Topoisomerase II from Chlorella Virus PBCV-1
CHARACTERIZATION OF THE SMALLEST KNOWN TYPE II TOPOISOMERASE*

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Type II topoisomerases, a family of enzymes that govern topological DNA interconversions, are essential to many cellular processes in eukaryotic organisms. Because no data are available about the functions of these enzymes in the replication of viruses that infect eukaryotic hosts, this led us to express and characterize the first topoisomerase II encoded by one of such viruses. Paramecium bursaria chlorella virus 1 (PBCV-1) infects certain chlorella-like green algae and encodes a 120-kDa protein with a similarity to type II topoisomerases. This protein was expressed in Saccharomyces cerevisiae and was highly active in relaxation of both negatively and positively supercoiled plasmid DNA, catenation of plasmid DNA, and decatenation of kinetoplast DNA networks. Its optimal activity was determined, and the omission of Mg2+ or its replacement with other divalent cations abolished DNA relaxation. All activities of the recombinant enzyme were ATP dependent. Increasing salt concentrations shifted DNA relaxation from a normally processive mechanism to a distributive mode. Thus, even though the PBCV-1 enzyme is considerably smaller than other eukaryotic topoisomerase II enzymes (whose molecular masses are typically 160–180 kDa), it displays all the catalytic properties expected for a type II topoisomerase.

Superhelical and interlocked DNA structures are generated by several cellular processes, including DNA replication and transcription, chromatin condensation and chromosome partitioning (1, 2). The topological state of the cellular DNA must be strictly controlled and adjusted to various requirements throughout the cell cycle (1, 2). Topoisomerases are enzymes that control cellular DNA topology (1, 3). They catalyze reactions in which individual DNA strands or DNA double helices pass through one another, altering DNA superhelicity or DNA chain interlocking.

Type I topoisomerases relieve superhelical tension by cleaving one strand of a DNA double helix and rotating the broken strand around the one that remains intact (4, 5). In contrast, type II topoisomerases pass an intact DNA duplex through a double strand break in another duplex or in another segment of the same DNA molecule. Eukaryotic type II topoisomerases are hypothesized to function as homodimers, in which each subunit of the dimer has a conserved N-terminal ATPase domain and a centrally located DNA binding/active site domain as well as a less conserved C-terminal dimerization/nuclear localization domain (6, 7). Whereas yeast and Drosophila possess only one type II topoisomerase, vertebrates encode two distinct isoforms of the enzyme, called topoisomerase IIα and -β (8, 9). Topoisomerases IIα and -β have similar primary structures and similar biochemical properties, but their patterns of expression differ; topoisomerase IIα is preferentially expressed in proliferating cells, whereas topoisomerase IIβ is expressed equally in proliferating and quiescent cells (10). Type II topoisomerases are essential for viability. They play indispensable roles in chromosome condensation and decatenation of daughter chromosomes at the end of replication (10). In addition, they are the targets of a variety of anticancer agents (6, 11). Despite rapidly emerging data about the structure and properties of these enzymes (6, 7), details of their catalytic mechanism are still missing.

Several eukaryotic type I topoisomerases have been characterized including enzymes encoded by some viruses that infect eukaryotes (12–15). For example, studies on topoisomerase I from vaccinia virus provided valuable insights into the structure-function relationships and the mechanism of type I enzymes (15). Although there are many studies on type II topoisomerases from eukaryotes (8, 9, 12, 16, 17), until now there has not been any characterization of a type II enzyme from a virus that infects an eukaryotic organism. Open reading frames (ORFs) with similarity to type II topoisomerases have been identified in only three viruses that infect eukaryotes, the Iridovirus Chilo iridescent virus (18), the unclassified African swine fever virus (19), and the Phycodnavirus Paramecium bursaria chlorella virus (PBCV-1) (20). The PBCV-1 virus contains a linear, 330-kilobase, non-permuted double-stranded DNA genome with covalently closed hairpin ends (21). The PBCV-1 genome encodes 376 ORFs that are believed to be protein-encoding genes. Approximately 50% of these 376 ORFs resemble previously identified proteins in data bases, including ten proteins predicted to have roles in DNA replication or DNA repair (22). A PBCV-1 encoded pyrimidine dimer glycosylase (23) and a DNA ligase (24) have been characterized previously.

Herein, we demonstrate that PBCV-1 ORF A583L encodes a novel type II topoisomerase. In addition to being the first char-
acterized topoisomerase II encoded by a virus that infects a eukaryotic organism, the predicted 1,061 amino acid size of the PBCV-1 ORF is smaller than any other known topoisomerase II.

**EXPERIMENTAL PROCEDURES**

**Materials**—Negatively supercoiled pBR322 DNA was prepared as described (25). Kinetoplast DNA (kDNA) was isolated from *Trypanosoma brucei* as described (26). Restriction endonucleases and the Klenow fragment of *Escherichia coli* DNA polymerase I were purchased from New England Biolabs; AmpliTaq DNA polymerase was from Perkin Elmer; pPICZC, pCR2.1, and pPICZaB were linearized by EcoRI. pPM231, pBT273 vectors, and *Saccharomyces cerevisiae* BJ5464 strain were a kind gift from Dr. Louise Prakash (University of Texas Medical Branch). D(+)-galactose and polymin P were purchased from Sigma; PMSF, peptatin, and leupeptin were from either Sigma or Roche Molecular Biochemicals; calf thymus topoisomerase I and histone H1 were purchased from Life Technologies, Inc.

**Polymerase Chain Reaction Cloning, Sequencing, and Expression of the Putative PBCV-1 Topoisomerase II Gene**—The entire putative topoisomerase ORF (A583L) of 3,186 base pairs was amplified from PBCV-1 genomic DNA using polymerase chain reaction. The 100-μl reaction contained 0.5 μg template DNA in 10 μl Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01% (w/v) gelatin, 200 μM of each dNTP, 10 μl of each primer (5'-GGGCGATATCPCGCGTTACCAAAAGCT-3' and 5'-CCGGGAAGCTTCTACAACGATTGCAGA-3'), and 4 units of AmpliTaq DNA polymerase. After initial denaturation (94 °C, 2 min), the reaction was subjected to 35 cycles of the following program: denaturation at 94 °C for 30 s, annealing at 64 °C for 30 s, and primer extension for 8 min at 72 °C. Amplified DNA was cloned using pCR2.1 vector and confirmed by DNA sequence analyses using dye terminator cycle sequencing and an automated sequencer (Perkin Elmer/Applied Biosystems). Two EcoRI restriction sites, flanking the target DNA in the pCR2.1 vector were used to excise it from the vector and to introduce it into pPICZaB vector. The inserted target DNA was then excised from the latter vector using a double digest with NdeI and XhoI restriction enzymes. The cohesive ends of the DNA generated by these enzymes were filled in with the Klenow fragment of *E. coli* DNA polymerase I, and the blunt-ended insert was introduced into the pPICZC PmlI restriction site. These end filling and blunt end ligation procedures produced a Kozak sequence AAXATG for optimal eukaryotic expression. The target DNA was excised from the pPICZC plasmid with EcoRI and, after cohesive end-filling, subcloned into the BglII site of pPM231 yeast shuttle expression vector. The resultant construct pPM231pelc1 was introduced into *S. cerevisiae* BJ5464 using an electroshock protocol (Perkin Elmer/Applied Biosystems). Two EcoRI restriction sites, flanking the target DNA in the pCR2.1 vector were used to excise it from the vector and to introduce it into pPICZaB vector. The inserted target DNA was then excised from the latter vector using a double digest with NdeI and XhoI restriction enzymes. The cohesive ends of the DNA generated by these enzymes were filled in with the Klenow fragment of *E. coli* DNA polymerase I, and the blunt-ended insert was introduced into the pPICZC PmlI restriction site. These end filling and blunt end ligation procedures produced a Kozak sequence AAXATG for optimal eukaryotic expression. The target DNA was excised from the pPICZC plasmid with EcoRI and, after cohesive end-filling, subcloned into the BglII site of pPM231 yeast shuttle expression vector. The resultant construct pPM231pelc1topop was introduced into *S. cerevisiae* BJ5464 using an electroshock protocol (Perkin Elmer/Applied Biosystems).

**Purification of the Recombinant Enzyme**—All enzyme purification was conducted at 4 °C. Cells from a 1.5-liter yeast culture were centrifuged for 10 min at 4,000 g, resuspended in 35 ml of buffer A (25 mM sodium phosphate, pH 6.8, 500 mM NaCl, 10% glycerol, 1 mM EDTA, 0.5 mM DTT, 0.2 mM PMSF, 1 μg/ml peptatin, 1 μg/ml leupeptin) and disrupted with a French press. Cell debris was removed by centrifugation at 20,000 × g for 10 min and 10% (w/v) polymin P was added slowly to the supernatant, with stirring to 0.1% final concentration. Stirring was continued for 30 min after all polymin P was added. This suspension was centrifuged at 20,000 × g for 10 min and a saturated solution of (NH₄)₂SO₄ was added slowly with stirring to the supernatant to 55% of saturation. After 30 min of stirring, precipitated proteins were centrifuged at 20,000 × g for 10 min and (NH₄)₂SO₄ was slowly added to the supernatant to 80% of saturation, the suspension was stirred for 30 min, and centrifuged. Precipitated protein was resuspended in a small volume of buffer B (25 mM sodium phosphate, pH 6.8, 10% glycerol, 0.5 mM DTT, 0.1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml peptatin) to produce the equivalent of 0.2 x (NH₄)₂SO₄

Resuspended protein was applied to a 9-mL hydroxylapatite column (internal diameter 1.5 cm), pre-equilibrated with buffer C (25 mM sodium phosphate, pH 6.8, 200 mM potassium phosphate, pH 6.8, 10% glycerol, 0.5 mM DTT, 0.1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml peptatin) and washed with three volumes of buffer C. Protein fractions were collected from the column, and eluted from the column with buffer D (40 mM sodium phosphate, pH 6.8, 300 mM NaCl, 1 mM EDTA, 0.5 mM DTT). An equal volume of glycerol was added to the concentrated recombinant enzyme preparation, and the enzyme was frozen at −80 °C. Protein concentration during the purification was estimated by absorbance measurement at 280 nm, by the Bio-Rad protein assay kit (Bio-Rad), and by SDS-polyacrylamide gel electrophoresis. The topoisomerase II activity in individual fractions was monitored by the plasmid catenation assay, described below. For all assays requiring enzyme dilution, PBCV-1 topoisomerase II was freshly diluted in enzyme dilution buffer (20 mM sodium phosphate, pH 6.8, 150 mM NaCl, 50% glycerol, 0.5 mM EDTA, 0.25 mM DTT).

**Relaxation of Negatively Supercoiled Plasmid DNA**—Unless otherwise noted, relaxation reactions contained 5 nM negatively supercoiled pBR322 plasmid DNA, 1 mM ATP, and 0.4 mM enzyme in the reaction buffer (10 mM Tris-HCl, pH 8.5, 62.5 mM NaCl, 62.5 mM KCl, 2.5% glycerol, 2.5 mM MgCl₂, 0.1 mM EDTA). The reactions were started by addition of the enzyme to the rest of the components, incubated at 25 °C for 10 min, and terminated by addition of the stop solution (3 μl of 0.77% SDS, 77 mM EDTA). After adding electrophoresis loading buffer (40% sucrose, 0.05% bromophenol blue, 0.05% xylene cyanole), the samples were heated at 70 °C for 2 min, loaded on 1% agarose gels and electrophoresed in 100 mM Tris borate, pH 8.3, 2 mM EDTA (TBE buffer) at 5 V/cm for 2.5 h. The gels were stained for 30 min in an aqueous solution of ethidium bromide (1 μg/ml). DNA bands were visualized by UV light, and photographed and quantitated using an Alpha Innotech digital imaging system. DNA relaxation was determined from loss of supercoiled DNA. The effects of ionic strength, pH, temperature, and magnesium concentration on PBCV-1 topoisomerase II DNA relaxation activity were measured under relaxation assay conditions, changing one of the above variables at a time. It should be noted that in experiments varying temperature, we made no attempt to control the pH of the reaction due to temperature effects on the pH of our Tris buffer. The level of enzymatic activity in this optimization study was expressed as the percentage of the supercoiled DNA that had been relaxed in the total amount of supercoiled DNA. For ionic strength experiments the NaCl/KCl ratio was always 1:1 (referred to as “salt” in the analysis). Thermal stability assays were conducted by incubating 0.4 mM PBCV-1 topoisomerase with 5 nM pBR322 DNA, but no ATP in the reaction buffer at each temperature. At appropriate times, 18-μl aliquots were removed, added to tubes containing 2 μl ATP, and incubated at 25 °C for 10 min. Reactions were stopped and analyzed as above. The effects of substituting nucleoside analogs for ATP or other divalent cations for Mg²⁺ were studied under described conditions, except that the indicated nucleoside analogs (DATP, GTP, CTP, and TTP) and divalent salts (CaCl₂, MgCl₂, CoCl₂) were used instead of ATP and MgCl₂ respectively.

**Relaxation of Positively Supercoiled Plasmid DNA**—Relaxation of positively supercoiled DNA was determined by the procedure of Osheroff et al. (29). Samples contained 5 nM relaxed pBR322 plasmid DNA, 2 mM PBCV-1 topoisomerase II, 0.5 mM ATP, and 10 μM ethidium bromide in 40 μl of reaction buffer containing 5 mM MgCl₂. Relaxed pBR322 DNA was generated by treating negatively supercoiled pBR322 with calf thymus topoisomerase I in reaction buffer. The enzyme was heat inactivated before the addition of other reaction components. After adding ethidium bromide and ATP, samples were incubated for 5 min at 25 °C. Topoisomerase II was added, and samples were incubated for up to 15 min at 25 °C. Reactions were stopped by adding an equal volume of
phenol-chloroform. Aqueous phase (20 μl) was removed from the reactions and mixed with 3 μl of stop solution followed by 2 μl of the loading buffer. Samples were subjected to electrophoresis in a 1% agarose gel in 40 mM Tris acetate, pH 8.3, 2 mM EDTA. DNA bands were stained, visualized and photographed as described above.

**Catenation of Plasmid DNA**—Catenation reactions contained 5 nM of pBR322 plasmid DNA, 1 mM ATP, 3 mg/ml of histone H1, and indicated amounts of the enzyme in the reaction buffer. The catenation assays were initiated by adding enzyme, incubated at 25 °C for 10 min, and stopped by addition of 3 μl of stop solution. Following proteinase K digestion (0.08 mg/ml final concentration) at 45 °C for 15 min, the samples were combined with the electrophoresis loading buffer and electrophoresed in a 1% agarose gel in TBE buffer, containing 0.5 mg/ml ethidium bromide. Decatenation products were visualized and photographed as above. One unit of PBCV-1 DNA topoisomerase catenation activity is defined as the amount of enzyme required to fully catenate 0.3 μg of supercoiled pBR322 plasmid DNA in 10 min at 25 °C in 20 μl of reaction buffer with 3 μg/ml histone H1.

**Decatenation of kDNA**—Decatenation was performed as described (30). The assays contained 0.3 μg kDNA, 1 mM ATP, and 0–8 nM topoisomerase II in 20 μl of reaction buffer. A control sample contained 24 units of calf thymus topoisomerase I in place of topoisomerase II. Reactions were initiated by the addition of PBCV-1 topoisomerase II, and samples were incubated for 10 min at 25 °C. Reactions were terminated by adding 3 μl of stop solution. Samples were mixed with loading buffer and electrophoresed in a 1% agarose gel in TBE buffer, containing 0.5 μg/ml ethidium bromide. Decatenation products were visualized and photographed as above.

**RESULTS**

**Expression and Purification of Recombinant PBCV-1 Topoisomerase II**—The PBCV-1 a583l gene, encoding a topoisomerase II homolog, was cloned into the GAL-inducible episomal expression plasmid pPM231, named pPM231pbcv1top, and transformed into a protease-deficient S. cerevisiae strain, BJ5464. Galactose induction resulted in the appearance of substantial amounts of a soluble protein with the predicted molecular mass (~120 kDa) of the PBCV-1 topoisomerase II-like protein (Fig. 1, lane 2). Polyclonal antibodies produced from short synthetic peptides, designed from the A583L amino acid sequence, reacted with the 120-kDa protein. Crude lysates from the transformed yeast had topoisomerase II activity, as judged by the ability to catenate circular DNA molecules (Table I). When yeast containing the vector alone were induced with galactose, the 120-kDa protein was absent, and DNA catenation activity was not detected from these lysates (data not shown). Therefore, it was concluded that the 120-kDa protein was indeed the PBCV-1 a583l gene product, the topoisomerase II-like protein. Further steps in the isolation of the recombinant enzyme from crude yeast lysates are summarized in Table I. As a final step, the recombinant enzyme was chromatographed on a hydroxylapatite column. The activity peak, as measured by the plasmid DNA catenation assay, coincided with the elution peak of the PBCV-1 topoisomerase-like protein (Fig. 2). Peak activity fractions were pooled, buffer exchanged, and concentrated. At this stage the enzyme was >90% pure (Fig. 1, lane 4). This expression/purification procedure produced 2–4 mg of protein/1.5 liter of culture.

**Relaxation of Negatively Supercoiled DNA**—The recodi-
nant PBCV-1 topoisomerase relaxed negatively supercoiled, covalently closed circular plasmid DNA in a time-dependent fashion (Fig. 3). For example, 5 nM pBR322 DNA became relaxed in about 16 min in the presence of 0.4 nM enzyme in a 20 μl reaction (Fig. 3B). DNA relaxation was linear until about 90% of the substrate were utilized; this linearity indicates that the relaxed DNA does not compete significantly for binding to the enzyme. Thus, supercoiled DNA is the preferred substrate.

**The Effects of Salt, pH, Temperature, and Mg²⁺**—The optimal salt, Mg²⁺, pH, and temperature for PBCV-1 topoisomerase catalysis are reported in Fig. 4. The optimal salt concentration (combined NaCl and KCl in equimolar ratio) of PBCV-1 topoisomerase activity was 125–150 mM (Fig. 4A). The enzyme was also active at 150–190 mM, albeit in a more distributive manner (see below). The PBCV-1 enzyme was inactive in Tris-HCl buffer at pH below 7.5 with the best activity at pH 8.5 and higher (Fig. 4B). PBCV-1 topoisomerase activity had a broad temperature maximum, between 20 and 30 °C (Fig. 4C); the enzyme was inactive at temperatures above 40 °C. PBCV-1 topoisomerase required magnesium ions for activity with the highest activity occurring at 2.5 mM Mg²⁺ (Fig. 4D). Ca²⁺, Mn²⁺, or Co²⁺ at concentrations ranging from 0.1 to 10 mM could not replace Mg²⁺ in the PBCV-1 topoisomerase reaction (data not shown).

**Processive and Distributive DNA Relaxation**—Processive DNA relaxation is a mode of topoisomerase catalysis, wherein the enzyme binds to DNA and removes many, if not all, supercoils before dissociating from the DNA (29). In contrast, in a distributive catalytic mode, the enzyme removes only one or a few superhelical turns per single binding event due to a greater tendency to dissociate from its substrate. Increasing the salt concentration to slightly more than the optimal 150 mM caused the normally processive PBCV-1 topoisomerase to become distributive (Fig. 5). The distributive relaxation was also supported by low ATP or high Mg²⁺ concentrations (not shown).

**ATP Dependence and Specificity**—Like other type II topoisomerases, PBCV-1 topoisomerase requires ATP for its DNA relaxation activity (Fig. 6). When ATP was omitted from reactions, no relaxed DNA product was formed. However, ATP concentrations as low as 100 μM supported the reaction with the optimal ATP concentration being 1 mM. dATP, but not GTP, CTP, or UTP, could substitute for ATP in the reaction (data not shown).

**Enzyme Stability**—To assess the temperature stability of PBCV-1 topoisomerase, DNA relaxation assays were performed with enzyme preincubated at 15, 25, and 35 °C in solutions containing all reaction components except ATP. At various time points, aliquots of these reaction solutions were combined with ATP and incubated at 25 °C for 10 min. The half-life of PBCV-1 topoisomerase II was approximately 50 min under relaxation reaction conditions (Fig. 7). Hence, the enzyme should retain most of its activity in the 10-min incubations at 25 °C used in the protocols as described.

**Positively Supercoiled DNA Relaxation**—A DNA unwinding assay was used to investigate relaxation of positively supercoiled DNA by PBCV-1 topoisomerase. Intercalative compounds such as ethidium bromide, locally unwind DNA and create compensatory positive superhelical twists in distal regions of a closed circular DNA molecule (2). Thus, a plasmid that is relaxed will appear to be positively supercoiled. When ethidium bromide-DNA complexes are treated with a topoisomerase capable of relaxing positively supercoiled DNA, the unconstrained positive DNA superhelical twists are removed. Subsequent extraction of the drug allows the local drug-induced unwinding to redistribute along the plasmid and manifest itself as net negative supercoiling. The overall result is the conversion of relaxed plasmids to negatively supercoiled molecules. In the presence of ethidium bromide, PBCV-1 topoi-
somerase converted relaxed pBR322 plasmid DNA to supercoiled forms (Fig. 8). Thus, like other type II enzymes, PBCV-1 topoisomerase is capable of relaxing positively supercoiled DNA.

DNA Catenation and Decatenation—Type II topoisomerases can interlock two or more circular DNA duplexes to form a higher order multimeric network in the presence of ATP and a DNA condensing agent, like histone H1 (1). As shown in Fig. 9A, PBCV-1 topoisomerase catenated pBR322 plasmid DNA. However, the catenanes failed to form if the high-energy cofactor was absent from the reaction (no ATP lane). DNA catenation by PBCV-1 topoisomerase required 20 times higher enzyme concentrations than those required for DNA relaxation.

Typically type II topoisomerases also catalyze decatennation of topologically linked DNA molecules in an ATP-dependent fashion. Fig. 9B demonstrates that PBCV-1 topoisomerase can decatenate kDNA networks and that the reaction is ATP-dependent. Consequently, as in the case of other type II topoisomerases, ATP is absolutely required for all the major activities of PBCV-1 topoisomerase. Because DNA networks can also be decatenedated by type I topoisomerases (if the DNA has single strand breaks), a topoisomerase I control was included in this experiment (Fig. 9B). The absence of decatennation with excess purified topoisomerase I indicated that PBCV-1 topoisomerase decatennates kDNA by a mechanism consistent with a type II enzyme.

DISCUSSION

The PBCV-1 topoisomerase II (1,061 amino acids) is the smallest euukaryotic topoisomerase II described to date and possibly the minimal type II enzyme, making it a convenient target for structure-function analysis, as compared with larger euukaryotic type II enzymes. Using S. cerevisiae topoisomerase II as a model, the smaller size of PBCV-1 enzyme results from the absence of about 260 amino acids at the C terminus. In larger topoisomerase II family members this region contains a nuclear localization signal and other regulatory regions. PSORT, a University of Osaka based cellular protein localization prediction program, predicts that PBCV-1 topoisomerase II is 5 times more likely to be a cytoplasmic protein than a nuclear protein. The same program predicts, for example, that the yeast topoisomerase II is a bona fide nuclear protein. Whether PBCV-1 topoisomerase II enters the nucleus of its host cell (e.g. via binding to another protein directed to the nucleus) or whether its function is limited to the cytoplasm is unknown. Interestingly, we have predicted that infecting PBCV-1 DNA and probably DNA-associated proteins are targeted to the nucleus where virus transcription occurs (22). The intracellular site of DNA replication remains to be determined.

This report demonstrates that the protein encoded by the chlorella virus PBCV-1 3,186-base pair intronless a583f gene has all the catalytic properties expected for a type II topoisomerase enzyme. The predicted amino acid sequence of the PBCV-1 enzyme has about 45% amino acid identity to type II topoisomerases from Arabidopsis thaliana, S. cerevisiae, Homo sapiens, and Drosophila melanogaster. The virus enzyme contains the topoisomerase II signature pattern LTEGDSAAT and a conserved active site tyrosine (residue 743). Like other type II topoisomerases, the PBCV-1 topoisomerase II can: 1) relax both negatively and positively supercoiled DNA (Figs. 3 and 8); 2) efficiently interlock circular DNA monomers into catenanes and unlink catenated circular DNA multimers (Fig. 9). PBCV-1 topoisomerase II removes negative superhelical twists from a plasmid DNA as efficiently as D. melanogaster topoisomerase II (29). Both over- and under-wound DNA are relaxed in a processive manner at optimal reaction conditions by the PBCV-1 enzyme; the S. cerevisiae and D. melanogaster type II enzymes behave in a similar manner (12, 29).

As detailed by Osheroff et al. (29), processivity or distributivity of a topoisomerase II is related to the ratio of rates of DNA relaxation and enzyme dissociation from DNA. The data in Fig. 5 demonstrate that the chlorella virus topoisomerase II...
relaxes negatively supercoiled pBR322 DNA distributively at salt concentrations higher than optimal. Eukaryotic type I topoisomerases do not require a high energy cofactor such as ATP for activity, and may even be inhibited by ATP (1, 32). Also, while it can have a stimulatory effect, Mg\textsuperscript{2+} is not essential for activity of type I topoisomerases (4). In contrast, like other type II topoisomerases, PBCV-1 topoisomerase II requires both Mg\textsuperscript{2+} ions and ATP for activity (Figs. 4 and 6).

Previous experiments on eukaryotic type II topoisomerases have demonstrated the importance of certain structural features of the nucleoside triphosphate cofactor. For example, the 2’-OH group is important but not essential for cofactor binding to the enzyme and enzyme turnover. Also the adenine moiety of the cofactor, and especially its N\textsuperscript{6} amino group, are critical for ATP-enzyme interaction and possibly enzyme catalysis (29, 31). As with other type II topoisomerases (12, 29), dATP, but not ribonucleoside-triphosphates other than ATP, satisfied the PBCV-1 topoisomerase II high energy cofactor requirement. Besides Mg\textsuperscript{2+}, none of the divalent cations examined (Mn\textsuperscript{2+}, Ca\textsuperscript{2+}, or Co\textsuperscript{2+}) supported activity of the enzyme. This result agrees with existing data on eukaryotic type II topoisomerases, although the Mg\textsuperscript{2+} optimum for the PBCV-1 enzyme is slightly lower (2.5 mM) than that for other topoisomerases (5–10 mM) (12, 29). The pH dependence profile of PBCV-1 topoisomerase II is similar to that of other family members. The virus enzyme activity has a wider temperature maximum than most type II topoisomerases: 20–30 °C.

In conclusion, our report extends the body of knowledge about activities of type II topoisomerases into the territory of eukaryotic virology. It provides characterization of the smallest known member of eukaryotic type II class of enzymes, while highlighting the overall similarity of its basic catalytic properties to other enzymes in its class. The study, however, raises questions about the biological role of this novel topoisomerase. First, if the primary structure and the function of this enzyme resemble those of its eukaryotic host, why would the virus encode its own topoisomerase? Is it needed to assist the host enzyme with its function, or does it have a unique role in viral replication? Second, how does the “truncation” in the C terminus of the PBCV-1 enzyme effect its targeting and regulation? Investigating these issues in vivo should enhance our understanding of this important class of enzymes.

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