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A topoisomerase from *Escherichia coli* related to DNA gyrase

(DNA relaxation/supercoiling/nalidixic acid/subunits/site-specific DNA cleavage)

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ABSTRACT **We have identified a topoisomerase activity from *Escherichia coli* related to DNA gyrase (topoisomerase II); we designate it topoisomerase II′. It was constructed of two subunits, which were purified separately. One is the product of the *gyrA* (formerly *nalA*) gene and is identical to subunit A of DNA gyrase. The other is a 50,000-dalton protein, which we have purified to homogeneity and call *ν*. *ν* may be a processed form of the much larger gyrase subunit B or may be derived from a transcript of part of the subunit B structural gene, because preliminary peptide maps of the two subunits are similar. Topoisomerase II′ relaxes negatively supercoiled DNA and, uniquely among *E. coli* topoisomerases, relaxes positive supercoils efficiently. It is the only topoisomerase that can introduce positive supercoils; these are stoichiometric with enzyme molecules. Topoisomerase II′ resembles gyrase in its sensitivity to oxolinic acid, the wrapping of DNA in an apparent positive supercoil around the enzyme, and the introduction in an aborted reaction of site-specific double-strand breaks in the DNA with concomitant covalent attachment of protein to both newly created 5′ ends. Unlike DNA gyrase, topoisomerase II′ has no negative supercoiling activity. Functional chimeric topoisomerases were constructed with the *α* subunit of the *Micrococcus luteus* gyrase and *ν* or gyrase subunit B from *E. coli*. We discuss the implications of the dual role of the *gyrA* gene product.**

Topoisomerases are enzymes that can alter the supercoiling of DNA (1, 2). One such enzyme is the *ω* protein of *Escherichia coli*, *Eco* topoisomerase I (topo I) (3). It relaxes negatively supertwisted (underwound) DNA, but is virtually unable to relax overwound DNA (4). Overwinding is generated by the untwisting of the double helix during replication and, unless relaxed, blocks further replication. DNA gyrase, another topoisomerase of *E. coli* (5), catalyzes the ATP-dependent introduction of negative superhelical turns into closed duplex DNA that is initially relaxed or positively supercoiled (6). Thus it could facilitate the separation of the template strands at a replication fork. Gyrase can also relax negatively (7, 8) but not positively (this report) supercoiled DNA in the absence of ATP.

Gyrase is composed of two subunits (9–11). Subunit A is encoded by the gene *gyrA* [the names *gyrA* and *gyrB* replace *nalA* and *cou*, respectively (12)] and is the target of the DNA synthesis inhibitor nalidixic acid and its analog oxolinic acid (9, 13). Gyrase subunit B is encoded by the unlinked *gyrB* gene, which determines sensitivity to the drugs novobiocin and coumermycin A_1 (9, 11, 12). The separately purified subunits can be mixed to reconstitute gyrase activity (9). The same ratio of subunits A and B is required to reconstitute the supertwisting and relaxing activities. However, the ratio of oxolinic acid-sensitive relaxation activity to supertwisting activity declines to a nonzero plateau value with increasing purity of the DNA gyrase (6, 7, 9). One explanation for this paradox would be contamination of less pure preparations with a relaxing enzyme distinct from gyrase but sharing with gyrase the *gyrA* product and thus oxolinic acid sensitivity.

We have identified and purified this enzyme, which we designate topo II′. It was constructed from subunit A and a 50,000-dalton subunit we call *ν* that appears to be related to subunit B. The subunits of topo II′ were resolved early in the purification and were purified separately; neither subunit alone had any detectable activity. Topo II′ resembled gyrase in that both enzymes relaxed negative supercoils, apparently wrapped their DNA substrate around them in a positive coil (6, 10), and carried out an aborted reaction in which double-strand breaks were introduced in the DNA at the same specific sites (7, 8, 14, 15). However topo II′ had no supercoiling activity. It also efficiently relaxed positive supercoils and in this respect is the only *E. coli* topoisomerase similar to eukaryotic topoisomerases (16).

MATERIALS AND METHODS

Except where indicated, materials and methods were as described (7, 9, 14, 15).

Enzyme Assays. Relaxation of negative supercoils was measured at 30°C in a 20-*μ*l reaction mixture containing 30 mM Tris·HCl (pH 7.4), 25 mM KCl, 5 mM dithiothreitol, 7.5 mM $MgCl_2$, 2 mM spermidine·HCl, bovine serum albumin at 50 *μ*g/ml, and, except where indicated, 25 fmol of native ColE1 DNA. The reaction was stopped with 5 *μ*l of a solution containing 5% (wt/vol) sodium dodecyl sulfate ($NaDodSO_4$), 25% (vol/vol) glycerol, and bromophenol blue at 0.25 mg/ml. The products were electrophoresed through a 1% agarose gel (7), which was stained with ethidium bromide and photographed. Assays of *ν* contained also 130 fmol of gyrase subunit A (concentrations of subunits always refer to the protomers); one unit of *ν* relaxed 12.5 fmol of ColE1 DNA in ½ hr.

Purification of the *ν* Protomer of Topo II′. The purification was from 500 g of H-560 (*polA*$^-$, *endA*$^-$) cells. Lysis, high-speed centrifugation, and Polymin P and $(NH_4)_2SO_4$ precipitations were as described for gyrase purification (9), except that 0.2 mM phenylmethylsulfonyl fluoride, an inhibitor of serine proteases, was added immediately after lysis. The $(NH_4)_2SO_4$ precipitate was dissolved in DE buffer (25 mM Tris·HCl, pH 7.5/10 mM 2-mercaptoethanol/1 mM EDTA/10% glycerol) and dialyzed against the same buffer until its conductivity equaled that of DE buffer plus 25 mM NaCl. The sample, containing 6.25 g of protein, was loaded onto a 25 cm × 24.2 cm^2 column of Whatman DE-52 DEAE-cellulose equilibrated with DE buffer plus 25 mM NaCl. The column was washed with 2 vol of this buffer and then eluted with a 0.025–0.3 M linear NaCl gradient in 6.6 liters of DE buffer. The *ν* activity eluting at 0.09 M NaCl (3×10^5 units in 110 mg of protein) was concentrated by precipitation with 60% saturated $(NH_4)_2SO_4$, dissolved in 3.8 ml of 20 mM potassium phosphate (pH 6.8)/10 mM 2-mercaptoethanol/1 mM EDTA/10% glycerol, then

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Abbreviations: topo, topoisomerase; $NaDodSO_4$, sodium dodecyl sulfate; DE buffer, 25 mM Tris·HCl (pH 7.5)/10 mM 2-mercaptoethanol/1 mM EDTA/10% glycerol.

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chromatographed on a 100 cm × 1.3 cm^2 column of Sephacryl S-200 (Pharmacia) equilibrated with 0.2 M potassium phosphate (pH 6.8)/10 mM 2-mercaptoethanol/1 mM EDTA/10% glycerol. Active fractions (partition coefficient, K_{av}, of 0.29) were pooled (10^5 units in 7 mg of protein), diluted with 3 vol of 10 mM 2-mercaptoethanol/10% glycerol, and loaded onto a 5 cm × 0.25 cm^2 column of hydroxylapatite (Bio-Gel HTP) equilibrated with 50 mM potassium phosphate (pH 6.8)/10 mM 2-mercaptoethanol/10% glycerol. This column was washed with 5 vol of the same buffer, then eluted with a 30-ml 0.05–0.5 M linear gradient of potassium phosphate (pH 6.8) in 10 mM 2-mercaptoethanol/10% glycerol. Active fractions, eluting at 0.16 M phosphate (50,000 units in 1 mg of protein), were diluted with 4 vol of 27% glycerol/10 mM 2-mercaptoethanol/1 mM EDTA and applied to a 4 cm × 0.5 cm^2 phosphocellulose (Whatman P-11) column that had been equilibrated with 20 mM potassium phosphate (pH 6.8)/10 mM 2-mercaptoethanol/1 mM EDTA/20% glycerol. The column was then washed with 4 ml of this buffer and eluted with a 30-ml, 0.02–0.8 M linear gradient of potassium phosphate (pH 6.8) containing 10 mM 2-mercaptoethanol, 1 mM EDTA, and 20% glycerol. The ν protein eluting at 0.14 M phosphate was >90% pure as determined by $NaDodSO_4$/polyacrylamide gel electrophoresis and was used in most of the experiments described here. Half of this fraction was concentrated to 0.2 ml by dialysis against 30% (wt/vol) polyethylene glycol/0.1 M potassium phosphate (pH 6.8)/10 mM 2-mercaptoethanol/0.1 mM EDTA and sedimented through a 4-ml 15–30% glycerol gradient in the same buffer at 49,500 rpm for 65 hr in a Beckman SW 56 rotor. Fractions sedimenting at 2.5 S contained ν activity (70,000 units/mg). Enzymes were stored at −20°C in 50% glycerol/0.1 M potassium phosphate (pH 6.8)/10 mM 2-mercaptoethanol/0.1 mM EDTA, at protein concentrations greater than 0.25 mg/ml and were stable for at least 12 months.

Other Enzymes. Subunits A and B of *E. coli* DNA gyrase were purified as described (9). *E. coli* ω protein and α and β subunits of *Micrococcus luteus* DNA gyrase (10) were a gift from J. Wang. Rat liver DNA untwisting enzyme (17) was a gift from J. Champoux.

RESULTS

Identification of a New Topoisomerase Activity. There was little hope of identifying the hypothetical new topoisomerase activity in crude extracts of *E. coli* because of the high background provided by known topoisomerases. We therefore attempted to resolve this activity chromatographically from previously identified topoisomerases. A preparation of *E. coli* proteins, separated from nucleic acids by Polymin P treatment and concentrated by precipitation with $(NH_4)_2SO_4$, was fractionated by DEAE-cellulose column chromatography. Fractions were assayed for relaxation of negative supercoils in the presence and absence of pure gyrase subunit A. In the absence of subunit A, the only relaxation activity detected was that of topo I, which eluted prior to the gradient. Inclusion of subunit A in the reaction mixture elicited an additional peak of relaxation activity, eluting at 0.09 M NaCl, slightly ahead of gyrase subunit B (Fig. 1). The protein responsible for this new activity was designated ν. Total relaxation activity due to ν exceeded that due to subunit B by an order of magnitude; consequently, subunit B had little effect on the profile of subunit A-complemented relaxation activity depicted in Fig. 1.

Purification and Physical Properties of ν. Using relaxation of negative supercoils in the presence of subunit A as an assay, we purified ν to homogeneity as described in *Materials and Methods*. Polyacrylamide gel electrophoresis in the presence of 0.1% $NaDosSO_4$ showed that ν consisted of a single 50,000-dalton polypeptide (Fig. 2). The quantity of this polypeptide paralleled ν activity throughout purification. The $s_{20,w}$ values obtained for ν in two glycerol gradient sedimentation experiments were 2.3 S and 2.7 S—strikingly low for a 50,000-dalton protein. The low $s_{20,w}$ suggests that ν is a monomer and highly asymmetric.

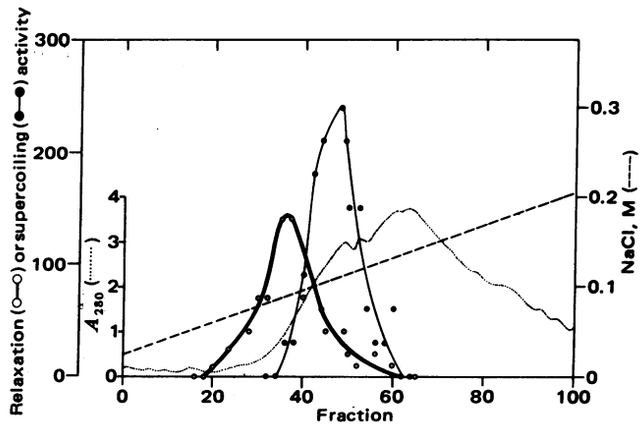

FIG. 1. Identification of topo II′. Protein extracted from *E. coli* strain H-560 (8.5 g) was applied to a 25 cm × 24 cm^2 column of DEAE-cellulose and eluted with a linear NaCl gradient in 25 mM Tris·HCl (pH 7.5)/10 mM 2-mercaptoethanol/1 mM EDTA/10% glycerol. The 25-ml fractions were assayed for relaxation (○) and introduction (●) of negative supercoils into ColE1 DNA in the presence of excess subunit A. Activity is the absolute value of the change in the linking number of the substrate, in pmol per min per ml of eluate. Relaxation activity due to topo I eluted before the start of the gradient and is not shown. Subunit A eluted at 0.19 M NaCl.

Preliminary one-dimensional peptide maps (18) of ν and subunit B showed substantial similarity.

Negative Supercoil Relaxation by Topo II′. Supertwist relaxation was optimal at approximately a 1:1 ratio of ν to subunit A protomers. Relaxation was processive (Fig. 3, lane d). Neither protein alone (Fig. 3, lanes b and c), even at 20 times the amount that gave full activity in the presence of the other (Fig. 3, lane f), showed relaxation activity. Optimal reaction conditions for topo II′ were very similar to those for DNA gyrase. The broad pH optimum centered at pH 7.5. Addition of EDTA or omission of a divalent cation abolished relaxation (Fig. 3, lane g). The optimal concentrations of $MgCl_2$, $CaCl_2$, and $MnCl_2$ were 7.5 mM, 1 mM, and 0.3 mM, respectively. Topo II′ activity was highest at 30 mM KCl; the reaction was inhibited about 50% by 100 mM KCl and was >90% inhibited at 150 mM KCl. Bovine serum albumin and spermidine were not required

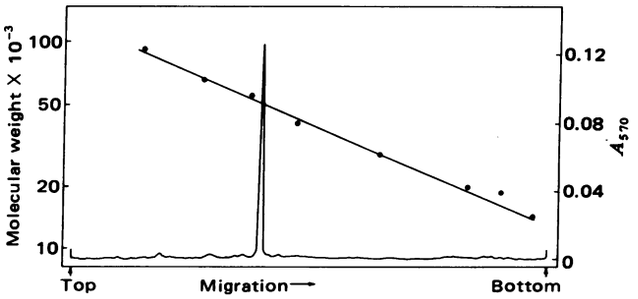

FIG. 2. Electrophoresis of 2.5 μg of ν through a 15% polyacrylamide slab gel containing 0.1% $NaDodSO_4$. Molecular weight standards (●) in adjacent channels were: rabbit muscle phosphorylase *b*, bovine serum albumin, bovine liver glutamate dehydrogenase, rabbit muscle creatine kinase, bovine erythrocyte carbonic anhydrase, soybean trypsin inhibitor, β-lactoglobulin, and egg lysozyme. After electrophoresis, the gel was stained with Coomassie blue and scanned at 570 nm with a Schoeffel model SD3000 spectrodensitometer. Shown is a scan of the purified ν and positions of the standards.

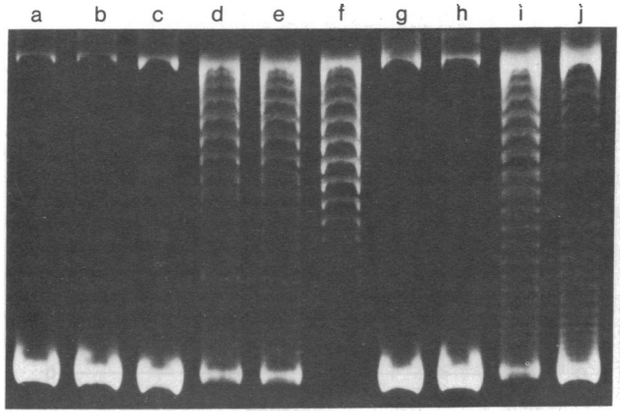

FIG. 3. Relaxation of negatively supercoiled DNA by topo II′. Reaction mixtures (20 μl) contained 50 fmol of native ColE1 DNA and: (a) no enzyme, (b) 20 pmol of *ν*, (c) 20 pmol of subunit A, (d) 0.5 pmol of *ν* + 0.5 pmol of subunit A, (e) same as d + 1 mM ATP, (f) 1 pmol of *ν* + 1 pmol of subunit A, (g) same as f + 8 mM EDTA, (h) same as f + 1 mM *N*-ethylmaleimide (dithiothreitol omitted), (i) same as f + 38 μM oxolinic acid, (j) same as f + 380 μM oxolinic acid. The reactions were stopped after 1 hr; 20 mM EDTA was added to mixtures i and j before stop solution to minimize cleavage. Samples were electrophoresed through a 1% agarose gel that was stained with ethidium bromide and photographed. Molecules with writhing numbers closest to zero are lowest in electrophoretic mobility and thus form bands nearest the top of the gel. Substrate DNA contained a small amount of nicked forms that migrate with fully relaxed DNA.

but enhanced activity. ATP obscures the relaxation activity of DNA gyrase (6) but had no effect on topo II′ (Fig. 3, lanes d and e). Novobiocin, a drug that specifically inhibits ATP-dependent gyrase reactions, also had no effect. Oxolinic acid (Fig. 3, lanes i and j) and *N*-ethylmaleimide (Fig. 3, lane h) inhibited topo II′ at concentrations comparable to those that inhibit gyrase (7).

In relaxation of negative supercoils, the specific activity of pure topo II′ was nearly identical to that of pure DNA gyrase. However, DNA relaxed by topo II′ had a higher electrophoretic mobility than gyrase-relaxed DNA (Fig. 4), indicating that the topo II′-relaxed DNA was more supercoiled. To determine the sign of this supercoiling, we compared the electrophoretic mobility of the product in two gel systems: a standard gel at 25°C (Fig. 4, lanes a–g) and a $MgCl_2$-containing gel at 4°C (Fig. 4, lanes a′–g′) (19). Closed duplex DNA is more negatively, or less positively, supercoiled in the 4°C gel. DNA circles relaxed by gyrase (lanes f and f′) or topo I (lanes g and g′) were slightly negatively supercoiled, so the magnitude of their supercoiling, and thus their electrophoretic mobility, was greater at the lower temperature. In contrast, the products of relaxation by topo II′ (lanes c–e and c′–e′) were positively supercoiled to an extent depending stoichiometrically on the ratio of enzyme to DNA (roughly +0.7 twists per protomer of the limiting subunit), and thus the magnitude of their supercoiling was reduced at the lower temperature.§ A plausible explanation for the positive supercoiling, based upon a model proposed by Liu and Wang (10) for DNA gyrase, is that topo II′, like gyrase, stabilizes positive supercoils when it binds to DNA, but unlike gyrase can relax the counterposing negative supercoils.

Relaxation of Positive Supercoils by Topo II′. Processes involving DNA strand separation generate positive twisting stress. This can be modeled by incubation of DNA with ethidium bromide, which on intercalation reduces the double-helical twist of DNA and thus induces compensating positive supertwists in closed duplex DNA. If these supertwists are relaxed, the linking number (2) is reduced and upon removal of the dye the DNA becomes negatively supercoiled.

§ At an intermediate stage in the reaction, the simultaneous presence of positively and negatively supercoiled product was sometimes seen on electrophoresis as a doubling of bands.

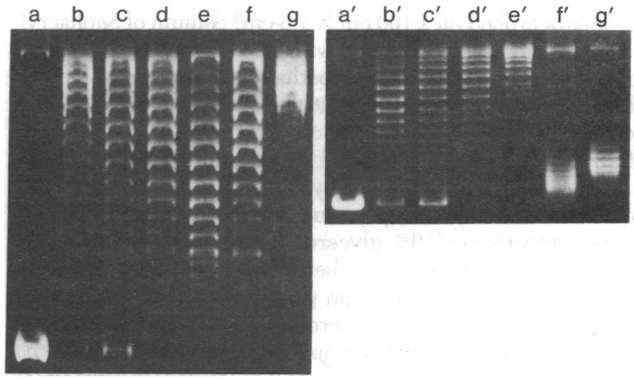

FIG. 4. Introduction of positive supercoils by topo II′. Native ColE1 DNA (100 fmol) was incubated for 1 hr with: (a and a′) no enzyme, (b and b′) rat liver DNA untwisting enzyme, (c and c′) 0.25 pmol of subunit A + excess *ν*, (d and d′) 0.5 pmol of subunit A + excess *ν*, (e and e′) 1 pmol of subunit A + excess *ν*, (f and f′) 1 pmol of subunit A + excess gyrase subunit B, (g and g′) 0.2 pmol of *E. coli* topo I. One half of each solution (a–g) was electrophoresed under standard conditions and the other half (a′–g′) was electrophoresed at 4°C through a 1% agarose gel containing 40 mM Tris·acetic acid (pH 7.8)/5mM $MgCl_2$/1 mM EDTA (19). Closed circular ColE1 DNA has roughly 10 additional negative (or fewer positive) supercoils under low-temperature gel conditions.

Topo I was essentially unable to relax positive twists (4), (Fig. 5, lane d). DNA gyrase did not relax these twists in the absence of ATP (Fig. 5, lane c), contrary to previous results with less highly purified enzyme (7, 8). However, gyrase introduced negative supercoils into the (initially) positively supercoiled substrate in the presence of ATP (6) (Fig. 5, lane e). Unlike gyrase, topo II′ relaxed positive supercoils in an ATP-independent reaction (Fig. 5, lane b), and, moreover, its positive supercoil relaxation activity measured as depletion of starting material appeared to match its activity on a negatively supertwisted substrate (cf. Fig. 5, lane b and Fig. 3, lanes d and f). Neither subunit A or *ν* by themselves relaxed positive supertwists.

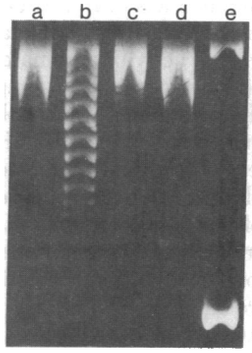

FIG. 5. Topo II′ relaxes positively supercoiled DNA. Reaction mixtures contained 50 fmol of relaxed ColE1 DNA and 1.5 μM ethidium bromide to induce positive supercoils in the DNA. When positive supercoils are relaxed, the linking number of the DNA is reduced. Because the ethidium was extracted with 2-butanol prior to electrophoresis, the starting material (a) migrated as relaxed DNA, and DNA relaxed in the presence of ethidium (b) was slightly negatively supercoiled and migrated faster. The lower band in lane e is fully supercoiled DNA. Reaction mixtures contained: (a) no enzyme, (b) 1 pmol of subunit A + 1 pmol of *ν*, (c) 2 pmol of subunit A + 2 pmol of gyrase subunit B, (d) 2 pmol of topo I, (e) 0.1 pmol of subunit A + 0.1 pmol of subunit B + 1 mM ATP.

Cleavage of DNA by Topo II′. Topoisomerases have transient reaction intermediates in which the enzyme is covalently linked to a break in the DNA. The structures of these intermediates are suggested by experiments in which breakage of the DNA, with concomitant formation of a protein–DNA bond, is uncoupled from the resealing reaction (1, 16). Topo II′, like DNA gyrase (7, 8, 14, 15), could be induced to make double-strand breaks in DNA at specific sites by successive addition of oxolinic acid and a protein-denaturing agent such as $NaDodSO_4$. The reaction was studied by using *Eco*RI nuclease-linearized ColE1 DNA as the substrate. The a and a′ and b and b′ bands (Fig. 6) represent products of cleavage at the *a* and *b* sites, respectively (14, 15). The sites at which topo II′ cleaved (Fig. 6, lanes a and b) appeared to be identical to those cut by gyrase (Fig. 6, lanes c and d), but the frequency of cleavage at a given site and the influence of reaction conditions differed. For example, ATP greatly augmented *b* site cleavage by gyrase and diminished somewhat its cleavage at the *a* site (Fig. 6, lanes c and d). In contrast, the cleavage pattern with topo II′ was not affected by ATP (Fig. 6, lanes a and b) but resembled more closely the +ATP than the −ATP pattern of gyrase cleavage.

M. luteus DNA gyrase contains two subunits, α and β, of heretofore unknown genetic specification (10), and will cleave DNA at low efficiency in an oxolinic acid-independent reaction when treated with $NaDodSO_4$ or OH^- (J. Wang, personal communication). The modular design of gyrase and topo II′ allows construction of heterospecific topoisomerases consisting of the α subunit of *M. luteus* gyrase and ν or gyrase subunit B from *E. coli*. These chimeric enzymes could relax or supertwist DNA, respectively, though less effectively than the corresponding *E. coli* enzymes. Subunits α and β of *M. luteus* gyrase thus are analogs of subunits A and B, respectively, of *E. coli* gyrase. Both chimeric enzymes cleaved DNA (Fig. 6, lanes f and g) much more efficiently than did *M. luteus* gyrase (Fig. 6, lane h). Cleavage required a denaturing agent such as $NaDodSO_4$, but not oxolinic acid. Remarkably, both chimeric enzymes, as well as *M. luteus* gyrase, cleaved at sites on the ColE1 DNA favored by the *E. coli* enzyme, notably the *b* site.

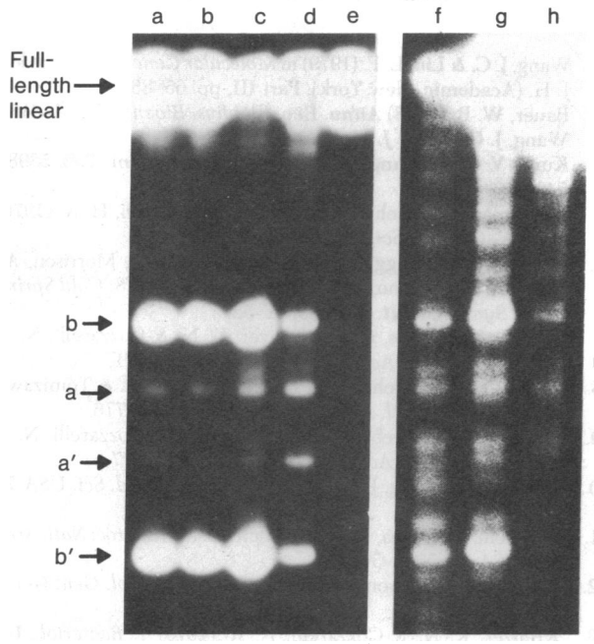

FIG. 6. Cleavage of DNA by topo II′. Reaction conditions were the same as for relaxation except the DNA substrate was 50 fmol of 5′-^{32}P-labeled *Eco*RI-linearized ColE1 DNA (15). ATP was added to reaction mixtures a and c after 55 min. Oxolinic acid was added to reaction mixtures a–d after 58 min. Reactions were stopped by addition of 1% $NaDodSO_4$ after a total of 60 min. Products were treated with proteinase K and electrophoresed through a 1% agarose gel, which was dried and autoradiographed. Reaction mixtures contained: (a) 0.16 pmol of subunit A, 0.2 pmol of ν, 1 mM ATP, and 0.39 mM oxolinic acid; (b) same as a − ATP; (c) 0.16 pmol of subunit A, 0.5 pmol of gyrase subunit B, 1 mM ATP, and 0.39 mM oxolinic acid; (d) same as c − ATP; (e) same as a − oxolinic acid; (f) 3 pmol of *M. luteus* gyrase subunit α + 1.5 pmol of ν; (g) 3 pmol of *M. luteus* subunit α + 0.3 pmol of *E. coli* subunit B; (h) 12 pmol of *M. luteus* subunit α + 10 pmol of *M. luteus* subunit β. Lanes a–e and lanes f–h are from two different gels. The b and b′ bands in lane f are depleted due to multiple cutting (compare the depletion of the full-length linear band in lanes f and g).

The topo II′-cleaved DNA had attached covalently to each new 5′ end a protein that caused it to be retained on nitrocellulose filters. The 5′ end was not labeled by polynucleotide kinase, whereas the 3′-OH terminus was unblocked and recessed and therefore could be extended by *E. coli* DNA polymerase I. The ends of topo II′-cleaved DNA thus appear to be identical to those produced by gyrase (7, 15). The protein attached to gyrase-cut DNA is the A protomer (unpublished result). We infer that a site-specific, staggered, double-strand break, with the A protomer covalently attached at each 5′-phosphate, is a reaction intermediate for both topoisomerases.

DISCUSSION

We have identified a topoisomerase activity from *E. coli* that consists of gyrase subunit A and subunit ν. ν is similar (but not identical) in its activities to gyrase subunit B, and initial peptide maps suggest a close relatedness in primary structure. It is therefore likely that ν is derived by proteolytic processing or is encoded by a portion of *gyrB* [cf. phage ϕX174 A and A′ proteins (20)]. Whatever is the ontogeny of ν, topo II′ probably exists normally in the cell. It is unlikely that B was processed to ν during purification because, in four separate purifications of ν carried out in the presence of phenylmethylsulfonyl fluoride and EDTA to minimize proteolysis, we found an order of magnitude more molecules of ν than of subunit B.

The ν subunit of topoisomerase II′ is a 50,000-dalton polypeptide. No contaminants were detected by $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 1), and the intensity of the 50,000-dalton band on polyacrylamide gels containing $NaDodSO_4$ paralleled the ν activity throughout purification. Because the molecular weight of ν calculated from its sedimentation coefficient of 2.5 S is even less than 50,000, ν is probably a monomer.

Both the *gyrA* gene product and ν were required for all known activities of topo II′. An early report from this laboratory suggested that a topoisomerase activity with properties similar to those of topo II′ was intrinsic to the *gyrA* gene product, then called PnaI (7). Our subsequent homogeneous preparation of subunit A had no intrinsic topoisomerase activity (6, 9), and thus PnaI may have been contaminated with ν. Such contamination could also explain the relaxation of positive supercoils by partially purified (7, 8) but not highly purified DNA gyrase (Fig. 5).

The three *E. coli* topoisomerases are compared in Table 1. All relax negative supercoils, but the total detectable activity of topo I greatly exceeded that of topo II′, which in turn was greater than that of topo II. Only topo II can negatively supercoil DNA. Topo II′ was the only activity from *E. coli* that efficiently relaxed positive supercoils (Fig. 5), although, in the presence of ATP, topo II can introduce negative supercoils into initially positively supercoiled DNA. Thus, topoisomerases II and II′ could function as active and passive versions, respectively, of the "swivel" postulated by Cairns (21) as essential for allowing unwinding of the DNA template during replication.

Table 1. *E. coli* topoisomerases

Property	Topo I (ω)	Topo II (gyrase)		Topo II′	
Subunits	Monomer	A	B	A	ν
Protomer $M_r \times 10^{-3}$	110	105	95	105	50
Structural gene	Unknown	*gyrA*	*gyrB*	*gyrA*	*gyrB* ?
Activities					
(−) Supercoil relaxation	Yes	Yes		Yes	
(+) Supercoil relaxation	No	No		Yes	
(−) Supercoil introduction	No	Yes		No	
Stoichiometric (+) supercoil introduction	No	No		Yes	
DNA wrapping around enzyme	None	(+)		(+)	
DNA site-specific binding	—*	Yes		Yes	
Denaturant-induced DNA breaks	Single strand	Double strand		Double strand	

* Some specificity has recently been observed by K. Kirkegaard and J. C. Wang (personal communication).

A comparison of the activities of topo II and topo II′ should help clarify specific structure–function relationships. Site specificity of DNA binding was similar for the two enzymes (Fig. 6), and DNA apparently wrapped around both enzymes in a positive coil (refs. 6 and 10 and Fig. 4). The similar number of coils may implicate the common subunit A as the spool for wrapping. If the sequence homology of ν and subunit B suggested by preliminary experiments is confirmed, then it appears that subunit B is divided physically into functional domains. One domain, represented by ν, is sufficient for binding subunit A and reconstitution of the double-strand breaking and rejoining activities of gyrase. The other domain contains the ATP binding site or allows its expression in energy-requiring reactions. Uncoupling of breakage-and-reunion from energy transduction due to the absence of this domain could account for some of the unique activities of topo II′.

The physiological relationship of topo II′ to gyrase is likely to be complex. The two enzymes have activities that are potentially either synergistic or antagonistic. Moreover, they might compete for specific binding sites on DNA and subunits B and ν might compete for the communal subunit A. The previously puzzling 10-fold excess of gyrase subunit A over subunit B now may be understandable in light of the approximately equal concentrations of subunit A and ν.

The presence of subunit A in two enzymes confounds the interpretation of physiological effects of drugs or mutations that affect its activity. Indeed, some of the striking differences between the physiological effects of nalidixic acid and coumermycin A_1 (22) may be a manifestation of the dual role of the nalidixic acid target protein.

Topoisomerases II and II′ are enzymes of modular design. A single protein, subunit A, can perform the concerted breakage and rejoining of both strands of the double helix as a reversibly associated component of alternative enzyme assemblies. Reversible subunit association can cause enzymatic activities to be overlooked. Obscuring activities often preclude crude extract assays, but purification away from interfering activities may in the process resolve loosely associated subunits. We have not yet detected any topo II′ activity other than that obtained by mixing the subunits *in vitro*, and we would have been unable to detect and purify ν, or subunit B, if we had not had on hand the complementary subunit A.

Topoisomerase activity is most conveniently assayed as relaxation of negatively supertwisted circular DNA, but this activity may not accurately reflect the function of a topoisomerase *in vivo*. Although positive supertwist removal aids any process in which the DNA double helix unwinds, few roles can be envisioned for relaxation of negative supertwists. The double-strand breakage-and-rejoining activity of topo II′ (or topo II) could also be employed, for example, in nonhomologous recombination or in resolution of catenated duplex DNA circles which might occur at the termination of DNA replication or as products of general recombination.

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1. Wang, J. C. & Liu, L. F. (1979) in *Molecular Genetics*, ed. Taylor, J. H. (Academic, New York), Part III, pp. 65–88.
2. Bauer, W. R. (1978) *Annu. Rev. Biophys. Bioeng.* **7**, 287–313.
3. Wang, J. C. (1971) *J. Mol. Biol.* **55**, 523–533.
4. Kung, V. T. & Wang, J. C. (1977) *J. Biol. Chem.* **252**, 5398–5402.
5. Gellert, M., Mizuuchi, K., O'Dea, M. H. & Nash, H. A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3872–3876.
6. Peebles, C. L., Higgins, N. P., Kreuzer, K. N., Morrison, A., Brown, P. O., Sugino, A. & Cozzarelli, N. R. (1978) *Cold Spring Harb. Symp. Quant. Biol.* **43**, 41–52.
7. Sugino, A., Peebles, C. L., Kreuzer, K. N. & Cozzarelli, N. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4767–4771.
8. Gellert, M., Mizuuchi, K., O'Dea, M. H., Itoh, T. & Tomizawa, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4772–4776.
9. Higgins, N. P., Peebles, C. L., Sugino, A. & Cozzarelli, N. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1773–1777.
10. Liu, L. F. & Wang, J. C. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2098–2102.
11. Mizuuchi, K., O'Dea, M. H. & Gellert, M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5960–5967.
12. Hansen, F. G. & von Meyenburg, K. (1979) *Mol. Gen. Genet.* **175**, 135–144.
13. Kreuzer, K. N. & Cozzarelli, N. R. (1979) *J. Bacteriol.* **140**, 424–435.
14. Sugino, A., Higgins, N. P., Brown, P. O., Peebles, C. L. & Cozzarelli, N. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4838–4842.
15. Morrison, A. & Cozzarelli, N. R. (1979) *Cell* **17**, 175–184.
16. Champoux, J. J. (1978) *Annu. Rev. Biochem.* **47**, 449–479.
17. Champoux, J. J. & McConaughy, B. L. (1976) *Biochemistry* **15**, 4638–4643.
18. Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106.
19. Shure, M. & Vinograd, J. (1976) *Cell* **8**, 215–226.
20. Linney, E. & Hayashi, M. (1974) *Nature (London) New Biol.* **245**, 6–8.
21. Cairns, J. (1963) *J. Mol. Biol.* **6**, 208–213.
22. Cozzarelli, N. R. (1977) *Annu. Rev. Biochem.* **46**, 641–668.