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Deoxyribonolactone formation in photoirradiation of 5-bromouracil-containing oligonucleotides by direct C1′ hydrogen abstraction

Kenzo Fujimoto, Yutaka Ikeda, Shigenori Ishihara and Isao Saito*

Department of Synthetic Chemistry and Biological Chemistry, Kyoto University, Kyoto 606-8501, Japan

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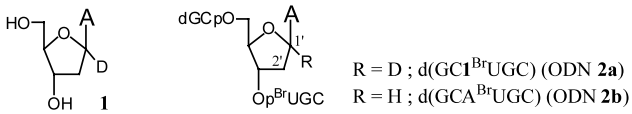
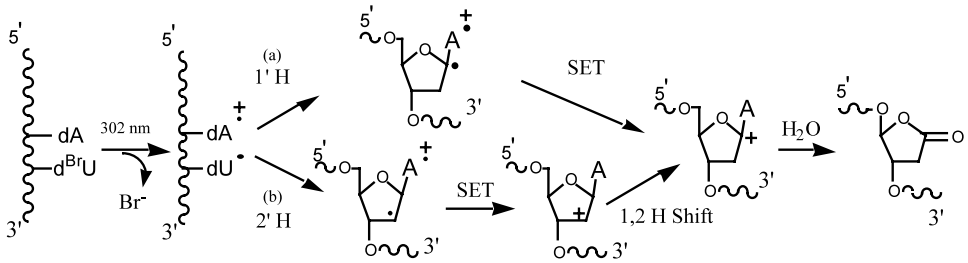
Abstract—Mechanistic studies on the formation of deoxyribonolactone by UV irradiation of hexamer d(GC**1**^{Br}UGC)$_2$ (ODN **2a**) containing 1′-deuterio-2′-deoxyadenosine **1** was examined. The kinetic isotope effect (k_H/k_D) for the formation of C1′ oxidation product **3** was 1.7±0.1. ESI-MS of d(pUGC) (**7**) obtained from the photoreaction of ODN **2a** indicated that 1′ deuterium was abstracted by uracilyl-5-yl radical and incorporated into **7** in 96% yield. © 2002 Published by Elsevier Science Ltd.

The formation of deoxyribonolactone structure in DNA has been reported for C1′ hydrogen abstraction of deoxyribose by γ and UV irradiation,[1] cationic manganese porphyrins[2] and neocarzinostatin.[3] We have reported that photoiraddiation of ^{Br}U-containing DNA hexamers induced C1′ and C2′-oxidation at the 5′ side of ^{Br}U residue via hydrogen abstraction at both C1′ and C2′ with a significant 5′-A^{Br}U-3′ sequence selectivity.[4] Recently, Greenberg et al. proposed an intriguing mechanism for C1′ oxidation that is derived from C2′ hydrogen abstraction followed by 1,2 H shift in photoirradiation of ^{Br}U-containing DNA (path b, Scheme 1).[5a,b] However, in our previous experiments on the photoreaction of stereospecifically C2′α deuterated hexanucleotide, no suppression of the formation of C1′ oxidation product has been observed.[4a] To better understand the mechanism of C1′ oxidation, we now examined a detailed analysis of the photoreaction of 1′-deuterio-2′-deoxyadenosine (**1**)-containing self-complementary hexamer d(GC**1**^{Br}UGC)$_2$ (ODN **2a**). Herein we report experimental evidence that the C1′ oxidation product results from direct C1′ hydrogen abstraction in photoirradiation of ^{Br}U-containing oligonucleotides.



Hexamer ODN **2a** was synthesized by automated DNA synthesizer using β-cyanoethylphosphoramidite of *N*-benzoylated-**1**,[6] which was synthesized in nine steps from D-(+)-ribonolactone.[7] ^{1}H NMR indicated that D content in *N*-benzoylated-**1** was more than 98%.[8] Fig. 1 shows an HPLC profile of the photoirradiated mixture of self-complementary ODN **2a**, indicating that a decreased amount of **3** as the C1′ oxidation product and an increased amount of **4** as C2′ oxidation product were produced in comparison with those of unlabeled



Scheme 1.

* Corresponding author. Fax: +81 75-753-5676; e-mail: saito@sbchem.kyoto-u.ac.jp

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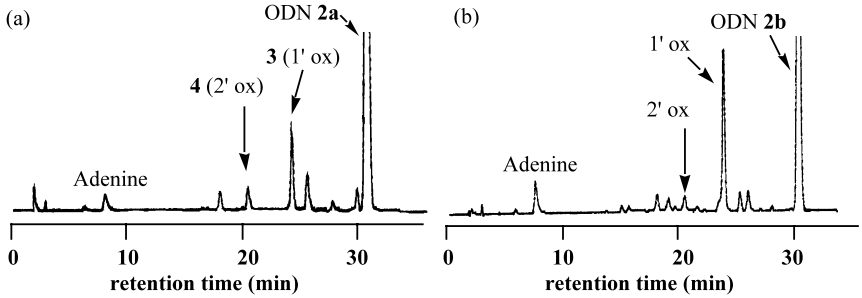
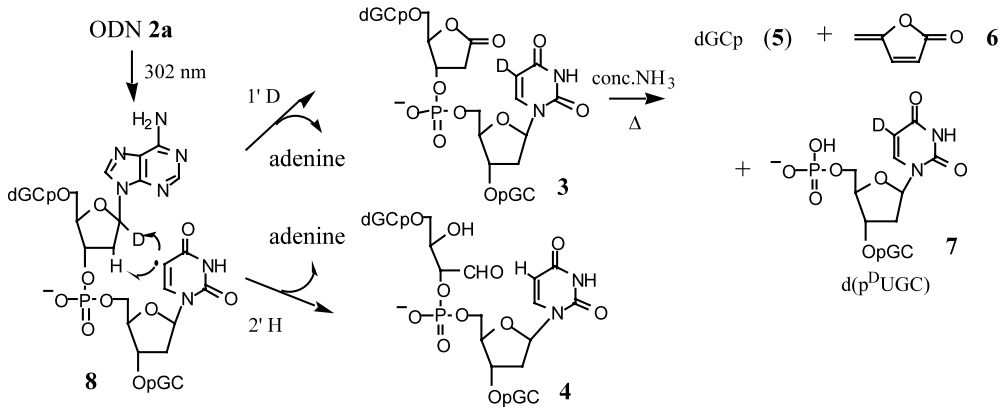


Figure 1. HPLC profile of the photoirradiation of (a) ODN **2a** and (b) ODN **2b**.

Table 1. Product analysis and kinetic isotope effect (KIE) of photoirradiated ODN **2a** and ODN **2b**

Hexamer	Conversion (%)	Product (μM)		Quantum yield ($\times 10^{-4}$)		k_H/k_D	
		3 (1′ ox)	**4** (2′ ox)	**3** (1′ ox)	**4** (2′ ox)	**3** (1′ ox)	**4** (2′ ox)
ODN **2a**	9.5±0.3	2.7±0.1	1.0±0.1	6.9±0.3	2.5±0.2	1.7±0.1	0.8±0.2
ODN **2b**	9.6±0.4	4.7±0.2	0.8±0.1	11.8±0.5	1.9±0.1	–	–



Scheme 2.

ODN **2b**.[9] Table 1 summarizes the product distribution in the photoreaction of the hexanucleotides together with the kinetic isotope effect (k_H/k_D) calculated based on the quantum yields for the formation of **3** and **4**.[10] The kinetic isotope effects (KIE) for the formation of **3** and **4** by photoirradiation of ODN **2a** as compared with ODN **2b** was found to be 1.7±0.1 and 0.8±0.1, respectively. This result indicated that the formation of **3** occurs via a rate-limiting abstraction of C1′ hydrogen of deoxyribose by adjacent uracilyl-5-yl radical as shown in Scheme 2. The magnitude of KIE (1.7±0.1) for the formation of **3** was in the range (1.0∼4.2) previously observed for direct C1′ H abstraction.[5a,b,11] The inverse KIE (0.8±0.1) for the formation of **4** also suggest the partitioning of C1′ and C2′ hydrogen abstraction by uracilyl-5-yl radical as a common intermediate in photoirradiation of ^{Br}U-containing DNA.

In order to determine D content of **3**, d(p^{D}UGC) fragment (**7**) was isolated by HPLC[12] after heating **3** with conc. aqueous ammonia as shown in Fig. 2a and subjected to ESI-MS. Fig. 2b shows the ESI-MS of d(pUGC) fragments derived from the photoreactions of both ODN **2a** and undeuterated ODN **2b**, indicating that the 1′ D was abstracted by uracilyl-5-yl radical and incorporated into **7** with 96% D content.[13] This result clearly indicated that **3** was caused from direct 1′ D abstraction by uracilyl-5-yl radical almost quantitatively. The formation of uracilyl hydroperoxide as proposed by Greenberg et al.[5b] has not been observed in our experiments.

In conclusion, by using ODN containing C1′-deuterated deoxyadenosine, we have proved that C1′ oxidation product in the photoirradiation of ^{Br}U-containing DNA is derived from direct 1′ hydrogen abstraction by uracilyl-5-yl radical.

Acknowledgements

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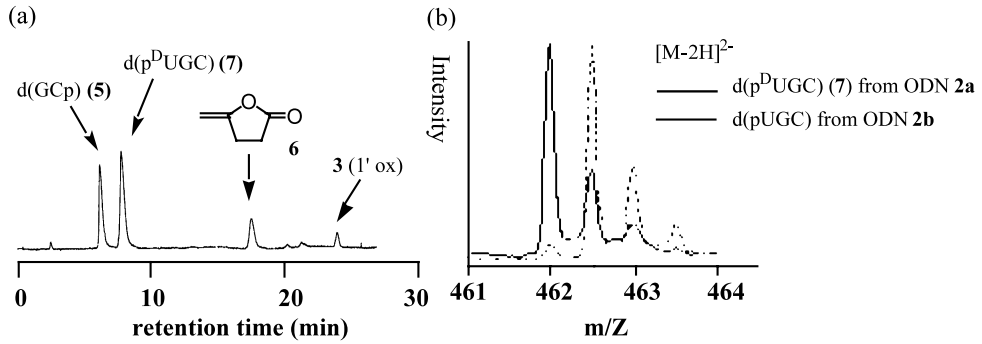

Figure 2. (a) HPLC profile of a mixture of isolated C1′ oxidation product **3** heated with conc. ammonia at 90°C for 10 min. (b) ESI-MS spectrum of d(pUGC) fragments obtained from ODN **2a** (dashed line) and ODN **2b** (solid line).

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6. ESI-MS: calcd for ODN **2a** ($C_{57}D_1H_{71}N_{23}O_{34}P_5$) ($M-H^-$) 1855.2405; found 1855.2640.
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8. The deuterium content was measured by comparison of the peak area corresponding to C1′ H and uracil C6 H of *N*-benzoylated-**1** by ^{1}H NMR spectrum.
9. The reaction mixture containing 0.15 mM (strand concentration) of oligonucleotide in 50 mM sodium cacodylate buffer (pH 7.0) in a Pyrex was irradiated at 0°C.
10. Quantum yield measurements were carried out at 0°C on a monochromator (302 nm) using ^{Br}U as an actinometer.[14]
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12. Compound **3** (retention time of 24 min) was collected by HPLC, and the residue was dissolved in conc. aqueous ammonia and heated at 90°C for 10 min. After neutralization, d(p^{D}UGC) fragment **7** (retention time of 8 min) was collected by HPLC and subjected to ESI-MS.
13. The deuterium content (r) of **7** was calculated from Eq. (1) where $A_{\mathrm{ODN}\ X}(Y)$ is a peak area for mass of ODN X corresponding to number (Y) in parentheses.

$$r\ (\%) = \frac{\left(A_{\mathrm{ODN\ 2a}}(462.5) - A_{\mathrm{ODN\ 2a}}(462.0) \times \frac{A_{\mathrm{ODN\ 2b}}(462.5)}{A_{\mathrm{ODN\ 2b}}(462.0)}\right) \times 100}{A_{\mathrm{ODN\ 2a}}(462.5) - A_{\mathrm{ODN\ 2a}}(462.0) \times \left(\frac{A_{\mathrm{ODN\ 2b}}(462.5)}{A_{\mathrm{ODN\ 2b}}(462.0)}\right)} \quad (1)$$

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