Site-Specific Topoisomerase I-Mediated DNA Cleavage Induced by Nogalamycin: A Potential Role of Ligand-Induced DNA Bending at a Distal Site†

Sai-Peng Sim,‡ Daniel S. Pilch,‡,§ and Leroy F. Liu*,‡,§

Department of Pharmacology, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, New Jersey 08854, and Cancer Institute of New Jersey, New Brunswick, New Jersey 08901

Received April 20, 2000; Revised Manuscript Received June 7, 2000

ABSTRACT: Many DNA binding ligands (e.g., nogalamycin, actinomycin D, terbenzimidazoles, indolocarbazoles, nitidine, and coralyne) and various types of DNA lesions (e.g., UV dimers, DNA mismatches, and abasic sites) are known to stimulate topoisomerase I-mediated DNA cleavage. However, the mechanism(s) by which these covalent and noncovalent DNA interactions stimulate topoisomerase I-mediated DNA cleavage remains unclear. Using nogalamycin as a model, we have studied the mechanism of ligand-induced topoisomerase I-mediated DNA cleavage. We show by both mutational and DNA footprinting analyses that the binding of nogalamycin to an upstream site (from position −6 to −3) can induce highly specific topoisomerase I-mediated DNA cleavage. Substitution of this nogalamycin binding site with a DNA bending sequence (A_5) stimulated topoisomerase I-mediated DNA at the same site in the absence of nogalamycin. Replacement of the A_5 sequence with a disrupted DNA bending sequence (A_2-TA_2) significantly reduced the level of topoisomerase I-mediated DNA cleavage. These results, together with the known DNA bending property of nogalamycin, suggest that the nogalamycin−DNA complex may provide a DNA structural bend to stimulate topoisomerase I-mediated DNA cleavage.

Eukaryotic DNA topoisomerase I (TOP1)[1] is a nuclear enzyme that is important for many DNA functions such as replication and transcription (reviewed in ref *1*). It catalyzes relaxation of supercoils by a cleavage and religation mechanism (*2*). One of the key reaction intermediates is a covalent topoisomerase I−DNA complex in which topoisomerase I is covalently linked to the 3′-phosphoryl end of the transiently broken DNA strand through a tyrosine residue at the active site (*3*).

Studies of a topoisomerase I binding and cleavage hotspot from *Tetrahymena* rDNA have demonstrated that DNA bending elements located both upstream and downstream of this topoisomerase I cleavage hotspot are important for topoisomerase I binding and cleavage (*4*). It has been suggested that preferential binding of topoisomerase I to curved DNA is the basis for its preferential relaxation of supercoiled DNA by sensing the writhe (*4*).

Many anticancer compounds (e.g., camptothecin, actinomycin D, and indolocarbazoles), known as topoisomerase I poisons, abort the cleavage and religation reaction of topoisomerase I, resulting in the accumulation of the covalent reaction intermediate, the cleavable complex (reviewed in ref *5*). Recent studies have demonstrated that, for some of these topoisomerase I poisons, their interactions with DNA are critical for effective trapping of topoisomerase I cleavable complexes. For example, the DNA minor groove binding ligands Ho33342 and the terbenzimidazoles are known to trap topoisomerase I cleavable complexes at specific DNA sites (*6*, *7*). Their stimulation of site-specific topoisomerase I-mediated DNA cleavage has been correlated with their DNA minor groove binding properties (*8*). Furthermore, these compounds preferentially bind and stabilize bent DNA domains, suggesting a possible correlation between drug-induced DNA bending and TOP1 poisoning (*9*). The ability of protoberberines to stimulate topoisomerase I cleavable complexes also is correlated with their DNA binding properties (*10*). The importance of ligand−DNA interaction in topoisomerase I-mediated DNA cleavage also is echoed from recent studies on the effects of various DNA lesions on topoisomerase I-mediated DNA cleavage. For example, UV dimers, DNA mismatches, and abasic sites have been shown to modulate topoisomerase I-mediated DNA cleavage (*11*, *12*). In the aggregate, these studies suggest that both covalent and noncovalent modifications of DNA can be effective mechanisms for the stimulation of topoisomerase I-mediated DNA cleavage. The prototypic topoisomerase I poison, camptothecin, appears to be an exception because it does not bind to DNA alone (*13*). However, studies with an alkylating camptothecin analogue have also suggested that it interacts with DNA in the drug−DNA−enzyme ternary complex (*14*).

Nogalamycin (structure as shown in Figure 1B) is a DNA binding ligand (*15*). Its aglycon chromophore intercalates into the DNA double helix, while its nogalose and the aminoglucose moieties interact with the DNA minor and major grooves, respectively (*16*). Nogalamycin exhibits a

† This research was supported by NIH Grants CA5043374 and CA39662.

* To whom correspondence should be addressed: Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854. Telephone: (732) 235-4592. Fax: (732) 235-4073. E-mail: lliu@umdnj.edu.

‡ University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School.

§ Cancer Institute of New Jersey.

[1] Abbreviations: TOP1, topoisomerase I; CPT, camptothecin; Ho33342, Hoechst 33342; rDNA, ribosomal DNA.

10.1021/bi000906h CCC: $19.00
Published on Web 07/18/2000

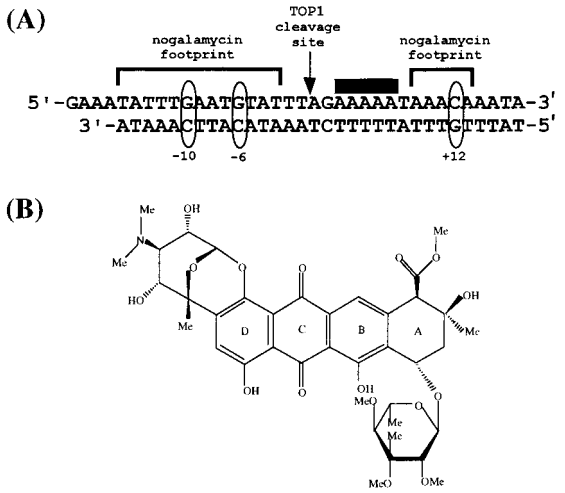

FIGURE 1: (A) Nogalamycin-stimulated TOP1-mediated DNA cleavage hotspot. The sequence represents the wild type DNA sequence used in this study. A TOP1-mediated DNA cleavage site is indicated by the vertical arrow. Previous DNase I footprinting studies (*18*) have revealed that nogalamycin binds to both upstream and downstream sites (see the bracketed areas). The circled G·C base pairs at positions −10, −6, and 12 are potential nogalamycin binding sites. The downstream A-tract is represented by a solid bar. (B) Chemical structure of nogalamycin.

strong preference for binding G·C base pairs embedded in AT-rich regions (*16*). NMR studies of a nogalamycin−oligonucleotide complex have revealed that nogalamycin binding induces a 22° bend at the binding site (*17*).

Our previous studies have demonstrated that nogalamycin can trap topoisomerase I cleavable complexes at specific sites on DNA (*18*). Studies of a nogalamycin-induced topoisomerase I cleavage hotspot have revealed that nogalamycin binds at distal regions both upstream and downstream from the site of cleavage (*18*) (also, see the nogalamycin footprints in Figure 1). However, the precise site for nogalamycin binding that is responsible for stimulation of topoisomerase I-mediated DNA cleavage was not determined. In the current study, we show that the nogalamycin binding site responsible for topoisomerase I cleavage is located upstream (positions −6 to −3) from the site of cleavage. When this upstream nogalamycin binding site was replaced with the DNA bending sequence A_5 (*19*), stimulation of topoisomerase I-mediated DNA cleavage at the same site was observed, in the absence of nogalamycin. Thus, a DNA bending sequence can substitute for the nogalamycin−DNA complex in stimulating topoisomerase I-mediated DNA cleavage. The possibility that nogalamycin stimulates topoisomerase I-mediated DNA cleavage by inducing a bend in the DNA within the ternary complex is discussed.

MATERIALS AND METHODS

Preparation of Oligonucleotide Substrates. Oligonucleotides were synthesized from Integrated DNA Technologies, Inc., and purified by denaturing polyacrylamide gel electrophoresis. Equal amounts of upper and lower strands in 50 mM Tris (pH 8.0), 10 mM $MgCl_2$, and 100 mM NaCl were heated to 90 °C and then allowed to cool slowly to room temperature. Fifty picomoles of annealed DNA substrate was labeled with [α-^{32}P]dATP using Klenow polymerase. Labeled DNA was purified using the QIAGEN nucleotide removal protocol.

TOP1-Mediated DNA cleavage. Calf thymus TOP1 was purified to near homogeneity by a procedure similar to that used for purification of calf thymus TOP2 (*20*). Annealed DNA substrate was incubated with different concentrations of nogalamycin in the reaction buffer [40 mM Tris (pH 7.5), 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM EDTA, and 30 μg/mL BSA] at 37 °C for 1 h. Camptothecin (CPT) was added to the control tube to a final concentration of 10 μM. Following addition of calf thymus TOP1, incubation was continued at 23 °C for 15 min. Reactions were terminated by adding SDS and proteinase K (1% SDS and 250 μg/mL proteinase K), followed by incubation at 37 °C for 1 h. Samples were extracted with a phenol/chloroform/isoamyl alcohol mixture and then precipitated with ethanol. Pellets were dissolved in formamide loading buffer (0.5× TBE, 0.1% bromophenol blue, 0.1% xylene cyanol, and 80% formamide), heated to 85 °C for 5 min, and then analyzed on a 15% denaturing polyacrylamide gel. The gel was transferred to a used X-ray film, covered with clear wrap, and exposed to X-ray film at −70 °C.

DNase I Footprinting. DNA substrate was incubated with different concentrations of nogalamycin in the presence of DNase I buffer [10 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 100 mM KCl] in a total volume of 7 μL at 37 °C for 1 h. Three microliters of diluted DNase I was added and incubation continued at 37 °C for 4 min. Thirty-five microliters of a precooled stop solution (92% ethanol and 0.54 M ammonium acetate) was added to stop the reaction. Ten micrograms of tRNA was added, and the mixture was kept in a dry ice/ethanol bath for 10 min. Samples were pelleted and dissolved in formamide loading buffer. After being heated at 85 °C for 5 min, samples were analyzed on an 8% denaturing polyacrylamide gel followed by autoradiography.

RESULTS

The G·C Base Pair Located at the −6 Position from the Site of Cleavage Is Critical for Nogalamycin-Induced Topoisomerase I-Mediated DNA Cleavage at the Hotspot. The DNA sequence surrounding the topoisomerase I cleavage hotspot induced by nogalamycin contains four G·C base pairs located at the −10, −6, 2, and 12 positions (refer to Figure 1A). These four G·C base pairs represent potential nogalamycin binding sites (*21*). Previous nogalamycin footprinting studies have ruled out the position 2 G·C base pair as a nogalamycin binding site (see Figure 1A and ref *18*). In the study presented here, we systematically mutated each of the other three G·C base pairs using the double-stranded oligonucleotides shown in Figure 2. As shown in Figure 2A, nogalamycin stimulates topoisomerase I-mediated cleavage at the hotspot (indicated by an arrow) in the wild type sequence. As shown previously, nogalamycin inhibited topoisomerase I-mediated DNA cleavage at other sites in a dose-dependent manner possibly due to its interference of topoisomerase I binding at these sites (*18*). Specific stimulation of topoisomerase I cleavage at the hotspot was also dose-dependent (Figure 2, lanes 4−8). It is interesting to note that this nogalamycin hotspot is only minimally stimulated by camptothecin (Figure 2A, lane 9, see the arrow). Mutation of the position −10 G·C to A·T (a G to A mutation on the top strand) had no effect on topoisomerase I cleavage at the hotspot (Figure 2B). Similarly, mutation of the position 12

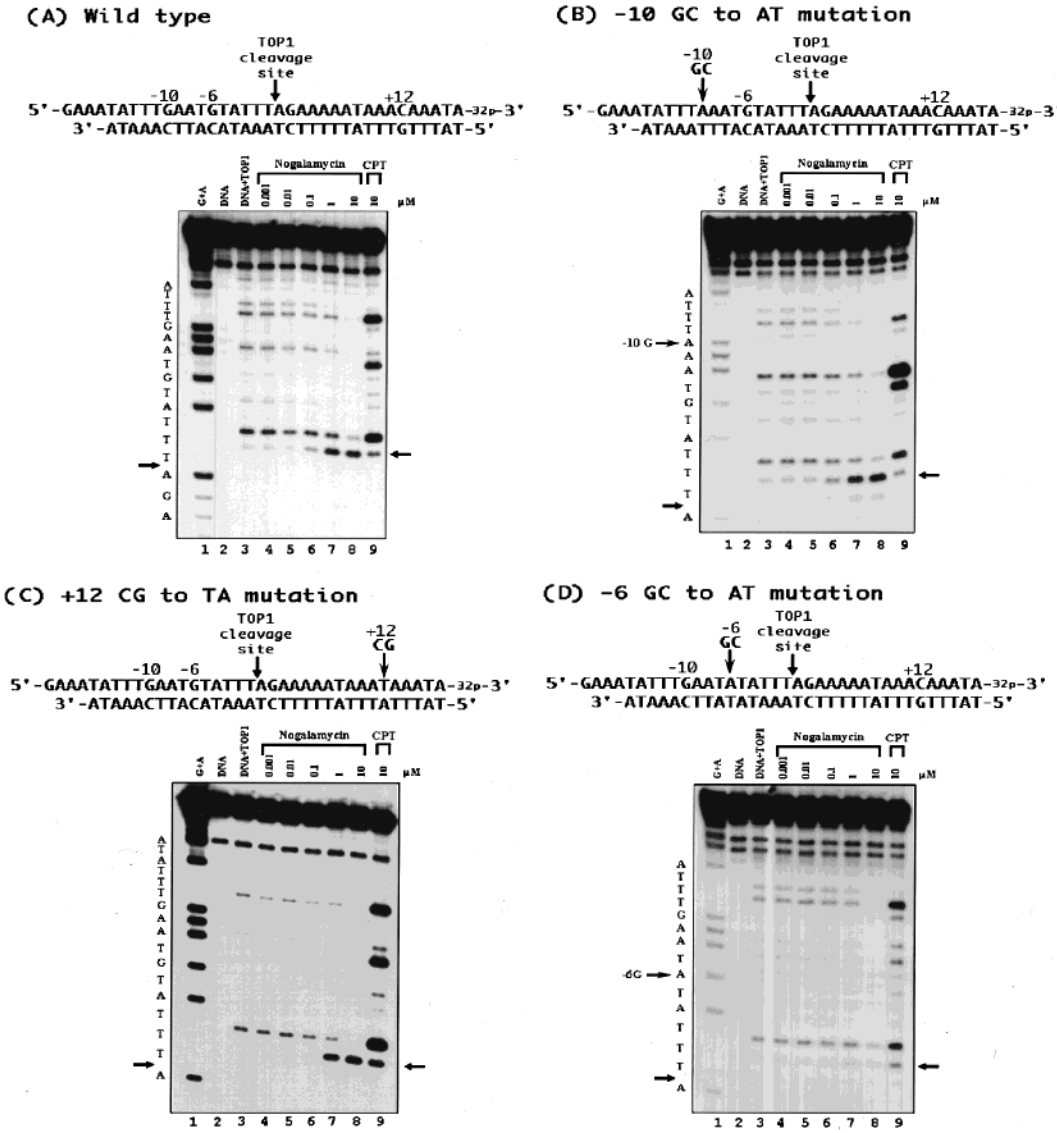

FIGURE 2: Sequential mutation of potential nogalamycin binding sites at positions −10, −6, and 12. TOP1-mediated DNA cleavage was performed (see Materials and Methods) using (A) the wild type sequence and sequences with sequentially mutated binding sites, namely, (B) the position −10 G·C to A·T mutation, (C) the position 12 C·G to T·A mutation, and (D) the position −6 G·C to A·T mutation. The right arrow indicates the nogalamycin-stimulated cleavage band, and the left arrow indicates the cleavage site in the sequence. The same cleavage site is also indicated on the respective sequences on the top of each gel. Increasing concentrations of nogalamycin are as shown, and CPT is included as a control.

C·G to T·A had no effect on topoisomerase I cleavage at the hotspot (Figure 2C). However, mutation of the position −6 G·C to A·T completely abolished nogalamycin-stimulated topoisomerase I cleavage at the hotspot (Figure 2D). These results suggest that the position −6 G·C base pair which is crucial for nogalamycin stimulation of topoisomerase I cleavage could be the critical nogalamycin binding site for topoisomerase I cleavage at the hotspot.

Mutational Analysis of DNA Sequences Downstream of the Cleavage Site. The nogalamycin-stimulated topoisomerase I cleavage sequence contains an A-tract sequence (A_5) located downstream from the site of cleavage at positions 3−7. This A-tract sequence has previously been shown to occur at the same position relative to the site of cleavage in the well-characterized *Tetrahymena* rDNA topoisomerase I binding and cleavage hotspot sequence (*4*). Studies of the *Tetrahymena* rDNA hotspot sequence have demonstrated that this A-tract sequence provides a DNA bend that is necessary for stimulation of topoisomerase I binding and cleavage. To test whether this A-tract DNA bending sequence is important for nogalamycin-stimulated topoisomerase I cleavage at the cleavage hotspot, the A_5 sequence was mutated to A_2TA_2. As shown in Figure 3A, this mutation did not affect nogalamycin-stimulated topoisomerase I cleavage at the hotspot. The same A_5 sequence together with its downstream sequence was also mutated to a nonbent sequence, $(TA)_6GA$ (Figure 3B). Again, this mutation did not affect nogalamycin-stimulated topoisomerase I cleavage at the hotspot. These results suggest that the downstream A-tract DNA bending sequence is not important for nogalamycin-stimulated topoisomerase I cleavage.

On the basis of the results from the mutational analysis described thus far, the critical element that is important for nogalamycin-stimulated topoisomerase I cleavage appears to be the position −6 G·C base pair. To test this possibility, multiple mutations were made on the hotspot sequence, including a position −10 G·C to A·T mutation, a position 12 C·G to T·A mutation, and substitution of the downstream

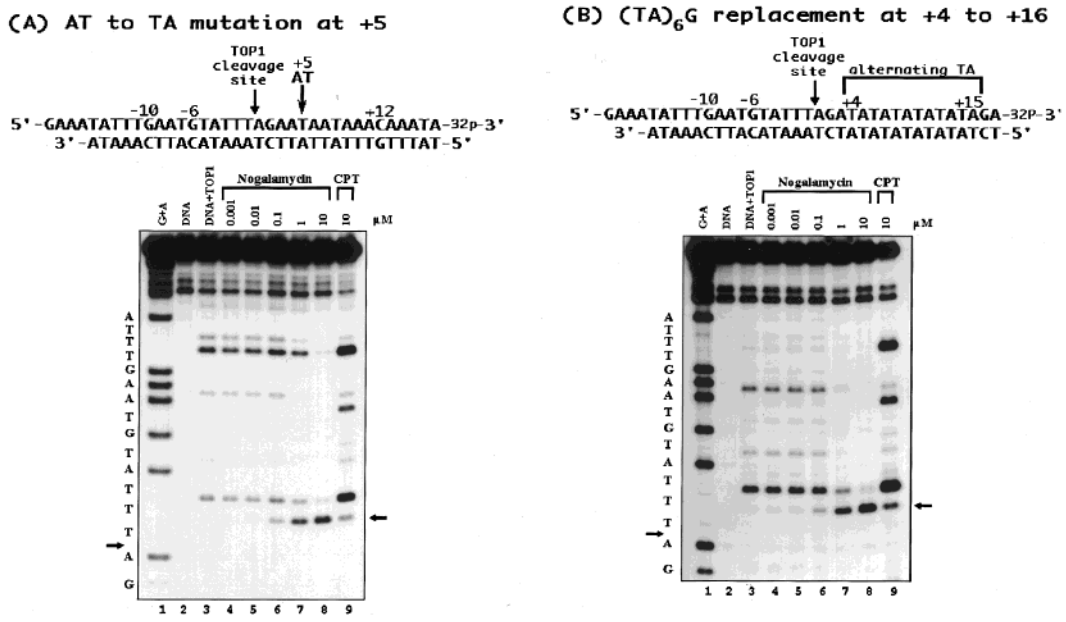

FIGURE 3: Mutational analysis of sequences downstream of the cleavage site. TOP1-mediated DNA cleavage was performed with increasing concentrations of nogalamycin using two sequences with a disrupted downstream A-tract: (A) the A·T to T·A mutation at position 5 and (B) the $(TA)_6G$ replacement at positions 4−16. The right arrow indicates the nogalamycin-stimulated cleavage band, and the left arrow indicates the cleavage site in the sequence.

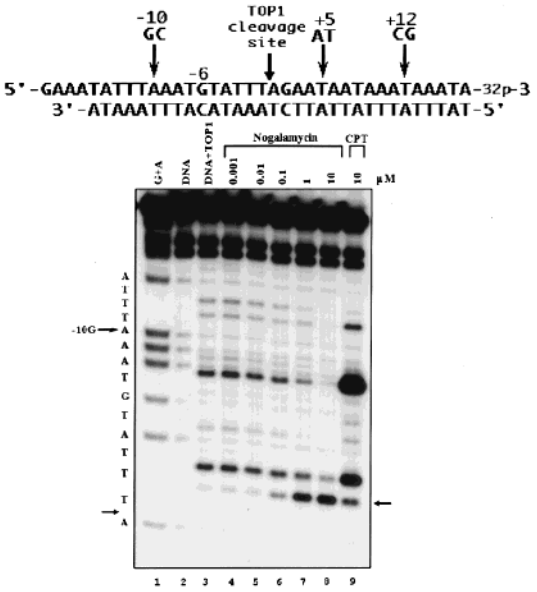

FIGURE 4: Potential nogalamycin binding site at position −6 as the only essential binding site for stimulating TOP1-mediated DNA cleavage. The sequence that is shown contains a single potential nogalamycin binding site at position −6. Binding sites at positions −10 and 12 were disrupted with G·C to A·T and C·G to T·A mutations, respectively. The downstream bend structure of the A-tract was abolished by an A·T to T·A mutation at position 5. The nogalamycin-induced cleavage band is denoted by the right arrow, and the corresponding cleavage site is denoted by the left arrow.

FIGURE 5: DNase I footprinting of nogalamycin binding to a sequence containing a single potential nogalamycin binding site. DNase I footprinting was carried out as described in Materials and Methods. Concentrations of nogalamycin are indicated at the top of each lane. Two concentrations of DNase I were used. The bracket encompasses the single nogalamycin footprint over the GTAT sequence.

A_5 sequence for A_2TA_2. As shown in Figure 4, nogalamycin-stimulated topoisomerase I cleavage at the hotspot was unaffected in the DNA sequence with these multiple mutations.

DNase I Footprinting Shows That Nogalamycin Binds to a Site That Encompasses the Position −6 G·C Base Pair. The importance of the position −6 G·C base pair in nogalamycin-stimulated topoisomerase I cleavage could be due to nogalamycin binding to the position −6 G·C base pair. To test this possibility, the multiply mutated sequence (see Figures 4 and 5) which contains the position −6 G·C base pair was used for DNase I footprinting in the presence of nogalamycin. As shown in Figure 5 (compare lanes 3−6, and 7−10), a single footprint extending from position −6 to −3 was observed (see the bracketed sequence marked nogalamycin footprint).

The Nogalamycin−DNA Complex Can Be Replaced with a DNA Bending Sequence To Stimulate Topoisomerase I Cleavage. Previous studies have suggested that DNA bending in both upstream and downstream sequences can stimulate

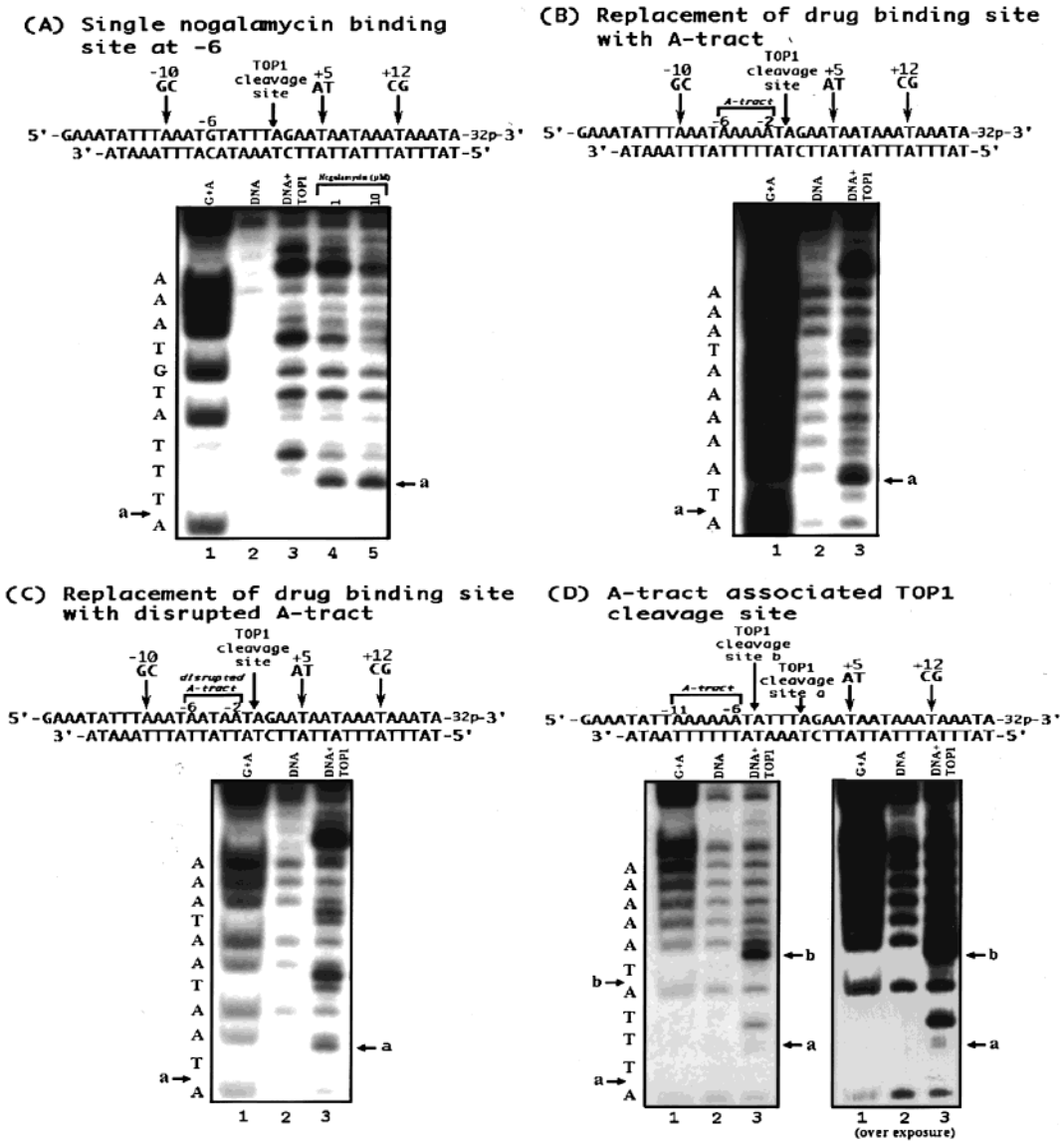

FIGURE 6: Upstream A-tract that induces TOP1-mediated DNA cleavage. TOP1-mediated DNA cleavage was performed using (A) a wild type sequence containing a single potential nogalamycin binding site at position −6, (B) a sequence with the single binding site replaced with an A-tract from position −6 to −2, (C) a sequence with the single binding site replaced with a disrupted A-tract A_2TA_2, and (D) a sequence with an A-tract further upstream from position −11 to −6. The right panel in panel D represents an overexposed film of the same gel shown on the left panel. The right arrow a denotes the cleavage band corresponding to the nogalamycin-induced cleavage hotspot. The right arrow b denotes an A-tract-associated TOP1-mediated DNA cleavage band. For the position of the A-tract, the TOP1 cleavage site a corresponding to band a and TOP1 cleavage site b corresponding to band b are as indicated on the sequence.

topoisomerase I cleavage (*4*). Nogalamycin is known to induce a DNA bend of 22° in a 2:1 complex with d(CGTACG)$_2$ (*17*). To test whether DNA bending may be responsible for nogalamycin-stimulated topoisomerase I cleavage, the nogalamycin binding site was replaced with a DNA bending A_5 sequence (GTATT was replaced with AAAAA from position −6 to −2) (see Figure 6B). The control sequence used in this experiment was the multiply mutated sequence containing a single nogalamycin binding site that includes the position −6 G·C base pair (see Figures 5 and 6A for the sequence). In the absence of nogalamycin, this insertion of the A_5 sequence in place of the nogalamycin binding site resulted in specific stimulation of topoisomerase I cleavage at the site marked with an arrow (labeled a in Figure 6B). This topoisomerase I cleavage site was mapped to the same site as the cleavage hotspot induced by nogalamycin in the control sequence (see the arrow labeled a in Figure 6A, lanes 4 and 5). Another mutant sequence, in which the nogalamycin binding site was replaced with a disrupted bending sequence (*22*) (GTATT was replaced with AATAA from position −6 to −2), also was tested (see Figure 6C). In the absence of nogalamycin, topoisomerase I cleavage at the hotspot (see the arrow labeled a in Figure 6C) was only weakly stimulated (compare lanes 3 in panels A and C of Figure 6). Compared with the A_5 sequence, the A_2TA_2 sequence was about 3-fold less effective in stimulating topoisomerase I cleavage at this hotspot in the absence of nogalamycin (compare lanes 3 in panels B and C of Figure 6). These results suggest that a DNA bending sequence located at the nogalamycin binding site can replace the nogalamycin−DNA complex to stimulate topoisomerase I cleavage.

To test whether a DNA bending A-tract sequence located at another upstream site can also replace the nogalamycin−DNA complex to stimulate topoisomerase I cleavage, a DNA bending A_6 sequence was created from position −11 to −6

by replacing TAAATG (positions −11 to −6) with AAAAAA (Figure 6D). Previous studies using *Tetrahymena* rDNA have suggested that a DNA bending A-tract at positions −11 to −6 can enhance topoisomerase I cleavage (*4*). As shown in Figure 6D, the DNA bending AAAAAA sequence located at positions −11 to −6 can only weakly stimulate topoisomerase I cleavage at the expected site (Figure 6D, right panel, see arrow a). Instead, a cleavage site (Figure 6D, left panel, see arrow b) located immediately downstream of the A_6T sequence is greatly enhanced. This result suggests that the location of the A-tract upstream of the cleavage site is important for specifying the site of topoisomerase I cleavage.

DISCUSSION

Sequence analysis of topoisomerase I cleavage sites induced by the topoisomerase I poison camptothecin has suggested that camptothecin binds with a preference for position 1 G from the site of cleavage (*23*). Studies with alkylating camptothecin have also supported such a conclusion (*14*). Uracil-containing DNA, abasic sites, and DNA mismatches have been shown to induce topoisomerase I-mediated DNA cleavage at sites immediately upstream from the structural alterations (*11*). In contrast, our studies of a nogalamycin−DNA complex have established that nogalamycin binds at a distal upstream site with a footprint covering nucleotides from position −6 to −3 on the scissile strand. This distal ligand binding may offer an advantage for studying the mechanism of topoisomerase I poisoning, since a DNA structural perturbation induced by ligand binding to a distal site is not likely to interfere directly with the cleavage and religation chemistry.

Using a topoisomerase I binding and cleavage hotspot sequence from *Tetrahymena* rDNA, the interaction between topoisomerase I and DNA has been analyzed by footprinting, modification interference, and in vitro cleavage using duplex DNA with defined length (*24*, *25*). These studies have suggested that the major interaction between topoisomerase I and DNA occurs from position −7 to 2 on the scissile strand, with positions −7 to −2 being the essential region of contact. X-ray crystallographic studies of a reconstituted topoisomerase I−oligonucleotide complex (in both covalent and noncovalent forms) have revealed an interaction between topoisomerase I and the phosphate groups from position −4 to 3 on the scissile strand (*26*). Our study reveals that the nogalamycin binding site extends from position −6 to −3 on the scissile strand, which partially overlaps with topoisomerase I−DNA contacts suggested from both biochemical and structural studies (*24*−*26*). On the basis of the known preference of nogalamycin binding for 5′-TG-3′ (*27*−*29*), the site of nogalamycin intercalation (through its aglycon chromophore) most likely occurs between the positions −7 and −6 bases upstream of the cleavage site. The nogalamycin footprint from position −6 to −3 on the scissile strand could reflect the interaction between the DNA helical grooves and the sugar moieties of nogalamycin.

Nogalamycin binding is known to alter the host DNA in a number of ways. It unwinds the DNA by 16° (*30*), and causes the base pairs to buckle, thereby expanding the minor groove of the DNA (*16*, *30*, *31*). In addition, NMR studies on a 2:1 nogalamycin−oligonucleotide complex have suggested that nogalamycin causes a DNA bend of 22° (*17*). Either of these structural alterations could, in principle, stimulate topoisomerase I-mediated DNA cleavage. Previous studies have demonstrated that topoisomerase I prefers bent DNA over normal DNA (*32*, *33*), as well as superhelical DNA over relaxed DNA (*34*). Consequently, we have studied the possible role of DNA bending in the induction of topoisomerase I-mediated DNA cleavage by nogalamycin.

In this study, we have shown that, in the absence of nogalamycin, replacement of the nogalamycin binding site GTATT (located at positions −6 to −2) with the DNA bending sequence A_5 results in the stimulation of DNA cleavage at the same site induced by nogalamycin. Insertion of the partially disrupted DNA bending sequence A_2TA_2 yielded a 3-fold lower stimulatory effect. These results are consistent with the notion that distal binding of nogalamycin may stimulate topoisomerase I-mediated DNA cleavage via the formation of a DNA bend located upstream of the site of cleavage.

The position of the DNA bend relative to the site of DNA cleavage is probably quite important. When a DNA bending A_6 sequence was used to replace the sequence TAAATG (located at positions −11 to −6), stimulation of DNA cleavage at the original nogalamycin cleavage hotspot was still observed, but the degree of stimulation was dramatically reduced (a more than 6-fold reduction relative to the A_5 sequence located at positions −6 to −2). Significantly, a cleavage site located immediately downstream of the A_6T sequence was greatly enhanced. The enhancement of TOP1-mediated DNA cleavage at this site could be due to the enhanced DNA bending propensity of the A_6T sequence relative to the TA_3TG sequence. Previous studies have demonstrated that topoisomerase I cleavage occurs immediately downstream of A_nT sequences (*35*).

Our results can be accommodated by a curvature model for the topoisomerase I−DNA interaction. In this model, topoisomerase I binds with high affinity to curved DNA and preferentially relaxed superhelical DNA by sensing the writhe (*4*). The interactions between topoisomerase I and the charged phosphate atoms of the DNA may be enhanced due to the curvature of the DNA in the topoisomerase I−DNA complex (*26*). The effect of the DNA bend may depend on both the sequence preference of topoisomerase I and the exact position of the bend relative to the site of cleavage, since the interaction between the phosphate atoms in the DNA backbone and the charged amino acid residues on topoisomerase I may not be uniform along the curvature. The curvature model also predicts that increased local DNA flexibility (e.g., mismatches, nicks, and gaps) within the topoisomerase I−DNA complex may enhance topoisomerase I binding. Clearly, more experiments are necessary to establish the role of DNA bending in topoisomerase I poisoning.

If the DNA bending model for topoisomerase I poisoning by nogalamycin is correct, it may be applicable to some other topoisomerase I-poisoning DNA binding ligands (e.g., bi- and terbenzimidazoles and actinomycin D) and/or DNA lesions (e.g., UV adducts, abasic sites, and mismatches). It is known that actinomycin D and UV adducts bend DNA by 16° and 21.7°, respectively (*36*, *37*). The terbenzimidazoles also have been shown to modulate DNA bending (*9*).

Abasic sites and DNA mismatches are likely to increase the local flexibility of DNA, and thereby could facilitate DNA bend formation within the topoisomerase I−DNA complex. However, it is also possible that multiple mechanisms exist for stimulation of topoisomerase I-mediated DNA cleavage. Unlike nogalamycin, some of the agents (e.g., camptothecin) and/or DNA lesions (e.g., abasic sites and mismatches) are known to act at or near the site of cleavage, which could suggest a different mechanism of action. For example, camptothecin has been proposed to displace the position 1 base pair immediately downstream of the cleavage site to prevent religation (*26*).

Topoisomerase I binding to bent DNA domains or to DNA with increased local flexibility may be biologically significant. One could speculate that cellular repair of damaged DNA sites may involve DNA repair helicases and/or chromatin remodeling. Recruitment of topoisomerase I to these local regions may be necessary to remove the excessive supercoils generated during repair. Further studies are necessary to establish the potential biological role of topoisomerase I binding to DNA bends and/or DNA with local flexibility. This study suggests that nogalamycin can be used as a model to study the interactions of topoisomerase I with bent DNA domains.

ACKNOWLEDGMENT

We are grateful to Dr. Barbara Gatto for her technical help in the course of this work.

REFERENCES

1. Wang, J. C. (1996) *Annu. Rev. Biochem. 65*, 635−692.
2. Champoux, J. J., and Dulbecco, R. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 143−146.
3. Champoux, J. J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3800−3804.
4. Krogh, S., Mortensen, U. H., Westergaard, O., and Bonven, B. J. (1991) *Nucleic Acids Res. 19*, 1235−1241.
5. Chen, A. Y., and Liu, L. F. (1994) *Annu. Rev. Pharmacol. Toxicol. 34*, 191−218.
6. Chen, A. Y., Yu, C., Gatto, B., and Liu, L. F. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 8131−8135.
7. Sun, Q., Gatto, B., Yu, C., Liu, A., Liu, L. F., and LaVoie, E. J. (1995) *J. Med. Chem. 38*, 3638−3644.
8. Xu, Z., Li, T. K., Kim, J. S., LaVoie, E. J., Breslauer, K. J., Liu, L. F., and Pilch, D. S. (1998) *Biochemistry 37*, 3558−3566.
9. Pilch, D. S., Xu, Z., Sun, Q., LaVoie, E. J., Liu, L. F., and Breslauer, K. J. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 13565−13570.
10. Pilch, D. S., Yu, C., Makhey, D., LaVoie, E. J., Srinivasan, A. R., Olson, W. K., Sauers, R. R., Breslauer, K. J., Geacintov, N. E., and Liu, L. F. (1997) *Biochemistry 36*, 12542−12553.
11. Pourquier, P., Ueng, L. M., Kohlhagen, G., Mazumder, A., Gupta, M., Kohn, K. W., and Pommier, Y. (1997) *J. Biol. Chem. 272*, 7792−7796.
12. Subramanian, D., Rosenstein, B. S., and Muller, M. T. (1998) *Cancer Res. 58*, 976−984.
13. Hsiang, Y. H., Hertzberg, R., Hecht, S., and Liu, L. F. (1985) *J. Biol. Chem. 260*, 14873−14878.
14. Pommier, Y., Kohlhagen, G., Kohn, K. W., Leteurtre, F., Wani, M. C., and Wall, M. E. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 8861−8865.
15. Neogy, R. K., Chowdhury, K., and Thakurta, G. G. (1973) *Biochim. Biophys. Acta 299*, 241−244.
16. Liaw, Y. C., Gao, Y. G., Robinson, H., van der Marel, G. A., van Boom, J. H., and Wang, A. H. (1989) *Biochemistry 28*, 9913−9918.
17. Robinson, H., Yang, D., and Wang, A. H. (1994) *Gene 149*, 179−188.
18. Sim, S. P., Gatto, B., Yu, C., Liu, A. A., Li, T. K., Pilch, D. S., LaVoie, E. J., and Liu, L. F. (1997) *Biochemistry 36*, 13285−13291.
19. Wu, H. M., and Crothers, D. M. (1984) *Nature 308*, 509−513.
20. Halligan, B. D., Edwards, K. A., and Liu, L. F. (1985) *J. Biol. Chem. 260*, 2475−2482.
21. Fox, K. R., and Alam, Z. (1992) *Eur. J. Biochem. 209*, 31−36.
22. Koo, H. S., Wu, H. M., and Crothers, D. M. (1986) *Nature 320*, 501−506.
23. Jaxel, C., Capranico, G., Kerrigan, D., Kohn, K. W., and Pommier, Y. (1991) *J. Biol. Chem. 266*, 20418−20423.
24. Stevnsner, T., Mortensen, U. H., Westergaard, O., and Bonven, B. J. (1989) *J. Biol. Chem. 264*, 10110−10113.
25. Svejstrup, J. Q., Christiansen, K., Andersen, A. H., Lund, K., and Westergaard, O. (1990) *J. Biol. Chem. 265*, 12529−12535.
26. Redinbo, M. R., Stewart, L., Kuhn, P., Champoux, J. J., and Hol, W. G. (1998) *Science 279*, 1504−1513.
27. Fox, K. R., and Waring, M. J. (1986) *Biochemistry 25*, 4349−4356.
28. Searle, M. S., Hall, J. G., Denny, W. A., and Wakelin, L. P. (1988) *Biochemistry 27*, 4340−4349.
29. Zhang, X. L., and Patel, D. J. (1990) *Biochemistry 29*, 9451−9466.
30. Searle, M. S., and Bicknell, W. (1992) *Eur. J. Biochem. 205*, 45−58.
31. Egli, M., Williams, L. D., Frederick, C. A., and Rich, A. (1991) *Biochemistry 30*, 1364−1372.
32. Caserta, M., Amadei, A., Di Mauro, E., and Camilloni, G. (1989) *Nucleic Acids Res. 17*, 8463−8474.
33. Camilloni, G., Caserta, M., Amadei, A., and Di Mauro, E. (1991) *Biochim. Biophys. Acta 1129*, 73−82.
34. Madden, K. R., Stewart, L., and Champoux, J. J. (1995) *EMBO J. 14*, 5399−5409.
35. Shen, C. C., and Shen, C. K. (1990) *J. Mol. Biol. 212*, 67−78.
36. Reinert, K. E. (1991) *J. Biomol. Struct. Dyn. 9*, 331−352.
37. Yamaguchi, H., van Aalten, D. M., Pinak, M., Furukawa, A., and Osman, R. (1998) *Nucleic Acids Res. 26*, 1939−1946.

BI000906H