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Research Article

Functional Expression of a DNA-Topoisomerase IB from *Cryptosporidium parvum*

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Cryptosporidium parvum, one of the most important causative organisms of human diarrheas during childhood, contains a monomeric DNA-topoisomerase IB (*CpTopIB*) in chromosome 7. Heterologous expression of *CpTopIB* gene in a budding yeast strain lacking this activity proves that the cryptosporidial enzyme is functional in vivo. The enzymatic activity is comprised in a single polypeptide, which contains all the structural features defining a fully active TopIB. Relaxation activity of the yeast extracts was detected only when *CpTopIB* ORF was expressed in a yeast expression system showing time and protein dependence under steady state kinetic conditions. The susceptibility of CpTopIB-transformed yeast to the irreversible inhibitor camptothecin and its water-soluble derivatives (topotecan and SN-38) was assessed.

1. Introduction

Cryptosporidium parvum is one of the most important causal agents of human diarrheas during childhood [1]. Infections occur by ingestion from poor quality supply water contaminated with oocysts (resistance form of this microorganism) [2]. In humans the infection is self-limiting in immunocompetent individuals but may be fatal in HIV-immunocompromised patients [3]. In ruminants the infection is particularly severe in neonatal lambs, which suffer from apathy, anorexia, abdominal cramps, diarrhoea, and a large elimination of oocysts in faeces [4].

The high prevalence of this disease may be a consequence of the resistance of oocysts to chemical water treatments, to the poor efficacy of the current drugs used or the lack of an effective vaccine. Therefore, it is an essential integrated approach in the management of this condition that involves the use of disinfectants, host immune-reinforcing agents, and new drugs [5, 6].

During last decade more than hundred antibiotics and antiparasitic drugs have been tested against *C. parvum* infections with little or no effect [7]. Paromomycin, a poorly absorbed aminoglycoside antibiotic, and more recently nitazoxanide were pointed as promising agents to treat cryptosporidial infections in acquired immunodeficiency syndrome (AIDS) patients [8]. Both compounds showed efficacy in animal models [9, 10] and paromomycin was prophylactic in neonatal calves, lambs, and goats. On the other hand, nitazoxanide—a nitazoxazole benzamide compound—has a wide range of antimicrobial activity against helminthic parasites. More striking is the drastic reduction in the prevalence of opportunistic *Cryptosporidium* spp. infections associated to AIDS patients treated with highly active antiretroviral therapy (HAART) [11]. Therefore, there is an urgent need for novel compounds that eradicate these infections with poor or no side effects on the host [12].

Enzymes responsible for DNA manipulation have been studied for long as potential pharmacological targets in proliferative processes such as microbial and parasitic infections and tumor processes. DNA topoisomerases (Top) are enzymatic machines catalyzing changes in the topological state of duplex DNA during replication, transcription, recombination, and DNA repair processes. There are two families and several subfamilies of these enzymes: Type I DNA topoisomerases (TopI) are ATP-independent enzymes with relaxation activity for both positively and negatively supercoiled DNA [13, 14]. Type II DNA topoisomerases (TopII) are multimeric proteins that hydrolyze ATP to generate temporary DNA double-strand breaks followed by passage and rejoining. TopII enzymes not only relax positively supercoiled DNA but also knot/unknot and catenate/decatenate close circular DNA [15].

Many compounds have been screened against Top enzymes, and some of them are being used nowadays. Fluoroquinolones were successfully tested as inhibitors of the DNA gyrases in bacteria and some of them are potent antibiotics of choice [16]. On the other hand TopIB is being targeted in the treatment of several tumor processes (Pommier, 2006). Water-soluble derivatives of camptothecin (CPT)—topotecan and the prodrug irinotecan—are part of the current pharmacopoeia against certain types of cancers, but the studies are much scarcer in relation to topoisomerases in protozoan parasites [17, 18]. The amazing finding of a bi-subunit TopIB in Kinetoplastids [19] has opened a new field in the chemotherapy of these parasites, but little is known about Top in apicomplexan. Only *Plasmodium falciparum* TopIB (PfTopIB) has been cloned, functionally expressed and targeted with CPT derivatives [20, 21].

An early report describing the cloning of a type II DNA topoisomerase from *C. parvum* was published years ago [22] but no further information about these enzymes has been reported at present. In this paper we describe the functional expression of *CpTopIB* gene from the apicomplexan protozoa *C. parvum*, as well as the heterologous expression, kinetic analysis of the recombinant enzyme and the inhibition by CPT and analogues.

2. Materials and Methods

2.1. Reagents. DNA modification and restriction enzymes were procured from Roche (Basel, Switzerland) and Amersham Biosciences. *Pyrococcus furiosus* (*Pfu*) polymerase was from Stratagene (La Jolla, Calif, USA). Cell culture media, chemicals and reagents were purchased from Sigma (St. Louis, Mo, USA). Primers for PCR amplification were from Sigma Genosys (UK). CPT and SN-38 were from Tocris (Tocris Bioscience, Ellisville, Mo, USA), topotecan from R&S Pharmachem (Co. Ltd. China).

2.2. Yeast and Cryptosporidial Strains. C. parvum ooquistes were kindly provided by Dr. Teresa Gómez Muñoz; Dpt. Atención Sanitaria, Salud Pública y Sanidad Animal; Universidad Cardenal Herrera (CEU) Valencia (Spain). For protein expression *Saccharomyces cerevisiae* strains (a gift from Dr. MA Bjornsti, St. Jude Children's Research Hospital, Memphis, TN) EKY3 [*MAT α ura3-52 his3Δ200 leu2 Δ1 trp1 Δ63 top1* Δ::TRP1] and MBY3 [*MAT α ura3-52 his3Δ200* leu2 Δ*1 trp1*Δ63, top1 Δ::TRP1 rad52 Δ::LEU2], both deficient in DNA topoisomerase I activity, were used [23].

2.3. Cloning of Cryptosporidial DNA Topoisomerase I. Cloning of *C. parvum* DNA-topoisomerase I (CpTopIB) was performed by PCR amplification using whole genomic cryptosporidial DNA as template [24] and the primers showed in Table 1. *CpTopIB* (GenBank accession number XM-628497) gene, was amplified using *Pfu*-polymerase and nucleotides, and the amplified band was loaded in a low-melting gel agarose, electrophoresed and taken from the gel for cloning. The 1923 nt amplified band was digested with SmaI and HindIII and ligated to YCpGAL (Leu-) vector and sequenced before expression in a TopIB-deficient yeast strain.

2.4. Yeast Expression System. S. cerevisiae strain, EKY3 was transformed with different constructs viz; wild type (*CpTopIB*); R318A; K358A; R416A; H489A and Y600F, as the different point mutations, by electroporation [25]. The GAL promoted expression vector carries the Leu- selectable marker and is maintained by selection in synthetic complete SC leu- medium. At least five independent clones were selected from each transformation. After 6-hour induction with 2% galactose in SC leu- raffinose medium, the cells were harvested by centrifugation, washed, and resuspended in TEEG buffer (50 mM Tris-HCl pH 7.4, 1 mM EDTA, 1 mM EGTA, 10% glycerol) supplemented with 0.2 M KCl, and protease inhibitors cocktail [1 × NaF, 1 × sodium bisulphite, 2 × Complete Mini (Roche Molecular Biochemicals)]. Cell extracts were prepared by disruption with acid-washed glass beads using a bead-beater chamber (5 cycles of 30 seconds on, 30 seconds off) according to a previously described procedure [26]. Briefly, cells were subjected to one freeze/thaw cycle at −80°C, lysed by vortexing with 425–600 μm glass beads and the extracts were cleared by centrifugation at 15000 xg for 30 minutes at 4°C.

2.5. Protein Purification. Purification of CpTopIB was done according to [27]. Briefly, overexpressing yeast extracts were loaded onto a phosphocellulose (P-11) column previously equilibrated as manufacturer indications. The recombinant proteins (wild-type CpTopIB and mutated forms) were eluted at 4°C with a discontinuous gradient of KCl (0.2, 0.4, 0.6, 0.8 and 1 M) in TEEG buffer, supplemented with 0.1 mg/mL sodium bisulphite, 0.8 mg/mL NaF and the protease inhibitors cocktail. The active fractions were mixed with equal volume of 2 M NH_2SO_4 and loaded onto a phenyl-sepharose column preequilibrated with TEEG containing 1 M NH_2SO_4 (Sigma-Aldrich, St. Louis, USA). The column was eluted with a discontinuous inverse gradient of NH_2SO_4 (1, 0.8, 0.6, 0.4 and 0.2 M). In order to reach an appropriate concentration for the different in vitro assays, the eluate from the phenyl-sepharose column was concentrated by Microcon YM-30 (Millipore, Co, Ma, USA). Purity of the samples was analyzed by SDS-PAGE. To store, 40% glycerol was added to preserve the activity and keep in a −20°C freezer. Protein concentration was determined colorimetrically [28].

2.6. DNA Relaxation Assays. CpTopIB activity was assayed by the relaxation of a negatively supercoiled plasmid DNA. The reaction mixture in a total volume of 20-μL contained 0.5 μg of supercoiled RF I (ΦX-174) DNA, 10 mM Tris-HCl buffer pH 7.5, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/mL bovine serum albumin, 50 mM KCl, and 0.2 μg purified protein. The reaction mixtures were incubated for 30 minutes at 37°C. The enzyme reactions were digested with the addition of 2 μg of proteinase K, incubating for 1 hour and adding up to 1% SDS. The extent of plasmid DNA relaxation was assessed by electrophoresis in a 1% agarose gel in 0.1 M Tris borate EDTA buffer pH 8.0 at 2 V/cm for 14 hours. The gels were visualized

Table 1: Sequences of the primers used in this study to clone and to mutate CpTopIB.

Specimen		Primers (5′-3′)
Wild type	FW	ATA AGA AT**G CGG CCG C**AT GGC ATC AGC TAC GGA TTC
	RV	G**GA CTA G**TT TAA AAT GAA AAA TCT GAT CTC G
R318A	FW	GAT TTT TTA GCT TTG **GCG** GTC GGA GGA GAA AAA G
	RV	C TTT TTC TCC TCC GAC **CGC** CAA AGC TAA AAA ATC
K358A	FW	CTT TAG ATT TTT TGG **GCG** CGG ATT CAA TTC GAT AC
	RV	GT ATC GAA TTG AAT CCG **CGC** CCA AAA AAT CTA AAG
R416A	FW	CT GCC AAG G TA TTT **GCG** ACA TTC AAT GCA TC
	RV	GA TGC ATT GAA TGT **CGC** AAA TAC CTT GGC AG
H489A	FW	GCG ATA TTA TGT AAC **GCG** CAG AGA TCA GTT CC
	RV	GG AAC TGA TCT CTG **CGC** GTT ACA TAA TAT CGC
Y600F	FW	CA TCG AAG ATT AAC **TTT** ATG GAT CCC AGG ATT TC
	RV	GA AAT CCT GGG ATC CAT **AAA** GTT AAT CTT CGA TG

under UV illumination after being stained with ethidium bromide (0.5 mg/mL) and a posterior electrophoresis in the presence of 0.1 mg/mL ethidium bromide, in order to separate the nicked DNA from the relaxed topoisomers.

2.7. In Vitro Cleavage Assay. To 0.5 μg supercoiled RF I DNA, in a total volume of 20 μL cleavage buffer (35 mM Tris HCl, pH 7.5, 50 mM KCl, 3 mM β-mercaptoethanol, 0.1 mM EDTA, and 50 mg/mL BSA), was added a solution of the appropriate drug dissolved in DMSO or water (final concentration of DMSO in the incubation mixture must not exceed 1%). The DNA-cleavage reaction was initiated by the addition of 2 units of CpTopIB purified as above. After incubation for 30 minutes at 37°C, the cleavable complex was trapped by the addition of 2 μL 10% SDS, followed by the digestion with proteinase K for a further 30 minutes period at 45°C. The mixture was purified with phenol/chloroform and electrophoresed in a 0.9% agarose gel containing ethidium bromide (0.5 μg/mL). The increase of nicked DNA was estimated as drug-induced CpTopIB-mediated DNA cleavage.

2.8. Site-Directed Mutagenesis. The procedure to obtain the mutated genes was developed according to the QuickChange method, following the instructions procedure; the ORF encoding *CpTopIB* gene was mutated and subcloned in the YCpGAL(Leu-) vector. Figure 1 describes the distinct mutations performed on CpTopIB.

The sequence of the primers used and their codon changes are listed in Table 1. The PCR reaction contained 20 ng of plasmid YCp-*CpTopIB* as template depending on the different mutations, 1 μM of each oligonucleotide, 10 μM dNTPs, 5 μL of 10 × *Pfu*-buffer, and 2.5 units of *Pfu*-polymerase for a total volume of 50 μL. Reactions were carried out in a Mastercycler gradient thermocycler (Eppendorf) as follows: a 5-minute first step at 94°C, followed by an amplification cycle at 95°C for 1 minute, 60°C for 1 min and 72°C for 1 minute; repeated for 16 times. Mutants were sequenced (Sistemas Genomicos, Spain) in order to confirm the absence of undesirable additional mutations.

2.9. In Vivo Sensitivity to CPT. Sensitivity of the MBY3 yeast cell lines transformed with va s CpTopIB constructs to CPT was determined, as described earlier. The individual transformed yeast cell lines grown in SC leu- medium plus dextrose were serially diluted 10-fold and 5-μL of each culture were spotted onto selective SC leu-agar plates supplemented with 25 mM HEPES, pH 7.2, 2% dextrose or galactose, supplemented with 30 μM CPT in a final 0.25% DMSO. The controls plates without the drug contained 0.25% DMSO. Viability (growth) of yeast cells was scored, as per the size of the culture spot, following incubation at 30°C for 24 hours.

3. Results

3.1. Cryptosporidium parvum Topoisomerase IB. The active form of CpTopIB (GenBankTM accession number XM-628497) is the expression product of a single 1923 bp ORF. The expressed polypeptide corresponding to the putative CpTopIB is constituted by 641 amino acids with a predicted molecular mass of 74.7 kDa and a pI of 8.7, showing a 60% identity to human DNA topoisomerase IB (hTopIB) (GenBankTM accession number J03250), 75% identity to baker yeast's (yTopIB) (GenBankTM accession number K03007), and 99.8% of identity with *C. homini*. The protein is much shorter than human's (765 amino acids) and baker yeast's (769 amino acids), but contains all the recognizable domains of a canonical TopIB protein. A recombinant active enzyme was obtained by cloning the PCR-amplified band into the yeast expression vector YCp-GAL(Leu-) driven by GAL1 promoter and expressed in a TopIB-lacking yeast system (EKY3).

Figure 1(a) shows a sequence alignment of CpTopIB with its homologous from human, *P. falciparum,* and the budding yeast *Saccharomyces cerevisiae* showing that, as expected, CpTopIB contains a putative nonconserved N-terminal

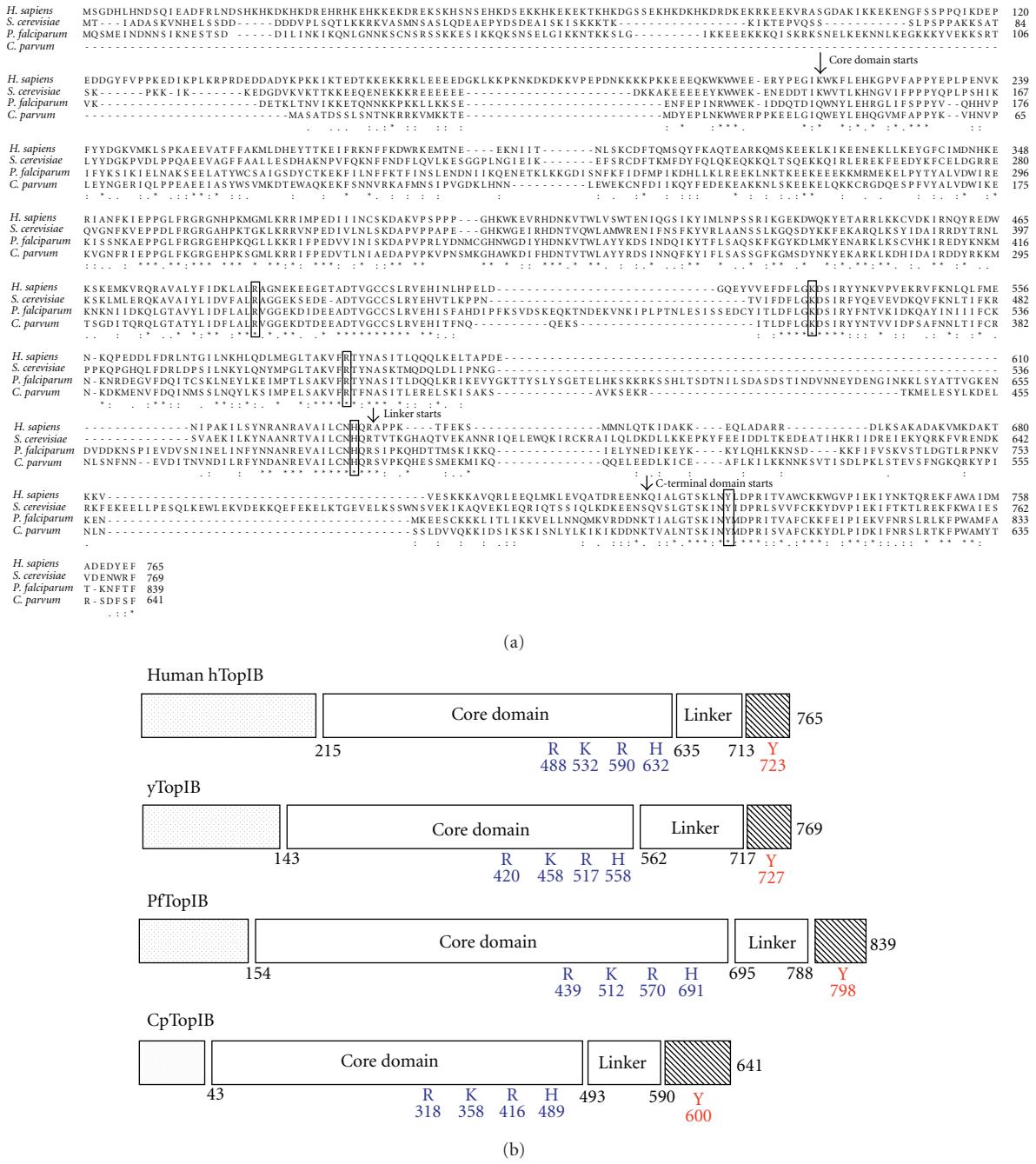

Figure 1: *Multiple amino acid sequence alignments of CpTopIB*. The amino acid sequences predicted for the *C. parvum*, *P. falciparum*, *S. cerevisiae*, and human topoisomerases were aligned using the ClustalX multiple sequence alignment program. *Symbols*: "∗" represents identical or conserved residues in all sequences in the alignment, ":" conserved substitutions and "." semiconserved substitutions. Complete genomic sequences of MAT proteins are available in GenBank, for *S. cerevisiae* accession number (K03007), *P. falciparum* (X83758), human (J-03250), and *C. parvum* (XM-628497). Arrowheads represent the points where a protein domain starts.

domain (start-Met-Gly 42), a conserved core domain (Ile-43-Val-492), and the C-terminal domain, (Thr-590-Phe-641) which shows high homology with other eukaryotic C-terminal ends [29] (Figure 1(b)). This region includes a phylogenetically conserved "SKINY" signature in which Tyr-600 (Tyr-723 in the human enzyme) plays role in DNA cleavage. The C-terminal domain is connected to the core by a poorly conserved coiled-coil putative linker (Val-493-Val-589). The putative cryptosporidial core domain has high homology with the one from the human enzyme (Gln 33-Ser 456) and other species, and it includes all the amino acid composing the so-called catalytic tetrad involved in the relaxing activity; Arg-318 (Arg-488 in the human enzyme), Lys-358 (Lys-532 in the human enzyme); Arg-416 (Arg-590

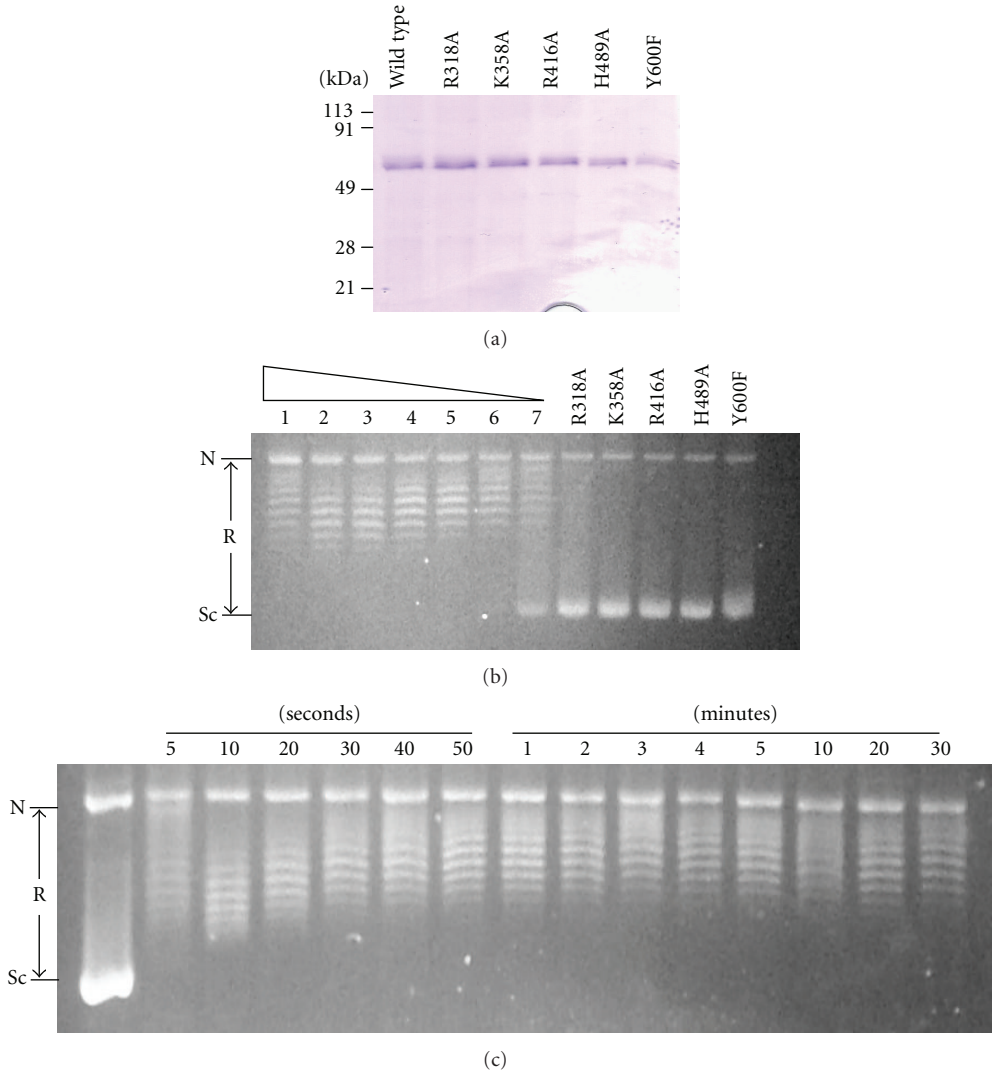

Figure 2: Expression and activity of CpTopIB and mutants into an EKY3 Top-IB-deficient yeast strain. (a) SDS-PAGE from the purified yeast extracts expressing wild type CpTopIB and the point mutations carried out into catalytic tetrad amino acids (R318A, K358A, R416A, and H489A) as well as the active site (Y600F). (b) Dilution assay 2-fold serial dilutions of wild type CpTopIB were assayed in a plasmid DNA relaxation assay for 30 minutes at 37°C (as described under "Materials and Methods"). (c) Equal concentrations of CpTopIB were incubated stepwise for 10 seconds to 30 minutes in a plasmid DNA relaxation assay. Reaction products were resolved in agarose gel and subsequently visualized by ethidium bromide staining. The reactions were incubated at 30°C for 30 minutes and then stopped with a mixture of 1% SDS and 6.1 μg of proteinase K. The reaction products were resolved in a 1% agarose gel and visualized by ethidium bromide staining. N: nicked; Sc: supercoiled DNA, R: relaxed DNA.

in the human enzyme) and His-489 (His 632 in the human protein).

When *CpTopIB* gene was expressed in the yTopIB-defective EKY3 yeast strain, using the YCp-GAL(Leu-) vector, significant relaxation activity was detected. Yeast extracts were pooled and purified as described above (Figure 2(a)). Purified CpTopIB was used for kinetic characterization. Relaxation activity of the supercoiled Rf I close circular DNA from the virus ΦX-174, was measured as a function of protein concentration (Figure 2(b)) and time (Figure 2(c)). Using 2 units of purified CpTopIB and 0.3 μg of supercoiled DNA substrate, a distributive relaxation activity with many relaxed topological isomers was obtained as early as 5 seconds, completing the process in 1 minute. Two-fold serially diluted CpTopIB-transformed EKY3 yeast extracts were assayed for relaxation activity (Figure 2(b)) starting from 0.2 μg protein (left lane). Significant activity was found after six 2-fold dilutions (0.03 μg protein) but no visible topoisomers were detectable beyond.

We further studied the relative in vitro DNA-cleavage potentials of CPT, topotecan, and SN-38—the active metabolite of irinotecan hydrolysis by cellular esterases [30]—at 10 and 100 μM final concentrations. The capability of these compounds to stabilize DNA-cleavable-complexes was determined by agarose gel electrophoresis, followed by SDS and proteinase K treatments that allow denaturation

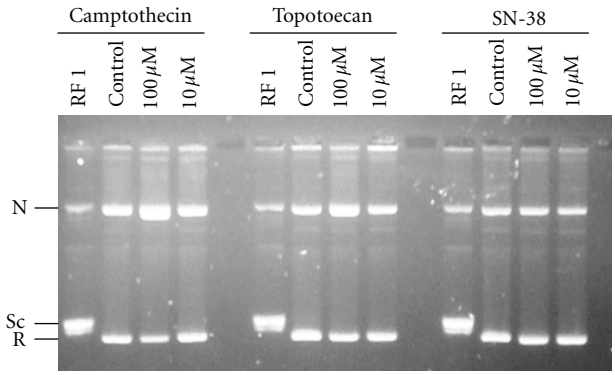

Figure 3: Effects of CPT, topotecan, and SN-38 on the stabilization of covalent TopIB-DNA cleavable complexes. Standard cleavage assay (Material and Methods) were carried out with final concentrations of CPT, topotecan, and SN-38 of 10 and 100 μM. CPT was added at a final concentration of DMSO of 1%. The reactions were incubated at 30°C for 30 minutes and then stopped with a mixture of 1% SDS and 6.1 μg of proteinase K. The reaction products were resolved in a 1% agarose gel and visualized by ethidium bromide staining. Sc: supercoiled DNA, R: relaxed DNA, N: nicked DNA.

and proteolysis of the binary complex, producing an increase in nicked DNA (Figure 3). There is a dose-dependent nicked-DNA increase for CPT and topotecan, but it could not be found for the irinotecan metabolite SN-38.

3.2. Site-Directed Mutagenesis Studies. The role of the amino acids of the catalytic tetrad was analyzed by site-directed mutagenesis, studying the relaxation activity of the mutated proteins. Based on these analogies, we replaced the residues sited at the core domain by the neutral amino acid alanine, which is not protonable at physiological pH, preventing the electrostatic contributions in substrate catalysis and hydrogen bonding (mutations R318A, K358A, R416A and H489A). On the other hand Tyr-600, placed at the C-terminal end, which aligns with a homologous Tyr at position 723 in the human enzyme, was replaced by Phe, which is sterically homologous but lacks the ability to cleave DNA (mutation Y600F). All these mutations were created in the pSK vector, carried to the yeast YCp-GAL(Leu-) plasmid, and heterologously expressed in the TopIB-deficient yeast system (EKY3). Figure 2(b) compares the residual relaxation activity of these mutants with the one found in the recombinant wild-type enzyme. As expected, none of the mutants of the active site created, displayed any significant ability to relax supercoiled DNA under standard assay conditions, using undiluted extracts (0.2 μg protein).

3.3. Topoisomerase-Deficient Yeast Sensitization to CPT. CpTopIB is conserved in terms of reaction mechanism and drug sensitivity. The heterologous expression of the wild type dimeric enzyme into a topoisomerase-deficient yeast strain (*top1*Δ) and defective in double-strand breaks repair (*rad52*Δ); MBY3 was able to confer sensitivity to 30 μM CPT when cultures where induced with 2% galactose (Figure 4).

The site-directed mutants prepared previously were transfected to the MBY3· yeast-deficient strain plated and induced with 2% galactose in absence and presence of 30 μM CPT. Pictures of Figure 4 show that none of the catalytic amino acids (R318A, K358A, R416A, and H489A), nor the active site mutant (Y600F), conferred susceptibility to camptotecin to the transformed yeasts. In addition CpTopIB MBY3—transformed yeast induced a significant drop in the number of viable cells when treated with CPT both in time course (Figure 5(a)) and dose-response (Figure 5(b)) experiments, thus showing the requirement of an active Top enzyme to be inhibited by the drug.

4. Discussion

There is no effective pharmacological treatment to eradicate cryptosporidiosis. It is well established that this disease can be fatal only when the immune system is challenged. Several antibiotics and antiprotozoal agents have been clinically or experimentally assessed, with very limited or no success. However, some drugs may be overviewed along with some new approaches attempted for new drug design and development against cryptosporidiosis. There is an increasing body of evidence that *Cryptosporidium* oocysts exposed to UV light are not able to regain the infectivity previous to exposure. Rochelle and coworkers explained that the mechanism of nucleotide excision repair (NER) is not sufficient to restore the damage caused to DNA due to mutations or the absence of repair genes [31]. Many Top inhibitors are DNA-damaging agents, causing stable single (SSB) and double strand breaks (DSB) [32]. This particular feature and the fact that these compounds are being used effectively as antineoplasic agents make Top enzymes promising targets for chemotherapy intervention against cryptosporidial infections.

We have cloned and functionally expressed the predicted CpTopIB gene (GenBank XM-628497), with *C. parvum* genomic DNA as a template. Like trypanosomatids, attempts to express this ORF in a routine bacterial expression system were not successful and a top-deficient yeast strain was required for this purpose [33]. Heterologous expression of *CpTopIB*-transformed *S. cerevisiae* strain under galactose pressure produced a single prominent band of 76 kDa, which is consistent with the estimated molecular mass of the putative ORF. Purified yeast extracts by a conventional low-pressure liquid chromatography procedure derived in a highly enriched solution of CpTopIB that was used to relax supercoiled DNA.

CpTopIB contains all the structural and enzymatic features described for all TopIB enzymes in a single polypeptide that relaxes supercoiled DNA distributively. The enzyme was targeted by CPT and the water-soluble derivative topotecan, which established irreversible bonds with DNA, but not by the irinotecan metabolite SN38 that surprisingly was not able to cleave DNA at the concentrations used. Site-directed mutagenesis of the amino acids composing the catalytic tetrad in the putative core domain yielded in no enzymatic activity at all, as well as the point substitution of the cleaving

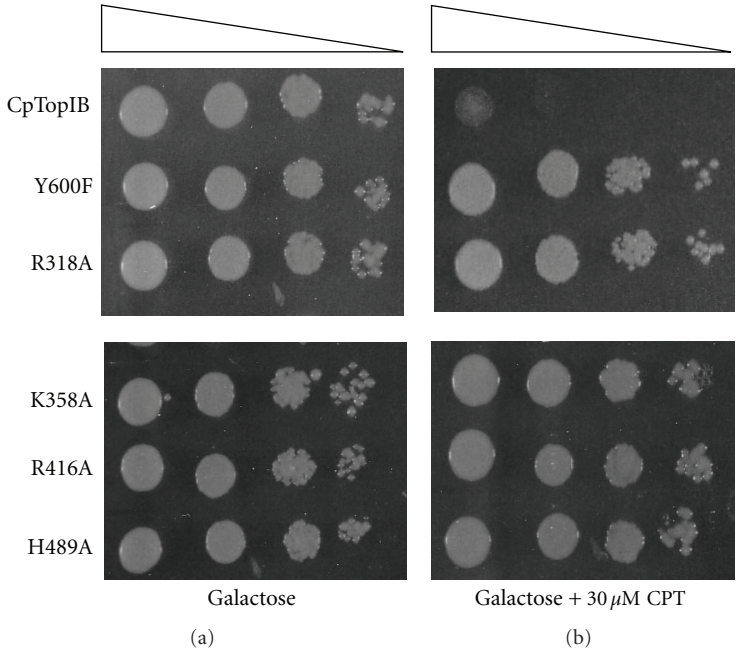

Figure 4: Expression of *CpTopIB* gene induces sensitivity to CPT-resistant yeast strain MBY3 (*rad52*Δ). Exponentially growing cells in dextrose, transformed with vectors carrying the cryptosporidial wild type and point mutations driven by GAL1 promoter, were serially 10-fold diluted starting from $OD_{595} = 0.5$. Five microliters were spotted on selective media under induced (2% galactose) conditions in the presence or absence of 30 μM CPT. All plates contained 0.375% DMSO. Empty assay was performed under similar assay conditions but expression the YCpGAL(leu-) vector without any insert.

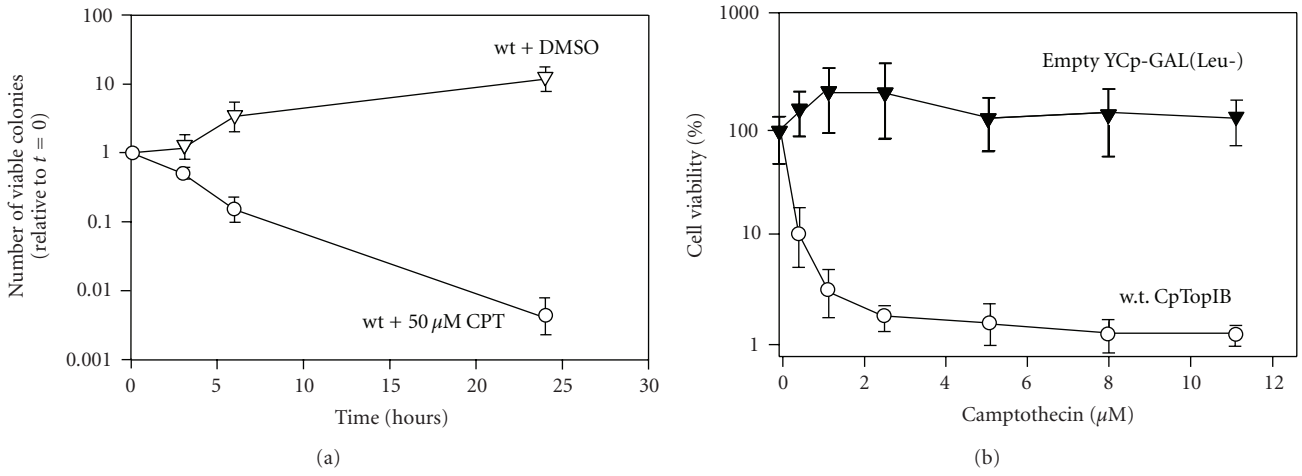

Figure 5: Yeast cell sensitivity to CPT depends on the expression of an active DNA topisomerase I from *Cryptosporidium* spp. (a) MBY3 yeast cells were transformed either with the YCp-GAL, vector empty, or carrying the wild type CpTopIB, in the presence or absence of 50 μM CPT (dissolved into 1% DMSO) at $t = 0$. After 3, 6, 24, and 48 hours aliquots were serially diluted and plated in Petri dishes containing yeast culture medium plus 1% dextrose. (b) Dose-response curve showing the inhibitory effect of CPT on yeast cell growing after 24 hours galactose induction. The number of colonies were determined at 30°C and plotted relative to the number of colonies obtained at $t = 0$. Data are the average of five different experiments.

Tyr-600 by Phe. All these results supported the existence of a conserved active centre that matched well with that reported in the human enzyme [29, 34].

Only one apicomplexan TopIB has been studied at present. Riou and coworkers, purified and characterized PfTopIB from infected erythrocytes (Riou et al., [35]). Like *C. parvum*, plasmodial enzyme is a monomeric protein of 104 kDa corresponding to a peptide of 839 amino acids which gene is located at chromosome 5 of the plasmodial genome, and it is developmentally regulated during the

different stages of *Plasmodium* life cycle [21]. In addition, the enzyme is effectively targeted by CPT that induces cleavable complexes with DNA in vitro [17].

In conclusion, there is an increasing resistance of *C. parvum* to such a broad range of chemotherapeutic agents, some of which are effective against other apicomplexan parasites. This might be related to the intracellular stages of the life cycle that are practically engulfed inside the host cytoplasm. Therefore, there is an urgent need of novel targets for drug screening. The above mentioned results show that *C. parvum* contains an active TopIB enzyme that is presumable involved in resolving structural problems in DNA during replication, transcription, and repair processes. Unlike other protozoa, the enzyme comprises a unique protein encoded by a single gene, which contains all the structural features defining a fully active TopIB. Understanding of distinct molecular characteristics of the CpTopIB and regulation of expression of the enzyme during parasite life cycle may be useful for development of novel selective inhibitors of CpTopIB as promising anticryptosporidial agents.

Abbreviations

Top:	DNA-topoisomerase
TopIB:	Type IB DNA-topoisomerase
TopII:	Type II DNA-topoisomerase
CpToPIB:	Gene encoding *Cryptosporidium parvum* topoisomerase I
hTOPIB:	Gene encoding human topoisomerase I
PfToPIB:	Gene encoding *Plasmodium falciparum* topoisomerase I
AIDS:	Acquired immunodeficiency syndrome
HAART:	Highly active antiretroviral therapy
DMSO:	Dimethylsulfoxide
CPT:	Camptothecin
NER:	Nucleotide excision repair
SSB:	Single strand breaks
DSB:	Double strand breaks

Acknowledgments

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