doi:10.1016/j.jmb.2006.01.022 J. Mol. Biol. (2006) 357, 1202–1210

JMB

Available online at www.sciencedirect.com

SCIENCE DIRECT®

ELSEVIER

The Structure of the Transition State of the Heterodimeric Topoisomerase I of *Leishmania donovani* as a Vanadate Complex with Nicked DNA

Douglas R. Davies[1], Adeel Mushtaq[1], Heidrun Interthal[2] James J. Champoux[2] and Wim G. J. Hol[1,3]*

[1]*Department of Biochemistry Box 357742, University of Washington, Seattle, WA 98195-7742, USA*

[2]*Department of Microbiology Box 357242, School of Medicine University of Washington Seattle, WA 98195-7242, USA*

[3]*Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195 USA*

**Corresponding author*

Type IB topoisomerases are essential enzymes that are responsible for relaxing superhelical tension in DNA by forming a transient covalent nick in one strand of the DNA duplex. Topoisomerase I is a target for anti-cancer drugs such as camptothecin, and these drugs also target the topoisomerases I in pathogenic trypanosomes including *Leishmania* species and *Trypanosoma brucei*. Most eukaryotic enzymes, including human topoisomerase I, are monomeric. However, for *Leishmania donovani*, the DNA-binding activity and the majority of residues involved in catalysis are located in a large subunit, designated TOP1L, whereas the catalytic tyrosine residue responsible for covalent attachment to DNA is located in a smaller subunit, called TOP1S. Here, we present the 2.27 Å crystal structure of an active truncated *L. donovani* TOP1L/TOP1S heterodimer bound to nicked double-stranded DNA captured as a vanadate complex. The vanadate forms covalent linkages between the catalytic tyrosine residue of the small subunit and the nicked ends of the scissile DNA strand, mimicking the previously unseen transition state of the topoisomerase I catalytic cycle. This structure fills a critical gap in the existing ensemble of topoisomerase I structures and provides crucial insights into the catalytic mechanism.

Keywords: trypanosomatids; transition state; sleeping sickness; Chagas' disease; leishmania

Introduction

Flagellated protozoa of the genus *Leishmania* are responsible for causing a group of parasitic diseases called leishmaniases. Of the order of 1.5–2 million new cases of leishmaniasis are estimated to occur annually, including approximately 500,000 cases of the visceral form of the disease, which is nearly 100% fatal if untreated.[1] *L. donovani* is the species of the parasite primarily responsible for causing the visceral form of leishmaniasis in areas of India, Bangladesh, Nepal, and Sudan. The geographic reach of leishmaniases is expanding, and resistance to traditional first-line drugs, the pentavalent antimonials, is now observed in a majority of cases in India,[2] emphasizing the urgent need for new treatments.

Type IB topoisomerases alter the topology of DNA by cleaving and religating a single strand of the DNA duplex.[3,4] In this reaction, DNA is cleaved *via* a transesterification step in which a catalytic tyrosine residue attacks the scissile phosphodiester, resulting in the formation of a DNA–3′-phosphotyrosyl enzyme covalent complex and release of a DNA strand with a free 5′ hydroxyl end. In the subsequent religation step, the 5′ hydroxyl group of the cleaved strand attacks the phosphotyrosyl moiety of the covalent complex, rejoining the ends of the nicked DNA strand and expelling the tyrosine residue of the topoisomerase I.

The catalytic activity of type IB topoisomerases is derived chiefly from five strictly conserved amino acid residues. In human topoisomerase I (hTopo I) these residues are Arg488, Lys532, Arg590, His632, and Tyr723 (Figure 1(a)). Several hTopo I structures have been solved and show the enzyme at

Present address: A. Mushtaq, Domainex, Malet Street, London WC1E 7HX, UK.

Abbreviation used: hTopo I, human topoisomerase I.

E-mail address of the corresponding author: wghol@u.washington.edu

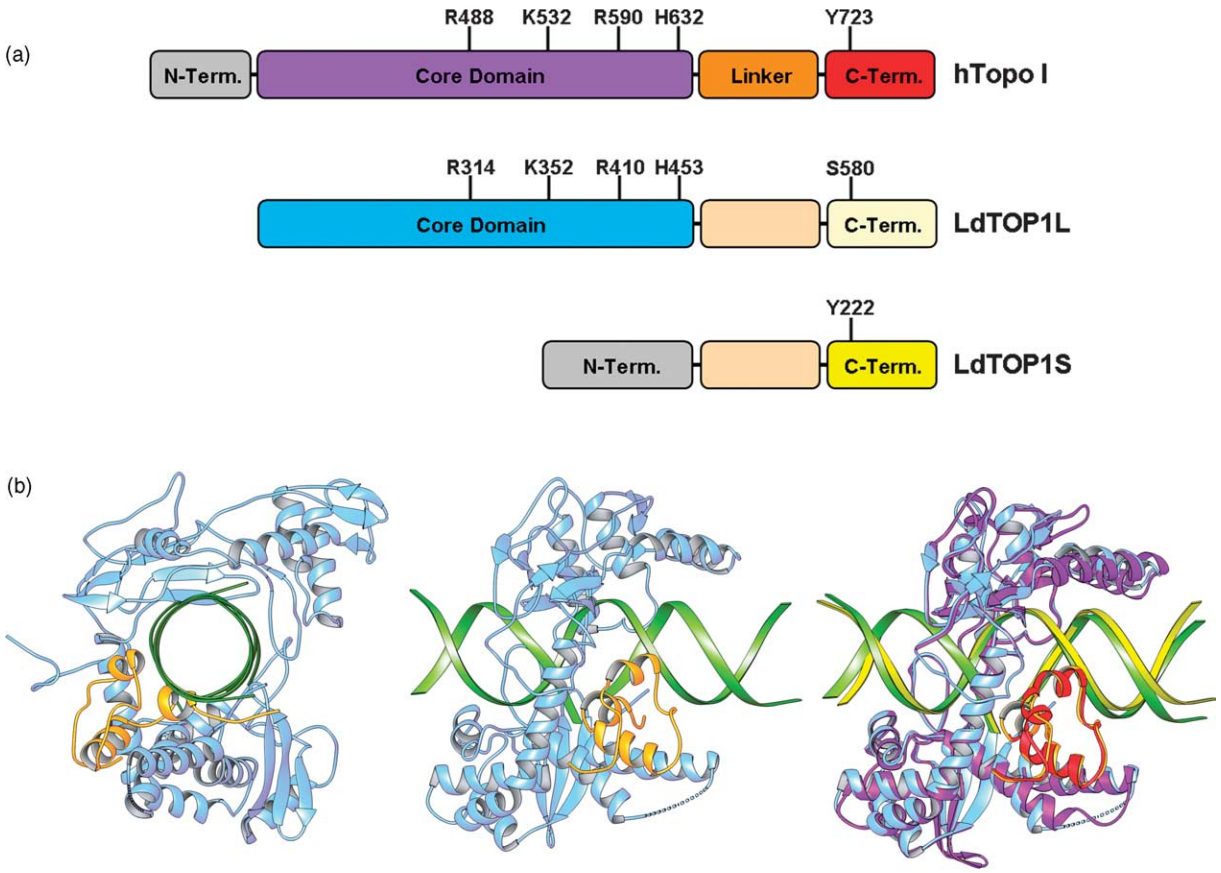

Figure 1. The overall structure of the LdTOP1LS–vanadate–DNA complex and general comparison with human topoisomerase IB. (a) Schematic alignment of human and *L. donovani* topoisomerase I subunits. Upper, human topoisomerase I; middle, LdTOP1L; bottom, LdTOP1S. Color-coding of domains matches that used for ribbon diagrams where the core subdomains of hTopo I are purple, the hTopoI C-terminal domain is red, the LdTOP1L core subdomains are blue and the LdTOP1S C-terminal domain is yellow. Vertical positioning represents approximate sequence alignment and catalytic residues are labeled. (b) Ribbon representations of the LdTOP1LS–vanadate–DNA complex. The LdTOP1L subunit is blue, the LdTOP1S subunit is yellow-orange, and the DNA backbone is green. The left-hand view is parallel with the DNA duplex axis; and the middle view is perpendicular to the DNA duplex axis. The right-hand view is a superposition of the LdTOP1LS complex with the human "reconstituted Topo I" (PDB 1A31), with the core subdomains purple, the C-terminal domain red and the DNA yellow. All Figures were created using Ribbons.[36]

the endpoints of the cleavage reaction. A Tyr723Phe mutant was used to obtain the structure of a non-covalent complex with DNA, representing the pre-reaction state.[5] A "suicide substrate" containing a 5′ bridging phosphorothiolate linkage was employed to arrest hTopo I as a covalent complex representing the DNA–3′-phosphotyrosyl intermediate following the cleavage reaction.[5] Furthermore, another Tyr723Phe structure containing a different base at the site of covalent attachment was solved that exhibited increased contacts between the basic side-chains and the central phosphate moiety, and was proposed to represent a state of the enzyme that was more advanced on the pathway to DNA cleavage (PDB ID 1EJ9).[6] Despite this impressive array of crystal structures, the catalytic mechanism of the type IB topoisomerases has still not been elucidated fully. The questions of whether there is a catalytic base responsible for activating the tyrosine nucleophile and the identity of a general acid for protonating the leaving group during cleavage remain unanswered.[3,6–8]

The structure and catalytic mechanism of type IB topoisomerases have been the subject of intensive study because topoisomerase I is critical for cellular processes such as DNA replication and transcription,[3,9,10] and because the nuclear topoisomerase I is the target of anticancer drugs such as camptothecin that poison the reaction by trapping the enzyme in the covalent enzyme–DNA intermediate state.[11,12,13] Camptothecin and its derivatives have been found to target the essential *L. donovani* topoisomerase I,[11,14] and these drugs are toxic to the parasite at concentrations that raise the intriguing possibility that such poisons may find a second life as anti-leishmaniasis drugs.

Although topoisomerase I has been confirmed to be the target of camptothecin in *L. donovani*,[11] the trypanosomal enzymes differ greatly from their human counterpart (Figure 1(a)). Whereas hTopo I