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Topoisomerase I interaction with SV40 DNA in the presence and absence of camptothecin

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ABSTRACT

Camptothecin is an antitumor drug, which is a specific inhibitor of eukaryotic topoisomerase I. Enzyme inhibition is related to the stabilization of cleavable complexes between topoisomerase I and DNA. The genomic and DNA sequence localization of L1210 topoisomerase I-mediated DNA breaks produced by camptothecin were determined in the SV40 genome. DNA cleavage was predominantly single-stranded and localized in selective regions of the DNA. A major cleavage site was found at nucleotide 4955 on the coding strand in the early transcription region. The DNA sequence was determined at prominent cleavage sites (nucleotides 127 and 199 in the two 72 bp repeats and nucleotide 4955). A DNA consensus sequence 5'-GATG-3' was found in SV40 DNA. Cleavage occured between the T and the G and topoisomerase I was linked to the 3'-DNA terminus at the T position. The sequence GATG is more frequent in the non transcribed strand of the early and late transcription of SV40 than in the transcribed strands. This finding is consistent with the role of topoisomerase I in transcription.

INTRODUCTION

Two types of eukaryotic DNA topoisomerases have been identified (For review see 1,2): type I DNA topoisomerases break transiently one DNA strand at a time and type II DNA topoisomerases both strands of duplex DNA in concert. DNA topoisomerase I is found in all eukaryotic cells and its activity seems required for DNA replication (3,4) and transcription (5,6).

Camptothecin is a cytotoxic plant alkaloid (7,8) which inhibits topoisomerase I by stabilizing a covalent enzyme-DNA complex, in which the enzyme is bound to the 3'-DNA terminus of a single-strand break (9,10). Camptothecin has also a strong antitumor activity against a wide range of experimental tumors (8). The antitumor activity is related to topoisomerase I inhibition (10).

The establishment of camptothecin as a specific inhibitor of topoisomerase I has been used to show the involvement of topoisomerase I in SV40 DNA replication (11) and transcription (12,13,14). Nevertheless, no consensus DNA sequence had been found at the DNA cleavage sites induced by camptothecin

(15) and the question was raised whether camptothecin produced DNA cleavage at preexisting topoisomerase I sites or at new drug specific sites.

In the present study, we find that camptothecin induces topoisomerase I-mediated DNA cleavage at specific sites of the SV40 genome, corresponding to a consensus sequence. This sequence is consistent with that found by Champoux et al. (16) for topoisomerase I in the absence of drug. Thus, the main effect of camptothecin is to increase DNA cleavage mediated by topoisomerase I itself.

MATERIALS AND METHODS

Materials, enzymes and drug:

SV40 DNA, Ban I and Hpa II restriction enzymes, and agarose were purchased from Bethesda Research Laboratories (Gaithersburg, MD). Fok I restriction enzyme and Klenow polymerase were purchased from Biolabs and Pharmacia, respectively. Polyacrylamide and [^{32}P]-α-dGTP were purchased from Bio-rad, Inc. (Richmond, CA) and New England Research Products (Boston, MA), respectively. Autoradiographies were performed with XAR-5 films (Eastman Kodak Company, Rochester, NY).

Type I and type II DNA topoisomerases were purified from mouse leukemia (L1210) cells, as described previously (17). One unit of topoisomerase I was defined as the amount of enzyme yielding 70% of closed circular-relaxed SV40 DNA (0.14 μg) in 10 min at 37°C (10). One unit of topoisomerase II was the amount of enzyme yielding 90% DNA relaxation in 30 min at 37°C.

Camptothecin was provided by Drs. Wani and Wall, Research Triangle Institute, Research Triangle Park, NC.

Labeling procedures of SV40 DNA:

[^{32}P]-end labeled SV40 DNA was prepared as follows: first, the DNA was linearized with Ban I restriction endonuclease at position 295 of the genome and its termini were labeled with [^{32}P]-α-dGTP and Klenow polymerase. Then, [^{32}P]-end-labeled DNA was cut with Hpa II restriction endonuclease at position 347. Such a procedure generates two [^{32}P]-3′-end-labeled fragments, one of 5191 base pairs and the other of 52 base pairs. Therefore, any DNA fragment longer than 53 base pairs could be localized unequivocally in the SV40 genome, as only one strand remains [^{32}P]-labeled.

In order to prepare the smaller DNA fragment (137 bp) containing the major topoisomerase I cleavage site, SV40 DNA was cut with Fok I restriction endonuclease, 3′-end labeled with [^{32}P]-α-dGTP and the 4912-5049 fragment was separated by electroelution after electrophoresis on a 6% acrylamide

gel. Since only one of the DNA termini (nucleotide 5049) of the 4912-5049 fragment was labeled, the isolated 4912-5049 fragment could be used directly for DNA sequencing.

DNA cleavage assays:

Reactions were performed in 30 µl reaction buffer (0.01 M Tris.Cl, pH 7.5, 0.15 M KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 µg/ml bovine serum albumin). Approximately 0.04 µg and 0.002 µg of [^{32}P]-3'-end-labeled Ban I-Hpa II fragment and Fok I fragment respectively were incubated with 220 units of topoisomerase I, or 10 units of topoisomerase II for 30 min at 37°C (see figures 4 and 6 legends for variations in salt, topoisomerase I concentrations, in time and temperature of incubation). Reactions were stopped by adding sodium dodecyl sulfate ($NaDodSO_4$), EDTA and proteinase K (1%, 20 mM and 0.5 mg/ml respectively) and incubated for an additional 60 min incubation at 37°C. After extraction with phenol-chloroform, samples were processed as described below.

DNA gels and autoradiography:

In the case of neutral agarose gels for non denatured samples, 4 µl loading buffer [10(x) solution consisting of 0.3% bromophenol blue, 16% Ficoll and 0.01 M Na_2HPO_4] was added to each sample, which was then heated at 60-70°C for 1-2 min. Equal counts were loaded into a 1% agarose gel made in TBE buffer. The gel was run at 2.8 V/cm for 15 h.

In the case of denatured samples run in neutral agarose gels, 6 µl alkaline loading buffer was added to each sample (145 mM NaOH, 7 mM EDTA, 2.5% Ficoll and 0.025% bromocresol green as final concentrations). Samples were heated at 80-90°C for 1-2 min and equal counts were loaded into a 1% agarose gel made in TBE buffer. The gel was run at 2.8 V/cm for 15 h.

In the case of DNA sequencing gels, samples were precipitated with ethanol, resuspended into 2.5 µl loading buffer (80% formamide, 0.01 M NaOH, 1 mM EDTA, 0.1% xylene cyanol and 0.1% bromophenol blue) and heated at 80-90°C for 1-2 min. Equal counts were loaded into a 6% wedge-shaped sequencing gel (29:1, acrylamide:bis; 7 M urea in TBE buffer). The gel was run at 2900 V (80 W) for 75 min.

At the end of electrophoresis, gels were transferred to 3 MM paper sheets, dried and autoradiographed with Kodak XAR-5 film. Autoradiographies of the agarose gels were scanned with a Beckman DU8B densitometer and analyzed by computer in order to determine the genomic localization of the DNA breaks (for details see ref.18).

RESULTS

Genomic localization of topoisomerase I-mediated DNA breaks induced by camptothecin in SV40 DNA:

Figure 1A shows the DNA single-strand breaks produced by topoisomerases I and II in the absence or presence of camptothecin. Very few DNA breaks were produced by topoisomerase I alone (lane 3). Camptothecin induced topoisomerase I-mediated DNA cleavage (lane 4), but produced no cleavage in the absence of enzyme (not shown). Camptothecin-induced cleavage was not random since it generated defined DNA bands. An arrow indicates the major cleavage site in SV40 DNA (figure 1A). Camptothecin had no effect upon the DNA cleavage pattern of topoisomerase II (compare lanes 5 and 6).

Figure 1B shows the DNA double-strand cleavage produced by topoisomerases I and II. Topoisomerase I by itself produced limited DNA cleavage (lane 3). Camptothecin increased this cleavage only slightly at a site corresponding to the major DNA single-strand break site (arrow, lane 4). As expected, topoisomerase II produced clearly detectable DNA double-strand cleavage upon which camptothecin had no detectable effect (lanes 5 and 6).

The genomic localization of the DNA cleavage sites observed in figure 1 was determined after densitometer scanning of the autoradiographies and computer analysis (figure 2). The major camptothecin-induced topoisomerase I-mediated site, seen in figure 1 (arrows), was localized at position 4928 (± 30 base pairs) under DNA denaturing conditions (figure 2B), and 4985 (± 30 nucleotides) under neutral conditions (figure 2D). The comparison of the DNA cleavage profiles under DNA denaturing conditions (panels A and B), showed that camptothecin increased DNA cleavage at preexisting topoisomerase I sites. However this increase was not equal at all sites and some cleavage sites seemed induced by camptothecin (1626, 2221, 4072, 4515 and especially 4928). Therefore, camptothecin appears to change the distribution pattern of topoisomerase I-mediated DNA breaks.

These experiments produced a clear delineation of the genomic distribution of topoisomerase I cleavage sites in SV40 DNA and prompted us to sequence the preferential cleavage site induced by camptothecin.

DNA sequence selectivity of topoisomerase I-mediated DNA cleavage induced by camptothecin:

In order to sequence the preferential topoisomerase I cleavage site produced by camptothecin, the same SV40 DNA fragment (3'-end-labeled at the Ban I restriction site) was first used. This fragment enabled us to study the location of topoisomerase I-mediated DNA breaks in the region of SV40 spanning the origin of replication and transcription.

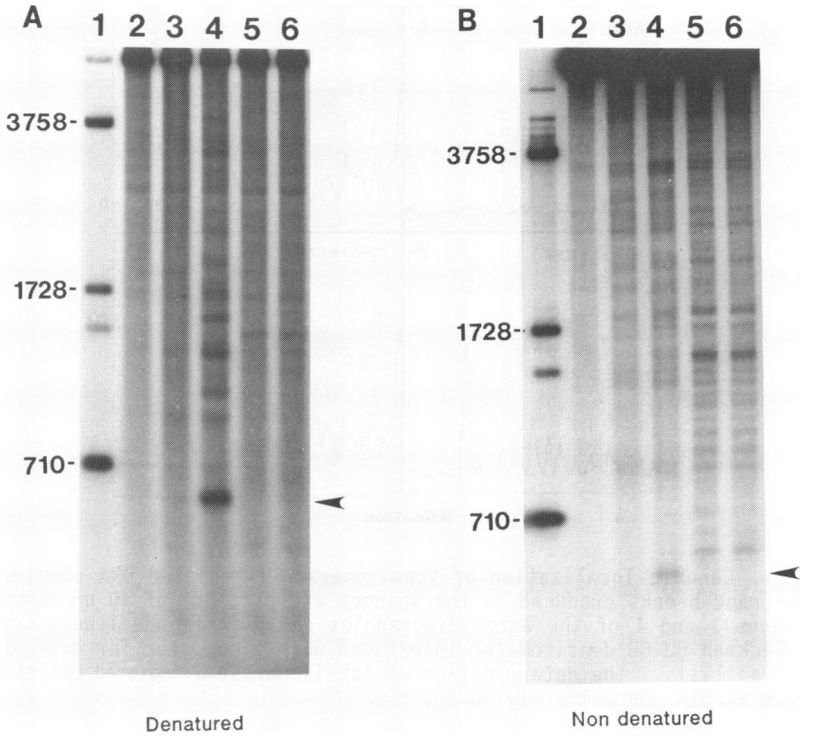

Figure 1. Topoisomerase I-mediated cleavage of SV40 DNA induced by camptothecin. The long Ban I-Hpa II SV40 DNA fragment (5191 bp) that had been [^{32}P]-end-labeled at the 3'-terminus of the Ban I cutting site (lanes 2: control) was reacted either with topoisomerase I alone (lanes 3) or in the presence of 10 μM camptothecin (lanes 4) or with topoisomerase II alone (lanes 5) or in the presence of 10 μM camptothecin (lanes 6). In panel A, samples were denatured in order to detect DNA single-strand cleavage. In panel B, the same samples were not denatured in order to detect DNA double-strand breaks. Markers (lanes 1) were labeled SV40 DNA fragments resulting from Hind III and EcoR I digestion. Their size in base pairs is indicated at the left of the pictures.

Figure 3A shows the pattern of DNA cleavage produced by topoisomerase I in this fragment. Three main sites were induced by camptothecin (arrows). Two of them were easily localized at positions 127 and 199 of the genome. These two sites have the same DNA sequence: 5'-AGAT / GCATGCTTTGC-3', and are at the same position in each of the two 72 bp repeats.

The third site of cleavage which was more intense was localized around position 4955 of the SV40 genome. It corresponded to the major site seen in the agarose gels (figures 1 and 2). The DNA sequence at this site could not

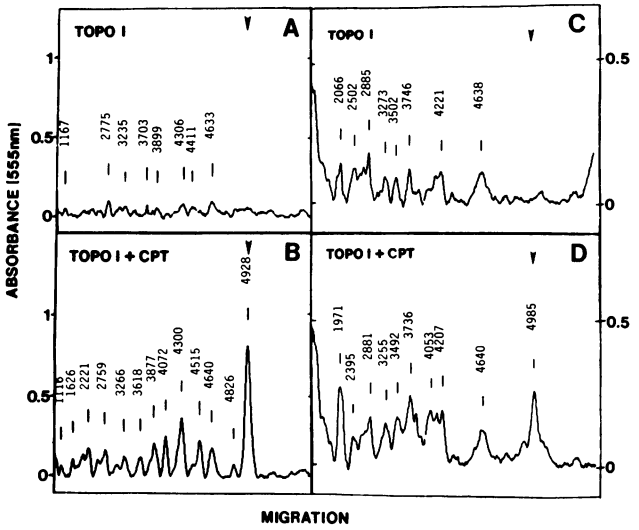

Figure 2. Genomic localization of topoisomerase I-mediated DNA single- and double-strand breaks produced in the absence and presence of 10 μM camptothecin. Lanes 3 and 4 of the autoradiographies shown in figure 1 were scanned with a Beckman DU-8B densitometer interfaced with a computer for data collection and analysis. The determination of topoisomerase I-mediated DNA cleavage sites was within approximately 30 bp.

be precisely determined (± 5 nucleotides) in the Ban I labeled DNA fragment because it was too far (approximately 600 nucleotides) from the labeling site. Thus, another DNA fragment was prepared. It was generated by a restriction digestion of SV40 DNA with Fok I; the 137 bp fragment (4912-5049) was labeled at the 3'-end on the same strand of DNA (upper) as previously and contained the DNA region of interest (between 4928 and 4985) (see figure 2).

Figure 3B shows the pattern of DNA cleavage obtained with this small fragment. The major site of cleavage was localized at position 4955 of SV40 genome (arrow) and sequenced: 5'-TGAT / GAGCATATTTT-3'.

Although topoisomerase I generates a 5'-OH DNA terminus during its catalytic reaction, instead of the 5'-P generated by the Maxam-Gilbert chemical reactions, no significant band shift could be detected between the localization of the topoisomerase I-mediated cleavage sites and the localization of the sequencing markers. This is due to the fact that the DNA cuts generated by topoisomerase I were 96 and 94 nucleotides away (sites 199 and 4955, figures 3A and B, respectively) from the labeling site. In this case, the expected shift to higher molecular weight is less than a half base (19).

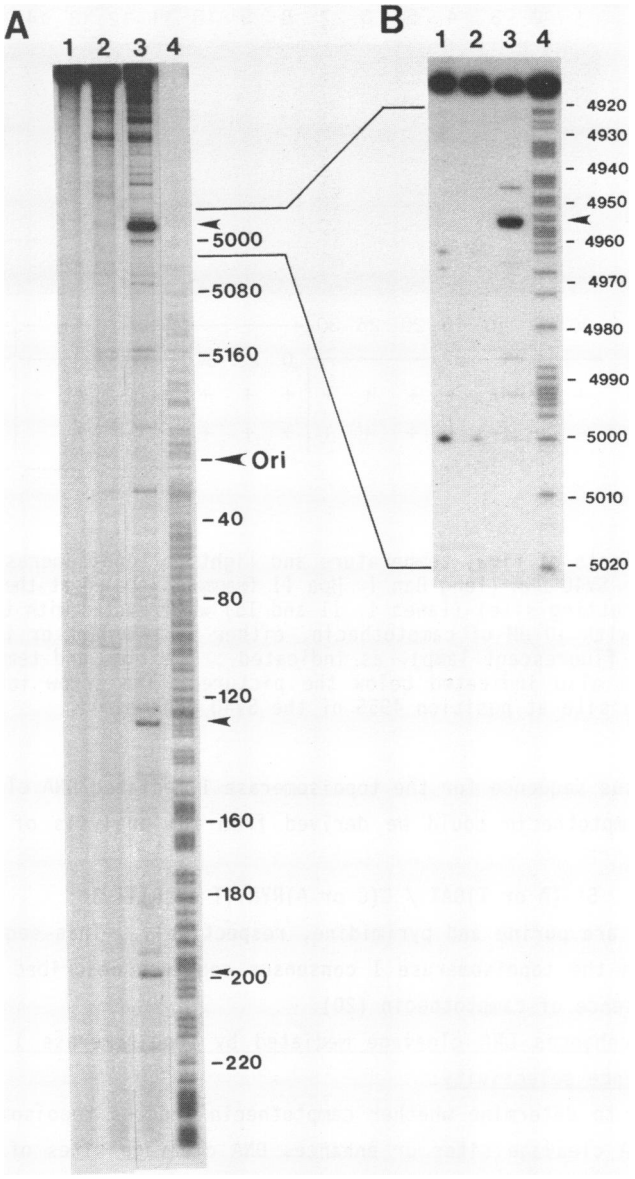

Figure 3. DNA sequence of camptothecin-induced topoisomerase I-mediated cleavage sites. In panel A reactions were performed with the long Ban I-Hpa II SV40 DNA fragment (5191 bp, genomic positions 295-347), and in panel B similar reactions performed with the 4912-5049 Fok I fragment (137 bp). In both cases the DNA fragments (lanes 1) were reacted with topoisomerase I in the absence (lanes 2) or in the presence of 10 μM camptothecin (lanes 3). Lanes 4 are purine sequencing lanes. Ori is the position of SV40 replication origin.

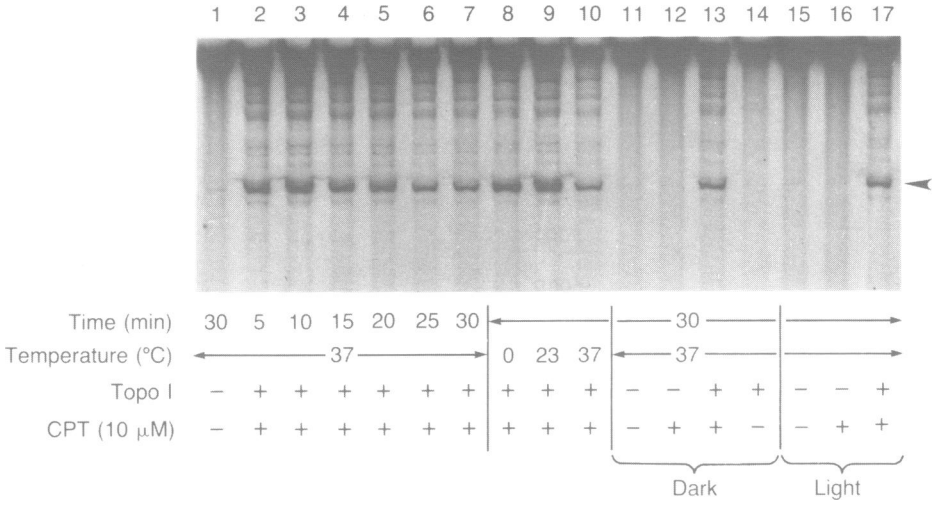

Figure 4. Effects of time, temperature and light on topoisomerase I-mediated DNA cleavage. SV40 DNA (long Ban I- Hpa II fragment labeled at the 3'-terminus of the Ban I cutting site) (lanes 1, 11 and 15) was reacted with topoisomerase I without or with 10 μM of camptothecin, either in the dark or in the light (10 cm from a fluorescent lamp), as indicated. The time and temperature of incubation are also indicated below the picture. The arrow indicates the major cleavage site at position 4955 of the SV40 genome.

A consensus sequence for the topoisomerase I-mediated DNA cleavage sites induced by camptothecin could be derived from the analysis of sites 127, 199, and 4955:

5'-(A or T)GAT / G(C or A)RYRY(T or A)TT-3'

where R and Y are purine and pyrimidine, respectively. This sequence is in agreement with the topoisomerase I consensus sequence described by Been et al. in the absence of camptothecin (20).

Camptothecin enhances DNA cleavage mediated by topoisomerase I alone with the same sequence selectivity:

In order to determine whether camptothecin induces topoisomerase I to create new DNA cleavage sites or enhances DNA cleavage sites of the enzyme alone, we tried to optimize the conditions of DNA cleavage.

Maximal cleavage corresponding to the intermediate state of the reaction was produced after 10 min of reaction at 37°C (figure 4, lane 3), therefore during the initial rate of the reaction. Longer times of incubation resulted in a progressive reduction of cleavage, which indicates the reversibility of the topoisomerase I-DNA complexes. When the incubation time was fixed to 30

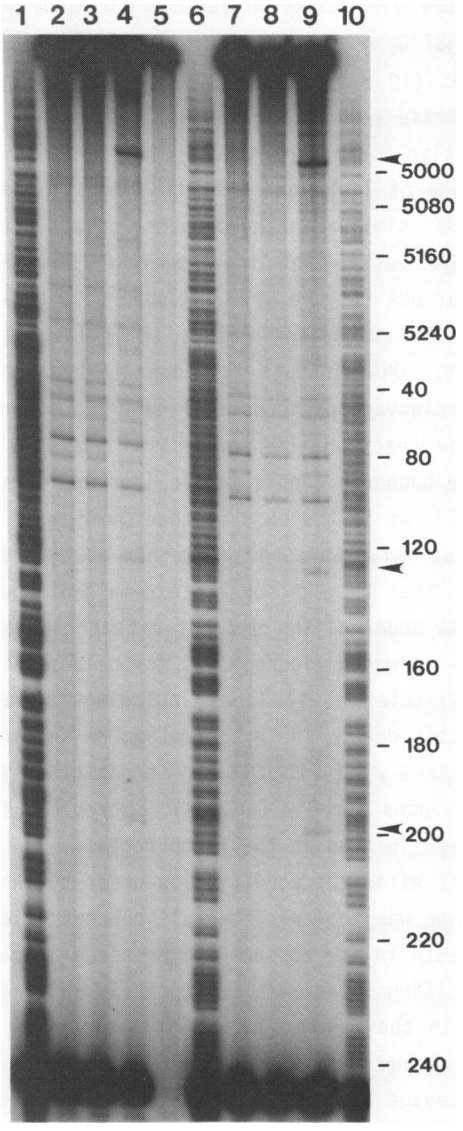

Figure 5. Comparison of topoisomerase I-mediated DNA cleavage induced by camptothecin in supercoiled and linear SV40 DNA. Lanes 2-5: supercoiled DNA was used in the topoisomerase I reactions; the DNA was then labeled with Klenow polymerase either after Ban I restriction (lanes 2-4) or directly (lane 5). Lanes 7-9: [^{32}P]-end labeled (linear) DNA was used. Both types of DNA (lanes 2 and 7) was reacted with 220 units topoisomerase I in the absence (lanes 3 and 8) or in the presence of 10 μM camptothecin (lanes 4 and 9). Lanes 1, 6 and 10 are purine sequencing lanes (method of Maxam and Gilbert).

min and the temperature of incubation varied, more DNA breaks were produced at 23 or 0°C than at 37°C, even though the cleavage intensity was not greater than at shorter times (10 min) at 37°C. This finding indicates the reversibility of topoisomerase I-DNA complexes at 37°C.

Figure 4 shows also that camptothecin by itself did not induce any DNA breaks in the presence of light and that the induction of topoisomerase I-mediated DNA breaks was similar in the presence or absence of light.

The DNA cleavage pattern of topoisomerase I was also examined in supercoiled and linear DNA (figure 5). DNA supercoiling stabilizes particular structures, such as DNA crossover points, hairpins, Z DNA, and locally denatured DNA regions. DNA cleavage was found to be similar quantitatively and to yield similar cleavage patterns in linear and supercoiled DNA. Under the conditions of the reaction, DNA relaxation was inhibited and SV40 DNA was either covalently bound to topoisomerase I or unprocessed and supercoiled (data not shown). If, as in the case of topoisomerase II cleavable complexes, the DNA strands do not swivel around each other at topoisomerase I cleavage sites, one can assume that the DNA substrate remained supercoiled during the reaction and that DNA supercoiling did not affect camptothecin-induced DNA cleavage. In the gel shown on figure 5, it may be noted that some non random cleavage sites are visible in all lanes including the untreated controls. These sites are probably due to the presence of DNA sequences similar to the Ban I recognition site G / G(CT)(AG)CC as at positions 43, 54, 64, 76, 85 and 97 where the sequences are G / GCGG(GA). Moreover, these sequences are repeated since they are located in the 21 bp repeats.

Finally, the most efficient condition in order to enhance topoisomerase I-mediated DNA cleavage was to lower the salt concentration. Such an enhancement was observed both in the absence and presence of camptothecin (figure 6). Under these conditions, the main camptothecin-induced DNA cleavage site (4955) was produced in the absence of camptothecin. This result indicates that camptothecin does not induce new topoisomerase I cleavage sites but only enhances DNA cleavage at sites generated by topoisomerase I alone (compare lanes 2 and 4, figure 6).

DISCUSSION

The following DNA sequence was found at the topoisomerase I cleavage sites induced by camptothecin in SV40 DNA: 5′-GATG-3′ (table I). Cleavage was between the T and the G, and therefore the enzyme was covalently bound to the T. A comparison of this sequence with those cleaved by topoisomerase

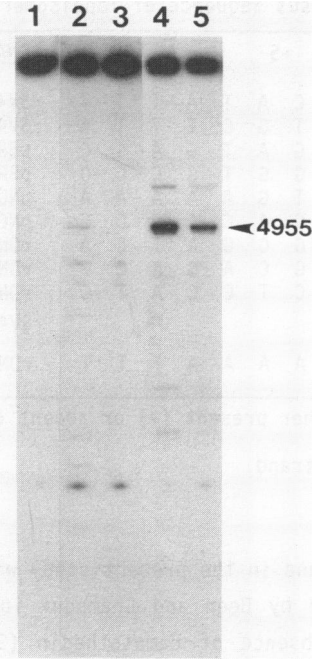

Figure 6. Comparison between topoisomerase I-mediated DNA cleavage at low salt concentration and camptothecin-induced DNA cleavage. SV40 DNA (Fok I fragment, [4912-5049] labeled at the 3'-terminus) (lane 1) was reacted with 145 units topoisomerase I in 50 mM NaCl (lane 2) or 150 mM NaCl (lane 3) and with 75 units of topoisomerase I in the presence of 10 μM camptothecin in 50 mM NaCl (lane 4) or 150 mM NaCl (lane 5). The arrow indicates the major cleavage site at position 4955 of the SV40 genome.

I plus camptothecin in pBR322, pNCI (15,21), and Xenopus oocyte rDNA (22) shows either a complete analogy or close similarity (table I). In all cases, a T was found at position -1. Thus, the covalent linkage between the tyrosine residue of topoisomerase I and the 3'-DNA terminus is through a phosphoryl bond next to a thymine and this T may not be replaced to conserve recognition by the enzyme (21). In most cases, a G was found at position +1 on the other side of the DNA break, and another one at position -3. However, the presence of a GATG sequence is not sufficient for topoisomerase I trapping by camptothecin. This sequence is found at position 81 of SV40 DNA and no DNA cleavage occurs at that position (see figure 3A). This result suggests that other DNA sequences are required for topoisomerase I recognition or that a particular DNA sequence nearby the 81 position inhibited the cleavage of the potential site.

Table I: DNA Consensus Sequence of Topoisomerase I cleavage sites.

-5			-1	+1			+5					+10	DNA (position)	CPT[1]	Ref.
T	T	G	A	T	G	A	G	C	A	T	A	T	T	T	SV40 (4955)	+	
G	A	G	A	T	G	C	A	T	G	C	T	T	T	G	SV40 (127,199)	+	(*)
A	G	G	A	T	G	A	C	G	A	T	G	A	G	C	pBR (113)[2]	+	(15)
A	G	G	G	T	G	A	C	G	G	T	G	C	C	G	pBR (128)[2]	+	(15)
A	T	G	G	T	G	C	A	T	G	A	A	A	A	A	pNCI (187)	+	(15)
C	C	G	T	T	G	A	G	C	A	C	C	G	C	C	pNCI (208)	+	(15)
C	A	G	A	T	G	G	A	G	C	G	A	T	C	A	rDNA (847)[2]	+	(22)
T	T	G	A	T	A	G	G	G	C	A	G	A	C		rDNA (1041)[2]	+	(22)
T	T	T	G	A	T	C	G	C	T	C	C	A	T	C	rDNA (840)	+	(22)
	A	G	A	T											SV40 (consensus)	-	(20)
	T	C	T														
G	A	C	T	T	A	G	A	A	A	A	A	T	T	T	rDNA	-	(21)

[1]CPT: camptothecin was either present (+) or absent (-) in the topoisomerase I cleavage reactions.
[2]indicates the lower DNA strand.
*present study.

The GATG sequence found in the present study with camptothecin is similar to the consensus reported by Been and Champoux in SV40 DNA for rat liver topoisomerase I in the absence of camptothecin (20). Moreover, the site found in the 72 bp repeats is common. Our major site was not analyzed in the study of Been and Champoux. The GATG sequence does not correspond to the consensus sequence reported by Busk et al. for Tetrahymena topoisomerase I in Tetrahymena rDNA (table I). However, the fact that the Tetrahymena sequence is found neither in the SV40 DNA that was used in the present study nor in the rDNA of higher eukaryotes (23) explains why the Tetrahymena consensus was not found in the other DNA studied.

The similarity between the topoisomerase I cleavage sites detected in the absence and presence of camptothecin (figures 3-6) indicates that camptothecin enhances topoisomerase I-mediated DNA cleavage at sites that are recognized by the enzyme alone. This result is consistent with the possibility that camptothecin inhibits topoisomerase I by trapping the normal intermediates of topoisomerase I reactions. Nevertheless this trapping shows some selectivity since not all the topoisomerase I-mediated DNA cleavage sites were equally sensitive to the action of camptothecin (see figures 2 and 3). This is in agreement with the 3 kinds of effects observed by Kjeldsen et al.: no change, increase and decrease of topoisomerase I-mediated DNA cleavage in the presence of camptothecin (15). We did not observe decreased DNA cleavage but this effect is probably observed only at saturating

topoisomerase I activities, which may not have been the case in our experiments.

The GATG sequence cut by camptothecin in the presence of topoisomerase I was found to predominate in the first half of the SV40 genome for the upper DNA strand: 14 sites occur between nucleotides 0 and 2620 versus 3 between nucleotides 2621 and 5243. The opposite was found for the lower strand: 2 GATG sequences occur between nucleotides 0 and 2620 versus 21 between nucleotides 2621 and 5243. This particular distribution coincides with the late and early transcription of SV40 DNA, which take place from left to right in the first half of the genome and from right to left in the second half of the genome, respectively. Although this particular distribution does not appear to be accidental, it is not a general feature of transcribing regions of eukaryotic genes (data not shown). Nevertheless, it suggests a role of the topoisomerase I GATG sequence in the transcription process of SV40.

It has been shown that the amount of topoisomerase I present in the cell is related to the genetic expression (12), that topoisomerase I is involved in the transcription process (12, 14, 21, 22, 24), and that the camptothecin-induced DNA breaks are located in transcribed region (12). In addition, Garg et al. have shown that camptothecin inhibits supercoiled rDNA transcription but not that of linear rDNA (14). Nevertheless, in our study, figure 5 shows similar patterns of DNA cleavage in supercoiled and linear DNA. These two results together suggest that this is not only the blockage of RNA polymerase by camptothecin-trapped topoisomerase I-DNA abortive complex but also the absence of a "swivel" in the DNA which blocks transcription.

Another interesting point is the similarity of the GATG recognition sequence for topoisomerase I with that of the transcription factor III_A ($TFIII_A$) (25). If topoisomerase I and $TFIII_A$ bind to DNA at the same site, they may participate together in gene activation.

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REFERENCES

1. Wang, J.C. (1985) Annu. Rev. Biochem. **54**, 665-697.
2. Wang, J.C. (1987) Biochim. Biophys. Acta **909**, 1-9.
3. Uemura, T. & Yanagida, M. (1984) EMBO J. **3**, 1737-1744.
4. Yang, L., Wold, M.S., Li, J.J., Kelly, T.J. & Liu, L.F. (1987) Proc. Natl. Acad. Sci. USA **84**, 950-954.
5. Weisbrod, S. (1982) Nucleic Acids Res. **10**, 2017-2042.
6. Fleischmann, G., Pflugfelder, G., Steiner, E.K., Javaherian, K., Howard,

G.C., Wang, J.C. & Elgin, S.C.R. (1984) Proc. Natl. Acad. Sci. USA **81**, 6958-6962.
7. Wall, M.E., Wani, M.C., Cooke, C.E., Palmer, K.H., Mc Phail, A.T. & Sim, G.A. (1966) J. Am. Chem. Soc. **88**, 3888-3890.
8. Wall, M.E. (1983) Med. and Pediatr. Oncol. **11**, 480A-489A.
9. Hsiang, Y.-H., Hertzberg, R., Hecht, S. & Liu, L.F. (1985) J. Biol. Chem. **260**, 14873-14878.
10. Jaxel, C., Kohn, K.W., Wani, M.C., Wall, M.E. & Pommier, Y. submitted for publication.
11. Snapka, R.M. (1986) Mol. Cell. Biol. **6**, 4221-4227.
12. Gilmour, D.S. & Elgin, S.C.R. (1987) Mol. Cell. Biol. **7**, 141-148.
13. Stewart, A.F. & Schutz, G. (1987) Cell **50**, 1109-1117.
14. Garg, L.C., DiAngelo, S. & Jacob, S.T. (1987) Proc. Natl. Acad. Sci. USA **84**, 3185-3188.
15. Kjeldsen, E., Mollerup, S., Thomsen, B., Bonven, B.J., Bolund, L. & Westergaard, O. (1988) J. Mol. Biol. **202**, 333-342.
16. Champoux, J.J., McCoubrey, W.K. & Been, M.D. (1984) Cold Spring Harbor Symp. Quant. Biol. **49**, 435-442.
17. Minford, J., Pommier, Y., Filipski, J., Kohn, K.W., Kerrigan, D., Mattern, M., Michaels, S., Schwartz, R. & Zwelling, L.A. (1986) Biochemistry **25**, 9-16.
18. Pommier, Y., Covey, J., Kerrigan, D., Mattes, W., Markovits, J. & Kohn, K.W. (1987) Biochem. Pharmac. **36**, 3477-3486.
19. Tapper, D.P. & Clayton, D.A. (1981) Nucleic Acids Res. **9**, 6787-6794.
20. Been, M.D. & Champoux, J.J. (1984) J. Mol. Biol. **180**, 515-531.
21. Busk, H., Thomsen, B., Bonven, B.J., Kjeldsen, E., Nielsen, O.F. & Westergaard, O. (1987) Nature **327**, 638-640.
22. Culotta, V. & Sollner-Webb, B. (1988) Cell **52**, 585-597.
23. Bonven, B.J., Gocke, E. & Westergaard, O. (1985) Cell **41**, 541-555.
24. Zhang, H., Wang, J.C. & Liu, L.F. (1988) Proc. Natl. Acad. Sci. USA **85**, 1060-1064.
25. Sakonju, S. & Brown, D.D. (1982) Cell **31**, 395-405.