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Crystal Structure of Full Length Topoisomerase I from *Thermotoga maritima*

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DNA topoisomerases are a family of enzymes altering the topology of DNA by concerted breakage and rejoining of the phosphodiester backbone of DNA. Bacterial and archeal type IA topoisomerases, including topoisomerase I, topoisomerase III, and reverse gyrase, are crucial in regulation of DNA supercoiling and maintenance of genetic stability.

The crystal structure of full length topoisomerase I from *Thermotoga maritima* was determined at 1.7 Å resolution and represents an intact and fully active bacterial topoisomerase I.

It reveals the torus-like structure of the conserved transesterification core domain comprising domains I–IV and a tightly associated C-terminal zinc ribbon domain (domain V) packing against domain IV of the core domain. The previously established zinc-independence of the functional activity of *T. maritima* topoisomerase I is further supported by its crystal structure as no zinc ion is bound to domain V. However, the structural integrity is preserved by the formation of two disulfide bridges between the four Zn-binding cysteine residues. A functional role of domain V in DNA binding and recognition is suggested and discussed in the light of the structure and previous biochemical findings. In addition, implications for bacterial topoisomerases I are provided.

Keywords: crystal structure; topoisomerase I; *Thermotoga maritima*; zinc ribbon

Introduction

DNA topoisomerases are a family of ubiquitous enzymes known to manage the topological state of DNA in cells.[1] They function by transiently breaking one or two strands of DNA, passing single or double-stranded DNA through the resulting gap and finally resealing the gap. Topoisomerases are crucial in a variety of cellular processes such as replication, transcription and recombination. A number of different enzymes has evolved and based on their biochemical properties, topoisomerases have been classified into type II topoisomerases, which break both strands of a DNA duplex in concert, and type I topoisomerases, which only break one strand of DNA. Type I topoisomerases can be further classified into two subfamilies, namely type IA enzymes, which form a transient covalent bond with the 5′-end of the cleaved DNA, and type IB enzymes forming a transient 3′-end covalent bond. While bacterial type II topoisomerases are well established drug targets in antibacterial therapy, bacterial type I topoisomerases have only recently emerged as potential new targets for therapy.[2,3]

Initial structural insight to the topoisomerase IA family was gained from crystal structures of a 67 kDa N-terminal fragment of *Escherichia coli* topoisomerase I,[4] an *E. coli* topoisomerase III[5] and a reverse gyrase from a hyperthermophilic archaeon[6] and has been a major breakthrough in understanding of this enzyme class: the structures revealed the presence of a common core element,

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comprising four protein domains, which is responsible for DNA cleavage and strand passage. The four domains are situated around a central hole where domains I and IV, comprising the base of the structural element, are linked to domain III *via* an extended, arch-like domain II. The conformation of the core element as observed in the crystal structures is characterized by intimate interactions between domains I and III. The current "enzyme-bridged" model of the enzymatic reaction[7–9] requires drastic conformational changes of the protein in the course of the catalytic cycle including the complete dissociation of domain III from domains I and IV. The active site of the enzyme is situated in domain I and III, and contains several conserved residues including an essential tyrosine. In addition, crystal structures of *E. coli* topoisomerases I and III in complex with short oligonucleotides have established the presence of a DNA binding cleft formed by residues of domain I and IV at the base of the protein.[10,11]

Despite the knowledge derived from the crystal structure analysis of *E. coli* topoisomerase I, the truncated protein of *E. coli* topoisomerase I employed for structure determination[4,11] only contained the well conserved N-terminal region of the protein, comprising the four domain central core element, but lacked the C-terminal domain harbouring putative zinc binding motifs. The C-terminal region of bacterial topoisomerase I varies in length and sequence with up to five copies of zinc binding motifs present in this region.[12] Although the truncated *E. coli* topoisomerase I is able to cleave single-stranded DNA, the relaxation of negatively supercoiled duplex DNA is impaired.[13] The zinc binding motifs have been implicated in substrate binding, strand passage or protein–protein interactions *in vivo*[14–16] although their precise function is largely unknown. Up to now, no crystal structure of a full-length bacterial topoisomerase I comprising the core domain and the zinc binding motifs has been reported. In order to support drug discovery efforts on topoisomerase I, we have determined the crystal structure of the fully functional topoisomerase I from *Thermotoga maritima*.

Topoisomerase I from *T. maritima*, a hyperthermophilic eubacterium with an optimal growth temperature of 80 °C, has been identified as a member of the type IA topoisomerase family.[17,18] Both *T. maritima* topoisomerase I and *E. coli* topoisomerase I are able to bind and cleave single-stranded DNA with a common cleavage preference for a cytosine in position −4 of the cleavage point.[18,19] During the catalytic cycle both enzymes alter the linkage number of DNA substrates in steps of one, indicating a common enzyme bridged, strand-passage mechanism.[20] In contrast, *T. maritima* topoisomerase I has an exceptional high DNA relaxation activity, which is at least 100-fold higher compared to *E. coli* topoisomerase I.[18] Moreover, the C terminus of the enzyme contains only a single zinc binding motif[18] and truncation of the whole C-terminal domain results in greatly reduced DNA relaxation activity, probably caused by inefficient substrate binding.[21] However, in contrast, mutations within the zinc binding motif, which lead to a loss of the zinc binding ability, do not affect DNA cleavage, relaxation or decatenation activity of *T. maritima* topoisomerase I.[18] In *E. coli* topoisomerase I, zinc depletion inactivates the enzyme[22] and a point mutation within the second zinc binding motif alters the cleavage efficiency and specificity.[16] In addition, truncated *E. coli* topoisomerase I, comprising solely the core domain, cannot relax negatively supercoiled DNA at all.[13]

Here we report the crystal structure of full length topoisomerase I from *T. maritima* at 1.7 Å resolution representing an intact and fully active bacterial topoisomerase I. In particular, we describe here structure and spatial orientation of the C-terminal zinc binding repeat, a structural motif conserved in most bacterial topoisomerases I, and attempt to relate this to its functional role.

Results and Discussion

Overall structure of *T. maritima* topoisomerase I

T. maritima topoisomerase I shares the typical topoisomerase type IA fold with the known members of this family. Structures of *E. coli* topoisomerase I (40% sequence identity) and *E. coli* topoisomerase III (18% sequence identity) can be superimposed on *T. maritima* topoisomerase I with RMS deviations of 1.5 Å and 4.9 Å using the C^{α} atoms of domains I to IV, respectively. The overall structure of *T. maritima* topoisomerase I (Figure 1) comprises five different protein domains. It exhibits electron density for most of the amino acid residues except for six N-terminal residues (residues 1–6), a loop at the surface of the protein (residues 319–332) and the 32 C-terminal residues (residues 602–633).

The domains I–IV (residues 1–542), constituting the protein core, share high structural similarity with cleavage/strand passage domains of other type IA topoisomerases, namely the 67 kDa N-terminal fragment of *E. coli* topoisomerase I (residues 1–581) and the cleavage/strand passage domain of *E. coli* topoisomerase III (residues 1–609). Similar to the *E. coli* structures domains I–IV in *T. maritima* topoisomerase I are arranged to form a torus of dimensions 95 Å × 60 Å × 45 Å with a hole of approximately 25 Å diameter in the centre. The torus is thought to accommodate single or double-stranded DNA after strand passage.[4,5] Although *T. maritima* topoisomerase I is efficient in decatenating nicked minicircles present in kinetoplastic DNA[18] it lacks a so-called "decatenation loop" in proximity to the central hole that is necessary for efficient decatenation of various substrates by *E. coli* topoisomerase III.[5,23] The mechanism of decatenation in *T. maritima* topoisomerase I is therefore likely