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Effects of Uracil Incorporation, DNA Mismatches, and Abasic Sites on Cleavage and Religation Activities of Mammalian Topoisomerase I*

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Abstract

Abasic sites and deamination of cytosine to uracil are probably the most common types of endogenous DNA damage. The effects of such lesions on DNA topoisomerase I (top1) activity were examined in oligonucleotides containing a unique top1 cleavage site. The presence of uracils and abasic sites within the first 4 bases immediately 5' to the cleavage site suppressed normal top1 cleavage and induced new top1 cleavage sites. Uracils immediately 3' to the cleavage site increased cleavage and produced a camptothecin mimicking effect. A mismatch with a bulge or abasic sites immediately 3' to the top1 cleavage site irreversibly trapped top1 cleavable complexes in the absence of camptothecin and produced a suicide cleavage complex. These results demonstrate that top1 activity is sensitive to physiological, environmental, and pharmacological DNA modifications and that top1 can act as a specific mismatch- and abasic site-nicking enzyme.

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CPT in standard reaction buffer (10 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM MgCl₂, 0.1 mM EDTA, 15 μg/ml bovine serum albumin). Reactions were stopped by adding either sodium dodecyl sulfate (SDS) (final concentration 0.5%) or NaCl (unless otherwise indicated, 0.5 M for 30 min at 25 °C followed by addition of 0.5% SDS). Kinetics of reversal were performed by adding NaCl (0.25 M final concentration) to the reactions and incubating the samples at 10 °C for indicated times. Time-course reactions were stopped with 0.5% SDS.

**Results**

**Effects of Uracil Incorporation and Mismatches**—The substrates used in these studies were derived from a Tetrahymena oligonucleotide (17) containing a top1 cleavage site where adenine in +1 position on the scissile (upper) strand was changed to a uracil to increase the effect of camptothecin and its derivatives (Fig. 1A) (8). We first investigated the effect of uracil incorporation at various positions in the nonscissile (lower) strand on the DNA cleavage/relation equilibrium induced by top1 in the presence or absence of CPT. Cleavage activity of top1 was significantly altered, depending on the uracil position. As shown in Fig. 2, three different effects were observed: (i) suppression of top1 cleavage when uracils were incorporated at positions −3, −2, and −1, and to a lesser extent at position −4. This suppression was observed even in the presence of 10 μM CPT. These results show that uracil misincorporation within the 4 bases immediately upstream from the top1 site (5' to the top1 cleavage site) suppresses DNA cleavage. Thus, modifications of DNA in this region can markedly alter top1 catalytic activity. (ii) Enhancement of top1 cleavage in the absence of CPT at the preexisting site was observed when uracils were incorporated at either the −6, −5, or +1 position (Fig. 2, compare lanes 2 for these positions and lanes 2 for the controls). This enhancement was 7–10-fold compared with control, and this effect was still observed for positions as far as −7 and −8 from the cleavage site (data not shown). In all cases, cleavage was reversible upon addition of 0.5 mM NaCl (Fig. 2, lanes 3 and 5). (iii) Induction of a new top1 cleavage site was observed when uracils were incorporated at positions −2 and −1 (Fig. 2A, white arrow). In the case of the −2 mismatch, the new top1 cleavage site was independent of CPT and was located immediately upstream from the mismatch, which is consistent with the enhancement produced by a mismatch at the +1 position. These data indicate that base mismatches can trap top1 cleavable complexes (18).

**Effects of Abasic Sites**—Modified oligonucleotides were used to investigate the effect of abasic sites at given positions on the top1 cleavage activity in the presence or absence of CPT. Depending on the position of the abasic site, top1 cleavage activity was differentially affected (Fig. 3). Abasic sites at positions −4, −3, −2, and −1 suppressed top1 cleavage at the normal site. New sites were also induced immediately upstream from the abasic site when the abasic site was at position −5, −4, or −2. top1 cleavage was enhanced (4–5-fold) in the absence of CPT when the abasic site was at positions −6, −2, and +1. This enhancement was associated with an inhibition of religation when the abasic site was at position −2 or +1. This can be seen in Fig. 3 as a persistent cleavage band (60–80% of the initial cleavage) after addition of salt (Fig. 3, compare lanes 4 and 5 and lanes 2 and 3). It should be noted that the persistent site observed with the oligonucleotide containing an abasic site at position −2 corresponds to an abasic site immediately downstream from the cleavage site. The results observed both with the abasic sites at positions +1 and −2 indicate that the presence of an abasic site immediately downstream from a top1 cleavage site enhances cleavage in the absence of CPT by inhibiting DNA religation and induces suicide-type reaction.

**Top1 Suicide Product Generated by a Single-stranded DNA Loop Immediately 3' from the Top1 Cleavage Site**—Enhancement of top1 cleavage in the absence of CPT was also observed using an oligonucleotide containing a displaced loop (bulge) next to the cleavage site on the nonscissile strand (Fig. 4A). Using different conditions of reversal, such as increased concentration of sodium chloride (N) or proteinase K (P) or heat treatment (H), we found that top1 cleavage persisted under these conditions. Using this mispair loop substrate, CPT had no further effect. With the control substrate, CPT-induced DNA cleavage was completely reversed by salt, proteinase K, or heat (Fig. 4B, left). Together, these results demonstrate that top1 can cleave efficiently DNA with a bulge but fails to religate the DNA, probably because of the stretching out of the loop and separation of the acceptor DNA from the top1. This would generate a suicide-type reaction.

**Effects of Mismatches and Abasic Sites at the +1 Position of the Scissile Strand on Top1 Cleavage Activity**—Because the guanine at position +1 on the scissile strand has been shown to increase the specificity of CPT derivatives for top1 cleavable complexes (19), we tested the effect of an uracil or an abasic site at this position. Uracil mismatch (U:C) or wobble base pair (U:G) increased top1 cleavage in the absence of CPT 8- and 7-fold, respectively (Fig. 5A, lanes 11, 12, 17, and 18). On the other hand, when an adenine was incorporated at the +1 position on the lower strand, leading to a U:A base pairing, no cleavage difference was noted as compared with the control and CPT was still active (Fig. 5A, lanes 5 and 6). All corresponding substrates containing an abasic site also increased top1 cleavage in the presence or the absence of CPT (Fig. 5A, lanes 8, 9, 14, 15, 20, and 21). Reversal of cleavage was studied in the presence of 0.25 mM NaCl to investigate the irreversible nature of the cleavage. The reaction rate of the top1-mediated religation process was decreased for the control oligonucleotide in the presence of 10 μM CPT, but reversal was complete after 15 min.
incubation (Fig. 5B), which is consistent with previous findings indicating that CPT reversibly inhibits the religation step of the top1 catalytic reaction (12, 20, 21). Uracil incorporation at the +1 position on the scissile strand did not affect the religation step in the absence of CPT, and reversal was complete after 5 min. In contrast, the presence of an abasic site at the same position inhibited the reversal of top1 cleavage, even when longer reversal times were used (Fig. 5B, right panel, arrow), suggesting the formation of a suicide product.

We further tested the effect of the abasic site at the +1 position by using a modified oligonucleotide synthesized with a tetrahydrofuran abasic site analogue at this position (Fig. 6A). The same irreversible trapping of top1 was observed in the absence or presence of camptothecin (Fig. 6B). Together, these results indicate that the presence of an abasic site immediately 3' to the top1 cleavage (position +1) generates a suicide product.
DISCUSSION

The present study demonstrates that uracil incorporation, DNA mismatches, and abasic sites can have profound and contrasting effects on top1 activity, depending on their position relative to the top1 cleavage site. Modifications within the first 4 bases immediately upstream of the cleavage site (positions −1 to −4) generally suppressed top1 cleavage, whereas modifications immediately downstream (position +1) generally trapped top1 cleavable complexes.

Uracil incorporation can result in true mismatches (C:U, T:U) or abnormal base pairs (G:U or A:U). Yeh et al. (18) have reported that the mammalian all-type mismatch nicking enzyme forms a cleavable complex with the 3′-DNA terminus 5′ to the eight possible types of DNA mismatches. They found that this mismatch nicking activity was in fact an intrinsic activity of top1 (18). Nash and Robertson (22) have also demonstrated that ΐ-Int topoisomerase specifically cleaves heteroduplex attachment sites containing mismatches. Consistent with these results, we found that the true mismatch U:C (Fig. 5A) resulted in enhanced top1 cleavage activity. However, we also found the same enhancing effect when base pairing was retained as in the case of the wobble base pairs G:U (Fig. 2B) or U:G (Fig. 5A). However, normal base pairing as in A:U had no effect on the enzyme activity (data not shown). These results demonstrate that abnormal DNA structure at the +1 position, immediately 3′ to the top1 cleavage site, is more important than the presence of uracil per se at this position. The study of Yeh et al. (18) also demonstrated the influence of DNA sequence immediately flanking the mismatch but did not investigate mismatches at specific sites relative to the top1 cleavage sites. Our study suggests that the top1 mismatch nicking activity exhibits selectivity for base mismatches immediately downstream of the preexisting top1 cleavage site: primarily at the +1 position and to a lesser extent at the +2 position because no cleavage enhancement was observed for mismatches at positions −1, −2, or −3 (23). This indicates that top1 can act as a mismatch-nicking enzyme only at limited sites on the DNA and that such sites are primarily determined by the enzyme. We also show for the first time that a mispaired single-stranded loop (bulge) immediately 3′ to the cleavage site leads to an irreversible cleavage complex.

The effects of uracil incorporation 5′ to the cleavage site depended on its position and on the structure of the resulting base pair (true mismatches C:U or T:U, wobble base pair G:U, or normal A:U base pair). When uracil was close to the top1 cleavage site (positions −1, −2, or −3) and resulted in T:U or C:U mismatches, top1 cleavage was suppressed. The lack of
Evidence for the close interaction of mammalian top1 with a tetramer oligonucleotide immediately upstream from the top1 site. The requirement of optimum enzyme-DNA contact with a tetranucleotides 2 at 25 °C.

Effects when they were located 5' to the top1 cleavage site, from 4 base pairs immediately upstream from the top1 cleavage site, to the top1 site, from 3 base pairs immediately downstream from the top1 cleavage site, appear to be critical for enzyme activity (23–26).

This result is consistent with a previous study (27) showing that cytosine methylation at position 3 on the scissile strand suppressed top1 cleavage, whereas no such suppression was observed at position 4. Together these observations indicate that both base pairing and major groove structure at each abasic site relative to the top1 cleavage site. A recent study of Osheroff and coworkers (16) showed that mammalian topoisomerase II enzyme activity (23–26). This could be attributed to the critical importance of the position of the abasic site relative to the top1 cleavage sites. Thus, the enhancing effects could have been masked by the suppressive effects in their global analysis using a large DNA fragment. An abasic site immediately 5' (position +1 on the scissile or uncleaved strand) to the cleavage site trapped irreversible top1 cleavable complexes (suicide products). Enhanced top1 cleavage in the absence of camptothecin was also observed with abasic sites at the +2 position and to a lesser extent at the +3 position. However, enhancement was less pronounced and cleavable complexes were more reversible than for the abasic sites at position +1. This is a camptothecin-mimetic effect (5).

Under these conditions, the religation step afterward is hindered by the abasic site. This could be interpreted as a requirement for base pairing immediately downstream from the top1-DNA linkage to align the cleaved strand for religation.

The presence of abasic sites had opposite (suppressive) effects when they were located 5' to the top1 cleavage site, from position −1 to position −4. This observation is consistent with the requirement of optimum enzyme-DNA contact with a tetramer oligonucleotide immediately upstream from the top1 site (23). Evidence for the close interaction of mammalian top1 with

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