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Escherichia coli DNA topoisomerase I catalyzed linking of single-stranded rings of complementary base sequences

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ABSTRACT

Eco DNA topoisomerase I (E. coli ω protein) has been observed to catalyze the formation of double-stranded, covalently closed DNA from complementary single-stranded DNA rings, a novel reaction which is topologically forbidden without the enzyme-catalyzed breakage and rejoining of DNA backbone bonds. Incubation of a mixture of single-stranded PM2 DNA rings of complementary base sequences with ω yields a species with a sedimentation coefficient in an alkaline medium characteristic of a covalently closed circular double-stranded DNA. Buoyant density measurements in CsCl at alkaline pH also identify the product as a covalently closed duplex ring. If the ω-catalyzed reaction is stopped short of completion, highly negatively supercoiled molecules are formed which sediment more slowly in an alkaline medium than the final duplex product. As the reaction proceeds the mean sedimentation rate of the intermediates increases. This is in agreement with the expectation that the linking number between the two complementary rings increases gradually during the course of the reaction from zero to that of a relaxed covalently closed circular DNA duplex. The possible role of DNA topoisomerases in genetic recombination is discussed.

INTRODUCTION

E. coli ω protein (Eco DNA topoisomerase I) is known to catalyze the removal of negative superhelical turns from covalently closed duplex DNA, by effecting the concerted breakage and rejoining of DNA backbone bonds (Wang, 1971; 1973). A mechanism has been proposed in which the breakage of a DNA backbone bond is accompanied by the simultaneous formation of a protein-DNA bond (Wang, 1971); the protein is linked, presumably covalently, to the 5' side of the newly generated break (Depew et al., 1978). The rejoining of the DNA backbone bond is the reverse of the bond breakage reaction (Wang, 1971). Enzymes displaying this activity of concerted breaking and rejoining of DNA backbone bonds are ubiquitous in nature (Champoux and Dulbecco, 1972; Baase and Wang, 1974; Keller, 1975; Pulleyblank and Morgan, 1975; Champoux and McConaughy, 1976; Bauer et al., 1977; Kung and Wang, 1977; Rosenberg et al., 1977; Yoshida et al., 1977; Poccia et al., 1978). Since such enzymes are invariably detected and studied by their catalysis of isomerization between

topological isomers of DNA, it has been proposed that they be termed DNA topoisomerases (Wang and Liu, 1978).

Eco DNA topoisomerase I, in addition to catalyzing the removal of negative superhelical turns from covalently closed duplex DNA, which is inhibited by single-stranded DNA, also promotes two types of reaction with single-stranded DNA itself. The ω-catalyzed interconversion between single-stranded DNA rings with and without topological knots has been reported recently (Liu et al., 1976). We report in this communication the second type of reaction: the ω-catalyzed formation of double-stranded, covalently closed duplex PM2 DNA from purified single-stranded rings of complementary base sequences. This reaction can be considered an extension of the ω-promoted relaxation of negative superhelical DNAs, since two complementary single-stranded rings are equivalent to a duplex ring with a linking number of zero. The same type of reaction catalyzed by rat liver DNA topoisomerase has been reported by Champoux (1977).

EXPERIMENTAL

Materials. Eco DNA topoisomerase I was the preparation described in Depew et al. (1978). Bovine pancreatic DNase I was purchased from Worthington. Agarose and diethylaminoethyl BioGel A (DEAE) were purchased from BioRad. Cesium chloride (optical grade) and ethidium bromide were purchased from Schwartz/Mann and CalBiochem respectively.

Purification of complementary single-stranded PM2 DNA rings.

PM2 DNA was extracted from the freshly purified phage according to the procedures of Espejo et al. (1969). Pancreatic DNase I was used to introduce one single chain scission per DNA molecule in the presence of a saturating amount of the dye (20 μg/ml of ethidium bromide at a DNA concentration of 50 μg/ml), as described previously (Hsieh and Wang, 1975).

To obtain complementary single-stranded PM2 DNA rings, the DNase treated sample was denatured by alkali, and separated into single-stranded rings and single-stranded linear molecules by alkaline agarose gel electrophoresis (McDonell et al., 1977). In a typical preparation, 300 μg of DNase I treated PM2 DNA was denatured by adding NaOH to a final concentration of 0.1 M, and loaded on a 14 cm wide 1 cm thick 10 cm long 1% agarose slab. Electrophoresis was carried out at 4°C in a medium containing 30 mM NaOH, 2 mM Na_3EDTA. A constant voltage gradient in the range 4-6 v/cm was usually applied, and electrophoresis was terminated after 40-50 hr. Two thin vertical strips were sliced from the two sides of the gel slab, and were stained in 0.04 M Tris·HCl, pH 8, containing 1 μg/ml of ethidium bromide. The positions of the

DNA bands in these slices were visualized under a uv lamp and were marked. The band which migrated more slowly consists of single-stranded rings. From the band positions in the stained slices, the horizontal band in the unstained gel containing single-stranded PM2 rings was excised. The stained slices were discarded, and the unstained DNA-containing agarose slice was homogenized in 30 ml of 100 mM sodium phosphate, pH 8.0, 1 mM Na_3EDTA, by passage through a 12 gauge needle. The suspension was quickly frozen, then allowed to thaw at 4°C for 6 to 8 hr. The agarose was pelleted by centrifugation at 20,000 g for 60 min in a Model L preparative ultracentrifuge (Type 30 rotor). The homogenization procedure was repeated once for the pellet, and the supernatants were pooled. The DNA was concentrated and purified by binding to a 1 ml bed volume DEAE column. After washing with 50 ml of 1 mM Na_3EDTA, 100 mM $NaHCO_3$, pH 9.6, the DNA was eluted with 1 mM Na_3EDTA, 100 mM $NaHCO_3$, pH 10.7, and collected in 0.5 ml fractions. The resulting 4 ml of DNA solution was passed through a Sephadex G-25 column, which had been pre-equilibrated in 10 mM pH 8.0 Tris·HCl, and 0.1 mM EDTA, and further concentrated by pelleting at 35,000 g for 12 hr in a Model L ultracentrifuge (SW50.1 rotor). The bulk of the supernatant was removed with a Pasteur pipet, and the pellet was resuspended in the remaining 0.5 ml of solvent. Approximately 30 µg of single-stranded DNA rings containing less than 10% contaminating linear species was obtained. It is known that pancreatic DNase I exhibits no strand specificity with PM2 DNA as the substrate, therefore the DNA ring preparation so obtained is expected to contain equal amounts of the complementary strands (Wang, 1974).

Analytical ultracentrifugation.

Band sedimentation was performed with 30 mm double-sector Type III centerpieces (Vinograd et al., 1965). The bulk sedimentation medium was 3 M CsCl, 0.10 M KOH, 0.01 M Na_3EDTA. Centrifugation was run at 29,500 rpm at 20°C in a Model E analytical ultracentrifuge (Spinco) equipped with a photoelectric scanner. The sedimentation coefficients measured were not corrected for buoyancy or viscosity effects.

CsCl density gradient centrifugation was performed with 12 mm double-sector cells at 42,000 revs/min at 20° for a minimum of 48 hr for both neutral and alkaline gradients.

RESULTS

(a) Treatment of complementary single-stranded PM2 DNA rings with Eco DNA topoisomerase I causes the appearance of a fast sedimenting species in an alkaline medium.

Fig. 1 shows two sedimentation profiles, in alkaline 3 M CsCl, of puri-

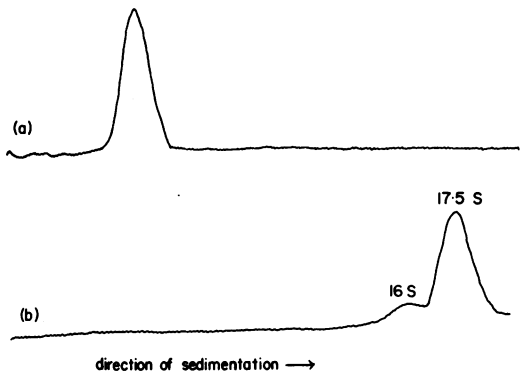

Fig. 1. Sedimentation of purified complementary single-stranded PM2 rings in an alkaline medium. Sedimentation patterns of single-stranded rings 20 min (a) and 120 min (b) after beginning of centrifugation. DNA preparation and band sedimentation runs in alkaline 3 M CsCl were performed as described in the EXPERIMENTAL SECTION.

fied single-stranded circular PM2 at different times in the sedimentation experiment. In the later scan, two peaks are resolved: the major peak consists of single-stranded circular PM2 DNA with a sedimentation rate of 17.5S, and a small amount of 16S single-stranded linear DNA. As displayed in Fig. 2, incubation of the sample with Eco DNA topoisomerase I gives two faster sedimenting species in the alkaline medium. The 27S species is most likely knotted single-stranded rings (Liu et al., 1976). The very high sedimentation coefficient of the other species, 57S, is characteristic of a covalently closed double-stranded PM2 DNA.

(b) DNA intermediates of lower linking numbers can be detected.

If single-stranded PM2 rings are incubated with a small amount of the topoisomerase and the reaction is terminated shortly after its initiation, the formation of a new species may be observed by alkaline sedimentation. Fig. 3 depicts such an experiment. After brief incubation with the enzyme, a very broad peak sedimenting more slowly than the final 57S product is noticed. As the reaction time is increased, the mean sedimentation rate of this broad distribution increases, until the peak disappears completely. We believe that this transiently appearing peak consists of duplex PM2 molecules of very high negative superhelical density, the logical intermediates of the conversion of two single-stranded rings to a relaxed double-stranded molecule. Highly twisted supercoils sediment more slowly than relaxed duplex molecules in an alkaline medium (Schmir et al., 1974). The extreme case, a twisted duplex DNA with a linking number of one, would denature to form interlocked single-stranded

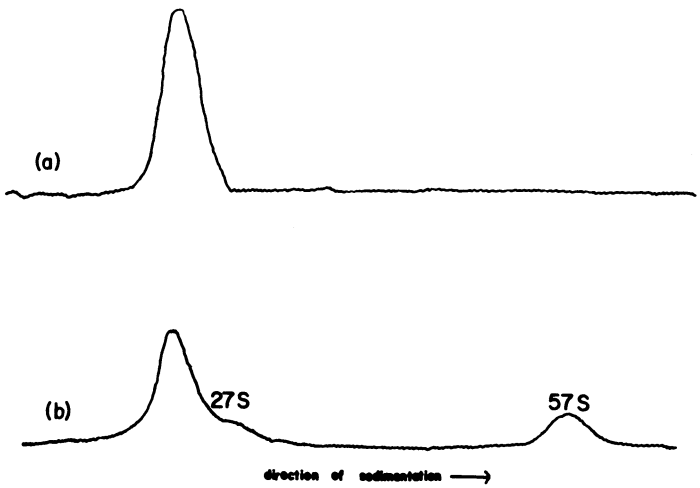

Fig. 2. Formation of covalently closed duplex DNA rings upon treatment of complementary single-stranded rings with Eco DNA topoisomerase I. Sedimentation patterns in alkaline 3 M CsCl before (a) and after (b) incubation of complementary PM2 DNA rings at a concentration of 10 µg/ml with 4 µg/ml of the enzyme in a medium containing 50 mM Tris·HCl (pH 8.0), 50 mM KCl, 2 mM $MgCl_2$, 50 µg/ml of bovine plasma albumin. The reaction was stopped after 30 min at 42° by adding Na_3EDTA to a final concentration of 0.02 M in the reaction mixture. One tenth volume of a saturated CsCl solution was then added to each sample, and sedimentation in alkaline 3 M CsCl was performed as described in the EXPERIMENTAL SECTION.

rings in alkaline solution; this species is expected to have a sedimentation coefficient 1.3 times that of a single-stranded DNA ring (Wang, 1970), or approximately 23S for interlocked single-stranded PM2 DNA rings. The observation that the mean sedimentation rate of the presumed intermediates increases with time strengthens the claim that we are visualizing the topoisomerase-catalyzed increase in linking number as the reaction proceeds.

(c) Identity of the covalently closed duplex DNA product is confirmed by buoyant density measurements.

Complementary single-stranded PM2 rings, incubated in the reaction medium in the absence of Eco DNA topoisomerase I, assume the profile shown in Fig. 4a after reaching equilibrium in a neutral CsCl density gradient. The pattern consists of a peak with two shoulders; the buoyant densities of these shoulders, 1.704 mg/ml and 1.719 mg/ml, correspond to double-stranded and single-stranded DNA respectively. Since there is no covalently closed duplex DNA in this sample, the double-stranded material presumably results from the renaturation of single-stranded rings with the small amount of single-stranded linear DNA present. More enigmatic is the buoyant density of the main peak itself, which,

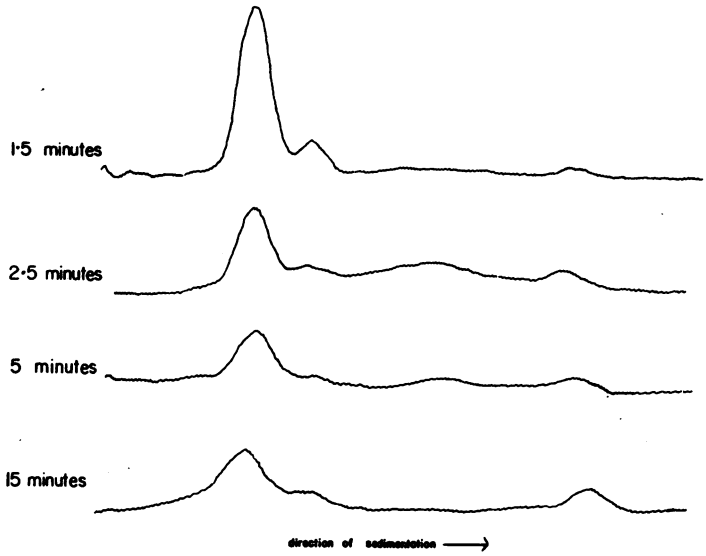

Fig. 3. Formation of species with intermediate sedimentation coefficients in alkaline CsCl. A reaction mixture (160 µl) containing 50 mM Tris·HCl (pH 8), 100 mM KCl, 2 mM $MgCl_2$, 50 µg/ml of bovine plasma albumin, 10 µg/ml of PM2 DNA rings and 1.5 µg/ml of Eco DNA topoisomerase I was incubated at 42°C. Forty µl aliquots were sampled at time intervals indicated in the figure. The reaction was stopped and the samples were analyzed by band sedimentation in alkaline CsCl as described in the legend to Fig. 2. Sedimentation coefficients of the species were calculated from at least nine scans obtained at different times during the sedimentation experiment. For all of the samples, within experimental error the sedimentation coefficients of the two species which sediment most slowly are 17.5S and the 27S respectively; the sedimentation coefficient of the fastest sedimenting species is 57S. Tracings of samples which had been incubated only briefly with the topoisomerase show a broad band sedimenting in between the 27S and the 57S species. The center of this broad band moves with a sedimentation coefficient of 40S, 43S and 48S respectively for the samples which had been incubated with the enzyme for 1.5, 2.5 and 5 min.

with a value of 1.713 mg/ml, suggests a structure with both single-stranded and double-stranded character. Complementary rings, renaturing to form only limited helical regions due to topological constraint, are expected to have a buoyant density intermediate between single-stranded and duplex DNA; we conclude that the main peak in Fig. 4a represents these partially renatured molecules.

After incubation with the enzyme, shown in Fig. 4d, there remains a small amount of unreacted single-stranded DNA. However, most of the DNA now has a buoyant density of 1.703 mg/ml, the buoyant density of both covalently closed and nicked duplex PM2 DNA. This is consistent with the observation from alkaline sedimentation experiments that ω catalyzes the formation of relaxed

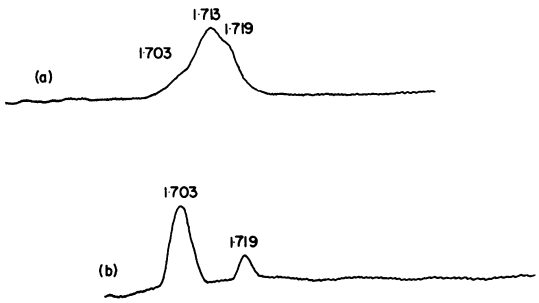

Fig. 4. Change in buoyant densities in neutral CsCl upon incubation of complementary single-stranded PM2 rings with Eco DNA topoisomerase I. (a) Buoyant density pattern of complementary single-stranded PM2 rings. (b) Buoyant density pattern of complementary single-stranded DNA rings after incubation with the enzyme. A reaction mixture (250 µl) consisting of 4 µg/ml DNA, 50 mM Tris (pH 8.0), 50 mM KCl, 2 mM $MgCl_2$, 50 µg/ml of bovine plasma albumin and 0.4 µg/ml of the enzyme was incubated for 1 hr at 42°C. After stopping the reaction with 25 µl of 0.2 M Na_3EDTA, the mixture was extracted first with buffer-saturated phenol, and then repeatedly with ether which had been freshly extracted with water. The samples in both (a) and (b) were adjusted to a density of about 1.70 mg/ml with solid CsCl. CsCl density gradient centrifugation was performed as described in the EXPERIMENTAL SECTION. The buoyant densities of the species are indicated in the figure. M. luteus DNA was used as an internal standard in separate experiments for the determination of the buoyant densities.

covalently closed double-stranded DNA from single-stranded rings. To demonstrate further that the duplex product is covalently closed, the buoyant densities of the DNA species in alkaline CsCl were determined. Fig. 5a shows the single-stranded rings banding in alkaline CsCl at a density of 1.753 mg/ml. After incubation with the enzyme (Fig. 5b), most of the DNA has a higher buoyant density, which is diagnostic of covalently closed duplex PM2 DNA.

DISCUSSION

Renaturation between single-stranded DNA rings of complementary nucleotide sequences has been observed by electron microscopy previously (Clayton et al., 1970; Kasamatsu and Wu, 1976; Broker et al., 1977). The renatured molecules, at least when viewed by the formamide spreading procedure, appear as rings with interspersed single-stranded "bubbles" and double-stranded segments. Intramolecular association between complementary single-stranded loops in a heteroduplex DNA has also been reported by a number of groups (Malamy et al., 1972; Kleckner et al., 1975; Berg et al., 1975; Mosharrafa et al., 1976; Broker et al., 1977).

Simple topology requires that the linking number between the two reassociated rings be zero, unless a transient break is introduced into one of

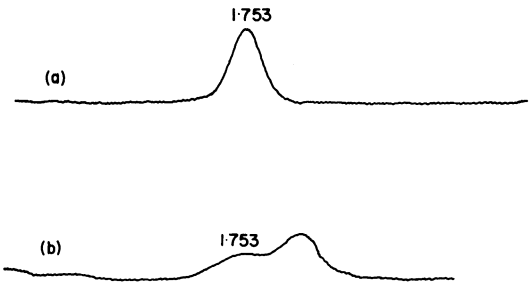

Fig. 5. Change in buoyant densities in alkaline CsCl upon incubation of complementary single-stranded PM2 rings with Eco DNA topoisomerase I. (a) Alkaline buoyant density pattern of single-stranded DNA rings. (b) Alkaline buoyant density pattern after incubation with the enzyme. The reaction was carried out as described in the legend to Fig. 4. The samples in both (a) and (b) were adjusted to a density of 1.76 mg/ml with solid CsCl; 2 M KOH was added to a final concentration of .05 M. CsCl density gradient centrifugation was performed as described in the EXPERIMENTAL SECTION. The buoyant density of single-stranded PM2 DNA was taken as 1.753 mg/ml. The buoyant density of the denser band is in the range 1.771-1.775 mg/ml depending upon the conditions of incubation.

the two rings. Since it is well established that DNA topoisomerases can introduce transient breaks into DNA, it is not surprising that in the presence of such an enzyme two complementary single-stranded DNA rings can be linked into a double-stranded ring. In fact, two complementary single-stranded DNA rings can be considered as a highly underwound double-stranded DNA ring, and their linking can be considered as a special case of the relaxation of an underwound or negatively superhelical DNA.

The ability of a DNA topoisomerase to topologically intertwine complementary single-stranded DNA rings hints a possible role for such enzymes in genetic recombination. Most discussions of genetic recombination invoke the generation of a single-stranded break in one or both of the recombining molecules as comprising the initial event (Holliday, 1964; Hotchkiss, 1974; Meselson and Radding, 1975). It is plausible, however, that topoisomerase-catalyzed intertwining of complementary strands of homologous duplexes might precede strand breakage. This possibility has also been pointed out by Champoux (1977). If both molecules undergoing recombination are duplexes with single-stranded gaps, topoisomerase-catalyzed intertwining could proceed by pairing complementary sequences in the gapped regions. The possibility also exists that both recombination participants are covalently closed circular duplex molecules. If the molecules are negatively superhelical, intermolecular intertwining of a segment of one strand of one molecule to the complementary

strand of another molecule, or the formation of two pairs of such intermolecular duplexes, is thermodynamically favorable under certain conditions (Hsieh and Wang, 1975). It is interesting to note that when DNA gyrase and a second topoisomerase such as Eco DNA topoisomerase I are both present, a closed circular duplex can be maintained in the negatively supercoiled state by gyrase and any topological constraint for the intertwining of strands can be overcome by the other topoisomerase. In several recent studies, gapped duplex DNA rings paired in single-stranded regions (Attardi et al., 1978) and covalently closed duplex DNA rings paired via intermolecular intertwining (Potter and Dressler, 1978) have been observed in in vitro recombination systems.

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