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Evidence for Intrastrand C2' Hydrogen Abstraction in Photoirradiation of 5-Halouracil-Containing Oligonucleotides by Using Stereospecifically C2'-Deuterated Deoxyadenosine

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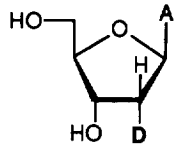
Abstract: In order to investigate the nature of deoxyribose C1' and C2' H abstraction by uracilyl-5-yl radical in DNA, we have examined the photoreaction of hexanucleotides $d(GCA^{Br}UGC)_2$ *in which one of the C2' hydrogens of the deoxyribose moiety was stereospecifically deuterated. Irradiation of (2'R)-[2'-*^{2}*H]-2'-deoxyadenosine-containing hexamer (1) gave only a smaller amount of 8 compared to that of the unlabeled hexamer, whereas (2'S)-[2'-*^{2}*H]-2'-deoxyadenosine-containing hexamer (2) gave essentially the same amount of 8 as that obtained for the unlabeled hexamer. The results indicated that the formation of C2' oxidation product 8 occurs via a rate-limiting abstraction of (2'α)-H of the deoxyribose moiety by uracilyl-5-yl radical. The kinetic isotope effects* (k_H/k_D) *obtained for the formation of 8 by photoirradiation of* ^{Br}U- *and* ^{I}U*-containing hexamers were 7.5 and 7.2, respectively.*

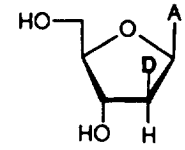
The DNA cleavage resulting from site-selective H abstraction from DNA deoxyribose by naturally occurring antitumor antibiotics[1] and designed synthetic DNA-cleavers[2] has been a subject of intense current interest. Understanding the mechanism of site- and regio-specific H abstraction provides important information for binding orientations of DNA-cleaving molecules onto duplex DNA.[2c,3] The incorporation of 5-halouracils into DNA has been known to enhance sensitivity to induce DNA strand cleavage and DNA-protein crosslinking upon photoirradiation by forming uracilyl-5-yl radical.[4] While this methodology has been utilized as a photocrosslinking or a photofootprinting method to identify binding sites in a DNA-protein complex,[5] detailed chemical events occurred via uracilyl-5-yl radical formation in DNA are not well understood. In addition to answering fundamental questions to the photochemistry of 5-halouracil-containing DNA, understanding the chemical reactivity of uracilyl-5-yl radical of fixed orientation in a DNA duplex would also be very important for elucidating the mechanism of H abstraction from DNA deoxyribose by other DNA-cleaving molecules. Thus, we initiated to investigate the photochemistry of 5-halouracil-containing hexanucleotides and have recently reported that photoirradiation of 5-halouracil-containing hexamers gives C1'- and C2'-oxidation products at 5' side of the 5-halouracil residue.[6] This result implies that uracilyl-5-yl radical formed in a DNA duplex can abstract C1' and C2' hydrogens of the deoxyribose moiety at 5' side competitively. In order to know the exact nature of deoxyribose C1' and C2' H abstraction by uracilyl-5-yl radical, we have

examined the photoreaction of hexanucleotides in which one of the C2' hydrogens of the target site was stereospecifically deuterated.

Stereospecifically deuterated 5-halouracil-containing hexanucleotides (**1-4**) were synthesized by automated DNA synthesizer using β-cyanoethylphosphoramidites of protected deuterated monomers **5** and **6**. The monomers, (2'R)-[2'-^{2}H]-2'-deoxyadenosine (**5**) and (2'S)-[2'-^{2}H]-2'-deoxyadenosine (**6**) were prepared as described previously.[7] ^{1}H NMR indicated that specific deuterium content of each monomer was more than 98%.



5 (2'α-D)



6 (2'β-D)

Photoirradiation of 5-halouracil-containing hexamers was performed at 0 °C with a transilluminator (302 nm) for 1 h and the photolysate was analyzed by HPLC. Figure 1a shows the HPLC profile of photoirradiated d(GCA^{Br}UGC)$_2$, showing the formation of ribonolactone **7** and C2' oxidation product **8** together with a release of adenine and a minor amount of dehalogenated product d(GCAUGC).[6b] Irradiation of (2'α)-deuterated hexamer (**1**) (Figure 1b) gave **8** but in a smaller amount compared to that for the unlabeled hexamer, whereas (2'β)-deuterated hexamer (**2**) gave essentially the same amount of **8** as that obtained for the unlabeled hexamer (Figure 1c).

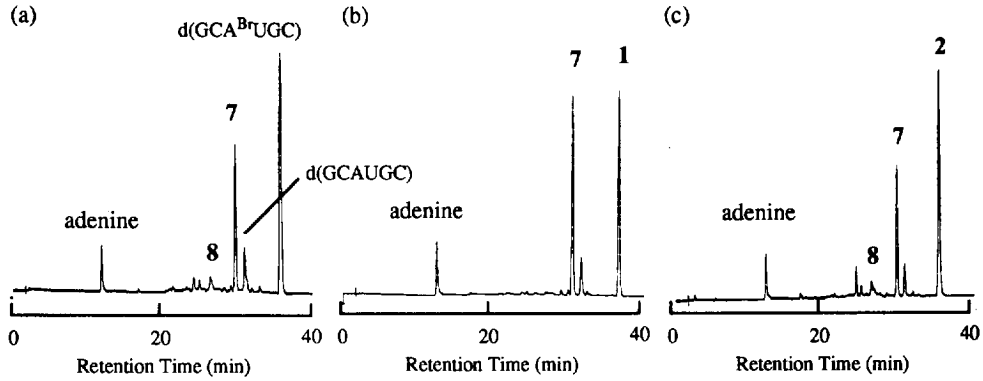


Figure 1. HPLC analysis of UV irradiated hexanucleotides, (a) d(GCA^{Br}UGC), (b) d(GC(2'α-D)A^{Br}UGC)$_2$ (**1**) and (c) d(GC(2'β-D)A^{Br}UGC)$_2$ (**2**). Each of the reaction mixtures (30 μL) containing hexamer (1 mM base concn) and NaCl (1 M) in 50 mM sodium cacodylate buffer (pH 7.0) in a quartz capillary cell was irradiated for 1 h at 0 °C with transilluminator (302 nm) under otherwise identical conditions. The reaction mixture was analyzed by HPLC on Cosmosil 5C$_{18}$ column (4.6 x 150 mm) detected at 254 nm; elution was with 0.05 M ammonium formate, 0 - 15 % acetonitrile, linear gradient, 40 min, at a flow rate of 1.0 mL/min.

A similar suppression of the formation of **8** was observed in the photoirradiation of ^{I}U-containing hexamers (**3** and **4**). Table 1 summarizes the product distribution in the photoreaction of the hexanucleotides together with the calculated kinetic isotope effect (k_H/k_D) for the formation of **8**. For accurate

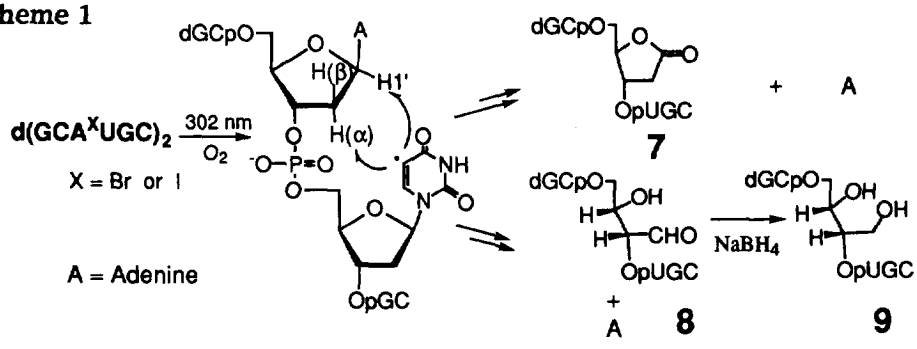
quantitation, C2' oxidation product **8** was reduced with $NaBH_4$ to erythritol-containing hexamer **9**[6b] which gave a more discrete peak under the present HPLC separation conditions. The results indicate that the formation of C2' oxidation product **8** occurs via a rate-limiting abstraction of (2'α)-H from deoxyribose moiety by uracilyl-5-yl radical as shown in Scheme 1. The kinetic isotope effects (KIE) obtained for **8** by photoirradiation of ^{Br}U- and ^{I}U-containing hexamers were 7.5 and 7.2, respectively. The KIE values ranging from 1.0-5.8 have been previously reported for C1', C4' and C5' H abstraction by several antitumor antibiotics.[8] The magnitudes of KIE obtained in the present study are substantially larger than those reported previously. This is presumably due to the energetically less favored H abstraction from C2' methylene group. In fact, recent *ab initio* calculation of deoxyribose radicals indicated that C2' radical is less favorable by 3-4 kcal/mol than C1', C3' and C4' radicals.[9] Concomitant increase in the formation of C1' oxidation product **7** in photoirradiation of (2'α)-deuterated hexamer **1** indicates that the rate of C1' H abstraction increases as a result of less favored competitive C2' D abstraction. These results clearly indicate the existence of partitioning of C1' and C2' H abstraction in the photoreaction of $d(GCA^{Br}UGC)_2$ and $d(GCA^{I}UGC)_2$. An analogous partitioning of C4' and C5' H abstraction from DNA by thiol-activated neocarzinostatin chromophore has been reported.[8c,10] Inspection of the structure of B DNA model implicates that 2'α H is more close to the C5 position of uracil at 3' side, suggesting that the distance and the easiness of H abstraction would control the ratio of competitive C1' and C2' H abstraction in the photoirradiation of 5-halouracil-containing B DNA.

Table 1. Product Distribution in Irradiation of 5-Halouracil-Containing Deoxyhexanucleotides (**1-4**)[a]

run	hexamer	adenine (μM)	**7** (%)	**8**[b] (%)	k_H/k_D for C2'ox
1	$d(GCA^{Br}UGC)_2$	105	38	6.4	-
2	$d(GC(2'\alpha\text{-D})A^{Br}UGC)_2$ (**1**)	102	43	0.85	7.5
3	$d(GC(2'\beta\text{-D})A^{Br}UGC)_2$ (**2**)	102	37	6.1	-
4	$d(GCA^{I}UGC)_2$	72	14	13	-
5	$d(GC(2'\alpha\text{-D})A^{I}UGC)_2$ (**3**)	69	17	1.8	7.2
6	$d(GC(2'\beta\text{-D})A^{I}UGC)_2$ (**4**)	69	16	13	-

[a]Photoirradiation and subsequent HPLC analysis of the mixtures were carried out under the conditions as described in Figure 1. [b]The amount of **8** was estimated as **9** after $NaBH_4$ reduction. To 10 μL of the photolysate, 1 μL of 1 M $NaBH_4$ was added. After 10 min incubation at room temperature, the reaction was quenched by addition of 1 mL of CH_3COOH, and the solution was subjected to HPLC analysis.

Scheme 1



In summary, we have proved for the first time that C2' oxidation products in the photoirradiation of 5-halouracil-containing DNA are arisen from 2'α H abstraction by uracilyl-5-yl radical.

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