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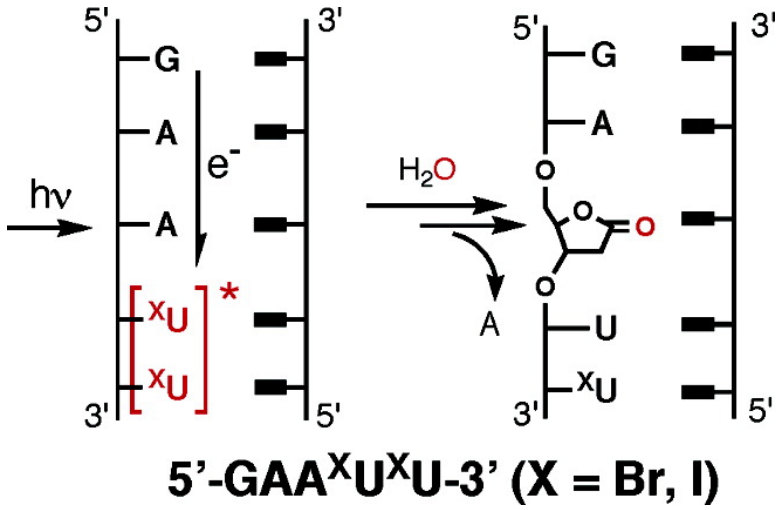

5'-GAA^{X}U^{X}U-3' (X = Br, I)

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Efficient Generation of 2′-Deoxyuridin-5-yl at 5′-(G/C)AA^{X}U^{X}U-3′ (X = Br, I) Sequences in Duplex DNA under UV Irradiation

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2′-Deoxyuridin-5-yl generated by the photoirradiation of 5-halouracil-containing DNA (d^{Br}U or d^{I}U) has been used as a probe to reveal the structures of nucleic acids and protein−DNA interactions.[1−9] Although there have been several reports of sequence specificity in the formation of strand breaks and alkali-labile sites in UV-irradiated d^{Br}U- or d^{I}U-containing DNA,[1,10,11] the molecular basis of the sequence-specific photoreactions of 5-halouracil-containing DNA is not well understood. Ito and Rokita recently examined excess electron transfer in DNA using d^{Br}U as an electron acceptor and a tetramethyldiaminonaphthalene donor.[12] Here, we report that 2′-deoxyuridin-5-yl is effectively generated in 5′-(G/C)AA^{X}U^{X}U-3′ and 5′-(G/C)A^{X}U^{X}U-3′ (X = Br, I) sequences in double-stranded DNA, which results in the selective formation of a 2′-deoxyribonolactone residue.

We first prepared 450 base pair (bp) DNA fragments containing ^{X}U by polymerase chain reaction (PCR), in which all thymine residues were substituted with ^{Br}U or ^{I}U. The DNA fragments were irradiated with monochromatic 302 nm UV light at 0 °C and then analyzed using 6% denaturing polyacrylamide gel electrophoresis (PAGE). The results are shown in Figure 1. Surprisingly, specific cleavage at 5′-(G/C)AA^{X}U^{X}U-3′ and 5′-(G/C)A^{X}U^{X}U-3′ sequences in both ^{Br}U- and ^{I}U-containing DNA fragments was observed in the top and bottom strands, even though slight differences in reactivity were observed in the case of ^{Br}U and ^{I}U, typically at site 2 and site 9. The same DNA sequence specificity in photoreactivity was observed in a different 450 bp DNA fragment (pET 28a). Importantly, bands reflecting DNA cleavage were observed only after heat treatment, indicating that heat-labile lesions are formed under these conditions (Supporting Information, Figure 1S).

To elucidate the structures of the lesions that caused specific thermal strand cleavage at 5′-(G/C)AA^{X}U^{X}U-3′ and 5′-(G/C)-A^{X}U^{X}U-3′ sequences, product analysis of photoirradiated 5′-CGAA^{I}U^{I}UCG-3′(ODN 1) and 5′-CGAA^{Br}U^{Br}UCG-3′(ODN 2) were investigated in detail. Photoirradiation of ODN 1 for 15 min resulted in the formation of two major products, **1** and **2**, with the concomitant release of free bases, A and ^{I}U (Figure 2a). Upon heating at 95 °C for 20 min, **1** was degraded to CGAp and pU^{I}-UCG, with the formation of 5-methylenefuranone, indicating that **1** was the ribonolactone-containing octamer, 5′-CGALU^{I}UCG-3′ (L = 2′-deoxyribonolactone).[13] The structure of **1** was further confirmed by electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) analysis (Figure 2S). Using a similar procedure, we found product **2** to be another L-containing octamer, 5′-CGAALUCG-3′. ESI-TOF-MS suggested that peaks a and b are reduced products (Figure 3S). Similarly, photoirradiation of ODN 2 produced L-containing octamers, 5′-CGALU^{Br}UCG-3′ (**3**) and 5′-CGAALUCG-3′ (**2**), as the major products, with the concomitant release of A and ^{Br}U.

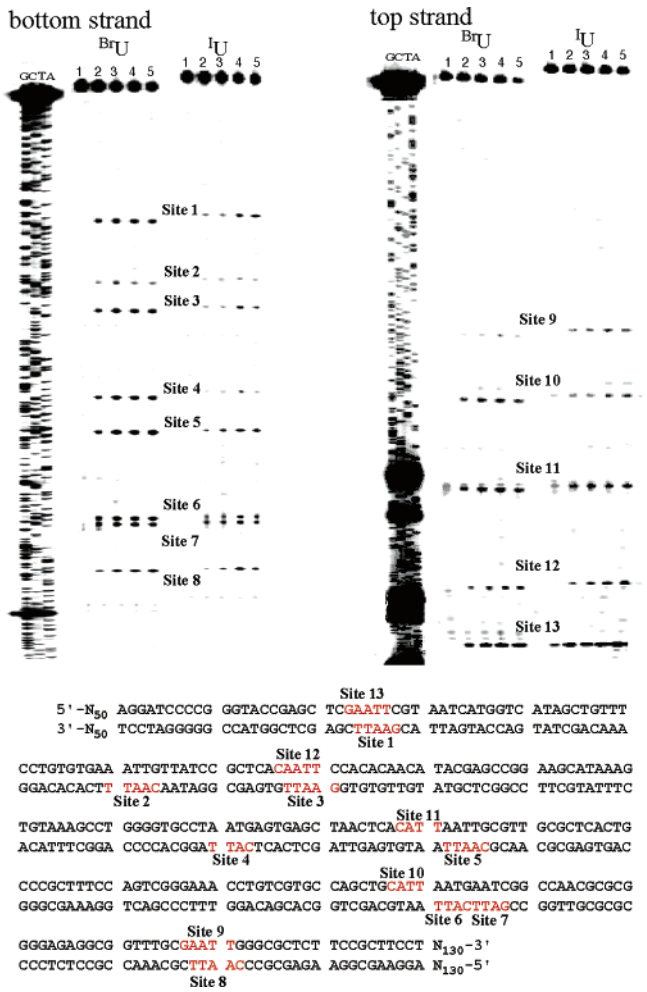

Figure 1. Denaturing polyacrylamide gel electrophoresis (PAGE) of Texas Red-labeled 5-halouracil-substituted 450 bp DNA fragments after photoirradiation. The reaction mixture contained 10 nM DNA fragments and 50 mM sodium cacodylate buffer (pH 7.0). Irradiation was performed using a monochromator (302 nm) at 0 °C. 5′-End-labeled top strand (pUC18 F378-827) and 5′-end-labeled bottom strand (pUC18 R1860-2309) DNA fragments were used. Lanes 1−5: DNA fragments (10 nM) were irradiated for 0, 15, 30, 45, and 60 s for ^{Br}U and 0, 45, 90, 135, and 180 s for ^{I}U (left, ^{Br}U-containing DNA fragments; right, ^{I}U-containing DNA fragments). Sequences containing cleavage sites are shown in red. All Ts were substituted with either ^{Br}U or ^{I}U.

Peaks c−e are assumed to be the photodimer, the 2′-hydroxylated product, and the reduced product, respectively (Figures 2b and 3S). Quantitative product analysis of photoirradiated ODNs 1 and 2 clearly indicates that the amount of free bases corresponds well to the amount of L-containing octamers **1**−**3** (Table 1).

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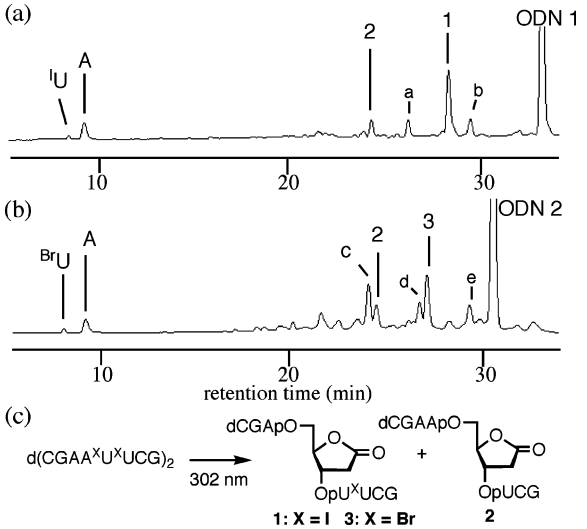

Figure 2. HPLC analysis of UV-irradiated 5′-CGAA^{I}U^{I}UCG-3′ (ODN 1) (a) and 5′-CGAA^{Br}U^{Br}UCG-3′(ODN 2) (b). Photoproducts (**1**−**3**) from ODN 1 and ODN 2 (c). The reaction mixture (total volume 10 μL) contained 10 μM (strand concentration) DNA oligomers and 20 mM NaCl in 50 mM sodium cacodylate buffer (pH 7.0). Irradiation was performed with a monochromator (302 nm) at 0 °C. The reaction mixtures (10 μL) were analyzed by HPLC on Wako 5-ODS-H columns (4.6 × 150 mm). Elution was performed with 50 mM ammonium formate (pH 6.5) containing 0−12% acetonitrile over a linear gradient for 40 min at a flow rate of 1.0 mL/min at 40 °C.

Table 1. Product Analysis of the Photoreaction of ODN 1 and 2[a]

	conversion	L-containing octamer yield, %			free base, μM (%)		
	(%)	**1**	**2**	**3**	A	^{I}U	^{Br}U
ODN 1	26	9	5		1.0 (10)	0.5 (5)	
ODN 2	30		5	13	1.4 (14)		0.5 (5)

[a] Yields were determined by HPLC. Numbers in parentheses are % yield of free bases, based on the strand concentrations of ODNs.

When ODNs 1 or 2 were irradiated (302 nm) in $H_2{}^{18}O$, incorporation of ^{18}O atoms from $H_2{}^{18}O$ into **1** and **3** was observed in both cases, indicating that H_2O is the source of the carbonyl oxygen of ribonolactone (Figure 2S). From these observations, we proposed that a possible mechanism for the efficient photoreactions at 5′-GAA^{X}U^{X}U-3′ sequences produces **1**−**3** (Scheme 1S). The initial electron transfer would occur from G to the electron-deficient, stacked ^{X}U^{X}U to provide the anion radical. Release of the halogen anion from ^{X}U^{X}U generates 2′-deoxyuridin-5-yl to abstract the C1′ hydrogen from the adjacent 5′ dA or d^{X}U. Electron transfer from the C1′ radical of dA or d^{X}U to the guanine cation radical gives rise to a C1′ cation and regeneration of guanine. Furthermore, G on the complementary strand (G/C to C/G base pair) also results in efficient electron transfer, inducing strand cleavage. Large interstrand stacking of G and A is assumed to contribute to the electron transfer from G to A. To examine the molecular basis of the efficient photoreaction, the photoreactivities of various oligonucleotides were investigated, and the results are summarized in Table 2. Since a change from ^{Br}U^{Br}U to ^{Br}UT (ODN 3) caused the photoreactivity to be substantially retained, and a single ribonolactone was formed (Figure 4S), further investigation was conducted using the ^{Br}UT sequence. The intervening A between G and ^{X}U^{X}U is considered to act as a bridge between the donor and acceptor, which may prevent rapid back electron transfer.[14] In fact, the number of intervening As strongly affects the yield of the L-containing oligomer. The G^{Br}UT-containing strands (ODN 4/5) did not show the reactivity, and single-A-containing strands (ODN 6/7) show lower photoreactivity than GAA^{Br}UT. This is probably due to the faster back electron transfer relative to the elimination of bromo anion. Also, the fact that the reactivity of GAAA^{Br}UT (ODN 10/11) is lower than that of GAA^{Br}UT indicates that intervening three As slightly weaken the efficiency of electron transfer from G. AA is the most suitable bridge for the charge separation after electron transfer from G to ^{Br}U (ODN 8/9). Furthermore, neither the single-strand (ODN 8) nor single ^{Br}U (ODN 12/13) produced L-containing oligomer. These results indicate that ^{Br}U^{Br}U or ^{Br}UT residues separated by an A bridge from an electron-donating G on the 5′ side in a duplex structure are essential for efficient ribonolactone formation.

Table 2. Product Analysis of the Photoreaction Using ODN 3−13[a]

ODN	conversion (%)	L-containing oligomer (%)
5′-CGAA^{Br}UTCG-3′ (ODN 3) 3′-GCT^{Br}UAAGC-5′	21	17
5′-CT*G*^{Br}*UT*ATC-3′ (ODN 4) 3′-GAC AATAG-5′ (ODN 5)	<1	<1
5′-CT*GA*^{Br}*UT*ATC-3′ (ODN 6) 3′-GACT AATAG-5′ (ODN 7)	15	4
5′-CT*GAA*^{Br}*UT*ATC-3′(ODN 8) 3′-GACTT AATAG-5′(ODN 9)	39	11
5′-CT*GAAA*^{Br}*UT*ATC-3′(ODN 10) 3′-GACTTT AATAG-5′(ODN 11)	28	5
5′-CTGAA^{Br}UTATC-3′ (ODN 8) 5′-CGAA^{Br}UGATC-3′ (ODN 12)	<1	<1
3′-GCTT ACTAG-5′ (ODN 13)	<1	<1

[a] Reaction mixture (total volume 10 μL) contained 10 μM (strand concentration) of DNA oligomers and 20 mM NaCl in 50 mM sodium cacodylate buffer (pH 7.0). Irradiation was performed with a monochromator (302 nm) at 0 °C for 5 min (ODN 3 and 4) or 10 min (ODN 5−14). The reaction mixtures (10 μL) were analyzed by HPLC.

In conclusion, we have identified two hot spot sequences, 5′-(G/C)AA^{X}U^{X}U-3′ and 5′-(G/C)A^{X}U^{X}U-3′, in 5-halouracil-containing DNA that induce damage to DNA under UV irradiation. These observations will be useful in studying the molecular basis of sequence dependence in the DNA-damaging process in UV-irradiated 5-halouracil-containing DNA.

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Supporting Information Available: PAGE analysis of a different 450 bp fragments (Figure 1S), product analysis of **1**−**3** (Figure 2S), peaks a−e (Figure 3S), and photoirradiated ODN 3 (Figure 4S), and the proposed mechanism of the photoreaction (Scheme 1S) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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