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Effects of Nucleosomes and Anti-tumor Drugs on the Catalytic Activity of Type II DNA Topoisomerase from Rat Testis

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ABSTRACT. To gain insight into the relative catalytic efficiencies of mammalian type I and type II DNA topoisomerases, in the cellular context, we have used naked DNA and DNA incorporated into nucleosomes as substrates. We observed that the relaxation activity of both the enzymes declined with DNA containing increasing densities of nucleosomes; however, kinetic analysis revealed that topoisomerase I seemed less affected than topoisomerase II. The addition of histone H1, in stoichiometric amounts, to naked DNA or minichromosomes lessened the activity of topoisomerase II, and required 7-fold less for complete inhibition when the latter was used as the substrate. To ascertain if the observed differences are specific to topoisomerase II from testis, we examined the effect of nucleosomes on the catalytic efficiency of its isoform from liver. Interestingly, the suppression of relaxation activity of liver topoisomerase II required substrates containing higher mass ratios of histone octamer/DNA. Studies on the effect of nucleosomes on the action of teniposide displayed significant differences in the kinetics of the reaction, in its IC_{50} values, and have provided biochemical evidence for the first time that nucleosomes increased inhibition caused by teniposide. Further, this feature appears to be specific for topoisomerase II-directed drugs and is not shared by the generic class of either DNA-intercalating or non-DNA-intercalating ligands. BIOCHEM PHARMACOL 53;9:1229–1238, 1997. © Elsevier Science Inc.

KEY WORDS. topoisomerases; nucleosomes; histone H1; unwinding; anti-tumor drugs

DNA topoisomerases are ubiquitous nuclear enzymes involved in catalyzing the conversion between different topological isoforms and orderly transmission of genetic material in both prokaryotic and eukaryotic cells. There are two major classes of topoisomerases, designated type I and type II, distinguished by their mechanism of action [1–3]. The eukaryotic topo II† is essential for a number of nuclear processes including the untwining of the parental and daughter chromosomes after replication, segregation of sister chromatids at mitosis and meiosis, and relaxation of supercoils generated during transcription [2, 4]. Topo II has also been suggested to constitute a component of two insoluble fractions from chromosomes and nuclei: the chromosome scaffold and the interphase nuclear matrix, where it is thought to play a key role in chromosome condensation and decondensation [5–10]. Upon binding to DNA, in the presence of ATP and Mg^{2+}, it acts as an ATP-dependent protein clamp around the duplex DNA, and catalyzes the transport of a segment of a DNA molecule through the DNA–enzyme gate [11]. After strand passage and turnover of enzyme, facilitated by hydrolysis of ATP, a second cycle of breakage–religation reaction ensues [12]. During this process, both strands of a DNA molecule are broken, thereby generating a transient covalent DNA–topo II complex, referred to as the "cleavable complex" [13, 14]. The "cleavable topo II–DNA complexes" have been recognized as the major molecular targets for a variety of potent anti-tumor drugs [13–16]. Several of these drugs act specifically by blocking the cleavage/religation step and others by irreversibly locking the exit gate of the protein clamp [14–18]. However, the information gained has been with naked DNA as the substrate.

A vast body of data suggests that the assembly of DNA into nucleosomes and higher-order chromatin structures imposes specific constraints on the ability of *trans*-acting factors to interact with DNA targets [19]. A host of strategies could be imagined of how *trans*-acting factors deal with nucleosomes and higher order chromatin structures. Progress in several experimental systems has demonstrated that the DNA targets are situated in accessible, nuclease-hypersensitive regions that punctuate the orderly array of nucleosomes. However, it has also been shown that nucleo-

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† *Abbreviations:* DTT, dithiothreitol; form I DNA, negatively supercoiled circular DNA as isolated from *Escherichia coli*; form IV DNA, relaxed DNA; MCs, minichromosomes; MNase, micrococcal nuclease; topo I, topoisomerase I; and topo II, topoisomerase II.

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somes, including histone H1, are present in actively transcribing genes [20]. Recent experiments have suggested that a class of evolutionarily conserved proteins, and some hormone receptors, facilitate transcriptional activation by antagonizing the inhibitory effects of chromatin proteins [21, 22]. There are at least three general attributes of the substrate DNA, *in vivo*, that are likely to affect the overall catalytic efficiency of topo II. These include the superhelical density of the substrate, nucleotide sequence, and both structural and regulatory proteins that organize DNA into chromosomes. A comparison of sequence specificity of cleavage patterns generated *in vivo* and *in vitro* by purified topo II with naked DNA and DNA incorporated into nucleosomes suggested that the potential sites of action of topo II are restricted inside the cell [23]. Further, these studies also demonstrated that very few strong sites on naked DNA were cleaved *in vivo*. The mechanism by which topo II gains access to DNA targets in chromatin remains poorly understood.

Previously, we reported the purification and functional characterization of topo II from rat testis and showed that it is both structurally and functionally distinct from its somatic isoform [24]. The focus of the present study was to gain insights into the relative catalytic efficiency of topo I and topo II, and to examine the action of topo II-directed clinically important anti-tumor drugs on DNA assembled into nucleosomes.

MATERIALS AND METHODS

Materials

Teniposide was a gift from Bristol–Myers Laboratories, Evansville, IN, U.S.A., and was used in the supplied form (10 mg/ml). Amiloride-HCl and amsacrine were gifts from the Merck Sharp & Dohme Co., Rahway, NJ, U.S.A., and the National Cancer Institute, Bethesda, MD, U.S.A., respectively. Amiloride and amsacrine were dissolved in DMSO, and diluted to appropriate concentrations in distilled water. Teniposide was stored at 4°; all the other drug stock solutions were stored frozen at −20°. Agarose, Zetaprobe membrane, and Coomassie brilliant blue were purchased from Bio-Rad Laboratories, Hercules, CA, U.S.A. ATP was purchased from US Biochemicals, Cleveland, OH, U.S.A. Trizma, BSA, SDS–PAGE marker proteins, ultra pure sucrose, and DTT were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. All other reagents were of analytical grade.

Enzymes, Proteins and DNA

Topo II from rat testis and liver was purified as described [24]. Calf thymus topo I was purchased from Bethesda Research Laboratories Inc., Grand Island, NY, U.S.A. MNase was obtained from the Worthington Biochemical Corp., Freehold, NJ, U.S.A., and *Hae*III from Pharmacia LKB Biotechnology, Uppsala, Sweden. Nuclei from adult rat liver were prepared as described [25]. Histone octamers and histone H1 were extracted from the nuclei with acid and salt, respectively. The purity of these preparations was ascertained by SDS–PAGE followed by Coomassie blue staining [26]. The histone preparations were devoid of detectable exonuclease and endonuclease activity. Phage M13 form I [^{3}H]DNA (negatively superhelical) was prepared as described [27].

Protein Analysis

Protein concentrations were determined by the method of Bradford [28] with BSA as the standard. Histone samples were analyzed by SDS–PAGE on 15% gels, followed by staining with Coomassie blue [26].

Preparation and Characterization of Minichromosomes

MCs comprising M13 form I [^{3}H]DNA and histone octamers were assembled as described [29, 30]. Briefly, histone octamers were mixed with form I DNA, at the specified mass ratios, in a buffer containing 10 mM Tris–HCl (pH 7.5), 0.2 mM EDTA, and 0.8 M NaCl in a total volume of 0.4 mL. Samples were incubated at 37° for 10 min, and the assembly was allowed to proceed at 4° for 15 hr. Salt was dialyzed gradually (0.8 M → 0.4 M → 0.02 M) at 4° using Centricon-10 microconcentrators. MCs were purified by ultracentrifugation on linear sucrose gradients. The repeated positioning of nucleosomes and the general protection of MCs were ascertained by digestion with micrococcal nuclease and *Hae*III, respectively, as described [25]. The digested products were analyzed on 1.6% agarose gels using a 1 kb ladder as a reference molecular weight marker.

Relaxation Assay

Topo II-promoted relaxation activity was monitored by the conversion of form I DNA to form IV DNA. Standard reactions (20 μL) contained 0.3 μg of naked form I M13 DNA or an equivalent amount of DNA in MCs [24]. One unit of purified topo II from testis or liver was added, and reaction mixtures were incubated at 37° for 30 min. The reaction was terminated by the addition of EDTA to 25 mM, SDS to 1%, and proteinase K to 0.2 mg/mL and then the mixture was incubated at 40° for 15 min. Reaction mixtures were processed for electrophoresis as described [24]. After electrophoresis, the gels were stained with ethidium bromide (0.5 μg/mL) and photographed under UV illumination. Alternatively, the reaction products were analyzed on agarose gels as described [24], then transferred to a nylon membrane, probed with ^{32}P-labeled M13 DNA, and visualized by autoradiography. Autoradiograms were scanned with an LKB Ultroscan XL laser densitometer. One unit of topo I or topo II activity was defined as the amount of enzyme required to relax 0.3 μg of form I to form IV DNA in 30 min at 37°.

For studies on the effect of histone H1 on the relaxation activity of topo II, histone H1 purified from rat liver was

added to the reaction mixtures at specified mass ratios. Reaction mixtures were processed as described above, and the products were visualized by autoradiography. To evaluate the action of various antineoplastic drugs on the activity of purified topo II, reaction mixtures were supplemented with the indicated amounts of the inhibitor in DMSO. Control experiments revealed that the final concentration of DMSO (0.4%) used in these experiments failed to show any detectable inhibitory effect on the relaxation of form I DNA (data not shown). Results of DNA cleavage experiments also indicated that DMSO alone failed to introduce breaks in form I DNA (data not shown).

Determination of IC_{50} Values

The inhibitory action of a number of antitumor drugs and distamycin was monitored as described above. The inhibitory potential of each of these drugs was expressed in terms of IC_{50} values that were obtained by densitometric scanning of individual lanes in the gel negatives or corresponding lanes in the autoradiogram. The relative amount of activity was plotted against drug concentration, and the concentration corresponding to 50% activity was taken as the IC_{50} value of the drug.

RESULTS

Construction of Minichromosomes

Synthetic MCs, containing increasing mass ratios of histone octamer/DNA, were prepared using form I DNA of bacteriophage M13 and histone octamers from rat liver. The rationale for using M13 DNA is that prokaryotic DNAs form nucleosomes as well or better than eukaryotic DNA [31]. MCs were assembled by mixing appropriate mass ratios of histone octamers/DNA in buffers containing high salt, dialyzed stepwise to attain an ionic strength of 0.02 M, and were purified by sucrose density centrifugation. The incorporation of DNA into nucleosomes was assayed by using two enzymatic probes: micrococcal nuclease and *Hae*III [25]. Under standard reaction conditions, naked DNA was completely cleaved by *Hae*III (Fig. 1A, lane 3), whereas MCs containing various mass ratios of histone octamer/DNA showed increasing protection from *Hae*III digestion (Fig. 1A, lanes 4–6). The presence of an array of nucleosomes on MCs was ascertained by digestion with MNase. As shown in Fig. 1B (lanes 4 and 5), naked DNA was completely digested by MNase to a mixture of oligo- and mononucleotides that ran out of the gel. Under identical experimental conditions, incubation of MCs containing a histone octamer/DNA mass ratio of 0.2 generated a fragment of DNA of 200 bp (Fig. 1B, lanes 7–9). Further, MCs containing higher mass ratios of histone octamer/DNA produced a discrete ladder of DNA fragments, suggesting repeated positioning of nucleosomes (Fig. 1B, lanes 10–15). The efficiency of assembly of nucleosomes on form I DNA is consistent with the data previously described [32].

Comparison of the Efficiency of Relaxation of Naked DNA and Minichromosomes by Type I and Type II Topoisomerases

Although topo II catalyzes conversion between different topological isoforms, relaxation of form I DNA to form IV DNA is the only reaction that it shares with topo I. Accordingly, to assay the effect of nucleosomes on the relative catalytic efficiencies of these enzymes, we carried out the relaxation assay. Reaction mixtures containing naked DNA or MCs with topo I or topo II were incubated for 30 min. Samples were deproteinized and analyzed by agarose gel electrophoresis. The extent of relaxation of naked form I DNA by topo I and topo II was comparable (Fig. 2). By contrast, with MCs containing histone octamer/DNA mass ratios of 0.1 and 0.15 the pattern of relaxation was different: nearly all of form I DNA was relaxed to form IV DNA by topo I, whereas topo II displayed a partial reaction. However, with MCs containing higher mass ratios of histone octamer/DNA, the relaxation activities of topo I and topo II were indistinguishable. Several control experiments corroborated the validity of these results. In one such experiment, we titrated the accurate amounts of enzymes required to relax naked DNA under conditions where some amount of form I DNA still persisted. We found no significant differences between topo I- and topo II-promoted reactions incubated for 15–30 min (data not shown). Consequently, these results suggest that nucleosomes either constrain the topological motions of duplex DNA or, alternatively, exclude a certain number of negative supercoils in the substrate. The former seems unlikely since the activity of DNA gyrase was unimpeded by the presence of higher mass ratios of histone octamer/DNA [33]. However, it is likely that wrapping of DNA on histone octamers may suppress a significant number of negative supercoils from relaxation by topoisomerases. The mass ratios of 0.3 to 0.4, which correspond to half of the nucleosomal density in somatic tissues, resulted in complete loss of relaxation. Although the actual mass ratios of histone octamers/DNA in rat testis are obscure, it is known that the chromatin structure is decondensed during spermiogenesis [34, 35].

Since the differences in the efficiencies of relaxation of MCs by topo I and topo II were discerned from incubations carried out for 30 min, it was important to compare the patterns of relaxation at early times of incubation to establish whether the kinetics indeed favored topo I. The time-dependent changes in the distribution of topoisomers with naked form I DNA suggested that both type I and type II enzymes catalyzed the reaction with similar efficiencies (Fig. 3A). Conversely, relaxation of MCs containing a histone octamer/DNA mass ratio of 0.2 by topo I and topo II showed notable differences: topo I catalyzed complete conversion of form I DNA to form IV DNA by 5 min, while topo II showed partial relaxation as evidenced by a broad distribution of topoisomers. Further, a small but significant amount of form I DNA persisted in the reactions after 5-min incubations (Fig. 3A, lanes 10 and 11). Densitometric scans of individual lanes displayed quantitative differ-

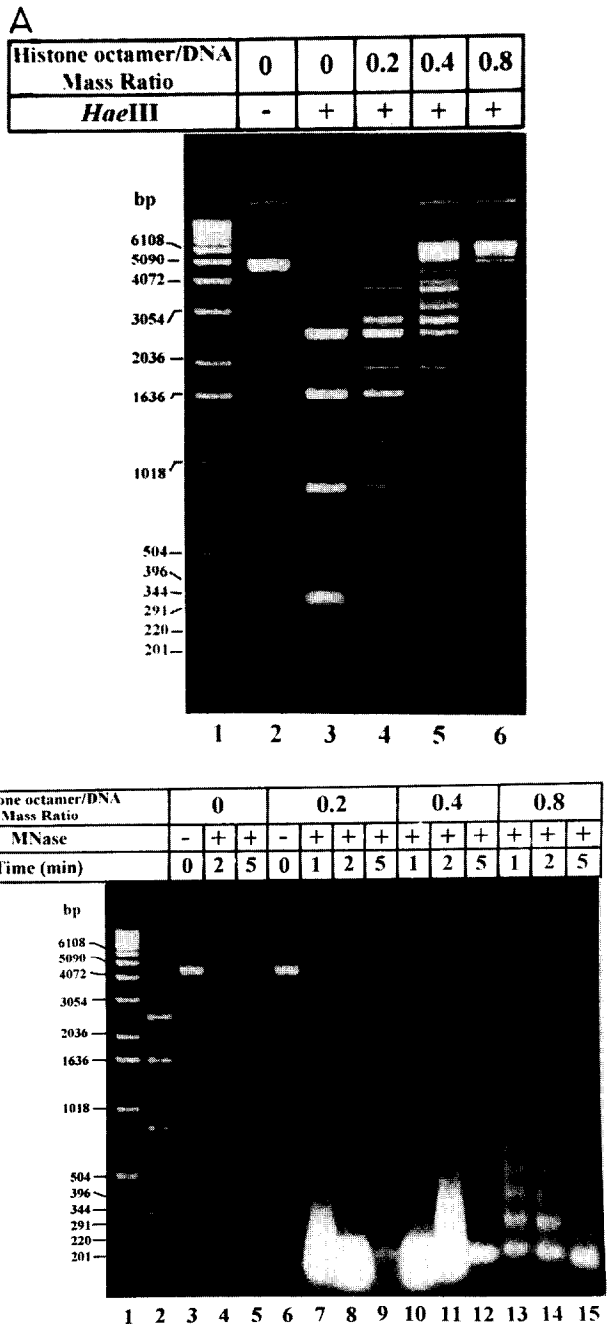

FIG. 1. Characterization of minichromosomes. (A) Protection of MCs from *Hae*III digestion. Naked form I DNA and synthetic MCs were digested with *Hae*III as described in Materials and Methods. Lane 1, 1 kb ladder; lane 2, naked form I M13 DNA (80% form I: 20% form II DNA); lane 3, same as lane 2 but digested with *Hae*III; lane 4, MCs containing a histone octamer/DNA mass ratio of 0.2 digested by *Hae*III; lane 5, MCs at a histone octamer/DNA mass ratio of 0.4, digested by *Hae*III; lane 6, MCs at a histone octamer/DNA mass ratio of 0.8, digested by *Hae*III. (B) Micrococcal nuclease digestion pattern. Naked form I DNA (1 μg) or an equivalent amount of DNA in MCs with specified mass ratios of histone octamer/DNA was incubated with MNase (10 units/mL) for the indicated time period. The samples were analyzed by electrophoresis as described in Materials and Methods.

ences in the distribution of topoisomers. At the end of 10-min incubations, about 30% of form I DNA persisted in the case of the topo II-driven reaction as opposed to the reaction catalyzed by topo I (Fig. 3B). However, it remained probable that the difference in the extent of relaxation of MCs by topo I and topo II was limited by the differential availability of recognition sites for each of these enzymes. To exclude such a possibility, we searched in the data base for consensus cleavage sites in M13 DNA [36] for both topo I [3, 37] and topo II [38, 39]. At degrees of homology > 90%, we detected at least 7 sites on M13 DNA, evenly spaced, for each enzyme. The enhanced activity of topo I on MCs is not due to stimulation by histone octamers, as it has been shown that histones, at the amounts used in this study, exert no positive effect [40]. Furthermore, under these experimental conditions, direct addition of histones to the reaction mixture had no discernible effect on the activity of topo I (data not shown). To establish the generality of the effect of nucleosomes on the catalytic activity of topoisomerases, we used topo I and topo II from calf thymus. In a similar manner, the activity of topo II, but not that of topo I, declined at lower mass ratios of histone octamer/DNA (data not shown).

Effect of Histone H1 on the Relaxation Activity of Topo II

The foregoing observations have illustrated that the relaxation of activity of topo II with MCs varies inversely to the

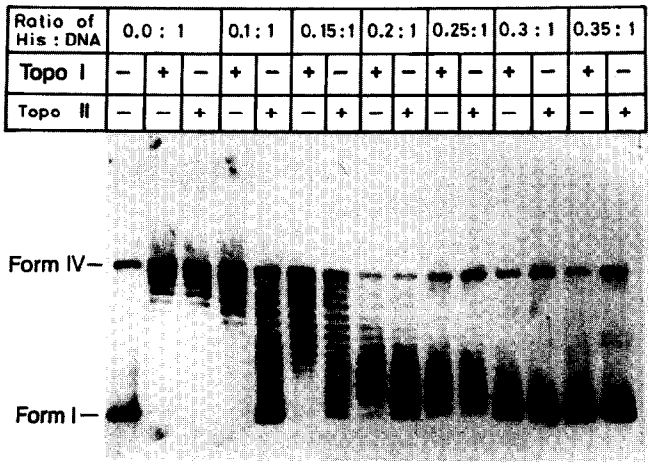

FIG. 2. Comparison of the efficiency of relaxation of naked DNA and MCs by topo I and topo II. Reaction mixtures containing 0.3 μg of naked form I M13 DNA, or MCs with the indicated mass ratios of histone octamer/DNA, were incubated with 1 unit of purified topo I (calf thymus) or topo II (rat testis), as described in Materials and Methods. Samples were incubated at 37° for 30 min, and the reaction was terminated by the addition of loading buffer. Individual samples were loaded onto a 1% agarose gel and electrophoresed as described in Materials and Methods.

mass ratios of histone octamer/DNA. Histome H1 is thought to mediate the formation of higher-order structure of eukaryotic chromatin in conjunction with histone octamers. We investigated the effect of varying amounts of histone H1 on the relaxation of naked DNA and MCs by topo II. Incubation of naked form I DNA with histone H1 inhibited relaxation in a concentration-dependent manner, and complete inhibition occurred at a histone H1/DNA mass ratio of 0.34 (Fig. 4). Likewise, histone H1 also caused a similar concentration-dependent inhibition with MCs; however, it required 7-fold less, and the extent of inhibition was more pronounced (Fig. 4, lanes 10–14).

Efficient Relaxation of Minichromosomes by Liver Topo II

Previously we showed that purified testis topo II differs both structurally and functionally from its counterpart in the liver [24]. Since the organization of chromatin in these two tissues is different [41], and to examine whether the observed differences are specific to the testicular enzyme, we analyzed and compared the catalytic efficiency of topo II from these two sources. Reactions were performed with topo II from testis or liver with naked DNA and at four ratios of histone octamer/DNA. Both the enzymes converted a significant portion of naked form I DNA to form IV DNA accompanied by a ladder of topoisomers (Fig. 5, lanes 2 and 3). However, at an identical amount of topo II, with MCs containing a histone octamer/DNA mass ratio of 0.1 and 0.2, topo II from liver caused complete relaxation, whereas the testis enzyme showed partial reaction (Fig. 5, compare lanes 5 and 7 vs 6 and 8). The relaxation activity of both the enzymes decreased with MCs containing increasing densities of nucleosomes. Further, the activity of testis topo II, but not that of the liver enzyme, was suppressed at a histone octamer/DNA mass ratio of 0.3 to 0.4 (Fig. 5, compare lane 10 vs 9).

Effect of Nucleosomes on the Action of Topo II-Directed Anti-tumor Drugs

By using cleavage assays, in conjunction with anti-tumor drugs, attempts have been made to map the recognition sites of topo II in SV40 minichromosomes and reconstituted chromatin [42, 43]. A caveat of these assays was that a single nick is sufficient to inflict DNA damage and trap the topo II–DNA intermediate; therefore, they do not provide details as to the overall catalytic efficiency of the enzyme. Consequently, we investigated the role of nucleosomes on the kinetics of relaxation of form I DNA by topo II in the presence of anti-tumor drugs such as teniposide, amsacrine, and amiloride. Amsacrine and amiloride belong to the DNA-intercalating group and teniposide to the non-DNA-intercalating group of inhibitors [15]. However, all of them modify topo II activity by stabilizing the covalent topo II–DNA intermediate, which appears to be the basis for their anti-tumor activity.

We first investigated the effects of increasing concentrations of amiloride and amsacrine on the ability of topo II to relax naked DNA and MCs. With both the substrates, relaxation activity of topo II was inhibited by amiloride and amsacrine in a concentration-dependent manner, and complete inhibition was achieved at 1 and 0.2 mM, respectively (data not shown). To explore further the effect of nucleosomes on the activity of topo II, in relation to the action of clinically important anti-tumor drugs, we used teniposide. The pattern of inhibition was different and intriguing: the presence of nucleosomes actually increased inhibition mediated by teniposide (Fig. 6). The IC_{50} required the inhibition of relaxation of naked DNA and MCs was in the range of 3.12 and 0.7 mM, respectively. To exclude the possibility that it is due to its non-intercalating nature, we studied the effect of distamycin, a non-DNA-intercalating and non-topo II drug, on relaxation of naked DNA and MCs by topo II. For both the substrates, the IC_{50} values were in the range of 50 μM (Fig. 7). It should be noted that the catalytic activity of topo II is enhanced by distamycin at low amounts (< 2 μM), whereas it is inhibited at higher concentrations [44, 45]. In this study, however, we used distamycin at inhibitory concentrations to assay its effect on the relaxation activity of topo II with naked DNA and DNA assembled into nucleosomes.

DISCUSSION

Our current understanding of the cellular function of mammalian topo II stems from *in vitro* studies with naked DNA and enzyme inhibitors [14–16]. The presence of nucleo-

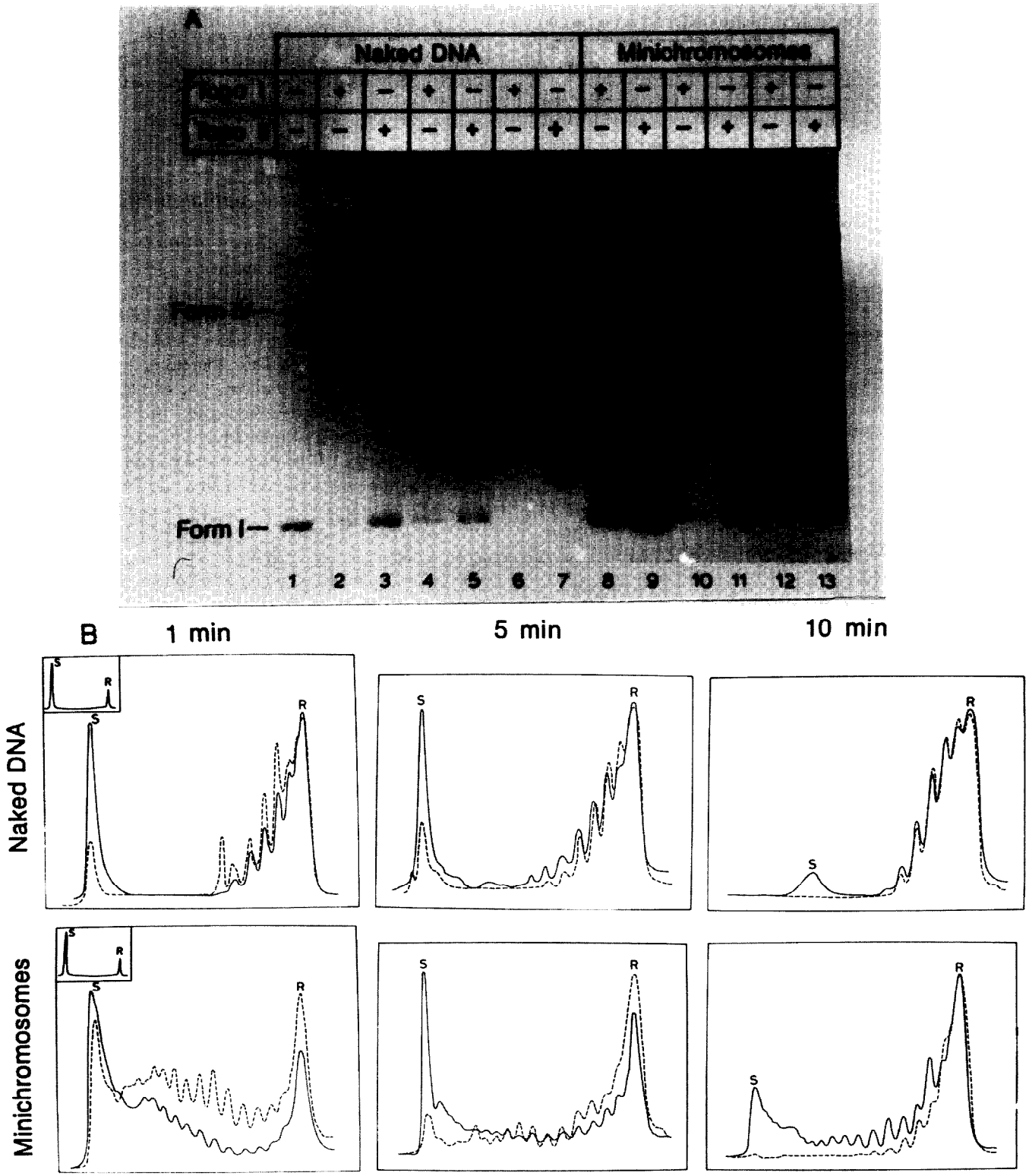

FIG. 3. Kinetics of topo I and topo II promoted relaxation of naked form I DNA and MCs. Reaction mixtures contained 0.3 μg of naked form I M13 DNA, or 0.3 μg of DNA in MCs, consisting of a histone octamer/DNA mass ratio of 0.2, with 1 unit of either topo I or topo II in a standard assay buffer as described in Materials and Methods. Samples were incubated at 37° for the times indicated below and were processed for electrophoresis as described in Materials and Methods. (A) Time course of relaxation. Lane 1, marker DNA (0.1 μg). Mixtures were incubated for 1 min (lanes 2, 3, 8, and 9); 5 min (lanes 4, 5, 10, and 11); or 10 min (lanes 6, 7, 12, and 13). (B) Densitometric tracing of individual lanes are shown for each sample from A. The autoradiogram was scanned as described in Materials and Methods. The dashed and continuous lines correspond to that of topo I and topo II reactions, and S and R represent the peak positions of negatively supercoiled and relaxed DNA, respectively. The upper panels represent the tracings of lanes 2–7 (naked DNA) and the lower panels show the tracings of lanes 8–13 (MCs). The inset depicts the tracing of lane 1.

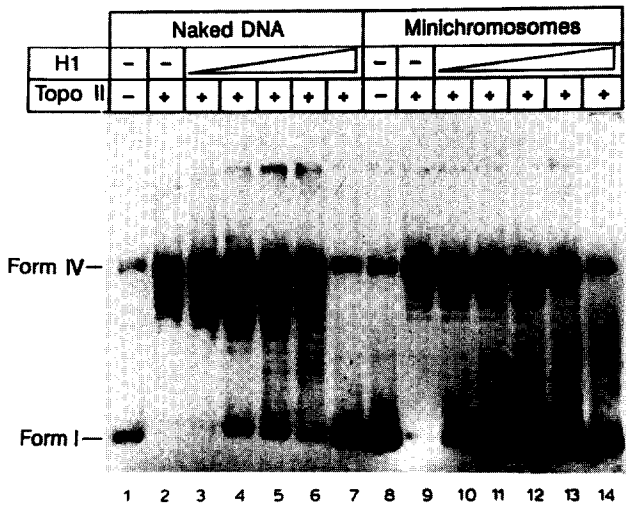

FIG. 4. Effect of histone H1 on relaxation of form I DNA and MCs promoted by topo II. Reaction mixtures contained 0.3 μg of naked form I M13 DNA or an equivalent DNA in MCs, at a histone octamer/DNA mass ratio of 0.2, in a standard assay buffer. Samples were incubated as described in Materials and Methods. Lanes 1 and 8, no enzyme; lanes 2 and 9, complete reaction in the absence of histone H1. Samples in the remaining lanes contained either naked DNA or MCs with histone H1/DNA at mass ratios of: 0.02/1 (lanes 3 and 10); 0.04/1 (lanes 4 and 11); 0.08/1 (lanes 5 and 12); 0.2/1 (lanes 6 and 13); and 0.34/1 (lanes 7 and 14).

somes and torsionally constrained superhelical domains in chromatin could present both steric occlusion and ionic barriers sufficient to make normal recognition of cognate DNA by topoisomerases rather unlikely. The understanding of the molecular processes of how topo II overcomes the many apparent impediments to its function, and the consequences for chromatin structure, are important issues. The assembly of nucleosomes by dialysis of mixtures of histone octamer and DNA from high salt to low salt has been established [29, 30]. The protection of MCs from *Hae*III, which has 10 cleavage sites that are periodically placed on M13 DNA, and a ~200 bp discrete repeating fragment of DNA generated by MNase is in good agreement with the presence of an array of nucleosomes [25].

Previous data have suggested that fragments of SV40 DNA reconstituted into chromatin or MCs were cleaved by topo I and topo II, albeit with reduced efficiency [38, 39, 46]. Under these experimental conditions, the relaxation activity was impeded with MCs containing similar mass ratios of nucleosomes. Nonetheless, these results are not necessarily at odds, and the differences appear to be due to the type of activity measured; unlike for the cleavage activity, the overall topological nature of DNA contributes to the relaxation activity of topoisomerases. The assembly of nucleosomes is highly favored on negatively superhelical DNA, and is driven by the progressive relaxation of the molecule until all the superhelical turns are titrated by nucleosomes [19, 30]. However, the data presented here suggest that form I DNA incorporated into nucleosomes is constrained topologically. It has been shown that the amount of negative supercoiling introduced by DNA gyrase was nearly identical for naked DNA and for DNA half-saturated with histone octamers [33]. Based on these findings, it has been proposed that at high levels of supercoiling, the nucleosomal structure is disrupted in such a way that the DNA bound to it is no longer constrained [33]. Further, by chemical modification and cross-linking of histones, Clark and Felsenfeld [30] have demonstrated that the conformation of histone octamers assembled on negatively superhelical DNA is unaltered. Thus, the features that contribute to the decline of relaxation of activity of testis topo II with MCs with higher mass ratios of histone octamer/DNA are obscure.

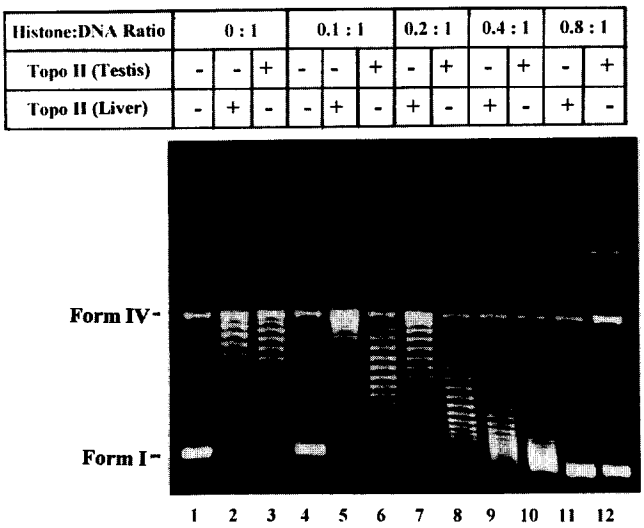

FIG. 5. Efficiency of relaxation of naked DNA and minichromosomes by topo II from testis and liver. Reaction mixtures contained 0.3 μg of naked form I M13 DNA or an equivalent amount of DNA in MCs, at the indicated mass ratios of histone octamer/DNA, with 1 unit of topo II from testis or liver. Samples were incubated at 37° for 30 min and then were analyzed by electrophoresis as described in Materials and Methods.

It has been demonstrated that the cleavage sites for topo I were suppressed in DNA incorporated into nucleosomes, but not in the linker regions [46]. By contrast, similar studies have not shown whether mammalian topo II cleaves DNA packaged into nucleosomes at the same sites as in naked DNA or in the linker regions. We found that the relaxation activity of testis topo II, but not that of the liver enzyme, declined with MCs as substrates. A number of studies have shown that the catalytic activity of topo II is enhanced by hyperphosphorylation as cultured cells enter the G_2/M phase of the cell cycle [47–49]. How can we reconcile the finding that testis topo II displays reduced activity compared with that of the liver enzyme with MCs as substrates? Although the phosphorylation status of topo II in meiotic cells is unknown, we think it is unlikely that the decrease in relaxation is due to lack of phosphorylation, as testis represents a bag of actively dividing germ cells. Further, with naked DNA the catalytic efficiency of testis topo II is similar to that of the liver enzyme, which excludes

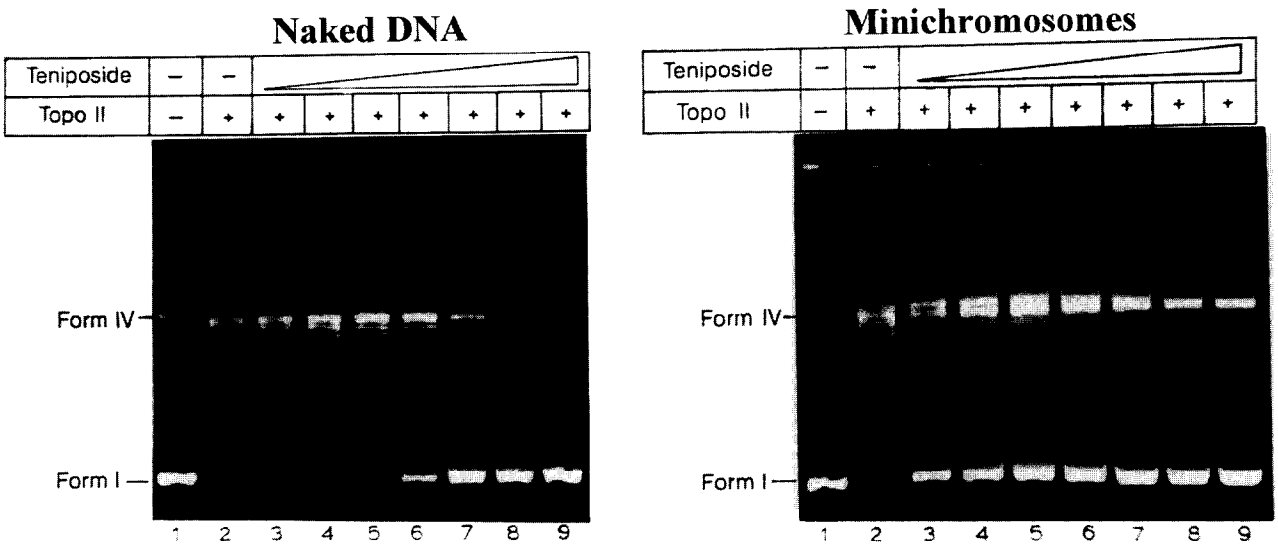

FIG. 6. Effect of teniposide on relaxation of naked form I DNA and MCs by topo II from testis. Reaction mixtures containing 0.3 μg of naked form I M13 DNA (left panel) or an equivalent amount of DNA in MCs (right panel), with a histone octamer/DNA mass ratio of 0.2, were incubated with topo II (1 unit) at 37° for 30 min. Samples were analyzed by electrophoresis as described in Materials and Methods. Lane 1, substrate DNA; lane 2, complete reaction in the absence of teniposide. The remaining lanes represent complete reactions containing teniposide at: lane 3, 0.78 mM; lane 4, 1.56 mM; lane 5, 2.34 mM; lane 6, 3.12 mM; lane 7, 3.9 mM; lane 8, 4.68 mM; and lane 9, 5.46 mM.

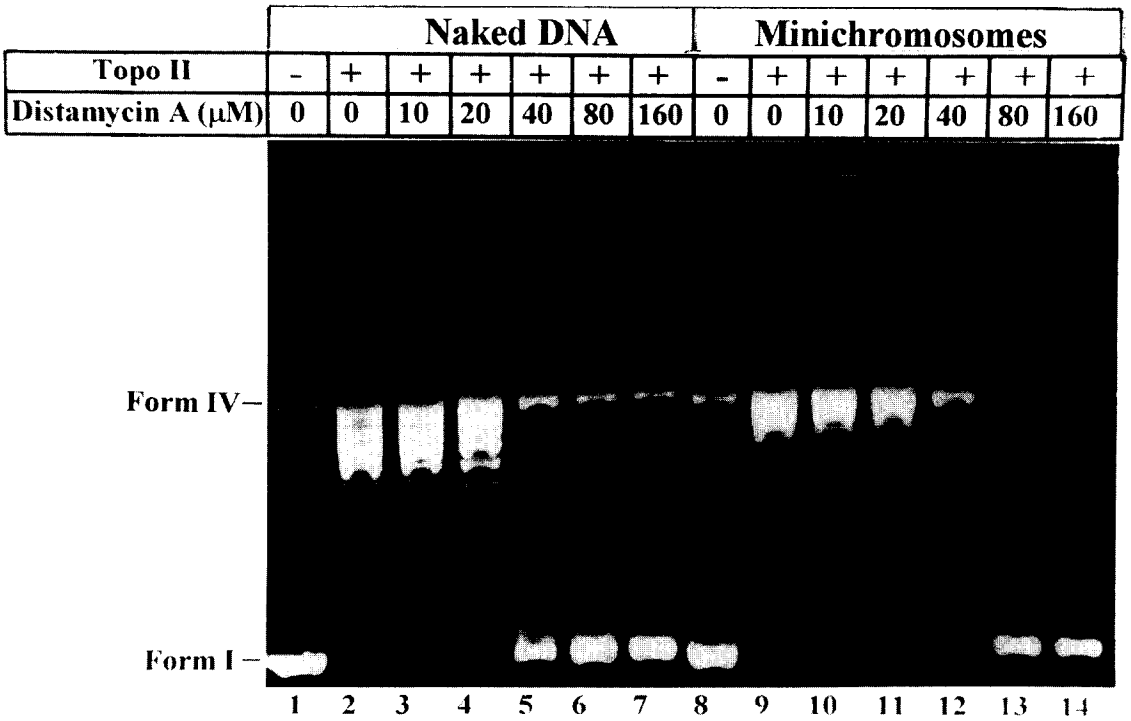

FIG. 7. Effect of distamycin on relaxation of naked form I DNA and MCs promoted by topo II from testis. Reaction mixtures contained 0.3 μg of naked form I M13 DNA (lanes 1–7) or 0.3 μg of DNA in MCs, with a histone octamer/DNA mass ratio of 0.2 (lanes 8–14). Samples were incubated with 1 unit of topo II at 37° for 30 min and then were analyzed by electrophoresis as described in Materials and Methods. Lanes 1 and 8, substrate DNA; lanes 2 and 9, complete reaction in the absence of distamycin. Samples in the remaining lanes represent complete reaction with distamycin: lanes 3 and 10, 10 μM; lanes 4 and 11, 20 μM; lanes 5 and 12, 40 μM; lanes 6 and 13, 80 μM; and lanes 7 and 14, 160 μM.

the possibility that lack of phosphorylation is the cause for decreased activity. However, it is possible that testis topo II binds preferentially to chromatin templates to assume a scaffolding role while diminishing its catalytic activity [6–8].

Several studies have suggested that a diverse group of drugs inhibit catalytic activity of topo II [13–16, 49]. To firmly establish the cellular role (s) of topo II, and to realize its chemotherapeutic potential, the kinetic and mechanistic aspects of the enzyme in relation to the action of antitumor drugs must be defined clearly. Correspondingly, studies with a few representative drugs and MCs have provided biochemical evidence for differences in their mechanism of action. Results of a series of comparative studies between naked DNA versus MCs in relation to the action of these drugs have displayed important differences. The differences appear to be significant with teniposide, which showed a 4-fold decrease in its IC_{50} dose between naked DNA and MCs. To our knowledge this is the first reported example where nucleosomes actually increased the teniposide-mediated inhibition of topo II. How could a nucleosomal DNA be better than naked DNA for the action of teniposide? It is possible that the interaction of teniposide with MCs is regulated by DNA structure, and higher IC_{50} values with naked DNA may be due to its non-specific interaction. Nonetheless, in view of a sizable difference in IC_{50} values between naked DNA and DNA incorporated into nucleosomes, caution should be exercised in extrapolating results of *in vitro* experiments to the *in vivo* situation. Further studies on the effect of topo II-targeted drugs *in vivo* and *in vitro* on the nucleohistone–nucleoprotamine transition should provide insights into the biological role of topo II in spermatogenesis.

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