Purification and characterization of a proteolytic active fragment of DNA topoisomerase I from the brine shrimp *Artemia franciscana* (Crustacea Anostraca)

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The ATP-independent type I topoisomerase from the crustacean *Artemia franciscana* was purified to near-homogeneity. Its activity was measured by an assay that uses the formation of an enzyme-cleaved DNA complex in the presence of the specific inhibitor camptothecin. The purification procedure is reported. Purified topoisomerase is a single-subunit enzyme with a molecular mass of 63 kDa. Immunoblot performed on the different steps of purification shows that the purified 63 kDa peptide is a proteolytic fragment of a protein with a molecular mass of 110 kDa. Similarly to the other purified eukaryotic topoisomerases, the crustacean enzyme does not require a bivalent cation for activity, but is stimulated in the presence of 10 mM-MgCl₂; moreover, it can relax both negative and positive superhelical turns. The enzyme activity is strongly inhibited by the antitumour drug camptothecin. The enzyme inhibition is related to the stabilization of the cleavable complex between topoisomerase I and DNA.

INTRODUCTION

DNA topoisomerases are enzymes that catalyse the interconversion of topological isomers of DNA by concerted breaking and rejoining of phosphodiester bonds [1]. Two distinct classes of DNA topoisomerase have been identified: type I enzyme mediates the strand passage step, acting on one DNA strand at a time, whereas type II breaks and rejoins both strands of DNA in concert. Reviews on the mechanism of action and functional aspects of topoisomerases have been published [2,3].

In vitro, these enzymes catalyse a variety of reactions such as relaxation and supercoiling, knotting and unknotting and catenation and decatenation of DNA [2]. Although detailed knowledge about the role of topoisomerases *in vivo* is still lacking, it is reasonable to assume that they are key enzymes in the control of DNA conformation with a consequent influence on replication, transcription and recombination [2,4]. Moreover, a number of clinically important anticancer drugs have been recently shown to kill tumour cells by affecting DNA topoisomerases, so correlating these enzymes with neoplastic cell proliferation [5,6].

Type I topoisomerases have been isolated from a variety of eukaryotic organisms, including plants [7,8], fungi [9,10], insects [11], amphibians [12], birds [13] and mammals [14].

Studies on the mechanism of topoisomerase I-catalysed reaction have shown that a covalent DNA–enzyme intermediate is formed [2,15]. Therefore a method allowing topoisomerase fixation on DNA (cleavable complex) has been devised. The method essentially consists in the termination of the topoisomerase intermediate reaction with the aid of a detergent or other protein-denaturing substances [16].

Recently analysis of these DNA–protein complexes has revealed a nucleotide sequence preference for the DNA–topoisomerase I interaction and the existence of consensus regions of cleavage [17,18]. To reinforce these data, camptothecin, a specific topoisomerase I inhibitor, was used [19,20]. The advantage of the use of the drug, which prevents the resealing of the nick, is the accumulation of the cleavable complex. This has opened up a new stage in mapping specific DNA–enzyme interaction [21].

In the present paper we describe a procedure for the isolation

and characterization of topoisomerase I from the brine shrimp *Artemia franciscana* (Crustacea Anostraca). The possibility of accumulating the cleavable complex, in the presence of camptothecin, gives us the opportunity to identify the polypeptide associated with the topoisomerase I activity during the purification steps. This assay is also used easily to quantify the enzyme activity and study its biological characteristics, such as molecular mass. Immunochemical properties, studied with polyclonal antibodies against the crustacean enzyme, are also presented.

EXPERIMENTAL

Materials

Unlabelled dNTPs were purchased from P-L Biochemicals; [α -³²P]dATP and [α -³⁵S]dATP were from Amersham. T7 DNA polymerase and Western Blot AP System were purchased from Promega. Camptothecin was from Sigma Chemical Co. Coumermycin and VP.16 (Etoposide) were kindly provided by Dr. Gambetta (Istituto Nazionale dei Tumori, Milano, Italy). Other chemicals were reagent grade.

Artemia

Cysts of *Artemia franciscana* were developed to nauplii as previously described [22]. Nauplii were collected and resuspended in 50 mM-Tris/HCl buffer, pH 8.0, containing 5 mM-MgCl₂, 1 mM-phenylmethanesulphonyl fluoride, 1% (v/v) dimethyl sulphoxide, 0.001 mg of pepstatin/ml and 80% (v/v) glycerol (0.1 ml/g) and used immediately or frozen at –80 °C.

Immunological reagents

Polyvalent antiserum against the *Artemia* topoisomerase was obtained by separating, by SDS/PAGE, 100 µg of the 63 kDa polypeptide from the DEAE-Sephadex purification step (see below). After brief staining with CuCl₂, the corresponding protein band was sliced out of the gel and homogenized in Tris-buffered saline (0.15 M-NaCl/10 mM-Tris/HCl buffer, pH 8), and polyacrylamide suspension was used to immunize a rabbit by multiple intradermal injections [23].

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DNA substrates

Supercoiled ColE1 DNA plasmid was isolated from *Escherichia coli* (strain A745 Met⁻Thy⁻) after chloramphenicol amplification [24], and further purified by equilibrium centrifugation in a CsCl/ethidium bromide gradient [19]. Relaxed DNA was prepared by allowing the supercoiled plasmid to react with purified topoisomerase I in a standard relaxation assay. After reaction, the DNA was purified by phenol extraction and ethanol precipitation. The 31-mer oligonucleotide 5'-A/TA/TA/TTTTT/CTCTAAGTT/CTTTTTTGGCTATAGCC-3' [18] used as substrate for the formation of the cleavable complex was synthesized by a 391 DNA Synthesizer (Applied Biosystems). The oligonucleotide was replicated with as primer the 3'-terminal nucleotides re-annealed to the complementary bases (positions 22–26). The replication mixture (final volume 50 μ l), containing 50 mM-Tris/HCl buffer, pH 7.5, 10 mM-MgCl₂, 50 mM-NaCl, 2 mM-dCTP, -dGTP and -TTP, 0.5 mM-dATP, 50 μ Ci of [α -³²S]thio]dATP (3000 Ci/mmol), 10 μ g of 31-mer oligonucleotide and 50 units of T7 DNA polymerase, was incubated for 60 min at room temperature and the reaction was stopped with 5 μ l of 0.5 M-EDTA, pH 8.0. The unincorporated nucleotides were then eliminated by microdialysis on a Millipore filter.

Relaxation activity assay

The assay is based on monitoring the relaxation of supercoiled ColE1 plasmid DNA by agarose-gel electrophoresis. Reactions were carried out in a volume of 10 μ l in the presence of 25 mM-Tris/HCl buffer, pH 8.0, 0.1 M-NaCl, 0.5 mM-EDTA and 1 μ g of supercoiled DNA. The samples were incubated at 30 °C for 10 min and the reaction was terminated by addition of 4 μ l of a mixture containing 6% (w/v) SDS, 67 mM-EDTA, 30% (w/v) sucrose and 0.1% Bromophenol Blue. The reaction products were separated by electrophoresis in 1% agarose gel in Tris/acetate buffer in a horizontal slab gel apparatus. DNA was detected by u.v. after ethidium bromide staining.

Positive supercoiled DNA was produced by using pre-relaxed DNA plus 1 μ g of ethidium bromide/ml.

Cleavable-complex formation assay

The cleavable-complex assay is based on the analysis by SDS/PAGE of the covalent nucleoprotein complex between topoisomerase I and DNA. The reaction was carried out as in the relaxation assay with plasmid DNA replaced by 0.1 μ g (10⁵ c.p.m.) of ³²P- or ³⁵S-labelled replicated oligonucleotide. After 5 min of incubation at 30 °C, 100 μ g of camptothecin/ml was added and the mixture was incubated for another 5 min. The reaction was stopped by adding an adequate volume of Laemmli sample buffer [25]. After electrophoresis by SDS/PAGE, the proteins were electroblotted on to nitrocellulose filter and the filter was exposed for autoradiography.

Analysis of immunoreactive peptides of DNA topoisomerase

Enzyme fractions were analysed for immunoreactive peptides by electrophoresis on 10% polyacrylamide gel in the presence of SDS [25]. Separated peptides were transferred on to nitrocellulose membrane and allowed to react sequentially with rabbit anti-(*Artemia* DNA topoisomerase I) serum and goat anti-(rabbit IgG) antibody conjugated to alkaline phosphatase and then detected as described by Knecht *et al.* [26].

Immunoprecipitation of DNA topoisomerase I

Topoisomerase I was mixed with anti-(*Artemia* DNA topoisomerase I) serum at a 3:1 ratio. After overnight incubation at 4 °C, immunoreaction was started by the addition of Protein A-Sepharose, and the mixture was mixed continuously for

60 min. Sepharose was then extensively washed with Tris-buffered saline, harvested by centrifugation and used as an enzyme source in a regular topoisomerase I assay.

Purification of *Artemia* DNA topoisomerase I

Preparation of nuclear extract (fraction I). DNA topoisomerase was purified from 100 g of fresh or frozen nauplii. All purification procedures were carried out at 4 °C. Nauplii were resuspended (1 g/ml) in 50 mM-Tris/HCl buffer, pH 8.0, containing 25 mM-KCl, 3 mM-MgCl₂, 2 mM-CaCl₂, 250 mM-sucrose, 1 mM-phenylmethanesulphonyl fluoride, 1% (v/v) dimethyl sulphoxide and 1 μ g of pepstatin/ml (homogenization buffer) and homogenized until the integrity of the cells, evaluated by optical microscopy, was disrupted. Homogenate was centrifuged at 15000 *g* for 10 min. The pellet was suspended in the homogenization buffer (1 ml/g), and the suspension was stratified on a 1.8 M-sucrose cushion and centrifuged at 30000 rev./min for 60 min in the 50.2 Ti rotor of the Beckman ultracentrifuge. The nuclear pellet was resuspended in homogenization buffer containing 50 mM-NaCl and nuclei were harvested by centrifugation at 15000 *g* for 10 min. The washed nuclei were finally extracted with 50 ml of 200 mM-NaCl in homogenization buffer.

The nuclear extract was clarified by ultracentrifugation at 45000 rev./min for 180 min in the 50.2 Ti rotor.

(NH₄)₂SO₄ precipitation (fraction II). (NH₄)₂SO₄ was added to fraction I to 55% saturation. The precipitate was removed by centrifugation at 10000 *g* for 20 min and a supplement of (NH₄)₂SO₄ was added to the supernatant up to 90% saturation. The harvested precipitate was dissolved in buffer A [50 mM-Tris/HCl buffer, pH 8.0, containing 5 mM-2-mercaptoethanol and 10% (v/v) glycerol] in the presence of 200 mM-NaCl and extensively dialysed against the same buffer.

Heparin-Sepharose chromatography (fraction III). Fraction II was loaded on to a 3 ml heparin-Sepharose column previously equilibrated with buffer A in the presence of 200 mM-NaCl. After being loaded, the column was washed with the same buffer and the proteins were eluted with a 60 ml linear gradient of 200–700 mM-NaCl in buffer A. The fractions containing active topoisomerase were pooled and dialysed against 50 mM-NaCl in buffer A.

DEAE-Sephadex chromatography (fraction IV). Fraction III was loaded on a 3 ml DEAE-Sephadex A-25 column equilibrated with 75 mM-NaCl in buffer A. The flow-through volume was dialysed against 50 mM-Tris/HCl buffer, pH 8.0, containing 50 mM-NaCl, 5 mM-2-mercaptoethanol and 50% (v/v) glycerol and stored at –20 °C into silicone-treated tubes.

RESULTS

Identification of DNA topoisomerase I

In order to identify which of the peptides present in the purification steps has topoisomerase activity, we used a modification of the procedure described by Trask & Muller [16]. Briefly, the cleavable complex was stably prepared by adding the topoisomerase I-specific inhibitor camptothecin to the reaction mixture containing the [³²P]oligonucleotide (25 bp) representing the topoisomerase I-recognition site ([18]; see the Experimental section). The reaction mixture was then analysed by SDS/PAGE. Fig. 1 shows the result of this cross-linking, obtained by partially purified topoisomerase of fraction II, analysed in the presence of different topoisomerase inhibitors. In the control reaction (lane A) a labelled band is detected with a molecular mass of about 78 kDa. Because the molecular mass of the covalent bound DNA ranges between 13 and 15 kDa, we estimate the protein forming the radiolabelled nucleoprotein complex to be a polypeptide of 62–65 kDa.

Fig. 1. Cleavable complex induced by DNA topoisomerase I-specific inhibitor

The reaction for the formation of cleavable complex is described in the Experimental section. The oligonucleotide 52-mer labelled with [32 P]AMP (10^5 c.p.m.) was used as probe. Lane A represents the reaction control; lanes B, C and D show the results of reactions involving respectively coumermycin, VP-16 and camptothecin (all at $100 \mu\text{g/ml}$). Positions of size marker proteins are indicated.

Table 1. Purification of DNA topoisomerase I from *A. franciscana*

Formation of the cleavable complex was used to quantify the enzyme activity. The nucleoprotein complex, formed in the presence of 0.1 mg of protein, was obtained as described in the Experimental section. The amount of radiolabelled probe, involved in the complex, was evaluated by scanning the autoradiographic tracing. One unit of enzyme is defined as the amount of protein required to bind 1 ng of synthetic oligonucleotide in 60 min .

Fractions	Protein		Total activity (units)	Specific activity (units/mg)
	(mg/ml)	(total mg)		
I Crude nuclear extract	2.50	225.0	180 000	800
II $(\text{NH}_4)_2\text{SO}_4$	0.70	7.0	97 000	14 000
III Heparin-Sephadex	0.07	0.56	50 000	100 000
IV DEAE-Sephadex	0.015	0.03	37 000	1 230 000

The experiment also shows that only camptothecin (lane D) increases the amount of the cleavable complex (by about 4–5-fold) with respect to the control reaction (lane A); in contrast, the topoisomerase II inhibitors [5], coumermycin (lane B) and VP-16 (lane C) do not have any effect.

Purification of DNA topoisomerase I

The purification scheme used to prepare topoisomerase I from the crustacean *A. franciscana* is summarized in Table 1.

Preliminary experiments (results not shown) demonstrate that an ATP- and Mg^{2+} -independent topoisomerase remains associated with the *Artemia* nuclei when they are prepared as described in the Experimental section. The topoisomerase activity is efficiently extracted from isolated nuclei by treatment with 0.2 M -NaCl at 4°C . After clarification of the nuclear extract by centrifugation the residual pellet contains the bulk of the inner histones and nucleic acids, whereas almost all of the topoisomerase activity is present in the supernatant. The second

Fig. 2. Heparin-Sephadex purification step of topoisomerase I

(a) Protein pattern eluted from the heparin-Sephadex column with an NaCl gradient (upper part of the panel). Portions ($50 \mu\text{l}$) of the indicated fractions were electrophoresed and the proteins detected by silver staining [26]. The same fractions ($2 \mu\text{l}$ each) were tested for cleavable-complex formation with the ^{35}S -labelled oligonucleotide 52-mer (b) and for the relaxation reaction (c). In (c) A represents the reaction containing pre-column protein fraction. S and R show respectively supercoiled and relaxed positions of the substrate plasmid.

step of purification uses $(\text{NH}_4)_2\text{SO}_4$ precipitations. More than 50% of the topoisomerase activity remains in solution to 55% saturation, but it is efficiently precipitated at 90% saturation.

The redissolved precipitate is applied to a heparin-Sephadex column from which topoisomerase is eluted at an ionic strength of 0.45 – 0.55 M -NaCl (Fig. 2). SDS/PAGE analysis of the eluted proteins shows that, corresponding to the fractions containing topoisomerase activity (Figs. 2b and 2c), a 63 kDa polypeptide is present as a major component (Fig. 2a).

To increase the purity of the topoisomerase further, the pooled fractions, containing the 63 kDa polypeptide, are loaded on to a DEAE-Sephadex column. Under the conditions described in the Experimental section, the polypeptide responsible for the

Fig. 3. Analysis by SDS/PAGE and immunodetection of purified topoisomerase I

A 50 μ l sample of purified DNA topoisomerase I (fraction IV) was analysed on 10% polyacrylamide gel in the presence of SDS. After electrophoresis, proteins were stained by the silver procedure (lane A). Lane B shows the immunoreactive polypeptides present in 20 μ l of fraction IV. The same amount of protein as for lane B was used in a reaction for the formation of the cleavable complex with 0.1 μ g (10^5 c.p.m.) of 35 S-labelled oligonucleotide 52-mer in the presence of 100 μ g of camptothecin/ml. After transfer to nitrocellulose membrane, the labelled nucleoprotein complexes were detected by autoradiography (lane C). Lane D shows the immunoreactive polypeptides present on the nitrocellulose strip of lane C.

Fig. 4. Biochemical properties of the purified topoisomerase I

Relaxed covalently closed DNA (lane 1) was prepared as described in the Experimental section and used as substrate for topoisomerase I (2 μ l of fraction IV) in the presence of 1 mM-ATP (lane 2) or 1 mM-ATP and 10 mM-MgCl₂ (lane 3). Supercoiled DNA was incubated with 2 μ l of enzyme in the presence of 100 mM-NaCl (lane 4), 10 mM-NaCl (lane 5), 300 mM-NaCl (lane 6) or 10 mM-MgCl₂ (lane 7). Lane 9 represents the action of topoisomerase I on pre-relaxed DNA plasmid in the presence of 1 μ g of ethidium bromide/ml; lane 10, as sample 9 in the absence of enzyme. Samples in lanes 8 and 11 contained respectively supercoiled and relaxed DNA. S and R indicate supercoiled and relaxed positions respectively.

topoisomerase activity flows through the column, whereas the majority of the contaminants bind to the resin. This procedure produces a purification of about 1500-fold starting from the nuclear extract (Table 1) and a purity of the topoisomerase of between 85 and 90% (Fig. 3, lane A).

Biochemical properties of purified topoisomerase I

The catalytic action of the topoisomerase I obtained by the purification described requires the presence of moderate ionic strength. In fact, maximum activity is observed in the presence of 0.15 M-NaCl whereas no activity is detected in the absence of NaCl or at NaCl concentration greater than 0.3 M. MgCl₂ is not required for enzyme activity if NaCl is held at 50 mM, but 10 mM

Fig. 5. Immunoreactive peptides present in the purification steps of topoisomerase I

Polypeptides contained in fraction I (lane A), fraction II (lane B), fraction III (lane C) and fraction IV (lane D) were fractionated on 10% polyacrylamide gel in the presence of SDS. After transfer of proteins to nitrocellulose membrane, the blot was incubated with the DNA topoisomerase I antiserum for detection of immunoreactive polypeptides as described in the Experimental section.

of the bivalent ion can restore the maximum enzyme efficiency (Fig. 4).

The interaction of topoisomerase and supercoiled DNA was examined in the relaxation reaction by varying the concentration of the enzyme in the presence of 1 μ g of supercoiled substrate. The results show that the rate of relaxation of DNA is directly proportional to the enzyme concentration, and therefore the interaction of the enzyme with supercoiled DNA follows classic first-order kinetics. Further, it was previously shown [1,2] that topoisomerase I extracted from different eukaryotic sources can remove negative as well as positive turns. We have confirmed this observation for the crustacean topoisomerase I, analysing the reaction products on agarose gel using positively supercoiled DNA plasmid produced by treatment of covalently closed relaxed DNA with ethidium bromide (Fig. 4, lanes 8–11). Moreover the purified topoisomerase cannot rewind relaxed covalently closed DNA in the absence or presence of ATP (lanes 2 and 3).

Immunological characterization of topoisomerase I

In order to clarify the real nature and structure of the 63 kDa polypeptide purified from *Artemia* cells, we made a rabbit polyclonal antiserum as described in the Experimental section. As shown in lane B of Fig. 3, the antiserum mainly recognizes the 63 kDa protein and a few minor polypeptides of lower molecular mass present in our final step of purification, probably representing products of proteolytic action occurring during the purification procedure. In contrast, no immunoreaction is detectable after incubation with pre-immune serum (results not shown). When the same experiment is carried out after cleavable-complex formation (see the legend of Fig. 3), one extra band of about 75 kDa appears (Fig. 3, lane D) corresponding to the major autoradiographic signal in a cleavable-complex assay (Fig. 3, lane C). The lower autoradiographic signals are in accordance with the result of the immunoblot experiment, confirming the presence of polypeptides related to DNA topoisomerase I still maintaining the enzyme activity. Moreover lane C shows two

Fig. 6. Action of anti-(*Artemia* topoisomerase I) serum on enzyme activity

DNA topoisomerase (20 μ l of fraction IV) was incubated overnight at 4°C with the same volume of rabbit serum against *Artemia* topoisomerase I, prepared as described in the Experimental section (samples on the left) or pre-immune serum (samples on the right). The two immunoreactions were then diluted twice to 80 μ l with topoisomerase I reaction mixture and incubated at 30°C. Samples (20 μ l) were withdrawn at the indicated times and the reaction was stopped by adding 8 μ l of topoisomerase stop solution. S and R indicate supercoiled and relaxed positions respectively.

Fig. 7. Immunoprecipitation of topoisomerase I

Samples (20 μ l) of purified topoisomerase I were mixed with 7 μ l of anti-(topoisomerase I) serum (samples on the left) or pre-immune serum (samples on the right) in a final volume of 40 μ l. After overnight incubation at 4°C, 80 μ l of the suspension of Protein A-Sepharose in Tris-buffered saline at a 1:1 ratio (v/v) was added to the two samples and processed as indicated in the Experimental section. The topoisomerase activity was tested to the indicated times as described in the legend to Fig. 5. S and R indicate supercoiled and relaxed positions respectively.

other bands with molecular masses of 90 and approx. 120 kDa; they could represent a small amount, not revealed by immunoblot, of a more native protein precursor. This hypothesis was confirmed by the Western blot experiment of Fig. 5 showing that topoisomerase I undergoes proteolytic action during the different steps of purification. The clarified crude extract (lane A) and the $(\text{NH}_4)_2\text{SO}_4$ precipitate (lane B) clearly show, above the 63 kDa polypeptide, four other more slowly migrating immunoreactive proteins with molecular masses of 72, 76, 80 and 110 kDa. These proteins are undetectable in the last two steps of purification (lanes C and D).

Activity of the polyclonal antibodies on DNA topoisomerase I

We have examined the effects of the polyclonal antibodies on type I topoisomerase-catalysed relaxation of supercoiled ColEI plasmid DNA. For this purpose, the purified crustacean topoisomerase was incubated at 4°C in the presence of a 1:1

ratio (v/v) of rabbit antiserum or pre-immune serum. Evaluation of topoisomerase activity was then carried out in the time-course experiment shown in Fig. 6. The result clearly shows that the antiserum against the 63 kDa polypeptide inhibits the relaxation of the supercoiled DNA. Indeed, only a small amount of supercoiled DNA is changed to the relaxed form after 12 min of incubation (Fig. 6, samples on the left); in contrast, only 5 min is sufficient to relax more than 50% of supercoiled DNA and 12 min to relax completely all the superhelicity of the plasmid DNA when the enzyme is preincubated with the pre-immune serum (Fig. 6, samples on the right).

The inhibition effect is a function of the amount of topoisomerase antiserum present in the preincubation step and also of the incubation time of the immunocomplex with DNA (results not shown).

These last observations allow us to devise an immunoprecipitation experiment the result of which is shown in Fig. 7. An immunocomplex, made by increasing to 3:1 the ratio enzyme/antiserum or enzyme/pre-immune serum, was complexed to Protein A-Sepharose and precipitated by centrifugation. The relaxation activity, tested by adding the Sepharose pellet to the reaction mixture, is clearly stabilized by the Protein A-Sepharose in the presence of antiserum (samples on the left) but not in the presence of pre-immune serum (samples on the right).

DISCUSSION

Type I topoisomerase seems to be a ubiquitous enzyme; it has been studied and purified from a variety of eukaryotic sources [8–14]. Years ago it was pointed out, by a biochemical approach by Liu & Miller [27] and by analysis of the gene coding topoisomerase I [28], that the native protein has a molecular mass of about 110 kDa. It was also suggested that proteins with topoisomerase activity having lower molecular mass may be products of proteolysis [27].

In the present paper we describe an improved procedure for the purification of topoisomerase I from crustacea. For ready realization of the project we monitor, during the purification, the topoisomerase activity using an assay that produces the accumulation of the cleavable complex by using the specific inhibitor camptothecin. This assay allows us to reveal the topoisomerase enzyme even in the presence of nuclease activities, which prevent the use of the relaxation assay, and also to check continuously the molecular structure of the protein. Moreover it allows us to evaluate the specific activity of the enzyme even if only a fraction of it, with a constant ratio in all the different steps of purification, is engaged in the formation of the cleavable complex.

Using the purification procedure described we have isolated a polypeptide of 63 kDa that shows topoisomerase activity. The purity of this protein, evaluated by a silver staining analysis, is about 85% with a minor contamination by a peptide of about 35 kDa. In contrast, the cleavable-complex analysis permits us to reveal, in the last step of purification, in addition to the main nucleoprotein complex of 75 kDa, other signs of the presence of proteins with topoisomerase activity but different molecular mass. The immunoblots obtained with antibodies against the 63 kDa protein demonstrate that the polypeptides forming the nucleoprotein complexes are all (or part) immunocorrelated to the 63 kDa topoisomerase. Moreover they confirm the hypothesis that the 63 kDa polypeptide is a proteolytic fragment of a protein with a higher molecular mass. The decrease in the protein mass clearly occurs during the purification procedure even in the presence of antiproteolytic compounds.

The enzymic properties of the crustacean topoisomerase I are

quite similar to those previously described for enzymes purified from higher eukaryotic organisms [2]. The enzyme relaxes both positive and negative supercoiled DNA in the absence of ATP and Mg^{2+} , with maximum activity at moderate ionic strength. Moreover, it is sensitive to the topoisomerase I-specific inhibitor camptothecin, but not to the drugs that specifically inhibit topoisomerase II from prokaryotic as well as eukaryotic cells. The similarity of the crustacean topoisomerase to other eukaryotic topoisomerases I is also suggested by cross-reaction between our antiserum and yeast and human enzymes (results not shown). This is in accordance with the comparative studies of topoisomerase I genes from different biological sources carried out by others [29,30].

We are indebted to the Artemia Reference Center of the University of Ghent (Belgium), which provided selected cysts. This research was supported in part by Consiglio Nazionale delle Ricerche (Grant no. CT 89.03380.14.115.02867) and by Progetto Finalizzato RAISA.

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Received 5 April 1991/11 July 1991; accepted 25 July 1991