Bolin, J. T., Filman, D. J., Matthews, D. A., Hamlin, R. C., & Kraut, J. (1982) *J. Biol. Chem. 257*, 13650–13662.
Braun, H., & Pfleiderer, W. (1973) *Liebigs Ann. Chem.*, 1082–1090.
Charlton, P. A., Young, D. W., Birdsall, B., Feeney, J., & Roberts, G. C. K. (1979) *J. Chem. Soc., Chem. Commun.*, 922–924.
Cocco, L., Groff, J. P., Temple, C., Jr., Montgomery, J. A., London, R. E., Matwiyoff, N. A., & Blakley, R. L. (1981) *Biochemistry 20*, 3972–3978.
Filman, D. J., Bolin, J. T., Matthews, D. A., & Kraut, J. (1982) *J. Biol. Chem. 257*, 13663–13672.
Fontecilla-Camps, J. C., Bugg, C. E., Temple, C., Jr., Rose, J. D., Montgomery, J. A., & Kisliuk, R. L. (1979) *J. Am. Chem. Soc. 101*, 6114–6115.
Freisheim, J. H., & Matthews, D. A. (1984) in *Folate Antagonists as Therapeutic Agents* (Sirotnak, F. M., Burchall, J. J., Emsinger, W. B., & Montgomery, J. A., Eds.) Vol. 1, pp 69–131, Academic Press, New York.
Gready, J. E. (1980) *Adv. Pharmacol. Chemother. 17*, 37–102.
Gready, J. E. (1984a) *THEOCHEM 109*, 231–244.
Gready, J. E. (1984b) *J. Comput. Chem. 5*, 411–426.
Gready, J. E. (1985) *J. Comput. Chem.* (in press).
Gupta, R. S., Flintoff, W. F., & Siminovitch, L. (1977) *Can. J. Biochem. 55*, 445–452.
Hehre, W. J., Stewart, R. F., & Pople, J. A. (1969) *J. Chem. Phys. 51*, 2657–2664.
Hitchings, G. H., & Smith, S. L. (1979) *Adv. Enzyme Regul. 18*, 349–370.
Huennekens, F. M., & Scrimgeour, K. G. (1964) in *Pteridine Chemistry* (Pfleiderer, W., & Taylor, E. C., Eds.) pp 355–376, Pergamon, Oxford.
Lai, P.-H., Pan, Y.-C. E., Gleisner, J. M., Peterson, D. L., Williams, K. R., & Blakley, R. L. (1982) *Biochemistry 21*, 3284–3294.
Mathews, C. K., & Huennekens, F. M. (1963) *J. Biol. Chem. 238*, 3436–3442.
Nixon, P. F., & Blakley, R. L. (1968) *J. Biol. Chem. 243*, 4722–4731.
Pfleiderer, W. (1979) *Chem. Ber. 112*, 2750–2755.
Pfleiderer, W., & Zondler, H. (1966) *Chem. Ber. 99*, 3008–3021.
Poe, M. (1977) *J. Biol. Chem. 252*, 3724–3728.
Pople, J. A. (1977) in *Modern Theoretical Chemistry* (Schaefer, H. F., III, Ed.) Vol. 4, pp 1–27, Plenum Press, New York.
Pople, J. A. (1978) *QCPE, No. 368.*
Pulay, P. (1979) *Theor. Chim. Acta 50*, 299–312.
Rokos, M., & Pfleiderer, W. (1971) *Chem. Ber. 104*, 739–747.
Singh, C. U., & Kollman, P. (1982) *QCPE, No. 446.*
Smith, S. L., Patrick, P., Stone, D., Phillips, A. W., & Burchall, J. J. (1979) *J. Biol. Chem. 254*, 11475–11484.
Williams, J. W., & Morrison, J. F. (1981) *Biochemistry 20*, 6024–6029.

Differential Modulation by Spermidine of Reactions Catalyzed by Type 1 Prokaryotic and Eukaryotic Topoisomerases†

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ABSTRACT: In the absence of DNA aggregation, spermidine inhibited the relaxation of negatively supercoiled DNA by *Escherichia coli* topoisomerase I at concentrations of the polyamine normally found intracellularly. Spermidine also curtailed the cleavage of negatively supercoiled $ColE_1$ DNA by the enzyme in the absence of Mg^{2+}. On the contrary, knotting of M13 single-stranded DNA circles catalyzed by topoisomerase I was stimulated by the polyamine. Relaxation of supercoiled DNA by eukaryotic type 1 topoisomerases, such as calf thymus topoisomerase I and wheat germ topoisomerase, was significantly stimulated by spermidine in the same range of concentrations that inhibited the prokaryotic enzyme. In reactions catalyzed by S_1 nuclease, the polyamine enhanced the digestion of single-stranded DNA and inhibited the nicking of negatively supercoiled DNA. These results suggest that spermidine modifies the supercoiled duplex substrate in these reactions by modulating the degree of single strandedness.

The natural trivalent cation spermidine is a universal component of all living cells and is present at millimolar intracellular concentrations (Cohen, 1971; Tabor & Tabor, 1976; Morris & Marton, 1981). Numerous studies involving microbial mutants and enzyme inhibitors have suggested that all cells must possess appropriate levels of polyamines, such as spermidine, for optimal growth and differentiation [reviewed in Morris (1981), Pegg & McCann (1982), Heby & Jänne (1981), and Cohn et al. (1978)]. In vitro, polyamines affect a myriad of biochemical processes, including stabilization and renaturation of DNA (Liquori et al., 1967; Christiansen & Baldwin, 1977) and the synthesis of all major macromolecules in the cell, but their sites of action in vivo have not been specifically defined. Spermidine is regularly included as a component of several in vitro reactions of nucleic acid metabolism, because of its positive influence on reactions catalyzed by enzymes like DNA and RNA polymerases, DNA ligase, DNA gyrase, and others (Fisher & Korn, 1979; Gumport, 1970; Gellert et al., 1976).

Topoisomerases, which are ubiquitous both in prokaryotes and in eukaryotes, alter the topological conformation of the

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DNA helix, without altering its primary structure, by concerted nicking and resealing of the sugar–phosphate backbone. DNA gyrase, together with topoisomerase I (ω protein or Eco topo I)[1] and Eco topo III, constitutes the known topoisomerases in *Escherichia coli* (Cozzarelli, 1980; Gellert, 1981; Srivenugopal et al., 1984). In *E. coli*, the degree of DNA supercoiling appears to be regulated by the opposing reactions of DNA gyrase and topo I (DiNardo et al., 1982; Gellert et al., 1982). Mechanistically, topoisomerases fall into two classes on the basis of the number of DNA strands broken and rejoined during a single catalytic event. Type 1 enzymes, such as Eco topo I and Eco topo III, are capable of removing superhelical turns in the absence of an added energy source; this is done by introducing a transient break in one strand and allowing rotation of the broken strand about the intact DNA chain, resulting in alteration of linking number in steps of one. Type 2 enzymes, like DNA gyrase, require ATP and alter the linking number in multiples of two by double-stranded breakage, strand passage, and reunion of DNA (Brown & Cozzarelli, 1979; Liu et al., 1980). Type 1 topoisomerases from *E. coli* and eukaryotic sources differ with respect to their requirements for Mg^{2+} ions, the effect of monovalent cations on the reactions, their ability to relax positive superhelixes, the details of the cleavage reactions, and the polarity of protein attachment to the broken strands (Liu, 1984). Several features of Eco topo I have been attributed to its reaction at single-stranded regions of supercoiled DNA; these include an inability to relax positively supercoiled DNA, strong dependence of the reaction on the degree of negative superhelicity of the DNA substrate, and incomplete relaxation of the substrate (Wang, 1971; Wang & Liu, 1979).

Spermidine has several effects on topoisomerase-catalyzed reactions. Aggregation and compaction of DNA manifested in the presence of spermidine (>4–5 mM) are requirements for catenation of double-stranded circles by Eco topo I and DNA gyrase (Krasnow & Cozzarelli, 1982). At concentrations below those required for catenation, spermidine stimulates the supercoiling reaction of DNA gyrase (Gellert et al., 1976; K. S. Srivenugopal and D. R. Morris, unpublished results). We report here that removal of negative superhelical turns by *E. coli* type 1 topoisomerases, viz., Eco topo I and Eco topo III (Srivenugopal et al., 1984), is inhibited by spermidine in the absence of DNA aggregation and at physiological ionic strength. In contrast, relaxation of supercoils catalyzed by type 1 eukaryotic enzymes (calf thymus and wheat germ topoisomerases) is stimulated by the polyamine. We suggest that spermidine influences the DNA substrate for Eco topo I reactions by modulating the degree of single strandedness in negatively supercoiled DNA. This interpretation is consistent with the effects of spermidine on reactions catalyzed by the single-strand-specific S_1 nuclease.

Experimental Procedures

DNA Preparations. Native $ColE_1$ DNA from *E. coli* JC411 (thy^-, met^-, leu^-, his^-, arg^-) was prepared by lysis with lysozyme and Triton X-100 followed by CsCl/ethidium bromide gradient centrifugation (Bolivar & Backman, 1979). Partially relaxed $ColE_1$ DNA was obtained by treating the supercoiled DNA with calf thymus topoisomerase I (Srivenugopal et al., 1984). $ColE_1$ DNA was tritium labeled by reducing the thymine concentration of the minimal media from 20 to 2 μg/mL and adding 1.0 mCi of [^{3}H]thymine (40 Ci/mmol) per 500-mL culture 30 min after the addition of chloramphenicol. The purified DNA had a specific activity of 30 000 cpm/μg. M13 phage was prepared from the growth medium of an overnight culture of infected cells by precipitation with poly(ethylene glycol) in the presence of NaCl, and the single-stranded DNA was purified by phenol/chloroform extraction and ethanol precipitation.

Chemicals. Stock solutions of spermidine trihydrochloride 3 HCl were neutralized to pH 7.0. The other products used were single-stranded calf thymus DNA–agarose (BRL), agarose (BRL), and gelatin (Difco).

Enzymes. Calf thymus type 1 DNA topoisomerase was purchased from BRL, and the wheat germ type 1 enzyme was a gift from Dr. James Champoux. S_1 nuclease was a product of Miles Laboratories. One topoisomerase unit in this paper is defined as the amount of enzyme required to fully relax 1 μg of DNA in 1 h. Eco topo I was purified from *E. coli* RR_1 containing a *top A* gene recombinant plasmid, pJW249 (Wang & Becherer, 1983). The procedure employed by Wang (1974) was slightly modified for this purpose. Briefly, instead of DEAE-cellulose chromatography, the dialyzed $(NH_4)_2SO_4$ fraction was applied to a phosphocellulose column, and the bound proteins were eluted with 0.4 M KCl. This eluate was passed through a single-stranded DNA–agarose column and washed with 0.4 M NaCl followed by 1.5 M NaCl in 20 mM potassium phosphate (pH 7.5)/0.5 mM EDTA/1 mM β-mercaptoethanol. The wash with 1.5 M NaCl removes DNase activity. Eco topo I was eluted with 4 M NaCl and 50 mM $MgCl_2$ in the above buffer, dialyzed, and concentrated on a small hydroxyapatite column (Otter & Cozzarelli, 1983). Topo I purified by this method was about 90% pure, as judged by SDS gel electrophoresis. Recovery was somewhat variable but on the order of 100–200 μg of protein from 50 g of *E. coli* cells. For this study, the important feature of this preparation is that it is free of detectable nuclease activity.

Relaxation of DNA by Topoisomerases. Eco topo I assays contained the following in a total volume of 30 μL: 20 mM Tris-HCl (pH 7.5), 3 mM $MgCl_2$, 50 μL of gelatin (autoclaved), 2.5% glycerol, 0.7 μg of native $ColE_1$ DNA, and 15–30 ng of purified topo I. KCl was added where indicated. After incubation at 37 °C for 10–20 min, the reaction was stopped by adding a Sarkosyl/EDTA mixture to a final concentration of 1% and 20 mM, respectively.

Eukaryotic topoisomerase reactions contained the following in 30 μL: 20 mM potassium phosphate (pH 7.5), 0.5 mM EDTA, 0.4 mM DTT, gelatin at 50 μg/mL, 0.7 μg of $ColE_1$, DNA, and 0.3–0.5 unit of topoisomerase. KCl or $MgCl_2$ was added in some assays at the concentrations indicated. Termination of the reactions was performed as described above for Eco topo I.

Single-Stranded DNA Knotting by Eco Topo I. The reaction components in a final volume of 30 μL were 20 mM Tris-HCl (pH 7.5), 3 mM $MgCl_2$, gelatin at 50 μg/mL, 2% glycerol, 0.5 μg of M13 DNA, and 100 ng of purified enzyme. Incubation was performed at 34 °C for 1 h, and the reaction was terminated by the addition of EDTA to 30 mM final concentration. The products were analyzed by alkaline agarose gel electrophoresis (see below).

To perform the unknotting reaction, samples of the knotting reaction mixtures were phenol extracted, and the DNA was precipitated with ethanol. The dried, knotted DNA was dissolved in 20 μL of a buffer that is optimal for unknotting: 20 mM Tris-HCl (pH 7.5), 30 mM KCl, 3 mM $MgCl_2$, and gelatin at 50 μg/mL. Incubation was carried out with 40 ng

[1] Abbreviations: SDS, sodium dodecyl sulfate; Eco topo I, topoisomerase I from *Escherichia coli*; Eco topo III, *E. coli* topoisomerase III; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; DTT, dithiothreitol.

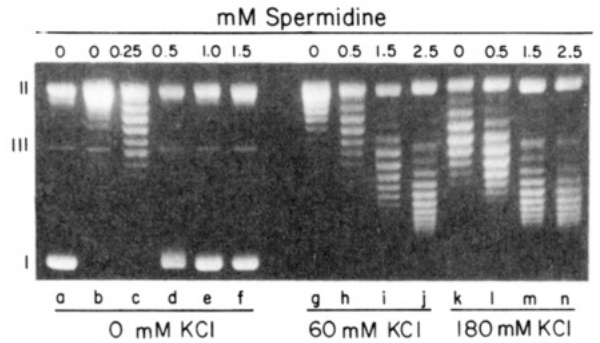

FIGURE 1: Effect of spermidine on the relaxing activity of *E. coli* topoisomerase I at low, moderate, and high ionic strength. Assays were performed as detailed under Experimental Procedures. Spermidine and KCl were added to the final concentrations indicated. No enzyme was added to reaction a, and 25 ng of Eco topo I was added to the others. Incubation period was 15 min. DNA aggregation (Experimental Procedures) was 4% under condition (d) and 100% in (e) and (f). It was not detected in any of the other samples.

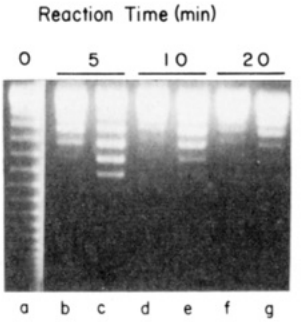

FIGURE 2: Inhibitory influence of spermidine during the progression of Eco topo I reaction with moderately supercoiled DNA. The reaction mixture contained 3 mM $MgCl_2$, 60 mM KCl, partially relaxed $ColE_1$ DNA, and 40 ng of Eco topo I. Spermidine at 1.0 mM was added to samples c, e, and g, and the reactions were terminated at the indicated times.

of Eco topo I for 4 h at 39 °C. EDTA was added to a final concentration of 30 mM, and alkaline gel electrophoresis was performed (see below).

S_1 Endonuclease Reactions. Single-stranded DNA digestion by S_1 nuclease was assayed by the disappearance of M13 DNA detected by agarose gel electrophoresis. The reaction mixture (30 μL) was composed of 30 mM sodium acetate (pH 6.5), 20 mM NaCl, 1 mM $ZnSO_4$, 1.5 μg of M13 DNA, and 6 units of S_1 nuclease. The samples were kept at 37 °C for 30 min, and the reaction was terminated with Sarkosyl/EDTA as above. For measurement of the nicking and linearization of negatively supercoiled DNA by S_1 nuclease, the assay components were the same as above, except that M13 DNA was replaced by 1 μg of $ColE_1$ DNA and S_1 nuclease was added at 70 units/assay. The incubation was at 41 °C for 1 h. The reaction products were analyzed on agarose gels after inactivation of the enzyme.

Gel Electrophoresis. A horizontal submarine agarose gel apparatus (0.6 × 14.5 × 18.8 cm) was used throughout. Agarose gels (1.3%) were cast in pH 7.5 electrophoresis buffer (50 mM Tris–phosphate and 1 mM EDTA). Samples were loaded in 0.03% bromophenol blue and 3% Ficol-600 (Pharmacia), and gels were run at room temperature at a constant 50 V for 13 h. The gels were stained with ethidium bromide (1 μg/mL) for 30 min, destained, and photographed under UV illumination. For the analysis of DNA knotting, agarose gels were prepared in 50 mM NaCl and 2 mM EDTA; the electrophoretic medium was 30 mM NaOH and 2 mM EDTA. The samples were made alkaline before loading (Maniatis et al., 1982).

DNA Aggregation Assay. The microcentrifuge assay described by Krasnow & Cozzarelli (1982) was used to monitor DNA aggregation. Conditions were as for the assays of topoisomerases or S_1 nuclease, with identical amounts of ^3H-$ColE_1$ DNA and boiled enzyme preparations added.

RESULTS

DNA Relaxation Catalyzed by Eco Topo I Is Inhibited by Spermidine. Figure 1 shows the influence of spermidine on the removal of negative superhelical turns by Eco topo I at low, moderate, and high ionic strength. Mg^{2+} (2–3 mM) is required for optimal relaxation by topo I. It is known that monovalent cations (Na^+ or K^+) inhibit relaxation by causing frequent dissociation of topo I from DNA (Wang, 1971, 1974; Wang & Liu, 1979). This effect is evident in Figure 1b,k. Spermidine cannot replace the requirement for Mg^{2+} in Eco topo I reactions; on the contrary, the results presented in Figure 1 clearly show that physiological concentrations of spermidine inhibit the reaction. The inhibitory effect of the polyamine is greater in reactions with low salt. At 180 mM K^+ and 3 mM Mg^{2+}, conditions that are close to physiological levels in *E. coli*, spermidine was still inhibitory, although less so than at low ionic strength.

Spermidine was inhibitory at 0.25 and 0.5 mM (Figure 1c,d), concentrations that gave no significant DNA aggregation. We failed to detect aggregation (see Experimental Procedures) under any of these conditions, except at 1–1.5 mM spermidine in low salt (see legend to Figure 1). Krasnow & Cozzarelli (1982) also did not observe spermidine-induced DNA compaction in the presence of 60–180 mM K^+ under conditions that were roughly equivalent to those employed here.

Moderately supercoiled DNA is a poor substrate for Eco topo I, requiring high levels of enzyme and prolonged incubation to achieve relaxation. The effect of spermidine on the reaction with this substrate has been examined (Figure 2). The assays were performed in the presence of 60 mM KCl, and no DNA aggregation was detected under these conditions. It can be seen that there is an initial phase of rapid relaxation and the reaction is almost complete within 5 min in the absence of spermidine (Figure 2b). The inhibitory effect of spermidine prevails at all time points (Figure 2, compare lanes b and c, d and e, and f and g). By comparing lanes b and g, one can estimate that a 4-fold increase in reaction time achieves approximately similar levels of relaxation in the presence of 1 mM spermidine.

Linearization of Native $ColE_1$ DNA by Eco Topo I. Eco topo I cleaves single-stranded and negatively supercoiled DNA in the absence of Mg^{2+} to form salt-stable and alkali-cleavable covalent complexes; these protein–DNA complexes are dissociated when exposed to Mg^{2+} in solutions of high ionic strength (Depew et al., 1978; Wang & Liu, 1979). With double-stranded DNA, complex formation depends strongly on the superhelical density, reminiscent of topo I catalyzed DNA relaxation. In the experiment detailed in Figure 3, complex formation was allowed between $ColE_1$ DNA and topo I in the absence of Mg^{2+}. Covalent complexes were isolated by coprecipitation with potassium dodecyl sulfate. The effect of spermidine in diminishing the reaction with negatively supercoiled DNA is apparent.

Spermidine Promotes Knotting of M13 DNA Circles. Eco topo I has been shown to introduce and remove knots in single-stranded circular DNA (Liu et al., 1976). The formation of knotted forms is favored at low temperatures and high ionic strength, while the reverse reaction, unknotting, is promoted at high temperatures and low ionic strength. In contrast to the inhibitory effect of salt seen in the relaxation reaction of

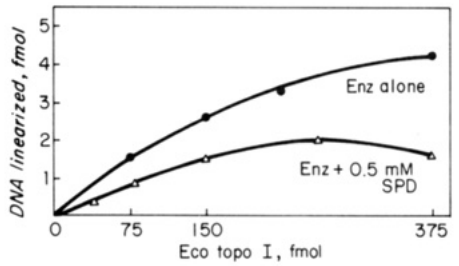

FIGURE 3: Linearization of negatively supercoiled ColE$_1$ [^{3}H]DNA by Eco topo I. Detection of DNA–protein complexes was by co-precipitation with potassium dodecyl sulfate (Trask et al., 1984). Reaction mixtures (20 μL) contained 20 mM Tris-HCl (pH 7.5), 20 mM KCl, 50 μg/mL gelatin, 50 ng of ColE$_1$ [^{3}H]DNA (1800 cpm, about 15% nicked circles), 2% glycerol, and Eco topo I at the amounts indicated. Spermidine was added at 0.5 mM, as indicated. After keeping the samples at 37 °C for 40 min, SDS was added to 1% final concentration followed by 200 μL of 2.5 M KCl to precipitate covalent protein–nucleic acid complexes at 4 °C. The precipitates were washed 3 times by resuspension in 1.5 mL of 10 mM Tris-HCl (pH 7.5) containing 100 mM KCl followed by centrifugation. The pellets were dissolved by adding 200 μL of 0.5 N HCl and then 200 μL of 0.5 N NaOH. These solutions were transferred to scintillation vials and counted by liquid scintillation spectrometry. Free DNA does not precipitate under these conditions; the background counts in the absence of enzyme were <6% of input. All assays were carried out in duplicate, and the averaged results are presented.

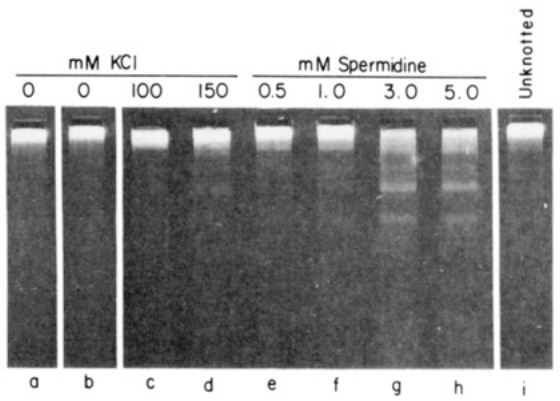

FIGURE 4: Spermidine promotes M13 DNA knotting by Eco topo I in the absence of KCl. Knotting reactions were performed as described under Experimental Procedures. KCl or spermidine was added at the indicated concentrations to reaction mixtures containing 3 mM Mg^{2+}. Lane a contains the substrate single-stranded circular M13 DNA after incubation in the absence of enzyme. To generate the DNA in lane i, duplicate reactions as in lane h were run, and the DNA was extracted from one reaction and reincubated under unknotting conditions (see Experimental Procedures).

this enzyme, 150 mM KCl seemed to be optimal for the knotting reaction (Liu et al., 1976). This novel topoisomerization reaction was proposed to result from the alternate nicking and closing action of the enzyme at small duplex or quasi-duplex regions arising from adventitious intramolecular base pairing.

Figure 4 shows an analysis of the knotting reaction of Eco topo I by alkaline agarose gel electrophoresis. Wang & Liu (1979) showed that knotted molecules of varying complexities have faster mobilities under these conditions than unknotted circles. Incubation in the presence of 100 or 150 mM KCl produced species of greater mobility than the substrate DNA (Figure 4c,d). Spermidine was even more effective than KCl in promoting the putative knotting reaction (Figure 4e–h). The bands of higher mobility were not products of the linearization reaction of topo I, since linearization does not require high KCl concentration (Depew et al., 1978; Wang & Liu, 1979; Figure 3 of this paper) as does knotting (compare lanes b and d of Figure 4). Further evidence that the higher mobility forms

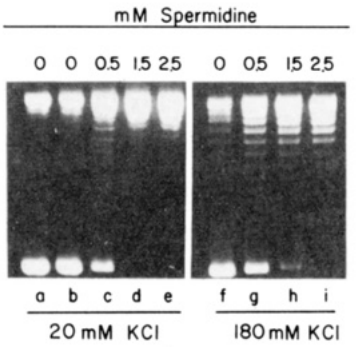

FIGURE 5: Calf thymus type 1 topoisomerase is stimulated by spermidine. The details of the assay are presented under Experimental Procedures. The reaction mixture in lane a contained no enzyme. In the reactions containing 20 mM KCl (b–e), the incubation was for 20 min with 0.7 unit of enzyme per assay. In the presence of 180 mM KCl (f–i), the reactions were carried out for 6 min with 0.2 unit of enzyme per assay.

are topoisomers comes from the fact that the reaction is reversed when the products are incubated with topo I under conditions known to favor unknotting (Figure 4i).

Activation of Eukaryotic Type 1 Topoisomerases by Spermidine. Eukaryotic type 1 enzymes do not require Mg^{2+} and are optimally active in the presence of 150–200 mM monovalent cations. Figure 5 presents the effects of KCl and spermidine on calf thymus topo I. In the reactions carried out at high salt, both the time of the reaction and the amount of enzyme added were reduced to less than one-third of those at low salt (see legend to Figure 5). Taking this into consideration, it would seem that the reaction is stimulated approximately 10-fold by high salt in the absence of spermidine (compare lanes b and f). At either concentration of KCl, increasing concentrations of spermidine dramatically stimulated the enzyme (Figure 5). Stimulation of the calf thymus enzyme by the polyamine still occurred in the presence of 5 mM Mg^{2+}. The activity of wheat germ type 1 topoisomerase also was enhanced in the presence of spermidine, although to a lesser extent than with the mammalian enzyme (data not shown).

Influence of Spermidine on Reactions of S_1 Endonuclease. Single-strand-specific S_1 nuclease is a zinc metalloprotein and has been used extensively to probe DNA secondary structures (Lilley, 1981; Hentschel, 1982; Singleton et al., 1982). The enzyme recognizes single-stranded regions in superhelical DNA and converts it first to a nicked circular molecule and then to a unit-length linear molecule. Like Eco topo I, the reaction of S_1 nuclease with supercoiled DNA is dependent on the degree of negative superhelicity; the enzyme cannot cleave molecules containing 30–40% or less of the natural superhelical density (Shishido, 1980). Nuclease S_1 was chosen to investigate the effect of spermidine on the secondary structure of ColE$_1$ DNA under reaction conditions similar to those used for Eco topo I.

As a control, Figure 6 (lanes a–e) shows the digestion of M13 DNA at various concentrations of spermidine. The polyamine clearly stimulates nucleolytic activity as measured by the disappearance of single-stranded circles.

Supercoiled ColE$_1$ DNA has a single major site for S_1 nuclease attack (Lilley, 1981). Nicking and linearization of ColE$_1$ DNA induced by nuclease S_1 are presented in Figure 6 (lanes f–j). Significant inhibition of nicking occurs in the presence of spermidine, even at low concentrations. With increasing concentrations of spermidine, the amount of unreacted substrate (form I) increased concomitantly with the decrease in nicked DNA (form II). DNA aggregation was

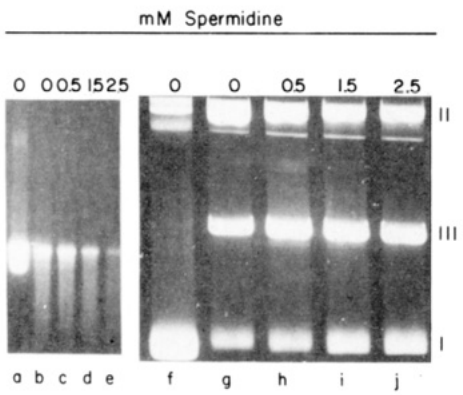

FIGURE 6: Activation of single-stranded DNA digestion and inhibition of supercoiled DNA nicking by S_1 nuclease in the presence of spermidine. Assay conditions are described under Experimental Procedures. Lanes a–e show digestion of M13 single-stranded DNA. Lane a is the M13 DNA substrate without enzyme treatment. Lanes b–e show DNA digestion in the presence of varying amounts of spermidine. Lanes f–j depicit the nicking and linearization of $ColE_1$ supercoiled DNA by S_1 nuclease. Lane f is the native $ColE_1$ DNA substrate used. Lanes g–j show the patterns of this reaction in the presence of spermidine at different concentrations. Roman numerals I, II, and III refer to negatively supercoiled, nicked, and linear forms of DNA, respectively. DNA aggregation did not occur in lanes h, i, or j.

not detected under these assay conditions. The increased amounts of linear DNA (form III) appearing in the presence of spermidine (Figure 6, lanes h–j) are expected, given the stimulation by the polyamine of the reaction of nuclease S_1 with single-stranded DNA.

Discussion

Spermidine exerts opposing influences on the type 1 and type 2 topoisomerases of *E. coli*. The relaxation activities of the two type 1 enzymes, Eco topo I and Eco topo III, are inhibited by physiological concentrations of spermidine (this paper; Srivenugopal et al., 1984). On the other hand, in the first report identifying DNA gyrase (Gellert et al., 1976), it was shown that the supercoiling activity of this enzyme was strongly stimulated by spermidine. Thus, on the basis of the influence of the polyamine on these enzymes, one would anticipate that a physiological effect of spermidine would be to increase the negative superhelical density of DNA in *E. coli* cells (Morris & Lockshon, 1981). Given the potential physiological significance of these effects of spermidine on DNA topoisomerases, we initiated the current studies on the nature of inhibition of the type 1 enzymes by the polyamine.

The contrasting effects of spermidine on the eukaryotic and prokaryotic type 1 topoisomerases are striking and also instructive. It was previously reported that the type 1 topoisomerase from *Trypanosoma cruzi* was activated by spermidine (Riou et al., 1983), and we report the activation of the mammalian and plant enzymes here. Both positively and negatively supercoiled DNA are substrates for the eukaryotic enzymes, implying that single-stranded character in the substrate is not necessary for activity [see Liu (1984) for a review of eukaryotic topoisomerases]. On the other hand, considerable evidence suggests that the prokaryotic type 1 topoisomerases act at sites with single-stranded character in the negatively supercoiled substrate (Gellert, 1981; Wang & Liu, 1979). The relaxation activity of prokaryotic type 1 enzymes is inhibited by single-stranded DNA and is strongly dependent on the degree of negative supercoiling of the substrate to the extent that these enzymes are inactive on positively supercoiled DNA. The fact that spermidine inhibits the prokaryotic enzymes, but not the eukaryotic type 1 proteins, could be explained if the polyamine were acting to reduce the single-stranded nature of the negatively supercoiled substrate; single strandedness is necessary for activity of the former but not the latter topoisomerases. Consistent with the interpretation that the influence of spermidine is on the substrate, rather than on the catalytic activity of the enzyme, is the finding that the knotting reaction of Eco topo I with single-stranded circular DNA, which requires the same strand scission and rejoining reactions as in the relaxation of negatively supercoiled double-stranded circules, is not inhibited, and if anything stimulated, by spermidine.

It is possible to exclude two interpretations of the inhibition of Eco topo I by spermidine. First, the inhibition manifests itself on the rate of the reaction, rather than on the extent of relaxation, as evidenced from the time course presented in Figure 2. Second, spermidine inhibition is not the result of DNA aggregation in the reaction mixture, at least under most of the conditions tested. In the presence of 60 or 180 mM KCl, no DNA aggregation was detected, and in the absence of KCl addition to the reaction mixture, insignificant DNA aggregation was observed below 0.5 mM spermidine. These conclusions are consistent with the phase diagram given in Krasnow & Cozzarelli (1982). Additionally, if spermidine were exerting its inhibitory influence on Eco topo I by way of an aggregation mechanism, one might have seen such a nonspecific inhibition extending to the eukaryotic topoisomerases as well. This clearly was not the case. Thus, spermidine inhibition of the rate of DNA relaxation catalyzed by Eco topo I occurs by a mechanism unrelated to substrate aggregation, leading to inaccessibility to the enzyme. In this regard, it is interesting to note that poly(ethylene glycol), at concentrations that promote both DNA aggregation and catenation by Eco topo I (Low et al., 1984), actually stimulates markedly DNA relaxation catalyzed by Eco topo I (unpublished results).

Spermidine is known to stabilize linear DNA duplexes [for example, see Christiansen & Baldwin (1977)]. However, the influence of spermidine on the single-stranded character of negatively supercoiled DNA has not been examined previously. Negative supercoiling of DNA duplexes promotes single-stranded character in regions that are enriched in dA + dT sequences (Funnell & Inman, 1979; Tong & Battersby, 1979). In this study, nuclease S_1 was used as a probe of single-stranded character of $ColE_1$ DNA as a function of spermidine concentration. A recent study of Shishido et al. (1983) with $ColE_1$ DNA showed a good correlation between sites of S_1 cleavage and sites at which *Hemophilus* topo I acted. Our results with S_1 nuclease digestion of superhelical $ColE_1$ DNA are best interpreted in light of the recent study of Carnevali et al. (1984) on topological transitions and superhelical density. There are two endonucleolytic events in S_1-mediated linearization: (1) introduction of the first nick leading to disappearance of superhelical forms, depending on superhelical density as well as the size of the DNA domain; (2) a cut on the second strand at the site of the nick to give the linear molecule. The second step is not dependent on superhelical density, since it takes place on a nicked, relaxed substrate. Our results with single-stranded phage DNA clearly show that spermidine profoundly stimulates this reaction. On the other hand, with superhelical duplex substrate, the nicking reaction (I → II, Figure 6) is inhibited by increasing concentrations of spermidine. The linearization reaction with nicked duplex substrate (II → III, Figure 6) was stimulated with increasing spermidine, as predicted from the results with single-stranded phage DNA. Thus, inhibition of S_1 nuclease activity on supercoiled DNA by spermidine seems to be due to a decrease

in single-stranded character of the substrate, rather than a direct inhibition of the nucleolytic activity of the enzyme.

To summarize our conclusions with regard to Eco topo I, those reactions of this enzyme that require single-stranded character in a negatively supercoiled substrate (relaxation of supercoils, formation of a covalent enzyme–DNA complex) are inhibited by spermidine. On the other hand, the knotting reaction with single-stranded phage DNA is not inhibited, but stimulated, by spermidine. These results, together with those using nuclease S_1 as a probe, are consistent with physiological concentrations of spermidine, at physiological ionic strength, causing sufficient decrease in single strandedness of negatively supercoiled DNA to reduce the reactivity of this substrate with these enzymes. Since type 1 eukaryotic topoisomerases do not require single-stranded regions for activity, it follows that these enzymes would not be inhibited by the polyamines. The mechanism of the profound stimulation of the eukaryotic enzymes by spermidine is not yet understood.

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REFERENCES

Bolivar, F., & Backman, K. (1979) *Methods Enzymol. 68*, 245–267.

Brown, P. O., & Cozzarelli, N. R. (1979) *Science (Washington, D.C.) 206*, 1081–1083.

Carnevali, F., Caserta, M., & DiMauro, E. (1984) *J. Biol. Chem. 259*, 12633–12643.

Christiansen, C., & Baldwin, R. (1977) *J. Mol. Biol. 115*, 441–454.

Cohen, S. S. (1971) *Introduction to the Polyamines*, Benjamin Hall, Englewood Cliffs, NJ.

Cohn, M. S., Tabor, C. W., & Tabor, H. (1978) *J. Bacteriol. 134*, 208–212.

Cozzarelli, N. R. (1980) *Science (Washington, D.C.) 207*, 953–960.

Depew, R. E., Liu, L. F., & Wang, J. C. (1978) *J. Biol. Chem. 253*, 511–518.

DiNardo, S., Voelkel, K. A., & Sternglanz, R. (1982) *Cell (Cambridge, Mass.) 31*, 43–51.

Fisher, P. A., & Korn, D. (1979) *J. Biol. Chem. 254*, 11033–11039.

Funnell, B. E., & Inman, R. B. (1979) *J. Mol. Biol. 131*, 331–340.

Gellert, M. (1981) *Annu. Rev. Biochem. 50*, 879–910.

Gellert, M., Mizuuchi, K., O'Dea, M. H., & Nash, H. A. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3872–3876.

Gellert, M., Menzel, R., Mizuuchi, K., O'Dea, M. H., & Friedman, D. I. (1982) *Cold Spring Harbor Symp. Quant. Biol. 45*, 391–398.

Gumport, R. I. (1970) *Ann. N.Y. Acad. Sci. 171*, 915–938.

Heby, O., & Jänne, J. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R., & Marton, L. J., Eds.) pp 243–310, Marcel Dekker, New York.

Hentschell, C. C. (1982) *Nature (London) 295*, 714–716.

Krasnow, M. A., & Cozzarelli, N. R. (1982) *J. Biol. Chem. 257*, 2687–2693.

Lilley, D. M. J. (1981) *Nucleic Acids Res. 9*, 1271–1288.

Liquori, A. M., Costanino, V., Crescenzi, V., Elia, E., Giglio, R., Puliti, M., DeSantis, M., & Vitagliano, V. (1967) *J. Mol. Biol. 24*, 113–122.

Liu, L. F. (1984) *CRC Crit. Rev. Biochem. 15*, 1–23.

Liu, L. F., Depew, R. E., & Wang, J. C. (1976) *J. Mol. Biol. 106*, 439–452.

Liu, L. F., Liu, C. C., & Alberts, B. M. (1980) *Cell (Cambridge, Mass.) 19*, 697–708.

Low, R. L., Koguni, J. M., & Kornberg, A. (1984) *J. Biol. Chem. 259*, 4576–4581.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning*, CSH Laboratory Publication, Cold Spring Harbor, NY.

Morris, D. R. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R., & Marton, L. J., Eds.) pp 233–242, Marcel Dekker, New York.

Morris, D. R., & Lockshon, D. (1981) *Adv. Polyamine Res. 3*, 299–308.

Morris, D. R., & Marton, L. J. (1981) *Polyamines in Biology and Medicine*, Marcel Dekker, New York.

Otter, R., & Cozzarelli, N. R. (1983) *Methods Enzymol. 100*, 171–180.

Pegg, A. E., & McCann, P. P. (1982) *Am. J. Physiol. 243*, C212–C221.

Riou, F., Gabillot, M., Douc-Rasy, S., Kayser, A., & Barrios, M. (1983) *Eur. J. Biochem. 134*, 479–484.

Shishido, K. (1980) *FEBS Lett. 111*, 333–336.

Shishido, K., Noguchi, N., & Ando, T. (1983) *Biochim. Biophys. Acta 740*, 108–117.

Singleton, C. K., Klysik, J., Stirdivant, S. M., & Wells, R. D. (1982) *Nature (London) 299*, 312–316.

Srivenugopal, K. S., Lockshon, D., & Morris, D. R. (1984) *Biochemistry 23*, 1899–1906.

Tabor, C. W., & Tabor, H. (1976) *Annu. Rev. Biochem. 45*, 285–306.

Tong, B. Y., & Battersby, S. J. (1979) *Nucleic Acids Res. 6*, 1073–1079.

Trask, D. K., Didonato, J. A., & Muller, M. T. (1984) *EMBO J. 3*, 671–676.

Wang, J. C. (1971) *J. Mol. Biol. 55*, 523–533.

Wang, J. C. (1974) *Methods Enzymol. 29*, 197–203.

Wang, J. C., & Liu, L. F. (1979) in *Molecular Genetics* (Taylor, J. H., Ed.) Part III, pp 65–88, Academic Press, New York.

Wang, J. C., & Becherer, K. (1983) *Nucleic Acids Res. 11*, 1773–1790.