. Comparative analysis of the various conformational states suggests a sequence of domain movements undertaken by the enzyme upon substrate binding.

Keywords: topoisomerase III; structure; complexes; ssDNA

Introduction

DNA topoisomerases perform a fundamental role in the cell by regulating DNA topology during a number of metabolic processes, including DNA replication, transcription, recombination, and chromosome condensation.[1–3] Topoisomerase-mediated transformation of DNA involves the transient cleavage of single-stranded (ssDNA) or double-stranded DNA (dsDNA), the passage of DNA through the resulting break, and the subsequent rejoining of the broken phosphodiester backbone. Topoisomerases use this basic reaction mechanism to catalyze a variety of complex DNA rearrangements, including the supercoiling and relaxation of DNA, and the catenation and decatenation of DNA molecules.

While all known DNA topoisomerases function through the formation of a covalent phosphotyrosine intermediate, several different subfamilies have evolved that differ in sequence, structure, and mechanism. Type I topoisomerases cleave a single strand of DNA while type II topoisomerases cleave both strands of a DNA duplex concurrently. Members of the type I family of enzymes are classified further as type IA topoisomerases, which form a 5′ phosphotyrosine linkage, or type IB topoisomerases, which function *via* a 3′ phosphotyrosine intermediate. Additionally, an archaeal type I enzyme, topoisomerase V, appears to define a new type I subfamily.[4] Type IA topoisomerases, exemplified by *Escherichia coli* DNA topoisomerase I and III, differ structurally and mechanistically from type IB enzymes, such as human topoisomerase I.[1] Type II enzymes bear no sequence similarity to type I topoisomerases, yet structural similarities observed between several type IA and type II topoisomerases suggest

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Abbreviations used: ss, single-stranded; ds, double-stranded; r.m.s.d., root mean square deviation.

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possible mechanistic similarities between the two families.[1]

Crystal structures of several type IA enzymes in the absence of DNA have been determined, including a 67 kDa N-terminal fragment of *E. coli* topoisomerase I,[5] *E. coli* topoisomerase III,[6] *Thermotoga maritima* topoisomerase I,[7] and *Archaeoglobus fulgidus* reverse gyrase.[8] The structures reveal that the core of the enzyme, which participates in the cleavage and strand passage events, adopts an overall four-domain, toroidal fold enclosing a central hole large enough to accommodate DNA. The active site of the apo enzyme is buried at the interface between two domains and is closed off to DNA. In the absence of DNA, the catalytic tyrosine participates in a hydrogen bond network with conserved residues from an adjacent domain, thereby remaining inaccessible to DNA. The variable C-terminal region, shown to be involved in DNA binding,[9,10] may be responsible in defining functional specificity of each enzyme.

There are only two structures of complexes of a type IA enzyme with DNA. The crystal structure of a catalytically inactive mutant of *E. coli* topoisomerase III (Y328F) complexed to an eight-base ssDNA molecule illustrated the conformational changes undertaken by the enzyme to accommodate its scissile ssDNA substrate.[11] In the structure of the non-covalent complex, the oligonucleotide binds along a groove that leads into the active site and is specific for ssDNA. DNA binding is accompanied by a domain reorientation resulting in the assembly of a catalytically competent active site where the Y328F substitution is ideally poised above the scissile phosphodiester bond and other conserved residues have realigned in preparation for catalysis. The proposed structure-based mechanism suggests that a conserved arginine (Arg330) and lysine (Lys8) serve to stabilize the phosphotyrosine transition state while an invariant glutamate (Glu7) is positioned to donate a proton to the 3′ leaving oxygen to facilitate cleavage. The second structure, a structure of a complex of wild-type *E. coli* topoisomerase I and ssDNA,[12] reveals a different conformational stage. In this structure, the enzyme remains closed and the active site is not formed, but part of the oligonucleotide is bound in the groove leading to the active site. A single helix forming part of this groove is displaced yet the rest of the enzyme maintains an overall apo-like conformation. The conformational stage observed appears to be an early intermediate just before the enzyme undergoes large domain rearrangements to open the active site and fully accommodate the ssDNA substrate.

While the structures of the *E. coli* topoisomerase III (Y328F)+ssDNA complex and *E. coli* topoisomerase I+ssDNA complex provided valuable insights into the pre-cleavage state of the enzyme, several questions remain to be addressed. The mechanism underlying activation of the catalytic tyrosine remains unclear. It is possible that the active site configuration seen in the structure of the DNA-bound topoisomerase III mutant does not accurately reflect the re-organization that occurs in the case of the active enzyme when the catalytic tyrosine is present. Furthermore, since the type IA topoisomerase reaction cycle proceeds through several different protein-DNA conformational intermediates, the capture of other enzyme-substrate intermediates, including a covalent enzyme-substrate complex containing the phosphotyrosine intermediate, is needed for a comprehensive understanding of the topoisomerase mechanism. We report here several crystal structures, ranging from 2.35 to 2.5 Å resolution, of the catalytically active form of *E. coli* topoisomerase III in complex with an eight-base ssDNA molecule at pH values ranging from 5.5 to 8. The structures reveal new enzyme+ssDNA conformational intermediates while further establishing key mechanistic aspects of the current type IA topoisomerase model for catalysis.

Results

Structure determination and overall structure

Initial crystals were obtained of wild-type *E. coli* topoisomerase III complexed to the same eight-base oligonucleotide used previously in structural studies with the *E. coli* topoisomerase III (Y328F) mutant.[11] The DNA sequence used for crystallization (5′-CGCAACT↑T-3′, where the arrow denotes the preferred site of cleavage) contains an asymmetric cleavage site recognized by *E. coli* topoisomerase III.[13] Surprisingly, the wild-type enzyme+ssDNA complex crystallized under the same conditions that produced crystals of the *E. coli* topoisomerase III (Y328F)+ssDNA complex yet the new crystals grow in a different crystal form. In the new crystals, there are two molecules in the asymmetric unit almost related by a 2-fold axis (~178°).

Interestingly, we discovered that the conformational state of each complex in the asymmetric unit depended on the cryoprotectant used prior to data collection. For crystals that had been cryoprotected in 25% glucose (Form I), the resulting structure shows both complexes in the same conformation. In

Figure 1. Overall structure of the open and closed complexes. (a) The diagram shows a schematic representation of the closed complex (Form I, pH 5.5). The four major domains of the protein are colored red, blue, purple, and green for domain I, II, III, and IV, respectively. The active site is found at the intersection of domains I and III. The ssDNA binding groove extends from domain IV to the active site. The ssDNA in the complex is shown in a ball and stick representation. (b) Schematic diagram of the open complex (Form II, pH 5.5), colored as in (a). (c) Stereo view showing the superposition of the closed (red), intermediate (blue), and open (green) complexes. The structures were aligned by superposing domain I only. The three structures correspond to Form I pH 5.5, Form II pH 8.0, and Form II, pH 5.5.