A Homogeneous Type II DNA Topoisomerase from HeLa Cell Nuclei*

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Using kinetoplast DNA networks as a substrate in a decatenation assay, we have purified to apparent homogeneity a type II DNA topoisomerase from HeLa cell nuclei. The most pure preparations contain a single polypeptide of 172,000 daltons as determined by sodium dodecyl sulfate-gel electrophoresis. The molecular weight of the native protein, based on sedimentation and gel filtration analyses, is estimated to be 309,000. These results suggest that the enzyme is a dimer of 172,000-dalton subunits. The enzyme is a type II topoisomerase as demonstrated by its ability to change the linking number of DNA circles in steps of two and to decatenate or unknot covalently closed DNA circles. No gyrase activity is detectable. ATP is required for the relaxation, decatenation, and unknotting of DNA, and a DNA-dependent ATPase activity is present in the most pure fractions. ATP is hydrolyzed to ADP in this reaction. This enzyme is very similar in its catalytic properties to T4 DNA topoisomerase (Liu, L. F., Liu, C. C., and Alberts, B. M. (1979) Nature 281, 456-461).

Topoisomerases are enzymes which introduce transient breaks in the DNA backbone and thereby participate in a number of genetic processes. Some reactions catalyzed by these enzymes which depend on the breakage and reunion mechanism are: 1) supercoiling or relaxation of closed circular duplex DNA; 2) catenation and decatenation of DNA circles; 3) knotting and unknotting of DNA circles; 4) viral integration or joining together of DNA strands to form novel sequences; and 5) complete renaturation of single-stranded circles of complementary sequence (for reviews see Refs. 1-4).

Topoisomerases have been found in both procaryotic and eucaryotic cells; they are present in the virion of vaccinia virus (5), and they are induced by several bacteriophages (6-8). Although the precise function of many of these enzymes is not clear, their participation in a variety of genetic processes such as replication (1-4), transcription (5-8), recombination (9, 10), integration (6), and transposition (11) has been suggested.

Topoisomerases have recently been divided into two classes which are distinguished by their reaction mechanisms (12, 13). The type I enzymes transiently break one strand of the helix, permitting the linking number to change in steps of one. Omega protein (14) from Escherichia coli and the eucaryotic nicking-closing enzymes (15) are examples of type I enzymes. Known type I enzymes do not require energy input for their reactions. The energy of the phosphodiester bond is conserved after strand breakage by the covalent attachment of the enzyme to one end of the DNA, allowing the DNA backbone to be reformed after topoisomerization (1, 2).

The type II topoisomerases catalyze the topological passing of two double-stranded DNA segments, presumably by introducing a transient enzyme-bridged double strand break on one of the crossing DNA segments (12, 16-18). This mechanism results in a change in linking number in steps of two (12, 18-20). A consequence of this mechanism is that type II enzymes can catenate and decatenate covalently closed circles as well as relax supercoils. Gyrase (21), which can also induce negative supercoiling, is the best characterized example of this class of enzymes (for reviews see Refs. 3 and 4). T4 topoisomerase, another well studied type II topoisomerase, has not been shown to supercoil DNA, but can catalyze relaxation, knotting, and catenation reactions (12). Based on genetic studies, the T4 enzyme is involved in DNA synthesis (reviewed in Ref. 7). Several eucaryotic enzymes, similar to T4 topoisomerase, have been identified by their ability to catenate and decatenate DNA (18, 22).

Because of the likely importance of type II topoisomerases in eucaryotic DNA replication, we are studying the topoisomerases present in HeLa cells. To search for a type II enzyme we have used as a substrate the mitochondrial DNA (kinetoplast DNA) of Crithidia fasciculata, an insect trypanosomatid. This DNA is in the form of networks consisting primarily of about 5000 covalently closed minicircles (2.5 kilobases) which are topologically interlocked (23). Type II topoisomerases, such as T4 topoisomerase and Micrococcus luteus DNA gyrase, decatenate this DNA to form individual minicircles which are easily detectable by gel electrophoresis (24). Type I enzymes cannot decatenate these networks, making a type II enzyme easy to detect even in the presence of a large amount of type I enzyme. Using this assay, we have purified to apparent homogeneity a new HeLa topoisomerase activity, which we call HeLa topoisomerase I. It is a type II topoisomerase. We have characterized its physical and enzymatic properties.

MATERIALS AND METHODS

Cell Growth and Nuclei Isolation—HeLa cells (S-3), a gift of Dr. Bernard Moss (National Institutes of Health), were grown at 37 °C in minimal essential medium for suspension cultures supplemented with 5% horse serum, 100 units/ml of penicillin, 0.1 mg/ml of streptomycin sulfate (all from Grand Island Biological Co.). Fresh cells (15 liters of culture; 5-6 × 10^6 cells/ml) were processed for each purification. The culture was chilled to 4 °C, and the nuclei isolation was carried out at 0-4 °C. The cells (about 20 g, wet weight) were centrifuged (3000 × g for 10 min), washed twice with 100 ml of 0.15 M NaCl, 10 mM sodium phosphate, pH 7.5, and then resuspended in 100 ml of extraction buffer (5 mM potassium phosphate, pH 7.0, 2 mM MgCl₂, 1 mM PMSF) added as a 100 mM solution in isopropanol alcohol, 1 mM mercaptoethanol, 0.5 mM dithiothreitol, 0.1 mM EDTA). After swelling at 0 °C for 30 min, the cells were broken by Dounce homogenization (about 20 strokes with a loose pestle). Cell disruption was monitored by

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The abbreviations used are: PMSF, phenylmethylsulfonyl fluoride; NaDodSO₄, sodium dodecyl sulfate; PEG, polyethylene glycol.
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RESULTS

Purification

A summary of the purification of HeLa topoisomerase II is presented in Table I. All steps of the purification were done at 0–4 °C, and the purification was completed in less than 3 days.

1. Preparation of the PEG Supernatant—Nuclei from 20 g of fresh cells were resuspended in 100 ml of nuclei wash buffer, and EDTA was added to a final concentration of 4 mM. After 15 min at 0 °C, the nuclei were lysed by the slow addition, with stirring, of 100 ml of 2 M NaCl, 50 mM Tris-HCl, pH 7.5, 10 mM mercaptoethanol, 1 mM PMSF. After another 15 min at 0 °C, 100 ml of 18% (w/v) PEG, 1 M NaCl, 50 mM Tris-HCl, pH 7.5, 10 mM mercaptoethanol, 1 mM PMSF were slowly added with constant stirring. This solution was incubated for 40 min at 0 °C with occasional stirring and then centrifuged at 12,000 × g for 30 min. The supernatant, fraction I (375 ml), was then used for further purification.

2. Hydroxylapatite Chromatography—Fraction I (375 ml) was loaded onto a hydroxylapatite column (1.5 × 15 cm) equilibrated with 6% PEG, 1 M NaCl, 50 mM Tris-HCl, pH 7.5, 10 mM mercaptoethanol, 1 mM PMSF. After loading, the column was washed with 100 ml of 0.2 M potassium phosphate, pH 7.0, in solution A (10% glycerol, 10 mM mercaptoethanol, 1 mM PMSF) and eluted with a gradient from 0.2 to 0.7 M potassium phosphate, pH 7.0, in solution A. The results of this column, showing assays for both topoisomerase I (B, bottom row) and topoisomerase II (B, top row) are shown in Fig. 1. The topoisomerase II activity elutes slightly ahead of the topoisomerase I. The two enzymes were pooled as indicated in Fig. 1A. The topoisomerase II pool is fraction II. Further purification of topoisomerase I has been described previously (25); further purification of topoisomerase II is described below.

3. Phosphocellulose Chromatography—Fraction II (35 ml) was diluted with an equal volume of 10% glycerol, 10 mM mercaptoethanol, 0.1 mM EDTA, and then loaded onto a phosphocellulose column (0.9 × 2.5 cm) equilibrated with 0.2 M potassium phosphate, pH 7.0, in solution A. The column was washed with the equilibration buffer (10 ml) and then eluted with five 10-ml steps of 0.3, 0.4, 0.5, 0.6, and 0.7 M potassium phosphate, pH 7.0, in solution A. Activity was eluted in the 0.4 M buffer and the active fractions were pooled and dialyzed for 3–5 h against 0.1 M KCl in solution B (40 mM Tris-HCl, pH 7.5, 10% glycerol, 10 mM mercaptoethanol, 1 mM PMSF). The dialysis buffer (500 ml) was changed twice. The dialyzed enzyme (6 ml) is fraction III.

4. DNA Cellulose Chromatography—Fraction III (6 ml) was loaded onto a DNA cellulose column (0.9 × 1.0 cm) equilibrated with 0.1 M KCl in solution B. The column was washed with 5 ml of the same buffer and eluted in 5-ml steps of 0.2, 0.3, 0.4, and 1 M KCl in solution B. The topoisomerase II activity eluted in the 0.3 M buffer and active fractions were pooled (fraction IV, 2 ml).

5. Gel Filtration on Bio-Gel A-1.5m—Fraction IV was loaded directly on a Bio-Gel A-1.5m column (1.5 × 85 cm) equilibrated with 0.2 M potassium phosphate, pH 7.0, in solution A, and the column was washed with the same buffer. The topoisomerase II activity eluted at 68 ml in a symmetrical peak (fraction V). Column fractions were also assayed by NaDodSO4 gel electrophoresis. The enzyme activity correlated with a single Coomassie blue-stained polypeptide, Rv, = 172,000, and no other polypeptides were detectable in the active fractions (Fig. 2). Since this polypeptide copurified with

| Table I |
|--------|
| Purification of topoisomerase II |
| Purification step | Protein (mg) | Specific activity | Activity recovered (%) |
|------------------|--------------|-----------------|-----------------------|
| 1. PEG supernatant | 250 | 1500 | 5.8 × 10^6 | (100) |
| 2. Hydroxylapatite | 8.7 | 560 | 6.4 × 10^6 | 30* |
| 3. Phosphocellulose | 1.4 | 224 | 1.6 × 10^6 | 15 |
| 4. DNA cellulose | 0.23 | 60 | 2.6 × 10^4 | 4 |
| 5. A-1.5m | 0.004 | 10 | 2.5 × 10^4 | 0.8 |

*An additional 30% of loaded activity trailed into the topoisomerase I pool. To reduce topoisomerase I contamination, this activity was discarded.

a Estimate of protein concentration was based on intensity of Coomassie blue-stained band on an NaDodSO4 gel.
Therefore, fraction V is virtually homogeneous.

In preliminary purifications of topoisomerase II we used frozen cells and found that the stability of all fractions, even in the presence of PMSF, was very poor. Total loss of activity in all fractions was observed after several days at 4°C. We believe that proteolysis makes a large contribution to the instability, as on NaDodSO₄ gels a number of smaller molecular weight bands are generated in fractions III and IV during storage at 4°C. By using fresh (never frozen) cells we found that the stability of the enzyme increased dramatically. Fraction IV is stable for 1 month at 4°C and then loses activity with a half-life of approximately 1 month. At -20°C, the enzyme is stable for at least 3 months. Fractions I-III have a half-life of approximately 3 weeks at 4°C. The tremendous loss of activity during the gel filtration step may not be due to instability. We believe it is due to adsorption of the enzyme to the Bio-Gel resin, as recoveries improved if the column was used several times.

### Calculation of Molecular Weight

As shown in Fig. 2, the subunit molecular weight of topoisomerase II is 172,000. We determined the native molecular weight from its hydrodynamic properties. A portion of fraction III (0.1 ml containing 3700 units) was mixed with catalase, aldolase, thyroglobulin, and ferritin (200 µg each) as markers and centrifuged on a 5-20% sucrose gradient. The sedimentation coefficient was determined by comparing the sedimentation rate of topoisomerase (measured by decatenation assay) with the sedimentation rate of the markers. The sedimentation coefficient is 9.2 s. In a separate experiment, the decatenation activity sedimented together with a polypeptide of $M_r = 172,000$, and no other detectable polypeptide cosedimented with activity (data not shown). The Stokes radius was determined by comparing the elution position on a Bio-Gel A-1.5m column of the topoisomerase with those of the same markers used for sedimentation. To calculate the Stokes radius, $(-\log K_v)^2$ was plotted against elution volume for the markers and a straight line was obtained (30). From the elution volume of topoisomerase II, its Stokes radius was found to be 78 Å. The molecular weight of the native protein was calculated from its sedimentation coefficient and Stokes radius by the method of Siegel and Monty (30) to be 309,000. Since the subunit $M_r = 172,000$, the active enzyme appears to be a dimer.

### Reactions Catalyzed by Topoisomerase II

Fig. 3 shows examples of the reactions catalyzed by HeLa topoisomerase II. Lanes a-c show the effect of the enzyme on negatively supercoiled pBR322 DNA. No relaxation is detectable in the absence of ATP (lane c), but relaxation is extensive in the presence of ATP (lane b). The enzyme will relax positive supercoils as well. If the pBR322 DNA in the standard assay is positively supertwisted by addition of ethidium bromide (2 µg/ml), this DNA is readily relaxed by topoisomerase II (data not shown).
Fig. 3. Reactions catalyzed by topoisomerase II. To demonstrate relaxation of negative supercoils, pBR322 DNA (lane a) was incubated with 8 units of HeLa topoisomerase II in the standard reaction mixture (lane b), or in an identical reaction mixture without ATP (lane c). To demonstrate decatenation, kinetoplast DNA (lane d) was incubated with 8 units of topoisomerase II in the presence (lane e) or absence (lane f) of ATP. Lanes g–j show the catenation reaction. Reactions (20 μl) contained 50 mM Tris-HCl, pH 7.9, 60 mM KCl, 10 mM MgCl₂, 0.5 mM ATP, 0.1 mM EDTA, 30 μg/ml of bovine serum albumin, 5 mM spermidine, 20 μg/ml of pBR322 DNA. Plasmid DNA (lane g) was incubated with 50 units of HeLa topoisomerase I (lanes h and j), or 40 units of HeLa topoisomerase II (lane i) for 30 min at 30 °C. Unknotting is demonstrated in lanes k–n. P4 knotted DNA (from phage heads) was incubated with (lane n) or without (lane l) HeLa topoisomerase II. As a control, unknotted P4 DNA (from mature phage) was incubated with (lane m) or without (lane k) the enzyme.

Table II

| Topoisomerase | Enzyme                | ATP Hydrolysis |
|---------------|-----------------------|----------------|
|               | units/min              | nmol/h         |
| T4            | 60                    | 0.04           | 0.34           |
| HeLa          | 16                    | <0.02          | 0.13           |
| HeLa          | 32                    | <0.02          | 0.23           |

Fig. 4. HeLa topoisomerase II changes the linking number of DNA in steps of two as shown by agarose gel electrophoresis. A topoisomer of pBR322 with a unique linking number, n (lane a), was incubated with 20 units of HeLa topoisomerase I (lanes b and d) or 8 units of HeLa topoisomerase II (lane c). Reaction conditions are described under “Materials and Methods,” except that the reaction volume was 60 μl, and the reaction products were precipitated with ethanol in the presence of 1 μg of tRNA as an carrier. The samples were resuspended in 20 μl of 90 mM Tris-borate, pH 8.3, 2.5 mM EDTA gel buffer, and 5 μl of 5% (w/v) NaDodSO₄, 25% (w/v) Ficoll, 25 μg/ml of bromphenol blue were added. The gel was electrophoresed, stained, and photographed as described under “Materials and Methods.” The band near the top of lane c is nicked circular DNA, indicating that there was a small amount of endonuclease in this particular preparation. Most preparations do not contain detectable endonuclease.

Inhibitors

We have studied the effect of several procaryotic topoisomerase inhibitors on HeLa topoisomerase II. In the standard assay, 50% inhibition is obtained with 100 μg/ml of oxolinic acid, 500 μg/ml of nalidixic acid, and 200 μg/ml of novobiocin. These concentrations are significantly higher than those re-
required to inhibit E. coli DNA gyrase by 50% (10 μg/ml for oxolinic acid (31), 200 μg/ml for nalidixic acid (31), and 1 μg/ml for novobiocin (32)).

**HeLa Topoisomerase II Is a Type II Topoisomerase**

Since this enzyme can decatenate and catenate covalently closed circular DNA, it is presumably a type II enzyme. To provide further evidence for this classification we treated a topoisomer of a unique linking number, n, with HeLa topoisomerase I or HeLa topoisomerase II. As shown by gel electrophoresis, the topoisomerase II products are topoisomers of linking numbers n + 2 (Fig. 4, lanes b and d). Linking number changes in steps of two are characteristic of a type II topoisomerase (12, 13). In contrast, the HeLa topoisomerase I products differ in linking number by steps of one (lanes b and d, Fig. 4).

**Discussion**

We have purified a type II topoisomerase from HeLa nuclei to apparent homogeneity, using an assay which depends on the ability of type II enzymes to decatenate covalently closed circular DNA. This assay has allowed us to detect and quantitate type II activity in crude extracts without interference from type I topoisomerases. Besides its ability to decatenate DNA circles, we have demonstrated that the enzyme can relax positive and negative supercoils and unknot topologically knotted DNA circles. No gyrase activity is detectable. The enzyme requires ATP for activity, and consistent with this requirement is the presence of a DNA-dependent ATPase. The most pure preparation contains a single polypeptide of Mr = 172,000 as measured by NaDodSO₄-gel electrophoresis. This polypeptide correlates well with activity throughout the purification and in sedimentation in a sucrose gradient. The native molecular weight, calculated from hydrodynamic properties, is 309,000, suggesting that a dimer of Mr = 172,000 subunits is the active species.

This enzyme shares several properties with other type II topoisomerases. The T4 topoisomerase, which has similar catalytic properties, probably has three subunits with Mr = 63,000, 52,000, and 15,000 (7). No data on molecular weight of the native T4 enzyme is available. Type II topoisomerases, which probably are similar to HeLa topoisomerase II and T4 topoisomerase, have been detected in *Drosophila* embryos (18) and *Xenopus* germinal vesicles (22). These enzymes differ from the prokaryotic DNA gyrase in that they are unable to induce negative supercoiling (7, 18, 22). Nevertheless, all the type II enzymes may be related in their reaction mechanisms. The mechanism involves the transient introduction of an enzyme-bridged double-stranded break and the passage of an intact DNA helix through the break. The ends of the broken DNA helix cannot rotate freely relative to one another during this event. This mechanism accounts for the observed change in linking number in steps of two (12, 17–19).

There is a clear need for energy input (ATP hydrolysis) in the supercoiling reaction catalyzed by gyrase. However, the requirement for ATP hydrolysis by other type II enzymes, which have not been shown to supercoil DNA, is less obvious and remains a matter of speculation. The ATPase activity in T4 topoisomerase is stoichiometrically related to strand passage (7). The HeLa topoisomerase II ATPase may also be related to strand passage, as we calculate that roughly the same number of molecules of ATP are hydrolyzed as strands passed. The HeLa and T4 enzymes may actually have some energy-requiring functions (*e.g.* gyrase activity) *in vivo*. Alternatively ATP may be required simply in the strand passage mechanism (3, 4, 7). One difference between gyrase and other type II enzymes may be that gyrase can bind the DNA exclusively in a positive supercoil (33). The other type II enzymes may not be able to wrap the DNA to generate a positively supercoiled domain, or may require factors such as a specific DNA sequence or other proteins to orient directionally on the DNA.

Type II topoisomerases may function in a number of important genetic processes. In replication they may play a role in initiation, elongation, and termination. In initiation they may serve to separate the strands at the replication origin in an origin-specific gyrase reaction (7). In elongation, they may relax positive supercoils which are generated by unwinding of the template strands. In termination, they may assist segregation of daughter molecules by passing one helix through another (34). Topoisomerases are thought to be important in transcription; for example, supercoiling may increase the activity of certain promoters (3). They may also facilitate assembly of DNA into its condensed chromosomal state. The ability of type II topoisomerases to pass one helix through another could be important in generating and maintaining this complex organization. Further studies will reveal the precise role played by HeLa topoisomerase II in these and other processes.

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**References**

1. Wang, J. C., and Liu, L. F. (1979) in *Molecular Genetics* (Taylor, J. H., ed) Part III, pp. 65–88, Academic Press, New York
2. Champoux, J. J. (1978) *Annu. Rev. Biochem.*, 47, 449–479
3. Gellert, M. (1981) *Annu. Rev. Biochem.*, in press
4. Cozzarelli, N. R. (1960) *Science* 137, 965–967
5. Bauer, W. R., Resecker, E. C., Kates, J., and Patzke, J. V. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74, 1841–1845
6. Kikuchi, Y., and Nash, H. A. (1979) *Proc. Natl. Acad. Sci. U.S.A.* 76, 3760–3764
7. Liu, L. F., Liu, C. C., and Alberts, B. M. (1979) *Nature* 281, 456–461
8. Meyer, T. F., and Geider, K. (1979) *J. Biol. Chem.* 254, 12642–12646
9. Champoux, J. J. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74, 3800–3804
10. Kirkegaard, K., and Wang, J. C. (1978) *Nucleic Acids Res.* 5, 3811–3820
11. Striglgranz, R., DiNardo, S., Voelkel, K. A., Nishimura, Y., Hirota, Y., Becherer, K., Zumsell, L., and Wang, J. C. (1981) *Proc. Natl. Acad. Sci. U.S.A.*, in press
12. Liu, L. F., Liu, C.-C., and Alberts, B. M. (1980) *Cell* 19, 697–707
13. Cozzarelli, N. R. (1980) *Cell* 22, 327–328
14. Wang, J. C. (1971) *J. Mol. Biol.* 55, 523–533
15. Champoux, J. J., and Dalbecco, R. (1972) *Proc. Natl. Acad. Sci. U.S.A.* 69, 143–146
16. Kreuzer, K. N., and Cozzarelli, N. R. (1980) *Cell* 20, 245–254
17. Mizuuchi, K., Fischer, L. M., O’Dea, M. H., and Gellert, M. (1980) *Proc. Natl. Acad. Sci. U.S.A.* 77, 1847–1851
18. Hasegawa, T., and Brugger, C. (1980) *Cell* 21, 115–125
19. Brown, P. O., and Cozzarelli, N. R. (1979) *Science* 206, 1081–1083
20. Fuller, F. B. (1978) *Proc. Natl. Acad. Sci. U.S.A.* 75, 3557–3561
21. Gellert, M., Mizuuchi, K., O’Dea, M. H., and Nash, H. A. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 3872–3876
22. Baldis, M. I., Benedetti, P., Mattoccia, E., and Tocchini-Valentini, G. P. (1980) *Cell* 20, 467
23. Englund, P. T. (1978) *Cell* 14, 157–168
24. Marini, J. C., Miller, K. G., and Englund, P. T. (1980) *J. Biol. Chem.* 255, 4976–4979
25. Liu, L. F., and Miller, K. G. (1981) *Proc. Natl. Acad. Sci. U.S.A.* 78, 3487–3491
26. Laemmli, U. K. (1970) *Nature* (London) 227, 680–685
27. Bradford, M. M. (1976) *Anal. Biochem.* 72, 248–254
28. Martin, R. G., and Ames, B. N. (1981) *J. Biol. Chem.* 236, 1372–1379
29. Alberts, B. M., and Herrick, G. (1971) *Methods Enzymol.* 21, 198–
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30. Siegel, L. M., and Monty, K. J. (1966) Biochim. Biophys. Acta 112, 346–362
31. Sugino, A., Peebles, C. L., Kreuzer, K. N., and Cozzarelli, N. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4767–4771
32. Gellert, M., O’Dea, M. H., Itoh, T., and Tomizawa, J.-I. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 4474–4478
33. Liu, L. F., and Wang, J. C. (1976) Proc. Natl. Acad. Sci. U. S. A. 75, 2098–2102
34. Sundin, O., and Varsharvsky, A. (1980) Cell 21, 103–114