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Characterization of a prokaryotic topoisomerase I activity in chloroplast extracts from spinach

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ABSTRACT

A topoisomerase I activity has been partially purified from crude extracts of spinach chloroplasts. This activity relaxes the supercoiled covalently closed circular DNA of pBR322. The enzyme requires Mg^{++}, but not ATP, and has an apparent molecular weight of about 115,000. It catalyzes a unit change in the linkage number of supercoiled DNA but cannot relax positive supercoiled DNA. These characteristics of the topoisomerase suggest it is of the prokaryotic type and would tend to support the endosymbiotic theory of plastid origin and evolution.

INTRODUCTION

The genome of chloroplasts exists as a closed covalently circular DNA of molecular weight from about 60 to 120 x 10^6 daltons for most of the plant species studied (1,2). The general organization of the chloroplast DNA is considered prokaryotic in nature even though intervening sequences in the genes for ribosomal RNA have identified in Chlamydomonas chloroplast DNA (3). The evolution of organelle genomes, either plastids or mitochondria, has been reviewed in a recent conference (4) but the true origin of these autonomously replicating structures remains unknown.

The mechanism of replication of the chloroplast DNA (ct DNA) is also unresolved, though electron microscope studies have suggested that there may be an analogy between the replication of ct DNA and that of mitochondrial DNA (5). In the latter case the supercoiled mitochondrial DNA is converted to an open circular form while strand displacement synthesis occurs (6).

In the course of study of in vitro DNA synthesis by isolated chloroplasts from maize (7) we have observed the presence of a topoisomerase capable of relaxing supercoiled plasmid DNA. This activity has also been detected in spinach chloroplasts which are the source of the

topoisomerase activity studied in this report. The characteristics of this partially purified enzymatic activity obtained from purified spinach chloroplasts, as reported here, suggest that it is a topoisomerase I of the prokaryotic type (8). This is in contrast to the only other report of a higher plant topoisomerase by Dynan et al., who isolated a topoisomerase I activity of the eukaryotic type from wheat germ (9).

MATERIALS AND METHODS

Reagents

DEAE-cellulose (DE52) was purchased from the Whatman Co., hydroxyapatite (Bio-Gel HTP) from Bio-Rad Laboratories, elutip-d from Schleicher and Schuell, Inc., Sephadex G-200, percoll and ficoll from Pharmacia Fine Chemicals, phenylmethylsulfonyl fluoride, adenosine triphosphate, deoxynucleoside triphosphate, dithioerithrytol (DTE), Orange G and ethidium bromide were from Sigma Chemical Co., p-toluenesulfonyl fluoride from Aldrich Chemical Co. Agarose (Seakem) was obtained from Marine Colloids Division-FMC Corp. [^{3}H]labeled deoxythymidine triphosphate, [^{14}C]bovine serum albumin and [^{14}C]ovalbumin were purchased from New England Nuclear Co. Bovine serum albumin (BSA) was purchased from Miles Laboratories, ovalbumin from Worthington Biochemical Corp., creatine phosphokinase and aldolase were from Sigma Chemical Co.

The plasmid pBR322 DNA was prepared according to Norgard et al. (10) and the plasmid pA03 DNA according to Oka et. al. (11). A single topoisomer of pA03 DNA was obtained from pA03 DNA by incubation with *E. coli* topoisomerase I (as described below) at room temperature for 30 min. The reaction mixture was loaded on a 0.8% low melting agarose gel and electrophoresed at 9 mA for 17 h using 89 mM Tris/boric acid, pH 8.3, 1 mM EDTA as a electrophoretic buffer. After staining the gel with 0.5 µg/ml ethidium bromide for 15 min. a single band, so visualized, was removed. The gel band was dissolved in 15 volumes of LS buffer (0.2 M NaCl, 20 mM Tris/HCl, pH 7.4, 1 mM EDTA) at 65°C for about 1 h, than placed at 37°C for 30 min. and passed through an elutip-d column. DNA was extracted from elutip-d with 400 µl of HS buffer (1 M NaCl, 20 mM Tris/HCl, pH 7.4, 1 mM EDTA), concentrated two fold in vacuo, and precipitated with three volumes of ethanol for 18 hours.

The precipitate was collected by centrifugation and dissolved in 30 mM Tris-HCl, pH 8.0, 1 mM EDTA. Salmon sperm DNA was purchased from

Sigma Chemical Co. and activated by nuclease treatment as described by Pedrali-Noy and Weissbach (12). Poly(A) and oligo $(dT)_{12-18}$ were obtained from PL-Biochemicals. Poly(A).oligo $(dT)_{12-18}$ was prepared as described by Siedlecki and Zmudzka (13).

E. coli topoisomerase I (ω protein) was a gift from Dr. J. C. Wang (Harvard University). Calf thymus topoisomerase I (10,000 u/ml) was purchased from Bethesda Research Laboratories.

Enzyme Preparation

a) Preparation of Chloroplasts

Field grown spinacia oleracea was purchased from local markets. The spinach leaves (100 g) were washed with cold deionized water containing 0.1% sodium hypochlorite and 0.05% Brij 58 and rinsed 3 times with cold distilled water. All subsequent operations were carried out at 4° using sterile solutions and glassware. The leaves were cut into small pieces and homogenized in 300 ml of STS buffer (330 mM sorbitol, 30 mM Tris-HCl, pH 8.0, 10 mM NaCl, 2 mM dithioerithrytol (DTE) with 3-4 second bursts in Waring Blender (maximum speed). The homogenate was filtered through 8 layers of sterile cheesecloth and centrifuged at 200 x g for 3 minutes. The pellet was discarded and the chloroplasts were pelleted by centrifugation at 1000 x g for 7 minutes. The crude chloroplast pellet was resuspended in 100 ml of STS buffer and centrifuged at 200 x g for 3 minutes (pellet discarded) and next at 2000 x g for 10 minutes. The resultant pellet was suspended in STS buffer (10 ml) and stored in liquid N_2 or placed on a 10-80% percoll gradient in STS buffer. In the latter case, 3 ml of the crude chloroplast suspension was layered onto a 26 ml gradient and centrifuged at 7500 x g for 20 minutes. The lower dark green chloroplast band was collected and microscopic examination showed that this band contained about 90% purified unbroken chloroplasts and the rest as broken chloroplasts. No nuclei were observed. This suspension was diluted 4 times with STS buffer and centrifuged at 10000 x g for 20 minutes.

b) Preparation of Crude Extract

The final pellet from (a), (∿2 g) was suspended in STS buffer (final volume 5 ml) and an equal amount of Buffer E (0.7 m NaCl, 20% glycerol, 30 mM Tris-HCl, pH 8.0, 2 mM DTE) containing 50 μg/ml phenylmethylsulfonyl fluoride (PMSF) and p-toluenesulfonyl fluoride (PTSF) and 0.2% of Triton X-100. After incubation for 30 minutes with occasional mixing at 4°C, the chloroplast lysate was centrifuged at 20000 x g for

20 minutes.

c) DEAE Cellulose Chromatography

The green supernatant from (b) was dialyzed 3 times against 2 liters of Buffer A (30 mM Tris-HCl, pH 8.0, 20 mM NaCl, 10% glycerol, 2 mM DTE, 20 µg/ml PTSF and PMSF) and loaded onto a 40 ml DEAE cellulose (DE52) column previously equilibrated with Buffer A. The column was washed with two columns volume of Buffer A and the enzyme eluted with a linear gradient from 20 to 400 mM NaCl in Buffer A using a total elution volume of 200 ml. Fractions (3 ml) were collected and assayed for DNA polymerase and DNA topoisomerase activity.

d) Hydroxyapatite Chromatography

The DEAE cellulose fractions containing topoisomerase activity were pooled, dialyzed 3 times (2 hours each) against 2 liters of Buffer B (20 mM KPO_4, pH 7.5, 8 mM DTE, 10% glycerol, 0.5 mM EDTA) and loaded onto a hydroxyapatite column (4 ml). To prevent the nonspecific absorption of protein to hydroxyapatite, the column had previously been washed with 5 ml of Buffer B containing 2 mg/ml BSA and then washed with 0.6 M KPO_4 in Buffer B and then equilibrated with Buffer B. The column was washed with 15 ml of 100 mM KPO_4 in Buffer B and the enzyme eluted with a linear gradient from 100 to 400 mM KPO_4 in Buffer B. Fractions of about 0.7 ml were collected and assayed. Fractions containing topoisomerase activity were pooled and dialyzed against 50 mM KPO_4, pH 7.5, 50% glycerol, 1 mM DTE and stored at -20°C without any loss of activity for 3 months.

DNA Topoisomerase Assay

a) Standard Assay

Two different assays were used. In the standard assay, topoisomerase activity was measured by separating the relaxed species of superhelical plasmid DNA in agarose gels. The standard assay contained in a final volume of 20 µl: 50 mM Tris-HCl, pH 8.0, 0.5 mM DTE, 10 mM $MgCl_2$, 100 µg/ml BSA, 25 µg/ml PMSF and PTSF, 35 µg/ml pBR322 DNA and enzyme as indicated in the figure legends. Incubations were carried out at 24°C for 30 minutes and the reaction was stopped by adding an equal amount of 20% ficoll containing 0.2% of Orange G and 20 mM EDTA. Fifteen µl aliquots from this mixture were removed and the circular DNA species were separated by electrophoresis in a 0.8% agarose gel in a horizontal slab apparatus at room temperature. The electrophoresis buffer contained 40 mM Tris-acetate, pH 7.8, 5 mM sodium acetate, 1 mM EDTA. After

electrophoresis at 100 mA for 3 hours or at 17 mA for 18 hours, the gels were stained for 30-60 minutes in 1 µg/ml of ethidium bromide and photographed in ultraviolet light.

b) Fluorescent Assay

Topoisomerase activity was also measured by the fluorescent method of Morgan and Pulleyblank (14). The reaction mixture contained in a final volume of 50 µl: 50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 2 µg pBR322 DNA, 0.1 mM DTE and 2-5 µl enzyme. Following incubation at 37°C for 30 minutes the reaction was terminated by chilling in an ice bath and the addition of 2 ml of a solution containing 20 mM K_3PO_4, 0.5 mM Na_3EDTA and 1 µg/ml of ethidium bromide. After heating in a 90°C bath for 2 minutes, the assay solutions were equilbrated to room temperature (1 h) and the fluorescence was determined. Fluorescence was measured at a wavelength of 584 nm using an excitation at 325 nm in an Aminco-Bowman fluorimeter. One unit of enzyme activity is defined as the amount of enzyme which catalyzes a 50% decrease in the fluorescence of supercoiled DNA in 30 minutes at 37°C. It should be mentioned that this assay is linear when the enzyme concentration is below 1 unit/50 µl assay.

DNA Polymerase Assay

The standard reaction mixture for DNA polymerase γ activity (15) contained, in a final volume of 60 µl: 50 mM Tris-HCl, pH 8.5, 1.0 mM DTE, 0.5 mM $MnCl_2$, 0.1 M KCl, 100 µM [^{3}H]dTTP (140 cpm/pmol), 160 µg/ml BSA, 50 µg/ml poly (A).$(dT)_{12-18}$ and 10 µl of the column fraction.

Incubations were carried out at 37°C for 15 minutes and 50 µl aliquots from the reaction mixture were placed on Whatman GF/C filter discs and batch washed with trichloroacetic acid as described by Bollum (16). Radioactivity was measured in a Beckman LS7500 scintillation counter.

Protein Determination

Protein was determined by dye binding method of Bradford (17).

Molecular Weight Determination by Sephadex G-200 Filtration

The hydroxyapatite fraction of the DNA topoisomerase (200 µl in 50% glycerol) was diluted two times with 0.1 M KPO_4, pH 7.5, 2 mM DTE and layered onto a Sephadex G-200 column (0.9 x 60 cm) which had been equilibrated with 70 mM KPO_4, pH 7.5, 25% glycerol and 1 mM DTE. The column was calibrated with the protein standards, rabbit muscle aldolase (M.W. 161000 ± 3000), rabbit muscle creatine phosphokinase (M.W. 81000), bovine serum albumin (M.W. 69000) and ovalbumin (M.W. 46000). The void

volume of the column was determined with dextran blue.

Glycerol Gradient Centrifugation

Two hundred µl of the enzyme was dialyzed overnight against 10 mM KPO_4, pH 8.0, 0.15 M NaCl, 1 mM DTT, 1 mM EDTA and layered onto a 10-30% glycerol gradient (5 ml) in 10 mM KPO_4, pH 8.0, 0.15 M NaCl, 1 mM DTE, 1 mM EDTA. [^{14}C]BSA was also included as an internal marker. The gradient was centrifuged at 39,000 rpm for 16 hours at 2°C in a SW-50.2 rotor (Spinco). Identical control gradients containing aldolase, creatine phosphokinase, [^{14}C]ovalbumin and [^{14}C]BSA as a molecular weight standards were run, simultaneously, in the same rotor. Molecular weight was estimated from the sedimentation data by the method of Martin and Ames (18).

RESULTS

Purification of Topoisomerase Activity

Topoisomerase activity can be detected in a crude extract from maize chloroplasts [Zimmermann and Weissbach (7)], or chloroplasts from the Wisconsin-38 line of cultured nicotiana tabacum cells (Siedlecki and Weissbach, unpublished data) and spinach leaves. Since the level of topoisomerase activity in chloroplasts is relatively low, we used spinach leaves, which were readily attainable commercially, as a source of chloroplasts from which to purify the enzyme activity.

The topoisomerase activity was purified by successive chromatography on DEAE cellulose and hydroxyapatite. Figure 1A shows the DEAE cellulose chromatography of the spinach chloroplast extract. There are two enzymatic activities shown in this panel, a DNA polymerase activity elating at 0.18 M NaCl and a topoisomerase activity which eluted at 0.28 M NaCl and which was detected by the standard electrophoretic assay as shown in the insert in Figure 1A. The DNA polymerase activity, which is merely shown as a marker, was detected with a DNA polymerase γ assay (cf. Methods) but showed no activity when assayed under DNA polymerase α and β conditions (12). This enzyme is presumably the same as that described by Sala et al. (19) and is the putative chloroplast DNA polymerase.

The peak of topoisomerase activity obtained from DEAE cellulose a indicated in Figure 1A was further purified on hydroxyapatite (Figure 1B). The overall recovery of the enzyme from the crude extract was about 16% with a final pufification of 110 fold (Table I). The peak of topoisomerase activity eluted from the hydroxyapatite chromatography at

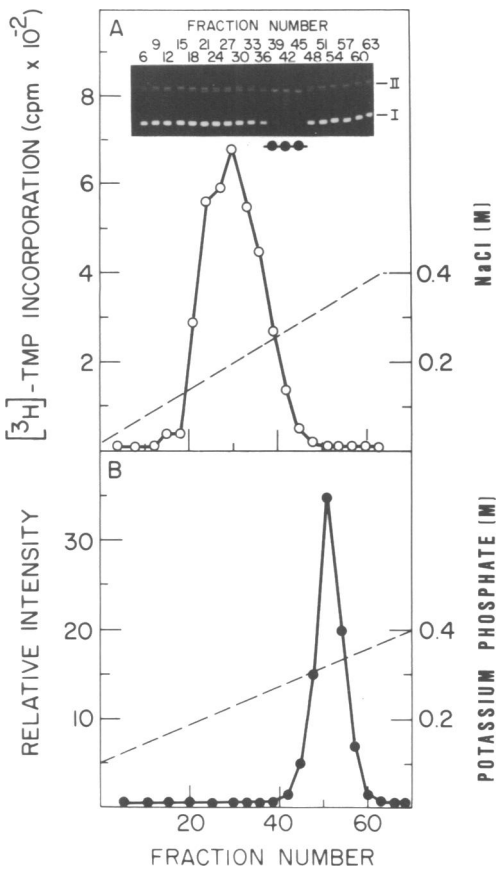

Figure 1 - Chromatography of Spinach Chloroplast Topoisomerase on DEAE Cellulose (A) and Hydroxyapatite (B)

Aliquots of 10 µl were assayed under standard electrophoretic conditions as described in "Materials and Methods" for DNA topoisomerase (Insert) and DNA polymerase γ-like (O) activity. Pooled fractions of topoisomerase activity from DEAE cellulose (-●-●-●-) were applied onto hydroxyapatite and 5 µl aliquots were assayed for topoisomerase activity (●-●) using the fluorescent method as described in Materials and Methods. Relative intensity (ordinate) refers to the fluorescence change at 584nm. I -supercoiled plasmid DNA, II - relaxed circular plasmid DNA. ---, elution buffer concentration.

Table I - Purification of Spinach Chloroplast Topoisomerase

Fraction	Volume (ml)	Total Protein (mg)	Units*	Specific Activity (U/mg protein)	Recovery
Crude Extract	70	53.24	17777	340	
DE52 Cellulose	31	2.94	11615	3950	65.3
Hydroxyapatite	4	0.08	2906	36325	16.3

*One unit of enzyme is defined as that quantity of enzyme catalyzing a 50% decrease in fluorescence under the standard reaction conditions at 37°C for 30 minutes (cf. Methods, Fluorescent Assay).

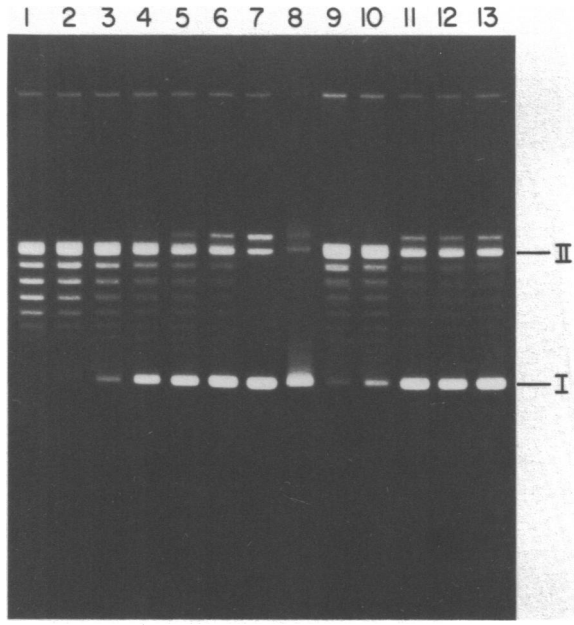

Figure 2 - Time Course and Temperature Effect of the Spinach Chloroplast DNA Topoisomerase Activity

The standard topoisomerase assays were performed as described under "Materials and Methods." Lanes 1-7, incubation under standard conditions for 90, 60, 30, 15, 10, 5 and 0 minutes, respectively. Lanes 8-13, incubation under standard conditions for 10 minutes at 55°, 42°, 37°, 24°, 16° and 10°C, respectively. The products of the reactions were analyzed electrophoretically as described in "Materials and Methods." I - supercoiled plasmid DNA, II - relaxed circular plasmid DNA.

0.32 M potassium phosphate was concentrated three fold by dialysis against 50% glycerol containing 50 mM KPO_4, pH 7.5, 1 mM DTE and used for further characterization of the enzyme.

Time Course and Temperature Requirements of hte Topoisomerase Activity

Under our standard assay conditions, the enzyme shows a roughly linear time course for at least 90 minutes (Figure 2, Lanes 1-7). In addition, the enzyme is active over a wide range of temperature from 10° to 42°C (Figure 2, Lanes 9-13). At 55°, there is no detectable activity (Figure 2, Lane 8).

Requirements for Topoisomerase Activity

The relaxing activity of the topoisomerase was found to be dependent on exogenously added Mg^{2+} ion. In the absence of Mg^{2+} no topoisomerase activity can be determined (Figure 3, Lanes 12, 13). When increasing

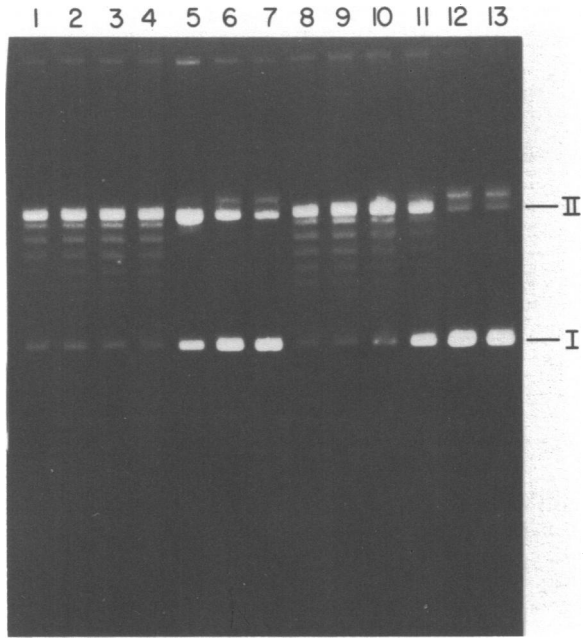

Figure 3 - ATP and Divalent Cation Requirements
The standard topoisomerase assay were performed as described in "Materials and Methods" except that: Lanes 1-4, contained ATP at 125, 50, 25, 0 μM, respectively; Lanes 5-7, minus Mg^{2+} plus 5, 1 and 0.5 mM Mn^{2+}, respectively; Lane 8-12, the Mg^{2+} concentration were 15, 10, 5, 2.5, 0 mM, respectively; Lane 13, minus Mg^{2+} plus 5 mM EDTA. The reactions were carried out at 24°C for 30 minutes and the products were analyzed electrophoretically as described in "Materials and Methods." I - supercoiled plasmid DNA, II - relaxed circular DNA.

amounts of Mg^{2+} are added, the enzyme activity reappears (Figure 3, Lanes 11 to 8). The optimal Mg^{2+} concentration for the topoisomerase activity is about 10 mM. Substitution of Mn^{2+} ions for Mg^{++} reduces the relaxing activity to almost zero (Figure 3, Lanes 5-7). Though the data is not shown, Ca^{2+} and Na^{+} are also ineffective in replacing the Mg^{2+} requirement of the topoisomerase.

We find that ATP is not required by the spinach chloroplast topoisomerase for its relaxing activity on supercoiled DNA. Figure 3, Lane 4 shows that topoisomerase activity is fully demonstrable in the absence of ATP and that addition of ATP up to 125 μm has no apparent effect on the reaction (Lanes 1-3).

The chloroplast DNA topoisomerase seems to require SH groups for

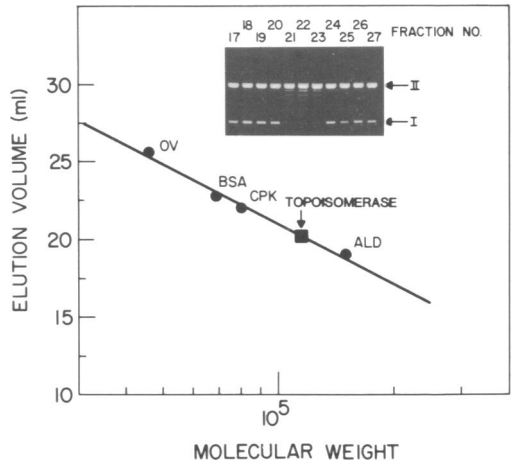

Figure 4 - Molecular Weight Determination by Sephadex G-200 Filtration

Chloroplast DNA topoisomerase (145 units) was applied to a Sephadex G-200 column as described under "Materials and Methods" using ovalbumin (OV), bovine serum albumin (BSA), creatine phosphokinase (CPK) and aldolase (ALD) as a molecular weight markers. The markers were assayed spectrophotometrically at 280 nm. The standard DNA topoisomerase assay and analysis of the supercoiled DNA products were performed as described under "Materials and Methods." I -supercoiled plasmid DNA, II - relaxed circular plasmid DNA.

activity since the addition of 2 mM N-ethylmaleimide causes a total inhibition of the enzyme (data not shown). The assay mixture for the enzyme, therefore, routinely contains 0.5 mM dithioerythritol.

Molecular Weight

Gel filtration of the chloroplast topoisomerase in 75 mM potassium phosphate, pH 7.5, through Sephadex G-200 suggest an apparent molecular weight of about 115,000 (Figure 4). This was confirmed by sedimentation of the enzyme through a 10-30% glycerol gradient in 0.15 M NaCl, in which solution it showed an S value of 6.1 corresponding to a putative molecular weight of 110,000 (data not shown).

Failure to Relax Positive Supercoiled DNA and Linkage Number

Wang (20) showed that ethidium bromide, in concentrations below 5 μg/ml, did not inhibit the relaxing activity of the E. coli topoisomerase (ω protein) for negatively supercoiled DNA. In the case of pBR322 DNA, ethidium bromide concentrations above 0.5 μg/ml cause the formation of positively supercoiled DNA (20,21). E. coli topoisomerase I cannot act on positively supercolied DNA (19,20) and this is confirmed in Fig. 5,

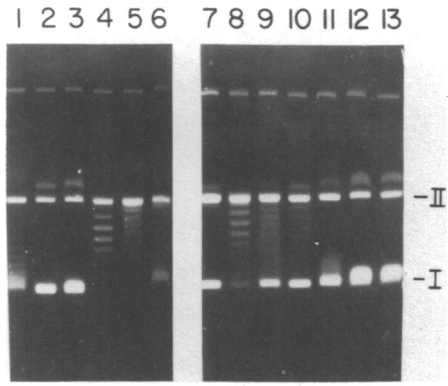

Figure 5 - Relaxation of Supercoiled DNA in Presence of Ethidium Bromide by Chloroplast Topoisomerase, E. Coli Topoisomerase I and Calf Thymus Topoisomerase I

The standard topoisomerase assays were performed as described under the Materials and Methods. Lanes 1-3, the standard assay with ethidium bromide at a concentration of 0, 1, 2 µg/ml and 20 ng of E coli topoisomerase I; Lanes 4-6, standard assay with ethidium bromide at a concentration of 0, 1, 2 µg/ml and 6 U of calf thymus topoisomerase I. Lane 7 - pBR322 DNA; Lanes 8-13, the standard assay with ethidium bromide at concentration 0, 0.25, 0.5, 1, 2, 5 µg/ml, respectively and with 20 ng of chloroplast topoisomerase. The reactions were carried out at 24°C for 30 minutes and products were analyzed electrophoretically I - supercoiled DNA, II - relaxed circular DNA.

Lanes 1, 2, 3. Furthermore, as seen in Lanes 11, 12, 13, the DNA relaxing activity of the spinach chloroplast topoisomerase also did not relax pBr322 positively supercoiled DNA, generated by ethidium bromide concentrations above 0.5 µg/ml. However, the enzyme can relax negatively supercoiled DNA in the presence of ethidium bromide at a concentration of 0.5 µg/ml or lower and was able to form the characteristic "ladder" of supercoiled DNA species with several different negatively supercoiled DNA substrates (Lanes 8, 9, 10). Fig. 5 also shows that calf thymus topoisomerase I (22) is capable of generating a spectrum of topoisomers from both negatively and positively supercoiled DNA (Lanes 4, 5, 6). The ability to relax a positive supercoiled DNA is one of the characteristics of a eukaryotic topoisomerase I not shared by the prokaryotic topoisomerase I.

The prokaryotic topoisomerases are known to catalyze a change in linkage number of n + 1 (20). Figure 6 shows a comparison of the superhelical forms of pA03 DNA generated by the E. coli ω protein with

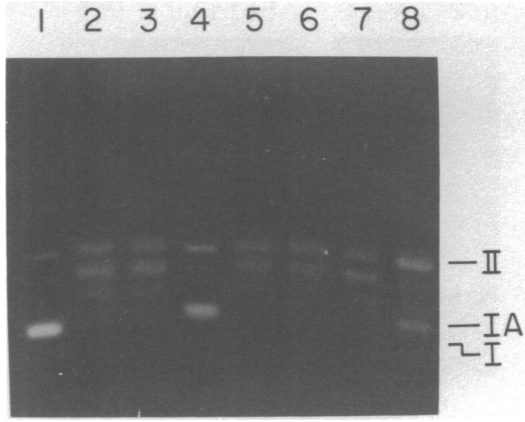

Figure 6 - Relaxation of Closed Circular pA03 DNA and a pA03 DNA Topoisomer by Chloroplast Topoisomerase, E. Coli Topoisomerase I and Calf Thymus Topoisomerase I

The standard topoisomerase assays were performed as described in Materials and Methods except that pBR322 DNA was replaced by pA03 DNA (Lanes 1-3) or a topoisomer of pA03 DNA (Lanes 4-8). Plasmid pA03 DNA (Lane 1) was incubated with 40 ng of E coli topoisomerase I (Lane 2) or with 40 ng chloroplast topoisomerase (Lane 3). A pA03 DNA topoisomer (Lane 4) was incubated with 40 ng (Lane 5) and 20 ng (Lane 8) of E. coli topoisomerase I, 40 ng of chloroplast topoisomerase (Lane 6) or a calf thymus topoisomerase I (Lane 7). The reactions were carried out at 24°C for 30 minutes and products were analyzed electrophoretically in a tris/borate buffer. I - supercoiled pA03 DNA, IA - pA03 topoisomer, II - relaxed pA03 circular DNA.

those obtained with the chloroplast topoisomerase. In these experiments both the supercoiled pA03 DNA and a topoisomer generated from it by action of the ω protein (cf. Methods) were used as templates. Figure 6, Lanes 2, 3 show the formation of topoisomers from pA03 supercoiled DNA by the ω protein and the chloroplast topoisomerase. These results can be compared to Lanes 5, 6, 7 and 8 which show that a topoisomer of pA03 DNA is also relaxed in the same manner by the ω protein, the chloroplast topoisomerase I and the calf thymus topoisomerase I. It is clear that the chloroplast enzyme, like the ω protein, produces a unit change in linkage number, characteristic of a topoisomerase I.

DISCUSSION

To our knowledge, this report is the first description of topoisomerase activity in chloroplasts. There are two classes of DNA topoisomerases which have been described (8). The type I enzymes convert topological forms of DNA by transient single strand breaks in the DNA

chain. Type II topoisomerases, such as DNA gyrase, act via double strand breaks in the DNA. Topoisomerase I enzymes are widely distributed in both prokaryotes and eukaryotes, including viruses (8,24,25). In animal cells, topoisomerase I is always associated with the nucleus though a separate mitochondrial enzyme has been reported (26). The prokaryotic topoisomerase I has been distinguished from the eukaryotic topoisomerase I by several criteria. The prokaryotic enzyme requires Mg^{++} for activity; the eukaryotic enzyme does not and will even function in the presence of a metal chelator (8). Eukaryotic topoisomerase can also relax both positively and negatively supercoiled DNA.

The data in this paper show that the chloroplast DNA topoisomerase requires Mg^{++} but not ATP for activity. This is strong presumptive evidence that the enzyme is of the prokaryotic group, though we recognize that a type II topoisomerase which can relax supercoiled DNA in the absence of ATP has been derived from E. coli DNA gyrase (27,28). Further evidence for the putative prokaryotic origin of the chloroplast topoisomerase I is shown by its ability to catalize a unit change in linkage number from a supercoiled DNA or a topoisomer derived from it, and its failure to unwind positively supercoiled circular DNA generated by ethidium bromide treatment. The finding of a "prokaryotic" topoisomerase in chloroplasts may be relevant to the origin of these autonomous organelles. It would support the endosymbiotic theory of prokaryotic invasion of the cell (29) rather than the non-symbiotic theory which predicts that chloroplasts arose by compartmentalization of part of the ancestral cells' genome (30). It will be of interest to examine the nucleus of the plant cell for topoisomerase activity. If such an enzyme is present in the plant nucleus, its relationship to the chloroplast topoisomerase should be examined. In this respect, the isolation of a "eukaryotic" type of topoisomerase with an apparent molecular weight of 110,000 from wheat germ by Dynan et al. (9) may be relevant, since other topoisomerases isolated from animal cells generally have molecular weights in the 60,000-75000 range (31). In addition, Miller has reported an uncharacterized DNA-topoisomerase activity in maize pollen (32).

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