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DNA topoisomerases from rat liver: physiological variations

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ABSTRACT

Besides the nicking-closing (topoisomerase I) activity, an ATP-dependent DNA topoisomerase is present in rat liver nuclei. The enzyme, partially purified, is able to catenate *in vitro* closed DNA circles in a magnesium-dependent, ATP-dependent, histone H1-dependent reaction, and to decatenate *in vitro* kinetoplast DNA networks to yield free minicircles in a magnesium-dependent and ATP-dependent reaction. It is largely similar to other eukaryotic type II topoisomerases in its requirements, and presumably belongs to this class of enzymes. Type I and type II activities were measured in rat liver nuclei as a function of regenerating time after partial hepatectomy : type I activity was not significantly changed during this process. In contrast, type II activity was considerably increased, suggesting a possible involvement of the enzyme in DNA replication.

INTRODUCTION

DNA topoisomerases are enzymes which catalyze the *in vitro* conversion of one DNA topological isomer to another. The existence of such a class of enzymes was revealed more than ten years ago, shortly after the discovery of superhelical DNA. However, their fundamental importance in nucleic acid metabolism has only begun to emerge as a consequence of the rapid accumulation of information on these proteins in recent years (for a review, see ref. 1-6). These enzymes catalyze a number of different *in vitro* isomerizations of circular DNA. These include relaxation of superhelical DNA (7), reassociation of complementary single-stranded DNA circles (8), knotting/unknotting (9) and catenation/decatenation of circular DNA (10,11). An additional reaction, the introduction of negative superhelical turns is performed by the bacterial gyrase (12). The simplest mechanism to explain these reactions is the transient breakage of phosphodiester bonds. Thus, two classes of topoisomerases are distinguished on this basis. Type I topoisomerases catalyze transient single-strand breaks, while type II enzymes catalyze transient double-strand breaks (9).

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However, the in vivo functions of these enzymes are still a matter of hypotheses. Studies of bacterial cells mutant in topoisomerase I (13) (14), (15) indicate that these mutants bear compensatory mutations reducing the gyrase activity and suggest that this enzyme is clearly involved in determining the level of supercoiling in bacteria and, consequently, plays an indirect role in DNA replication, transcription and transposition. Biochemical and genetic experiments with bacterial mutants in type II topoisomerases (DNA gyrase and topoisomerase II') indicate that these enzymes are required for DNA replication (16). However, it remains to be established in which stage of DNA replication, initiation (17), DNA chain elongation (18), termination (19), the type II topoisomerases play a role, and whether or not these enzymes are involved in other functions like DNA repair (20) and recombination (21). Moreover, the catenation/decatenation reaction may have biological significance as a part of the mechanisms which control the condensation/decondensation of chromosomal DNA.

Type I and type II topoisomerases have been isolated from a variety of eukaryotic organisms, including plants (22,23), fungi (24,25), insects (26) amphibians (11,27) birds (28) and mammals (29 to 32). In most cases, both classes of enzymes are found together in the nuclei. In rat liver, ATP-independent topoisomerase was isolated and purified to homogeneity several years ago (33), and its in vitro properties were established in details (34,35). Since this enzyme belongs to type I, we further assume that ATP-independent relaxation found in rat liver nuclei is due to topoisomerase I.

Here we describe the isolation and some of the properties of an ATP-dependent DNA topoisomerase which presumably belongs to the type II class of enzymes. Finally, in search for possible function of topoisomerases in eukaryotic DNA replication, we have followed the enzyme activities of type I and type II topoisomerases in rat liver nuclei during the process of regeneration which takes place after partial hepatectomy. This system has been often used to study the appearance of proteins of the replication machinery in a tissue where cells are in an active phase of DNA synthesis (36,37).

MATERIALS AND METHODS

Proteins, Nucleic acids, nucleotides and drugs : histone H1 was purchased from Boehringer (Mannheim). Staphylococcal V8 protease was from Miles laboratories (UK). Kinetoplast DNA from Trypanosoma cruzi was a generous gift from Dr G. Riou (Institut Gustave Roussy). Supercoiled pBR322 DNA was prepared from Escherichia coli strain HB101 by the method of Clewell and Helinski (38) and purified by cesium chloride-ethidium bromide (EtBr) density centrifugation. SV40 DNA was

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prepared from virions as described by May et al. (39) and purified by CsCl-EtBr density centrifugation. Nucleotides (the four ribonucleoside triphosphates, dATP, and ATP-γ-S were purchased from Boerhinger (Mannheim), as well as ethidium bromide, novobiocin, and nalidixic acid. Oxolinic acid was from Substantia (Paris).

Enzyme purification. Purified nuclei from rat liver were prepared by a method derived from that of Blobel and Potter (40). All operations were at 0°C in the presence of 1 mM phenylmethylsulfonyl fluoride (PMSF). In a standard preparation, 100 g of liver tissue from normal or 2/3 hepatectomized rats were homogenized in a Potter homogeneizer with 400 ml of 50 mM Tris-HCl, pH 7.5, 25 mM KCl, 3 mM $MgCl_2$, 2 mM $CaCl_2$ (TKCM Buffer) containing 0.25 M sucrose and 1 mM PMSF. The homogenate was filtered through cheesecloth and centrifuged for 10 min at 4000 g. The nuclear pellet was suspended in TKCM-1.62 M sucrose, layered over a cushion of TKCM-2.3 M sucrose and centrifuged for 80 min at 27.000 rpm in a SW27 Beckman rotor. The pellet of purified nuclei was resuspended in TKCM 0.25 M sucrose containing 0.5% Triton X 100 and allowed to stand for 10 min at 0°C. The nuclei, freed of nuclear membranes, were collected by centrifugation, washed twice with TKM-0.25 M sucrose, 1 mM PMSF buffer (identical to TKCM but containing 5 mM $MgCl_2$ instead of 3 mM $MgCl_2$, 2 mM $CaCl_2$) and finally resuspended in TKM. Fraction II was prepared as described by Champoux and Mc Conaughy (33) : lysis was obtained by adding 1/10 volume 0.2 M EDTA and 2 volumes of 80 mM Tris-HCl, pH 7.5, 1 mM DTT, 2 mM EDTA, 1 mM PMSF, 2.0 M NaCl and 20% glycerol. After 45 min at 0°C with gentle stirring, 2 volumes of buffer (40 mM Tris-HCl , pH 7.5, 1 mM EDTA, 1.0 M NaCl, 0.5 mM DTT, 10% glycerol) containing 18% polyethylene-glycol (PEG 6000) and 1 mM PMSF were added and stirring was prolonged for another 45 min at 0°C. The precipitate containing nucleic acids was pelleted by centrifugation at 17,000 g for 20 min. The supernatant so obtained is refered as Fraction II. Fraction II (about 20 mg protein) was equilibrated by dialysis against 0.1 M potassium phosphate, pH 7.4, 1 mM EDTA, 2 mM 2-mercaptoethanol, 10% glycerol and 1 mM PMSF, and loaded onto a phosphocellulose column (4 ml bed volume). The column was developped with a linear gradient from 0.2 M to 0.6 M potassium phosphate. Active fractions were pooled, dialyzed against 0.1 M potassium phosphate pH 7.0, 2 mM 2-mercapto-ethanol, 0.5 mM EDTA, 20% glycerol to give Fraction III. This fraction was further purified by using hydroxyapatite (Fraction IV) and DNA cellulose (Fraction V) columns. The details of the purification proeedures will be published elsewhere.

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Enzyme assays. ATP-independent relaxation of a supercoiled DNA was tested according to (41) in a 30 µl reaction mixture containing 10 mM Tris-HCl, pH 7.9, 0.2 mM EDTA, 0.5 mM DTT, 0.2 M NaCl, 0.6 µg pBR322 DNA I, and 2 µl of the fraction to be assayed. After 30 min at 37°C, 1% sodium dodecylsulfate was added for 5 min and the DNA products were analyzed on agarose gels. One unit of topoisomerase I is defined as the amount of enzyme necessary to relax 0.6 µg (1 nanomole) of supercoiled DNA in 30 min at 37°C (33).

ATP-dependent catenation of PBR322 DNA form I was assayed as described by Liu (3) in a total volume of 30 µl containing 50 mM Tris-HCl, ph 7.9, 43 mM KCl, 25 mM $MgCl_2$, 0.5 mM DTT, 30 µg/ml BSA, 1 mM ATP, 0,9 µg. pBR322 DNA I, 0.3 µg histone H1 and 2 µl of the fraction to be assayed. After 30 min at 30°C, the mixture was processed as indicated above and the DNA was analyzed on agarose gel. One unit of catenation activity is defined as the amount of enzyme necessary to catenate 20% of the 0.9 µg input DNA in 30 min at 30°C. The same incubation was used to measure ATP-dependent relaxation. One unit of activity is defined as the amount of enzyme necessary to relax 0.6 µg (1 nanomole) pBR322 DNA in 30 min at 30°C.

ATP-dependent decatenation was assayed in a total volume of 30 µl with the same reaction mixture as for catenation, except that the DNA substrate was 0.4 µg kinetoplast DNA. Histone H1 was omitted and the KCl concentration was raised to 85 mM. One unit of topoisomerase II activity (decatenation) is defined as the amount of enzyme necessary to decatenate 50% of the 0.4 µg input k.DNA in 30 min at 30°C (31).

In all three assays, the reaction was stopped by addition of SDS to a final concentration of 1% and the DNA was analyzed by agarose gel electrophoresis in horizontal slab gels (1% agarose) prepared and run in 36 mM Tris, 30 mM NaH_2PO_4, 1 mM EDTA, pH 7.8. Migration was for 17 h at 1 V/cm at room temperature. After the gels were stained with ethidium bromide, photographs of the gels were taken under short wave UV light Ilford HP4 film. In each case, densitometric profiles of the negatives were made by using a Joyce Loebl densitometer and peak areas were measured for serial dilutions of the enzyme fraction assayed. This quantitation was found to be linear up to only 20% of the input DNA transformed in the case of catenation and 50% of the input DNA in the cases of relaxation and decatenation. Finally, we estimated the quantitation of the activities not better than 50% accurate in the case of catenation and 30% in the case of relaxation and decatenation. The visualized DNA bands were eventually cut out of the gel under UV light and electroeluted in dialysis bags at 5 V/cm for 17 h in 40 mM Tris-acetate, pH 7.8,

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2 mM EDTA buffer. Eluted DNA was ethanol precipitated, taken in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA and diluted in distilled water for electron microscopy.

Electron microscopy. Aliquotes of DNA were phenol extracted and further diluted to 0.2 to 0.5 μg/ml with distilled water. Samples were prepared for microscopy by the formamide technique of Davis, Simon and Davidson (1971) (42) and rotary shadowed with platinum at an angle of 5-8°. Micrographs were taken with a Philipps E.M. 400 electron microscope at 80 KV. Lengths were measured with a Hewlett-Packard 9864 A digitizer interfaced with a 9820 calculator.

Nuclei, protein and DNA quantitation. Nuclei, dispersed in TKCM buffer containing 0.5% Triton X-100 were counted in a Malassez chamber under the microscope. The average of 4 determinations was used to estimate nuclei concentration. Protein determination was performed by the method of Bradford (43). DNA concentrations were measured by a fluorimetric assay, using diaminobenzoic acid (DABA) as described by Fiszer-Szafarz et al. (44).

RESULTS

Type I and type II topoisomerases are present in rat liver nuclei:

Topoisomerase activities were assayed on the polyethyleneglycol supernatant (fraction II) prepared after high salt extraction of purified nuclei (see Materials and Methods). Topoisomerase I was measured by the method described by Keller (41), i.e. The relaxation of a supercoiled DNA in a magnesium-independent, ATP-independent reaction described under Materials and Methods. Topoisomerase II was assessed by the production of catenanes from monomeric, double-stranded DNA circles in a magnesium-dependent, ATP-dependent, histone H1-dependent reaction. Alternatively, topoisomerase II was measured by the decatenation of the large networks of kinetoplast DNA from *Trypanosoma cruzi* in a reaction which is dependent on magnesium and ATP (31) as detailed under Materials and Methods. The catenation/decatenation assays are specific for type II topoisomerases, since ATP is absolutely required in the conditions of the reaction. Using these assays, we have found large amounts of type I and type II topoisomerase activities in purified rat liver nuclei. In addition, such activities have been detected in the polyethyleneglycol supernatant (fraction II) of nuclear extracts isolated from mouse embryos and from a number of different mouse and hamster cells grown *in vitro* (45).

Further copurification of topoisomerases I and II activities from rat liver :

By using both the catenation and the decatenation assays, the ATP-dependent topoisomerase (topoisomerase II) was further purified about 100 fold from the polyethyleneglycol supernatant (fraction II) free of nucleic acids.

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The purification procedure included phosphocellulose, hydroxyapatite, and DNA cellulose columns as described under Materials and Methods. The most purified fraction (DNA cellulose, fraction V) was not homogeneous, based on SDS-polyacrylamide gel electrophoresis. It was free of deoxyribonuclease activity, but still contained large amounts of contaminating ATP-independent relaxation activity

Analysis of topoisomerase I and II activities in vitro :

Fraction II, or more purified enzyme fractions (fractions III to V), from rat liver nuclei were able to catalyze several topological isomerizations of a covalently-closed, circular DNA, as reported for other eukaryotic topoisomerases (24 to 30). These reactions included relaxation of supercoils, catenation of double-stranded DNA circles, and finally decatenation of DNA networks.

Relaxation of a supercoiled DNA occurred in the absence of magnesium and ATP by virtue of the topoisomerase I activity contained in the fraction. In the presence of ATP, relaxation was stimulated to a variable extent, due to the amount of topoisomerase II activity present in the preparation.

Catenation of covalently-closed circles of the plasmid pBR322 DNA is illustrated in fig. 1. Incubation of such supercoiled DNA with rat liver fraction II in the presence of ATP and histone H1 gave rise to relaxed monomeric DNA circles and catenated DNA products, i.e. dimers, trimers, tetramers... and networks. The reaction was followed by the electrophoretic mobility of the products on agarose gels and by direct observation in the electron microscope of the DNA species electroeluted from the gels (fig. 1). Thus, more than 80% of the molecules extracted from the dimer band had the appearance illustrated in the middle right of fig. 1. The 20% remaining comprised monomeric circles and linear DNA presumably generated by breakage during extraction, and a few concatemeric dimers which migrated in the gels as a band slightly slower than catenated dimers. As observed in the case of gyrase and other topoisomerases, catenanes composed of different circular DNA's can be generated efficiently by mixing various unrelated DNA's in the incubation medium, indicating that homology is not necessary for the reaction. In all cases including catenation of a single DNA species, the extent of the reaction was limited to about 40% catenated products, even with excess enzyme and long incubation time. This was presumably due to the reverse reaction, i.e. decatenation. The balance between catenation and decatenation could be modified by KCl concentration and the amount of histone H1 present. As shown in fig. 2, optimum catenation was obtained with about 45 mM KCl, whereas the reaction

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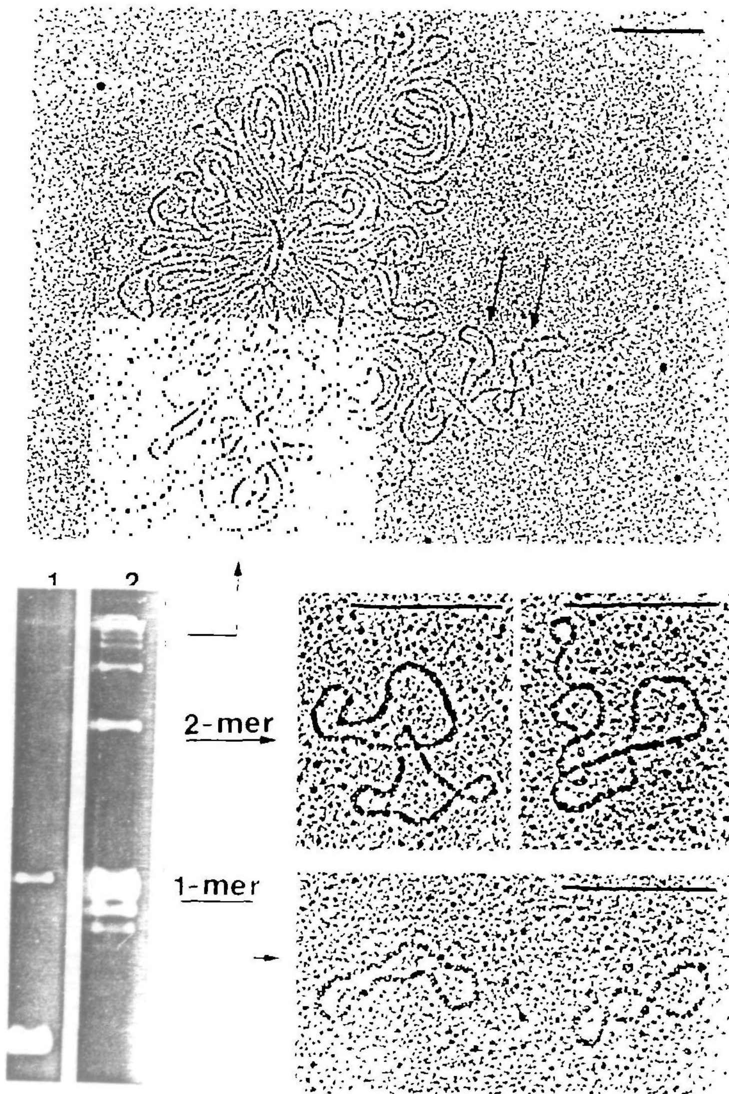

Fig. 1 : Catenation of pBR322 DNA. Supercoiled pBR322 DNA (0.9 µg) was incubated with 5 units of topoisomerase, decatenation units, under the standard conditions and the products of the reaction were analyzed on agarose gels. Lane 1: pBR322-control; lane 2 : products of incubation. The bands corresponding to monomers, oligomers and networks were cut out of the gel, electroeluted and visualized in the electron microscope. The upper part of the figure shows a network composed of pBR322 circles. The arrows indicate some of these circles visible at the edge of the network. Middle right : catenated dimers ; lower right : monomers. The bar represents 1µ.

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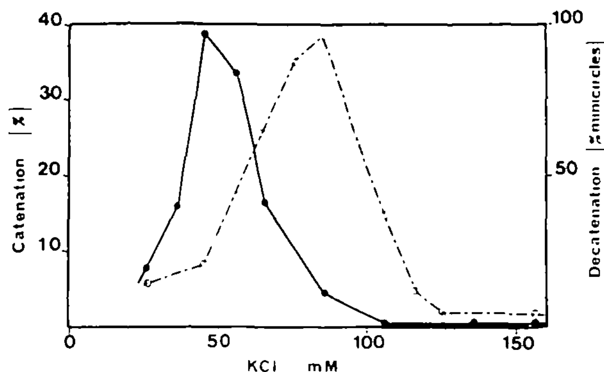

Fig. 2 : Effect of [KCl] on the catenation and decatenation reactions. Catenation ●—● and decatenation ○—○ were performed respectively with pBR322 and with kinetoplast DNA in the standard reaction mixtures specific for these assays. Incubations were for 45 min in the presence of different KCl concentrations. The extent of each reaction was determined by densitometric quantitation of the products on agarose gels.

was completely inhibited above 100 mM KCl. Histone H1 is required for catenation (fig. 3A). The optimum histone H1 to DNA ratio was 0.3 (w/w). Higher proportions of histone (above 0.5) completely abolished the formation of catenanes (not shown). ATP is absolutely essential for the catenation. It can be replaced by dATP, but by no other ribonucleoside triphosphate (fig. 3B). However, with GTP, the reaction yielded a small amount of catenated dimer (fig. 3B lane 5, band b), indicating that GTP might be a cofactor with a lower affinity than ATP. Not only the binding of ATP but its hydrolysis was necessary for the reaction, as indicated by the absence of catenation with ATP- γ-S, a non-hydrolyzable analogue of ATP. We have tested the effect of different topoisomerase inhibitors on catenation. Novobiocin, but not nalidixic or oxolinic acids inhibited the reaction. Novobiocin behaved as a competitive inhibitor of ATP, since catenation was 50% inhibited only by high novobiocin concentrations (330 μg/ml≃ 500 μM) in the presence of 1 mM ATP, and by 60 μg/ml (90μM) novobiocin in the presence of 0.25 mM ATP (data not shown).

Decatenation of kinetoplast DNA networks from Trypanosoma cruzi was examined according to the legend of fig. 4. The results shown indicate that incubation of these networks, comprising several thousand catenated circles, with rat liver fraction II in the presence of ATP resulted in its almost complete conversion to 1.48 kilobases minicircles. As indicated in

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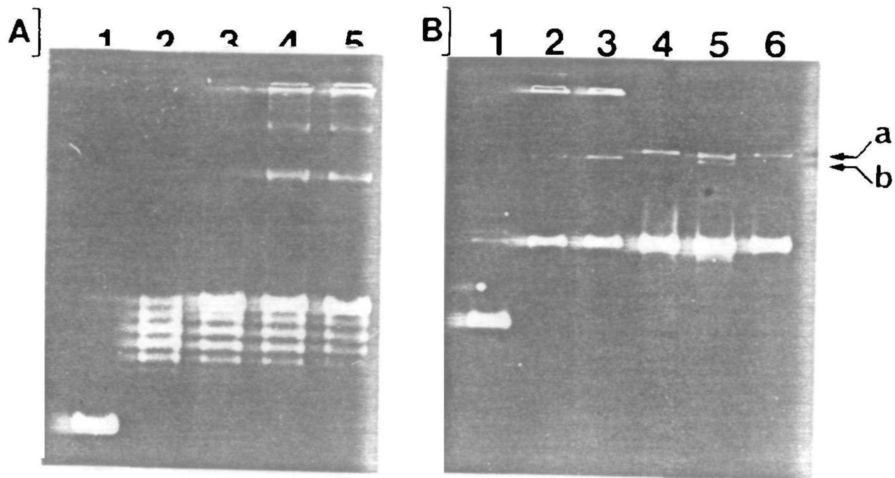

Fig. 3 : Requirements of the catenation reaction. Supercoiled pBR322 (0.9 μg) was incubated with 5 units of topoisomerase, decatenation units, in the standard catenation mixture for 45 min at 30°C, in the presence of various amounts of histone H1 (A) or various nucleoside triphosphates (B) and analyzed on agarose gels.
(A) histone H1. Lane 1 : pBR322 control; lanes 2 to 5 : histone H1 to DNA ratio of (2) 0, (3) 0.05, (4) 0.1, and (5) 0.3.
(B) Nucleoside triphosphates. Lane 1 : pBR322 control; lane 2 : complete reaction mixture with ATP; lane 3 : dATP (1 mM) in place of ATP : lanes 4 to 6 have respectively CTP, GTP, and UTP (1 mM each) in place of ATP. Band a is concatemeric dimer; band b catenated dimer.

fig. 4, lane 1, the starting material, i.e. the k.DNA networks, failed to enter the gel. Electron microscopy of this material is presented in fig. 4A. The products of incubation (fig. 4, lane 2) are shown in fig. 4B and C. The reaction yielded 90-98% of fully decatenated minicircles (fig. 4C) and 2-10% of partially-decatenated networks (fig. 4B). Decatenation, as indicated above for catenation, was found sensitive to KCl concentration. The optimum activity was obtained with 85 mM KCl and decatenation was completely inhibited above 120 mM KCl (fig. 2). The equilibrium between catenation and decatenation is influenced not only by KCl concentration (fig. 2) but by the presence of histone H1. As shown in fig. 5, lane 4, decatenation did not require histone H1, but was inhibited by H1. As for catenation, decatenation required magnesium, the binding of ATP and its hydrolysis (fig. 5, lane 3 and 8), and was completely inhibited by high novobiocin concentration (500 μg/ml) in the

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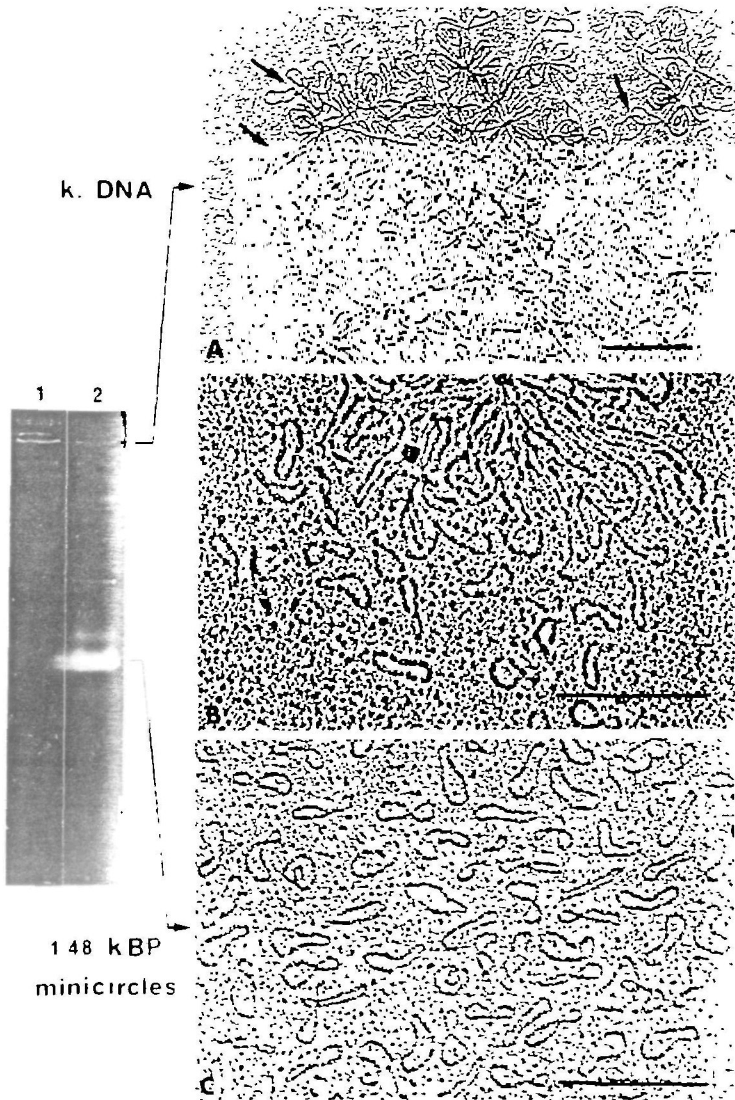

Fig. 4 : Decatenation of kinetoplast DNA networks. Kinetoplast (k.) DNA from *Trypanosoma cruzi* (0.4 μg) was incubated with 5 units of topoisomerase in the standard decatenation mixture for 45 min at 30°C. The products were analyzed on agarose gels and in the electron microscope. Lane 1 and A : k. DNA before reaction, the arrows indicating some catenated minicircles clearly resolved at the edge of the network. Lane 2 and C : products of full decatenation. B : residual partially decatenated networks. The bar represents 1μ.

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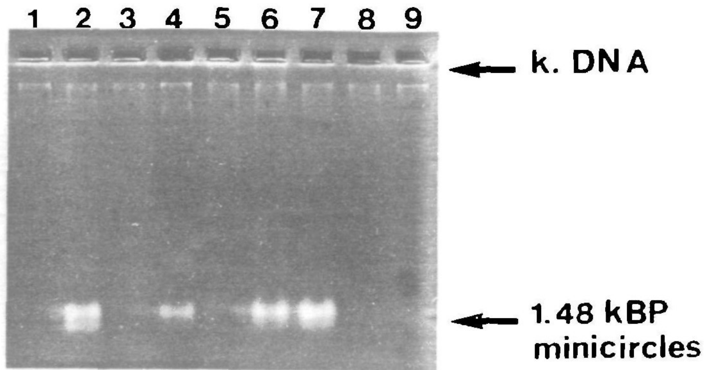

Fig. 5 : Requirements for the decatenation reaction. Kinetoplast (k.) DNA was incubated with topoisomerase in the standard decatenation mixture, except that additions to and omissions from this mixture were made. Lane 1 : k. DNA control; lane 2 : complete reaction; lane 3 : ATP omitted; lane 4 : addition of histone H1 (histone to DNA weight ratio of 0.3); lane 5 to 7 : addition of novobiocin (500 μg/ml), oxolinic acid (250 μg/ml) and nalidixic acid (250 μg/ml); lane 8 : ATP replaced by ATP-γ-S (1 mM); lane 9 : topoisomerase fraction was preincubated with staphylococcal V8 protease.

presence of 1 mM ATP but not by oxolinic or nalidixic acids (250 μg/ml each) fig. 5, lanes 5-7).

Topoisomerase activities in regenerating liver

Fraction II was prepared as described under Materials and Methods from rat livers which were harvested various times after partial hepatectomy and subsequent liver regeneration. Each fraction was assayed as described in Materials and Methods for the following activities : (i) relaxation of supercoiled pBR322 DNA in the absence of ATP (topoisomerase I); (ii) relaxation of supercoiled pBR322 DNA in the presence of ATP (topoisomerase I and II); (iii) ATP-dependent catenation of pBR322 DNA (topoisomerase II); and (iv) ATP-dependent decatenation of kinetoplast DNA (topoisomerase II). Fig. 6 shows a considerable increase in topoisomerase II activity (catenation and decatenation) in the course of regeneration of rat liver. Maximum activity was found in fraction II from rat liver removed 40h after partial hepatectomy (fig. 6A lanes 12 to 16 and fig. 6B lanes 8-10).

The quantitative aspects of this study are presented in Table I. The activities of the various fractions have been expressed in units per milligram of protein, and in units per 10^8 nuclei. The comparison of the data of Table I led to two major conclusions. First, type I topoisomerase activity was not significantly changed during regeneration of the liver, given the

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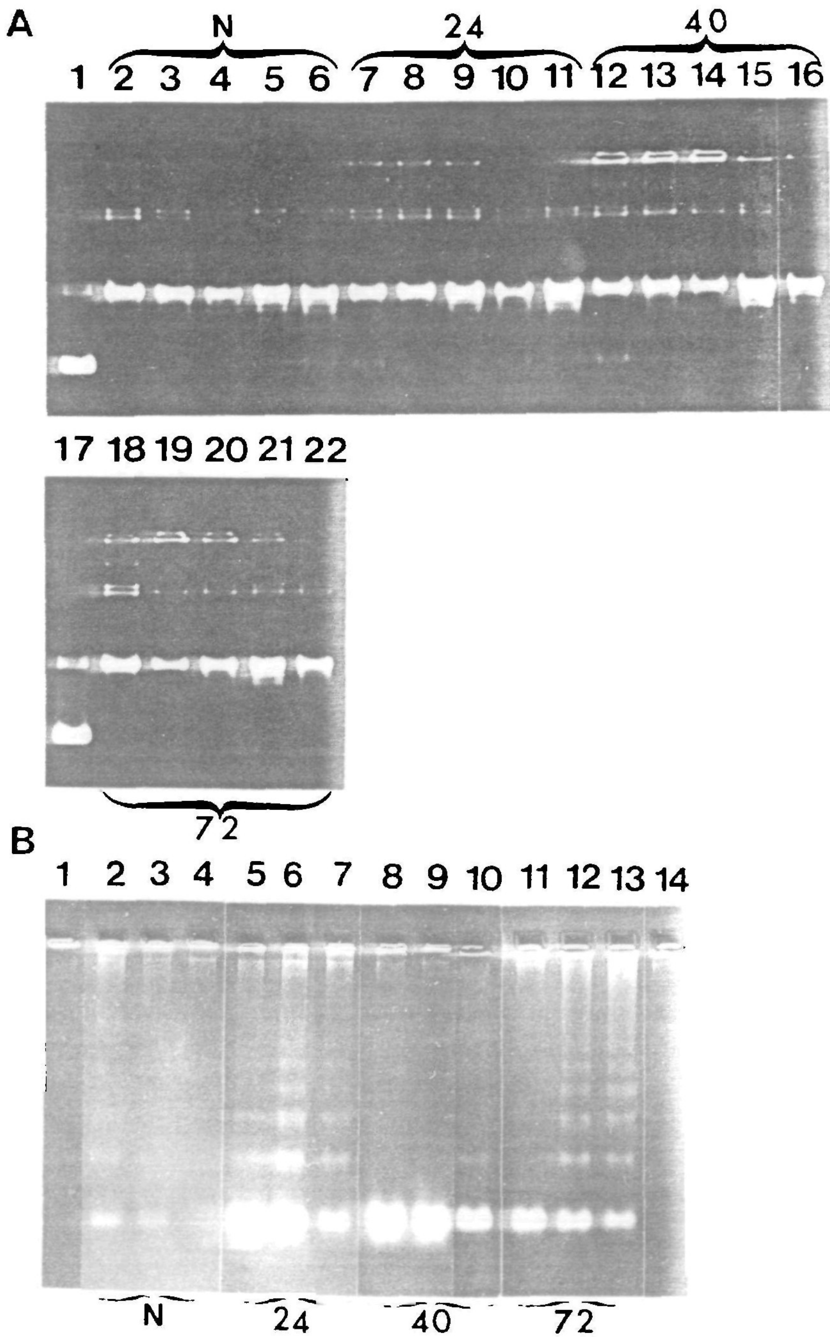

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relatively low accuracy of the assay. Second, type II topoisomerase increased considerably during the regeneration process, with maximum activity being detected about 40h after partial hepatectomy. This conclusion was supported by the parallel increase of activity measured by the three different assays for topoisomerase II : (i) ATP-dependent relaxation; (ii) catenation; (iii) decatenation.

DISCUSSION

The experiments described in this paper demonstrate the existence of an ATP-dependent DNA topoisomerase in rat liver nuclei, in addition to the topoisomerase I previously isolated by Champoux (30). This new topoisomerase was found to catalyze in vitro the catenation of closed DNA circles in a magnesium dependent, ATP-dependent, histone H1 dependent reaction, and the decatenation of kinetoplast DNA networks in a magnesium dependent and ATP-dependent reaction. It is presumably similar to the ATP-dependent DNA topoisomerases recently isolated from other mammalian cells, Hela cells (31) and Calf thymus (46); and refered to type II enzymes.

However, since our preparations are contaminated by the type I topoisomerase, we have not been able to prove that the enzyme changes the linking number of a circular DNA by steps of two, a criterium for classification as a type II topoisomerase (9). The experiments that we have performed on different cell lines in culture (45) suggest that this topoisomerase activity is ubiquitous and therefore is presumably essential for one or several functions in Nucleic Acids metabolism.

Purification of the ATP-dependent topoisomerase from rat liver has been followed both by the catenation and the decatenation assays. However, quantitation of the activities is easier and more accurate in the case of

Fig. 6 : Catenation and decatenation activities of fractions II from rat liver nuclei isolated at various times after hepatectomy. A) Catenation activities were tested under standard conditions on serial dilutions of fraction II. Four series of 5 dilutions (1/5, 1/10, 1/20, 1/50, 1/100) were made. Lanes 1 and 17 : pBR322 control; lanes 2 to 6 : incubations with fraction II from normal liver (protein concentration 0.59 mg/ml); lanes 7 to 11 : fraction II from 24 h regenerating livers (1.24 mg/ml); lanes 12 to 16 : fraction II from 40 h regenerating livers (0.78 mg/ml); lanes 18 to 22 : fraction II from 72 h regenerating livers (1.20 mg/ml). B) Decatenation activities were tested under standard conditions on serial dilutions (1/20, 1/50, 1/100) of fraction II preparations. Lanes 1 and 14 : k. DNA control; lanes 2 to 4 : fraction II from normal rat liver. Lanes 5-7 : fraction II from 24 h regeneration; lanes 8-10 : fraction II from 40 h regeneration; lanes 11-13 : fraction II from 72 h regeneration.

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TABLE I

Enzymatic activity	Fraction II from rat liver: time after hepatectomy			
	Non hepa-tectomized	24h	40h	72h
Relaxation in the absence of ATP (topoisomerase I activity)				
10^3 Units/mg protein	254 140 233	167	203 186 125	104
10^3 Units/10^8 nuclei	92	95	58 82	77
Relaxation in the presence of ATP (topoisomerase I + topoisomerase II activities)				
10^3 Units/mg protein	171 230	134	854 850 -	208
10^3 units/10^8 nuclei	98	77	265 -	153
ATP-dependent catenation (topoisomerase II activity)				
10^3 Units/mg protein	5.1 4.3 8.5	8.1	31.0 97.0 -	25.0
10^3 Units/10^8 nuclei	3.3	4.6	30.2 -	18.5
ATP-dependent decatenation (topoisomerase II activity)				
10^3 Units/mg protein	6.8 8.6	22.6	94.6 62.0	19.8
10^3 Units/10^8 nuclei	3.4	13.2	29.6 41.0	14.6

Variations of topoisomerase activities in regenerating rat liver. Topoisomerase activities were measured in a series of Fractions II from rat liver removed various times after partial hepatectomy. In each fraction, the activities were measured by four different assays, as described in Materials and Methods. The results were expressed in Units per milligram of protein and in Units per 10^8 nuclei. The activities of several preparations are eventually shown for fractions from normal and 40 h regenerating rat liver.

decatenation. After fractionation on phosphocellulose, hydroxyapatite, and DNA-cellulose, the active fraction obtained still contained contaminating ATP-independent relaxation activity. This copurification can be interpreted either in considering that the column fractionation was not enough resolutive to separate the enzymes, or by the existence of protein-protein interactions between type I and type II topoisomerases in rat liver.

As reported for other eukaryotic type II topoisomerases (31), histone H1 is an essential component of the catenation reaction, but, in contrast with Drosophila (26) and Calf thymus enzymes (46), a nearly crude

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fraction (fraction II) from rat liver nuclei dit not contain any protein factor replacing histone H1. Nevertheless, it is not known whether or not this dependence on histone H1 has any biological significance and moreover whether or not the reaction of catenation itself plays any role in vivo.

Inhibitors of the bacterial DNA gyrase, novobiocin, nalidixic and oxolinic acids, were tested on rat liver topoisomerases : as found for Drosophila (26) and Calf thymus (46), and in contrast with Hela cells (31), only novobiocin at high concentrations is inhibitor of type II activity. In the case of Drosophila, it has been suggested that oxolinic acid, even if it has poor inhibitory effect on catenation/decatenation activities, does bind to the enzyme and stimulates the double-strand breakage (47).

We have focused our attention on the activity of type I and type II topoisomerases in rat liver nuclei during the process of regeneration which follows partial hepatectomy : the results suggest that only the type II enzyme is considerably increased in regenerating liver. This increase paralleled the increase of DNA polymerase α observed in the same system (36,37) with again a maximum 40 h after hepatectomy. However, due to the low accuracy in the quantitation of the activities (see Materials and Methods), we cannot exclude a small variation in topoisomerase I activity. Champoux found that topoisomerase I remains unchanged in regenerating liver, as well as in S phase cultured cells or virus-infected cells (48). In contrast, Rosenberg et al.(49) found a considerable increase in topoisomerase I activity of S phase lymphocytes compared to G_o.

Little is known on the functions of type II topoisomerases in vivo. These enzymes may be involved in various processes of DNA metabolism, including replication, recombination, repair, transcription, and chromatin condensation/decondensation (1). Our findings on rat liver enzymes emphasize a possible role of topoisomerase II in DNA replication, or in the control of the mechanisms which change chromatin structure during replication.

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