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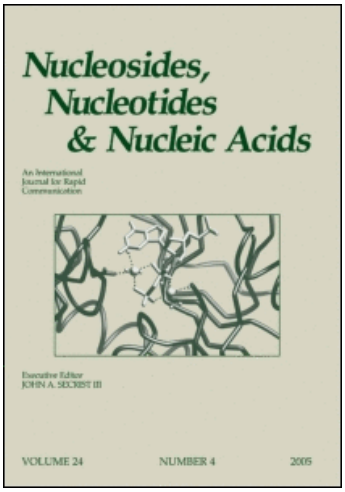

Nucleosides, Nucleotides and Nucleic Acids
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To cite this Article Arslan, Tuncer , Abraham, Anil T. and Hecht, Sidney M.(1998) 'Structurally Altered Substrates for DNA Topoisomerase. I. Effects of Inclusion of a Single 3'-Deoxynucleotide Within the Scissile Strand', Nucleosides, Nucleotides and Nucleic Acids, 17: 1, 515 — 530
To link to this Article: DOI: 10.1080/07328319808005195
URL: http://dx.doi.org/10.1080/07328319808005195

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NUCLEOSIDES & NUCLEOTIDES, 17(1-3), 515-530 (1998)

STRUCTURALLY ALTERED SUBSTRATES FOR DNA TOPOISOMERASE I. EFFECTS OF INCLUSION OF A SINGLE 3'-DEOXYNUCLEOTIDE WITHIN THE SCISSILE STRAND†

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ABSTRACT: A partial DNA duplex containing a high efficiency topoisomerase I cleavage site was substituted singly at each of three sites with 3'-deoxyadenosine. Depending on the site of substitution, the facility of the topoisomerase I-mediated cleavage or ligation reactions was altered. Inclusion of the modified nucleoside at the 5'-end of the acceptor oligonucleotide diminished the rate of religation following substrate cleavage by the enzyme.

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INTRODUCTION

Eukaryotic DNA topoisomerases play an important role in controlling DNA higher order structure.[1,2] Through the introduction of transient breaks in the DNA phosphate ester backbone, the topoisomerases effect relaxation of supercoils in DNA structure, and thereby facilitate DNA replication and transcription.[3,4] They are also implicated in DNA recombination.[5-7] Because of their essential role in critical cellular processes, the topoisomerases have become important targets for the development of antitumor agents.[8-12]

Eukaryotic DNA topoisomerases can be classified into groups according to their mode of action. The type I DNA topoisomerases effect the transient breakage of a single strand of DNA, while type II DNA topoisomerases mediate the breakage of both DNA strands; the latter enzymes require ATP.[1,2,13]

The cleavage of DNA by topoisomerase I may be envisioned as a nucleophilic attack of an active site tyrosine OH group on a phosphodiester bond in

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†This paper is dedicated to the memory of our friend and colleague, Prof. Tsujiaki Hata

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DNA, resulting in the covalent attachment of the enzyme to the DNA substrate via a 3'-*O*-phosphorotyrosine bond, with concomitant relase of a DNA oligonucleotide having a 5'-OH group (FIG. 1).[14] The cleavage and religation reactions are in equilibrium. While the ligation reaction is normally strongly favored,[15] the position of equilibrium can be altered experimentally, e.g. by the manipulation of experimental conditions[16,17] or alteration of DNA substrate structure.[18,19]

The cleavage and ligation reactions can also be studied separately, for example by site-specific cleavage of single-stranded DNA,[20,21] the use of double-stranded DNA substrates having nicks in the noncleaved strand[22] or partial DNA duplexes.[23,24] The covalent topoisomerase I - DNA binary complex resulting from uncoupling of the cleavage and ligation reactions can be purified[19] and undergo ligation with DNA acceptors to afford DNA products.[20-25] In addition to religation to complementary acceptors,[23-25] nonhomologous acceptors have also been observed to afford ligation products albeit often with lesser efficiency.[25-27] Henningfeld et al. have recently demonstrated the ligation of acceptor oligonucleotides modified at the 5'-end such that the ligation products had altered connectivity.[19]

In our earlier study, one interesting finding was that an acceptor oligonucleotide having a 5'-terminal deoxyadenosine analogue with a homologated sugar at the 5'-position (**I**) was capable of acting as a substrate for religation with a topoisomerase I-DNA binary complex having a single-stranded region on the noncleaved strand complementary to the modified acceptor. Presently, we have extended this finding by employing a modified acceptor oligonucleotide having the 5'-terminal deoxyadenosine linked to the remainder of the oligomer through a 2' → 5' linkage (**II**) (FIG. 1).

In addition, three modified partial duplexes, each containing a single 3'-deoxyadenosine within the DNA topoisomerase I binding region on the scissile strand, were utilized to further characterize the mechanism of topoisomerase I-mediated cleavage and ligation. Depending on the location of the 3'-deoxyadenosine moiety relative to the cleavage site, the forward or reverse reaction was altered.

RESULTS AND DISCUSSION

While 3'-deoxyadenosine (cordycepin) is commercially available, it is also quite expensive so we chose to prepare this nucleoside by chemical synthesis. 3'-Deoxynucleosides have been prepared from commercially available ribonucleosides and also by the coupling of suitably protected nucleobases to modified DNA sugars.[31,32] Because the later strategy affords considerable flexibility in the range of modified nucleosides that can be prepared, we chose to prepare 3'-deoxyadenosine by the latter strategy (FIG. 2).

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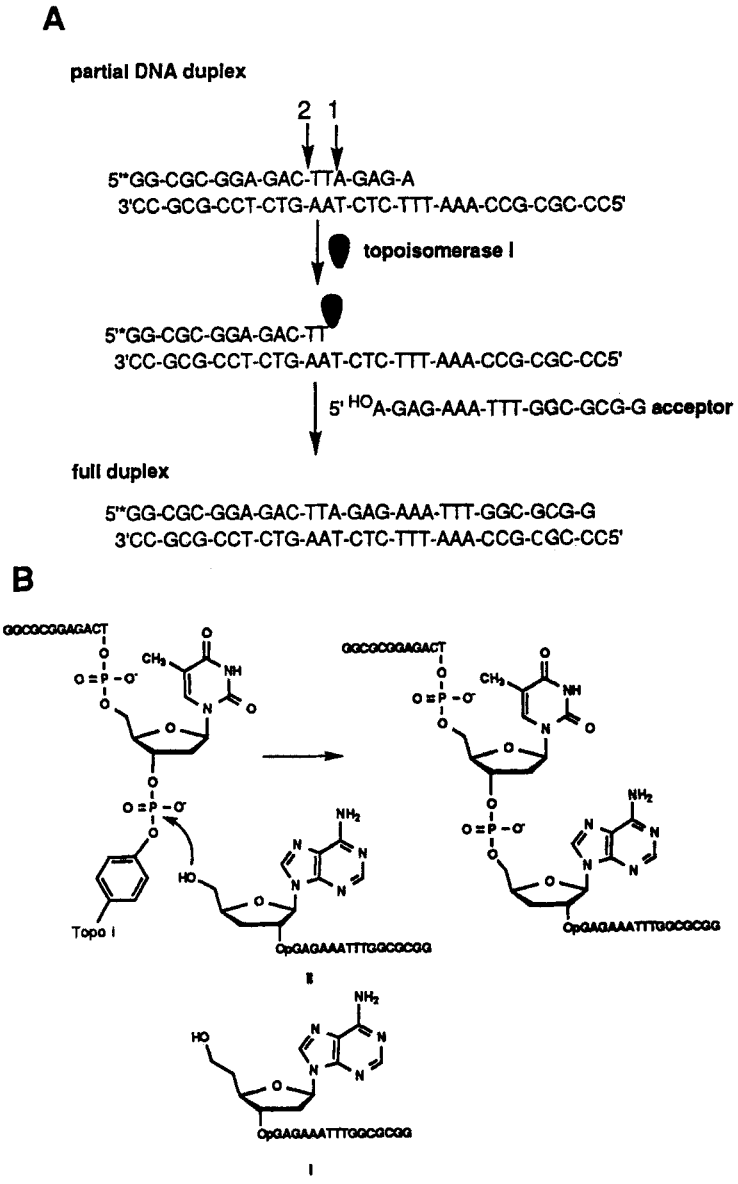

FIG. 1. (A) DNA substrate utilized to uncouple the cleavage and ligation reactions of topoisomerase I, thereby allowing DNA strand exchange. (B) Topoisomerase I-mediated ligation reaction.

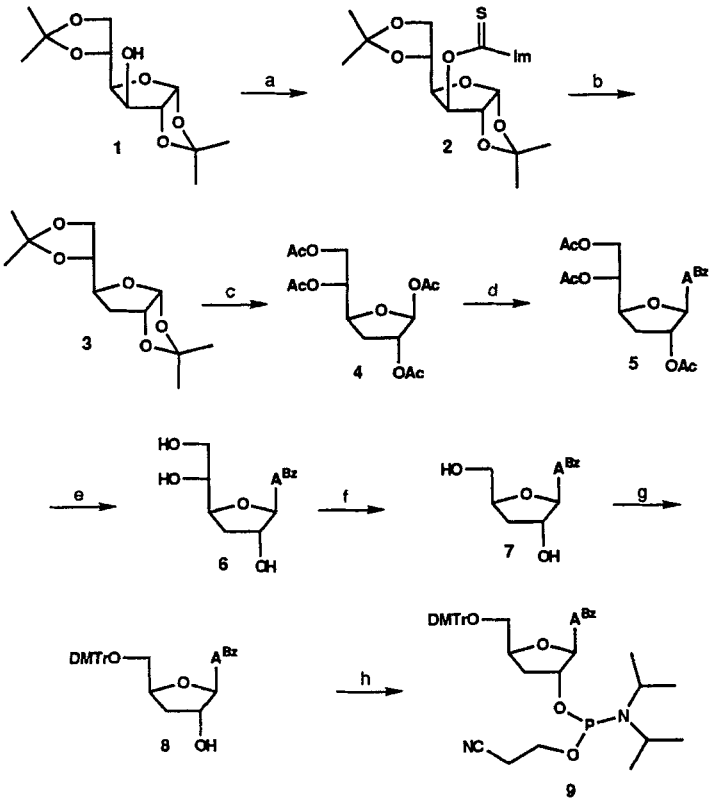

FIG. 2. (a) Im_2CS, THF, 65 °C, 97%; (b) Bu_3SnH, AIBN, toluene, 110 °C; (c) Ac_2O, AcOH, TsOH, 79%; (d) bisTMS-A^{Bz}, TMSOTf, CH_3CN, 55 °C, 12 h, 61%; (e) 2N NaOH, EtOH-pyridine, 77%; (f) $NaIO_4$, THF-H_2O, $NaBH_4$, MeOH, 72%; (g) DMTrCl, pyridine, 69%; (h) $NCCH_2CH_2ClPN(iPr)_2$, $EtN(iPr)_2$, CH_2Cl_2, 70%.

Preparation of N^6-Benzoyl-3'-deoxyadenosine. The synthesis started from diacetone glucose (**1**). The hydroxyl group of **1** was thioacylated[33-35] by treatment with thiocarbonyldiimidazole in THF to give **2** in 97% yield. The thioacylated sugar **2** was deoxygenated by the Barton procedure[36,37] to afford **3** in 98% yield. Treatment of **3** with $HOAc/Ac_2O$ in the presence of catalytic *p*-TsOH at reflux afforded tetraacetate **4** in 79% yield. Introduction of the adenine moiety was carried out by modification[38,39] of the Hilbert-Johnson reaction.[40,41] In this fashion, all acidic protons of the nucleobase were replaced with silyl groups, increasing the nucleophilicity such that reaction with the sugar acetal obtains in the presence of a Lewis acid such as trimethylsilyl trifluoromethane-sulfonate (TMSOTf). In the case of

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2-*O*-acyl protected ribose, the acyl group stabilizes the oxonium ion from the α face of the molecule, so the incoming nucleobase can attack only from the β face; therefore, the β anomer is the sole product.[42,43] Thus, *bis*-TMS-A^{Bz} was incorporated into tetraacetate **4** to give modified nucleoside **5** in 61% yield as a single isomer. The acetyl groups of **5** were then selectively deprotected by treatment with sodium hydroxide to give triol **6** in 77% yield.[44] The diol moiety of **6** was cleaved by treatment with sodium periodate in aqueous THF to afford the 5'-aldehyde; this intermediate was not isolated due to possible epimerization at C-4,[45] but was reduced directly with $NaBH_4$ to afford 3'-deoxyadenosine derivative **7** in 72% yield.[46] That the structure of **7** actually had the isomeric structure shown was established by detailed analysis of the ^1H NMR and UV spectra, and by comparison of **7** with an authentic sample derived from commercially available 3'-deoxyadenosine. The primary hydroxyl group of **7** was selectively protected with DMTrCl in pyridine to give **8** in 69% yield. The activated monomer **9** suitable for oligonucleotide synthesis was then obtained in 70% yield by treatment of **8** with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite.

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Oligonucleotide Synthesis. The modified monomer **9** was incorporated into the 5'-termini of the acceptor oligomer and topoisomerase I binding region on the scissile strand of the partial duplex using standard solid phase chemistry.[47] Cleavage from the solid support and removal of base-sensitive protecting groups were effected using concentrated NH_4OH for 12 h at 55 °C. The DMTr-protected oligomers were purified using Nensorb preparative chromatography; detritylation with trifluoroacetic acid then afforded the fully deblocked oligonucleotides. All oligonucleotides were purified on a preparative 20% denaturing polyacrylamide gels; the DNA was recovered by crush and soak, then by precipitation.

Topoisomerase I-Mediated Coupled Cleavage-Ligation Reaction Using a Modified Acceptor. To permit further definition of the ability of modified oligonucleotides to participate as acceptors in topoisomerase I-mediated cleavage-ligation reactions, we prepared an acceptor oligonucleotide 17 nucleotides in length and having 3'-deoxyadenosine at the 5'-end (FIG. 1B). The ability of this acceptor oligonucleotide to undergo topoisomerase I-mediated ligation was investigated in a coupled cleavage-ligation assay. The 5'-^{32}P end labeled partial duplex shown (FIG. 1A) was treated with calf thymus DNA topoisomerase I in the presence of acceptor oligonucleotide **II** at 37 °C. Aliquots of the reaction mixture were quenched after predetermined times by the addition of 1% SDS and then proteolyzed with 1 mg/mL proteinase K (1 h; 37 °C) to digest the covalently bound enzyme. The reactions were then analyzed on a 20% denaturing polyacrylamide gel (FIG. 3). The acceptor oligonucleotide containing 3'-deoxyadenosine at its 5'-terminus afforded a single

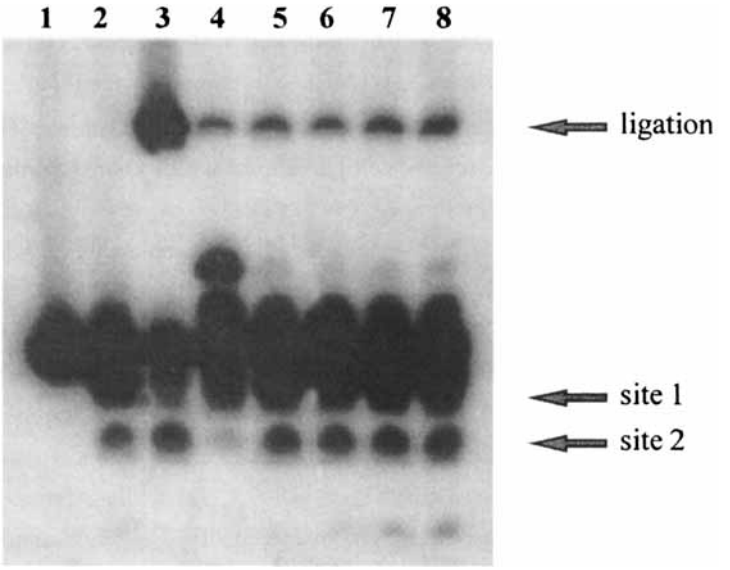

FIG. 3. Autoradiogram of a 20% denaturing polyacrylamide gel illustrating the ligation of modified acceptor oligonucleotide **II** to the 5'-^{32}P end labeled partial duplex. Lane 1, DNA alone; lane 2, DNA + topoisomerase I; lane 3, DNA + topoisomerase I + unmodified acceptor; lanes 4-8, DNA, topoisomerase I + modified acceptor **II** incubated at 1, 2, 4, 9 and 13 h, respectively. Following incubation at 37 °C for the specified times, the reactions were treated with proteinase K and SDS prior to analysis.

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ligated product (FIG. 3, lanes 4-8) that co-migrated with the ligated unmodified acceptor (FIG. 3, lane 3). Phosphorimager quantification indicated that ligation with the modified acceptor oligonucleotide (**II**) afforded ligated product to the extent of 9% after 1 h reaction time, relative to the amount of product obtained with the unmodified acceptor after 1 h. Longer incubation with the modified acceptor oligomer resulted in steadily increased yields of ligation product; after 13h, for example, the yield of ligated product was 20% (FIG. 3, lane 8).

We have demonstrated previously that incubation of modified acceptor oligonucleotide **I** with the purified topoisomerase I-DNA binary complex shown in FIG. 1A resulted in yields of ligated product up to 30% within 1 h.[19] However, when treated in the coupled assay system utilized here, oligomer **I** gave only 4-5% ligated product within 1 h relative to the amount of product obtained with unmodified acceptor oligonucleotide (data not shown). It is interesting that **I** and **II** have the same total number and types of bonds connecting the ultimate 5'-OH nucleophile to the

unmodified structural element that the two oligomers share in common, although **II** has more atoms that are "misaligned" relative to the unmodified oligomer. The somewhat lesser efficiency of **I**, as compared with **II**, in forming ligated product may well reflect the greater flexibility of **I** in proximity to the nucleophilic OH group.

It may be noted that cleavage of the DNA substrate also obtained at site 2 (FIG. 1A), but no ligation product was formed from this intermediate in the presence of the unmodified or modified acceptor oligonucleotides. Also apparent at longer incubation times was a band corresponding to cleavage of the labeled DNA closer to the 5'-end (FIG. 3, lanes 7 and 8). This is believed to reflect hydrolysis of the enzyme - DNA covalent binary complex.

Topoisomerase I-Mediated Coupled Cleavage Reaction Using Modified Partial Duplexes. 3'-Deoxyadenosine was also incorporated into the scissle strand of the partial DNA duplex to permit assessment of its effect on the cleavage and ligation reactions by topoisomerase I. Three modified duplexes were prepared; each had a single 3'-deoxyadenosine at positions +1, -4 or -6, relative to cleavage site 1 (FIG. 4A). As shown in FIG. 4B, the extent of substrate cleavage and ligation was altered, and depended importantly on the position of the 3'-deoxyadenosine moiety. Relative to the unmodified DNA substrate, the DNA partial duplex containing 3'-deoxyadenosine at position +1 suppressed cleavage at site 1 and resulted in a concomitant increase in cleavage at site 2 (FIG. 4B, cf lanes 1-3 and 4-6). No ligation product was detected. As might have been anticipated, inclusion of 3'-deoxyadenosine at position -4 had less effect on cleavage at site 1 by topoisomerase I, and a significant amount of ligation product was formed from this cleavage intermediate. Perhaps more surprising was the observation that cleavage at site 2 was still enhanced, given that this site is fairly close to the modified position in the substrate (FIG. 4B, lanes 7-9). More remarkable still were the results obtained using the substrate having 3'-deoxyadenosine at position -6. Cleavage was observed exclusively at site 1 (FIG. 4B, lanes 10-12), and there was about three-fold enhancement in the yield of ligation product (cf lanes 3 and 12).

Previous studies have pointed to mutations in the recognition sequence as deleterious to abortive cleavage as a consequence of reduced DNA binding ability of topoisomerase I.[48] However, in the present case it is unlikely that the decrease in cleavage is due to altered binding affinity, as compared with altered accessibility of the active site tyrosine residue to the target nucleotides. Support for this view derives from the substitution at position +1, which resulted in decreased cleavage at site one, but was compensated by a substantial increase in cleavage at site two. In addition, the cleavage at site one in the DNA substrate altered at position -4 was comparable to that in the

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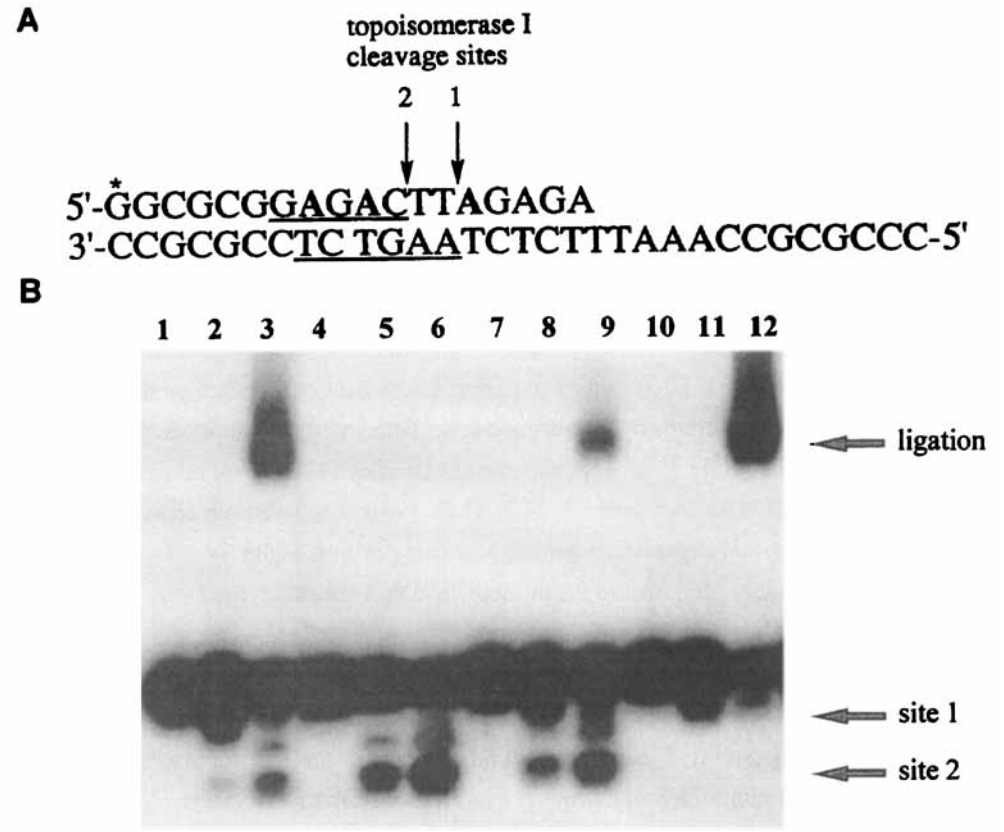

FIG. 4. (A) Topoisomerase high efficiency substate depicting conserved region (underlined) and modified adenosines (bold). (B) Audiogram of a 20% denaturing polyacrylamide gel illustrating the ligation of the acceptor oligonucleotide to the 5'-^{32}P end labeled partial duplex. Lane 1, DNA alone (unmodified 18-mer); lane 2, DNA (unmodified 18-mer) + topoisomerase I; lane 3, DNA (unmodified 18-mer) + topoisomerase I + unmodified acceptor; lane 4, DNA alone (modified at position + 1); lane 5, DNA (modified at position +1) + topoisomerase I; lane 6, DNA (modified at position + 1) topoisomerase I + unmodified acceptor; lane 7, DNA alone (modified at position -4); lane 8, DNA (modified at position -4) + topoisomerase I; lane 9, DNA (modified at position -4) + topoisomerase I + unmodified acceptor; lane 10, DNA alone (modified at position -6); lane 11, DNA (modified at position -6) + topoisomerase I; lane 12 (modified at position -6) + topoisomerase I + unmodified acceptor. The heterogeneity in the cleavage bands resulted from incomplete proteolytic digestion of the bound enzyme by proteinase K.

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unmodified oligomer, suggesting that binding affinity per se was not affected by this modification within the recognition sequence. The present results are in better agreement with those obtained for substrates having methylated nucleotides in the same region, which had little effect on the cleavage activity of topoisomerase I.[48] Although nucleotides -4 to +1 are thought to interact intimately with the enzyme and are important for enzyme recognition,[33] the present data suggest that these nucleotides are important for controlling accessibility to the cleavage site and not for enzyme binding. The substitution at position -6, further removed from the cleavage site, resulted in selective cleavage at site one. In contrast to the consequences of depurination of nucleotides in this region,[48] the results obtained with the substrate having 3'-deoxyadenosine at position -6 demonstrate that inhibition of topoisomerase I cleavage need not result form substrate alteration in this region. In the aggregate, the present results are consistent with earlier studies concerning the structural elements in the DNA substrate that may be critical to enzyme function.[48,50-53]

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Differential effects on ligation were also apparent depending on the location of the 3'-deoxyadenosine moiety. The absence of ligation product obtained from the substrate modified at position +1 may simply reflect the increased cleavage at site 2 in lieu of site 1. However, the absence of any ligation product in this case also indicates that the enzyme-DNA binary complex formed at site 2 could not undergo religation across the two nucleotide gap that would result from binding of the acceptor oligonucleotide to the binary complex.[27] Substitution at position -4 partially restored ligation ability. It may be noted, however, that the extent of ligation of this substrate was still less than that of the unmodified substrate in spite of the fact that the efficiency of cleavage at site 1 was comparable for the two substrates. Conceivably, this may be attributed to helix instability, which has been shown to reduce the ligation efficiency of topoisomerase I.[54] The specific effects on DNA structure of the introduction of 3'-deoxyadenosine at different sites have not been determined. Nonetheless, the fact that alteration of substrate structure several nucleotides from the site of cleavage substantially altered the course of enzymatic cleavage and ligation argues for a critical role of DNA conformation in controlling topoisomerase I-mediated alteration of DNA structure.

EXPERIMENTAL SECTION

General Methods

Experiments requiring anhydrous conditions were performed under an argon atmosphere. Reactions were carried out at 25 °C unless otherwise indicated. Solvents were J.T Baker p.a. and were used without further purification unless mentioned

otherwise. THF and diethyl ether were distilled from potassium/benzophenone. Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, GA. Melting points were measured on a Thomas Hoover apparatus and are not corrected. ^{1}H NMR and ^{13}C NMR were recorded on General Electric QE-300; all δ values are given in ppm relative to tetramethylsilane and J values are in Hz. Thin layer chromatography (TLC) was run on Merck silica gel F_{245} pre-coated plates; spots were visualized by dipping the plates in a Ce-Mo staining reagent. For column chromatography, Fluka silica gel 60, mesh size 230-400 was used.

T4 polynucleotide kinase and proteinase K were purchased from United States Biochemicals. Nensorb prep nucleic acid purification cartridges were from DuPont-New England Nuclear and [γ-^{32}P]ATP (7000 Ci/mmol) was obtained from ICN Radiochemicals. Scintillation counting was performed on a Beckman LS-100C instrument using Beckman Ready Safe scintillation fluid.

Polyacrylamide gel electrophoresis was carried on 20% gels [19% (w/v) acrylamide, 1% (w/v) *N, N*-methylenebisacrylamide, 8 M urea] in 90 mM Tris-borate buffer, pH 8.3, containing 5 mM EDTA. Gels were visualized by autoradiography at -80 °C with Kodak XAR-2 film and quantified using a Molecular Dynamics Phosphoimager using ImageQuant version 3.2 software. Distilled, deionized water from a Milli-Q system was used for all aqueous manipulations.

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3-*O*-(Imidazolethiocarbonyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (2). Thiocarbonyldiimidazole (2.3 g, 12.9 mmol) was added quickly to a stirred solution containing 3.0 g (11.5 mmol) of **1** in 75 mL of THF. The reaction mixture was stirred at 65 °C for 16 h in the dark and the solution was then concentrated under diminished pressure. The resulting residue was dissolved in 200 mL of 5:1 ethyl acetate-CH_2Cl_2. The solution was washed successively with 50 mL of cold 0.5 N HCl, 75 mL of saturated $NaHCO_3$ and 75 mL of brine, then dried over $MgSO_4$. The solution was concentrated under diminished pressure to afford **2** as a thick oil: yield 4.10 g (97%); ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 3 H), 1.27 (s, 3 H), 1.35 (s, 3 H), 1.50 (s, 3 H), 3.96-4.22 (m, 4 H), 4.69 (d, 1H, J = 4 Hz), 5.76 (d, 1 H, J = 2.5 Hz), 5.89 (d, 1 H, J = 4 Hz), 6.99 (s, 1 H), 7.54 (s, 1 H) and 8.30 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.5, 25.6, 27.0, 27.3, 67.9, 72.7, 80.2, 83.1, 84.9, 105.5, 110.2, 113.1, 118.3, 131.5, 137.2 and 182.8.

3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (3). To a stirred solution containing 4.5 mL (17.0 mmol) of tributyltin hydride and 300 mg (1.8 mmol) of 2,2'-azo-*bis*-(2-methylpropionitrile) (AIBN) in 200 mL of toluene was added dropwise a solution of 4.07 g (11.0 mmol) of **2** in 50 mL of toluene at 110 °C. The combined solution was stirred at 110 °C for 30 min and then concentrated under

diminished pressure to afford a residue which was purified by silica gel chromatography. Elution with 1:1 ethyl acetate-hexanes afforded **3** as a colorless oil: yield 2.64 g (98%); ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 3 H), 1.24 (s, 3 H), 1.31 (s, 3 H), 1.40 (s, 3 H), 1.64 (m, 1 H), 2.07 (dd, 1 H, J = 4, 13.5 Hz), 3.70 (m, 1 H), 4.03 (m, 3 H), 4.64 (m, 1 H) and 5.69 (d, 1 H, J = 3.5 Hz); ^{13}C NMR ($CDCl_3$) δ 25.5, 26.5, 26.8, 27.1, 35.7, 67.5, 77.2, 79.0, 80.8, 106.0, 109.9 and 111.6.

3-Deoxy-1,2:5,6-tetra-*O*-acetyl-β-D-glucofuranose (4). To a stirred solution containing 2.44 g (10.0 mmol) of diacetonide **3** in 150 mL of acetic acid was added 4.8 mL (69 mmol) of acetic anhydride, followed by 200 mg (1.15 mmol) of *p*-toluenesulfonic acid. The reaction mixture was heated at reflux for 30 min and then the solvent was co-evaporated with two 150-mL portions of toluene under diminished pressure. The residue was dissolved in 150 mL of ethyl acetate, washed with 50 mL of saturated $NaHCO_3$, and then dried over $MgSO_4$. The solution was concentrated under diminished pressure to afford a residue which was purified by silica gel chromatography. Elution with 3:7 ethyl acetate-hexane afforded **4** as a colorless oil: yield 2.62 g (79%) (9:1 anomeric ratio). The diastereomeric ratio was determined according to the signal of the anomeric protons in the crude product. The spectral data belongs to the major anomer. ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3 H), 2.04 (s, 3 H), 2.06-2.10 (m, 2 H), 2.12 (s, 3 H), 2.14 (s, 3 H), 4.04 (m, 2 H), 4.40 (m, 1 H), 5.02-5.13 (m, 2 H) and 6.10 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.1, 21.3, 32.5, 63.1, 73.2, 79.4, 99.6, 169.5, 170.3, 170.4 and 171.0; Anal. Calcd for $C_{14}H_{20}O_9$: C, 50.60; H, 6.07. Found: C, 51.06; H, 6.18.

***N*^{6}-Benzoyl-9-[(2*R*, 3*R*, 5*R*)-3-acetoxy-5-[(1*R*)-1,2-(diacetoxy)-ethyl]tetrahydrofuran-2-yl]adenine (5).** Trimethylsilyl trifluoromethanesulfonate (362 μL, 1.81 mmol) was added to a stirred solution containing 2.5 g (7.52 mmol) of tetraacetate **4** and 2.88 g (7.52 mmol) of *bis*-TMS-*N*^{6}-benzoyladenine in 200 mL of CH_3CN at room temperature. The combined solution was heated at 55 °C for 12 h. After cooling to 0 °C, the reaction was quenched by the addition of 50 mL of saturated $NaHCO_3$ followed by the addition of 150 mL of ethyl acetate. The organic layer was separated, washed successively with two 50-mL portions of saturated $NaHCO_3$ and two 50-mL portions of brine, then dried over $MgSO_4$. The solution was concentrated under diminished pressure to afford a residue which was purified by silica gel chromatography. Elution with 9:1 CH_2Cl_2-methanol afforded nucleoside **5** as a colorless foam: yield 2.34 g (61%); mp 108-112 °C; ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3 H), 2.07 (s, 3 H), 2.15 (s, 3 H), 2.29 (dd, 1 H, J = 4.5, 13.5 Hz), 2.93 (m, 1 H), 4.07 (dd, 1 H, J = 5.5, 12.5 Hz), 4.47 (dd, 1 H, J = 3, 12.5 Hz), 4.55 (m, 1 H), 5.37 (dd, 1 H, J = 5.5, 9 Hz), 5.81 (d, 1 H, J = 6 Hz), 6.07 (d, 1

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H, J = 1 Hz), 7.47-7.63 (m, 3 H), 8.01 (d, 2 H, J = 7.5 Hz), 8.07 (s, 1 H), 8.80 (s, 1 H) and 8.95 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.1, 21.2, 21.3, 33.4, 62.8, 71.7, 79.8, 90.2, 124.1, 128.4, 129.1, 133.1, 134.0, 142.3, 150.3, 151.7, 153.0, 165.5, 170.2, 170.5 and 170.9; Anal. Calcd for $C_{24}H_{25}N_5O_8$: C, 56.34; H, 4.93. Found: C, 56.15; H, 5.08.

***N*^{6}-Benzoyl-9-[(2*R*, 3*R*, 5*R*)-3-hydroxy-5[(1*R*)-1,2-(dihydroxy)-ethyl]tetrahydrofuran-2-yl]adenine (6).** A solution of 2.2 g (4.3 mmol) of nucleoside **5** in 10 mL of 2:1 ethanol-pyridine was treated with 3.3 mL of 2 N NaOH in 3.3 mL of ethanol at room temperature. The combined solution was stirred at room temperature for 5 min, then cooled to 0 °C. The solution was adjusted to pH 7 with 1N HCl, then added to 250 mL of ethyl acetate. The organic layer was washed successively with 100 mL of saturated $NaHCO_3$ and 100 mL of brine, then dried over $MgSO_4$. The solution was concentrated under diminished pressure to afford a residue which was purified by silica gel chromatography. Elution with 9:1 CH_2Cl_2-methanol afforded nucleoside **6** as a colorless foam: yield 1.28 g (77%); mp 228-231 °C; ^1H NMR (CD_3OD) δ 1.94-2.02 (m, 1 H), 2.36 (m, 1 H), 3.35 (m, 2 H), 3.95 (m, 1 H), 4.47 (m, 1 H), 4.67 (m, 1 H), 6.04 (s, 1 H), 7.42-7.60 (m, 3 H), 8.01 (d, 2 H, J = 7 Hz), 8.62 (s, 1 H) and 8.67 (s, 1H); ^{13}C NMR (CD_3OD) δ 47.4, 63.6, 72.7, 75.9, 81.9, 92.1, 124.4, 128.4, 128.7, 132.9, 133.9, 143.3, 151.8, 152.6, 152.9 and 167.1; mass spectrum (FABMS) *m/z* 386.147 ($C_{18}H_{20}N_5O_5$ requires 386.146).

***N*^{6}-Benzoyl-3'-deoxyadenosine (7).** Sodium periodate (378 mg, 1.77 mmol) was added to a stirred solution containing 460 mg (1.18 mmol) of nucleoside **6** in 10 mL of 9:1 THF-H_2O. The suspension was stirred vigorously at room temperature for 2 h, then concentrated under diminished pressure. The residue was dissolved in 25 mL of methanol and filtered. To the filtrate was added 75.6 mg (2.0 mmol) of $NaBH_4$ at 0 °C. The solution was stirred at room temperature for 30 min and then cooled to 0 °C. The solution was adjusted to pH 7 with 1N HCl, then extracted with 150 mL of ethyl acetate. The organic layer was washed successively with 50 mL of saturated $NaHCO_3$ and 50 mL of brine, then dried over $MgSO_4$. The solution was concentrated under diminished pressure to afford a residue which was purified by silica gel chromatography. Elution with 9:1 CH_2Cl_2-methanol afforded diol **7** as a colorless foam: yield 326 mg (72%); mp 220-223 °C; ^1H NMR (CD_3OD) δ 1.96-2.07 (m, 1 H), 2.29-2.38 (m, 1 H), 3.65 (dd, 1 H, J = 2.5, 12.5 Hz), 3.88 (dd, 1 H, J = 2.5, 12.5 Hz), 4.49 (m, 1 H), 4.70 (m, 1 H), 6.07 (d, 1 H, J = 1.5 Hz), 7.48-7.62 (m, 3 H), 8.02 (d, 2 H, J = 8 Hz), 8.66 (s, 1 H) and 8.70 (s, 1 H); Anal. Calcd for $C_{17}H_{17}N_5O_4$: C, 57.44; H, 4.82. Found: C, 57.28; H, 4.85.

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N^6-Benzoyl-3'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)adenosine (8). To a solution containing 155 mg (0.436 mmol) of diol **7** in 2 mL of pyridine at 0 °C was added slowly 222 mg (0.654 mmol) of dimethoxytrityl chloride in 1 mL of pyridine. The combined solution was stirred at 0 °C for 12 h, then extracted with a mixture of saturated $NaHCO_3$ (25 mL) and ethyl acetate (100 mL). The organic layer was washed with 25 mL of brine, then dried over $MgSO_4$. The dried solution was concentrated under diminished pressure to afford a residue which was purified by silica gel chromatography. Elution with 9:1 CH_2Cl_2-MeOH afforded **8**, which was isolated as a colorless powder: yield 197 mg (69%); mp 160-164 °C; ^1H NMR ($CDCl_3$) δ 2.23-2.35 (m, 2 H), 3.26 (dd, 1 H, J = 4 , 11 Hz), 3.40 (dd, 1 H, J = 3, 10.5 Hz), 3.77 (s, 3 H), 3.78 (s, 3 H), 4.51 (m, 1 H), 4.69 (s, 1 H), 4.95 (m, 1 H), 5.97 (d, 1 H, J = 3 Hz), 6.76 (m, 4 H), 7.17-7.70 (m, 12 H), 8.02 (d, 2 H, J = 7 Hz), 8.27 (s, 1 H), 8.79 (s, 1 H) and 9.02 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 34.7, 55.7, 65.3, 76.5, 76.9, 77.0, 80.9, 86.9, 93.5, 113.6, 123.8, 127.4, 128.3, 128.4, 128.5, 129.3, 130.4, 130.5, 133.3, 134.1, 136.1, 136.2, 141.7, 145.0, 150.0, 151.3, 152.7, 159.0 and 165.1; mass spectrum (FABMS) *m/z* 658.265 ($C_{38}H_{36}N_5O_6$ requires 658.266).

N^6-Benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-3'-deoxyadenosine 2'-*O*-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (9). To a stirred solution containing 72 mg (0.109 mmol) of **8** in 5 mL of CH_2Cl_2 was added successively 49 μL (0.28 mmol) of diisopropylethylamine and 24.2 μL (0.109 mmol) of 2-cyanoethyl *N,N*- diisopropylchlorophosphoramidite at 0°C. The reaction mixture was stirred at room temperature for 1 h, then diluted with 50 mL of ethyl acetate. The solution was washed with 15 mL of saturated $NaHCO_3$, dried over $MgSO_4$, and then concentrated under diminished pressure to afford a residue which was purified by silica gel chromatography. Elution with 45:45:10 CH_2Cl_2-ethyl acetate-Et_3N afforded monomer **9** as a colorless foam: yield 65.4 mg (70%) as a 1:1 mixture of diastereomers; ^1H NMR ($CDCl_3$) δ 1.15-1.39 (m, 12 H), 2.05-2.83 (m, 4 H), 3.32-3.75 (m, 4 H), 3.77 (s, 6 H), 3.80-3.98 (m, 2 H), 4.64 (m, 1 H), 5.01 (m, 1 H), 6.21 (s, 0.5 H), 6.27 (s, 0.5 H), 6.74-6.82 (m, 4 H), 7.17-7.63 (m, 12 H), 8.00 (m, 2 H), 8.27 (s, 0.5 H), 8.28 (s, 0.5 H), 8.76 (s, 0.5 H), 8.77 (s, 0.5 H) and 8.93 (s, 1H); mass spectrum (FABMS) *m/z* 858.372 ($C_{47}H_{53}N_7O_7P$ requires 858.374).

Oligonucleotide Substrates. Synthetic oligonucleotides were purchased from Cruachem Inc. or synthesized on a Biosearch 8600 series DNA synthesizer using standard phosphoramidite chemistry.[47] The oligonucleotides synthesized on the Biosearch DNA synthesizer were deblocked and cleaved from the solid support by treatment with concentrated NH_4OH at 55 °C for 12 h and then concentrated by vacuum centrifugation. The modified acceptor oligomer was purified by Nensorb

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chromatography. The Nensorb cartridge was activated with 10 mL of methanol and then preequilibrated with 5 mL of 0.1 M triethylammonium acetate (TEAA) buffer, pH 7.0. Deblocked oligonucleotides dissolved in 4 mL of 0.1 M TEAA, pH 7.0, were pipetted onto the resin. The resin was then washed with 10 mL of 1:9 acetonitrile-0.1 M TEAA, pH 7.0. The oligonucleotides were detritylated with 25 mL of 0.5% trifluoroacetic acid and washed with 10 mL of 0.1 M TEAA, pH 7.0. Elution of the deblocked oligonucleotides was accomplished with 5 mL of water containing 35% methanol (v/v). All oligonucleotides were purified on preparative 20% denaturing polyacrylamide gels; the DNA was recovered by crush and soak, then by precipitation. The DNA was 5'-^{32}P end labeled with T4 polynucleotide kinase + [γ-^{32}P]ATP.[55]

Hybridization of Substrates. Oligonucleotides were hybridized in a solution (100 μL total volume) containing 10 mM Tris-HCl, pH 7.6, 40 mM NaCl, 5 mM $MgCl_2$ and 5 mM $CaCl_2$. The solution was heated to 80 °C for 5 min, then cooled slowly to room temperature under ambient conditions (~ 3 h). Due to the low DNA strand concentrations, hybridization mixtures contained 65 fmol of the labeled strand and a 100-fold excess of the unlabeled strands to ensure complete hybridization of the labeled DNA.

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Topoisomerase I-Mediated Reaction with a Modified Acceptor Oligonucleotide. The labeled duplex (6.5 fmol, 100,000 dpm) was incubated with topoisomerase I[11] (8.8 ng) in the presence of 1000-fold excess of the acceptor oligomer in a reaction mixture (20 μL total volume) containing 20 mM Tris-HCl, pH 7.5, 40 mM NaCl, 0.5 mM EDTA, 5 mM $MgCl_2$, 5 mM $CaCl_2$ and 1 mM DTT. The reaction was incubated at 37 °C and aliquots were removed 1, 2, 4, 9 and 13 h, quenched by the addition of 1% SDS and proteolyzed with 1 mg/mL proteinase K (1 h; 37 °C). The reactions were dissolved in 12 μL of loading solution (10 M urea, 1.5 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol). The reactions were heat-denatured at 90 °C for 5 min and quick chilled on ice; 6.5 μL was applied to a 20% denaturing PAGE.

Topoisomerase I-Mediated Reaction Involving Substrates with a Modified Scissile Strand. The labeled duplex (6.5 fmol, 100,000 dpm) was incubated with topoisomerase I[11] (8.8 ng) in the presence of a 1000-fold molar excess of the acceptor oligomer in a reaction mixture (20 μL total volume) containing 20 mM Tris-HCl, pH 7.5, 40 mM NaCl, 0.5 mM EDTA, 5 mM $MgCl_2$, $CaCl_2$ and 1 mM DTT. The reactions were incubated for 1 h at 37 °C, quenched by the addition of 1% SDS and proteolyzed with 1 mg/mL proteinase K (1 h; 37 °C). The reactions were dissolved in 12 μL of loading solution (10 M urea, 1.5 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol). The reactions were heat-denatured at 90 °C for 5 min and quick chilled on ice; 6.5 μL of each was applied to a 20% denaturing PAGE.

ACKNOWLEDGMENT

This work was supported by Research Grant CA53913, awarded by the National Cancer Institute.

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