Conformation-Dependent Photochemistry of 5-Halouracil-Containing DNA: Stereospecific 2′α-Hydroxylation of Deoxyribose in Z-form DNA

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It is well-recognized that DNA has a remarkable structural heterogeneity.[1] Such local DNA conformations are thought to play an important biological role in processes such as gene expression by altering DNA−protein interactions.[2] Although the left-handed Z-form DNA is one of the characteristic and significant local structures of DNA,[3] the precise biological functions of Z-DNA have not been fully understood presumably due to the lack of an appropriate detection method in a living cell system.[4] Since the Z-form region is assumed to appear in a very short period of time in a living cell, utilization of a photochemical reaction which directly reflects the DNA local conformation would be infinitely useful. Previously, we have demonstrated that photoirradiation of 5-halouracil-containing B-form and DNA−RNA hybrid oligonucleotides results in a conformation-dependent H abstraction, i.e., the competitive C1′ and C2′ hydrogen abstractions were observed in the B-form DNA, whereas predominant C1′ hydrogen abstraction occurred in the DNA−RNA hybrid.[5] Inspection of the molecular model of Z-form DNA suggested that the 5-halouracil in the Z-form DNA would have a unique photoreactivity compared with B-form DNA due to differences in the manner that purine bases stacked on the 3′ pyrimidine base, e.g. the ribose C2β hydrogen of the 5′ side purine is very close to C5 of uracil, whereas the C1′ hydrogen is very far from C5 of uracil. In addition, the increase in the UV absorption at 308 nm by Z-DNA was expected to enhance the reactivity. In this study, we examined the photoreaction of the ^{I}U-containing Z-form octanucleotide and found that a novel stereospecific 2′α-hydroxylation occurs efficiently in the Z-form DNA.

To date, only few chemical or photochemical reactions that are specific to the Z-form DNA have been developed. Most of the available experimental data are limited to the investigation on poly(dG-dC) which exists in a Z-form only at a high salt condition.[3a,6] We have already demonstrated that the incorporation of a methyl group at the guanine C8 position (m^8G) dramatically stabilizes the Z-conformation of short oligonuceotides in a variety of sequences.[7] For example, ^{I}U-containing octanucleotide d(CGCG^{I}UGCG) (ODN **1**) mixed with complementary m^8G-containing oligonucleotide d(Cm^{8}GCACm^{8}GCG) (ODN **3**) forms a typical Z-conformation at a 2 M NaCl concentration as evidenced by the CD spectrum. On the other hand, when unmodified oligonucleotide **2** was used as a complement for **1**, ODN **1**−**2** showed a typical B-form CD spectrum. These two systems, ODN **1**−**2** and ODN **1**−**3**, were used to investigate their photoreactivity.

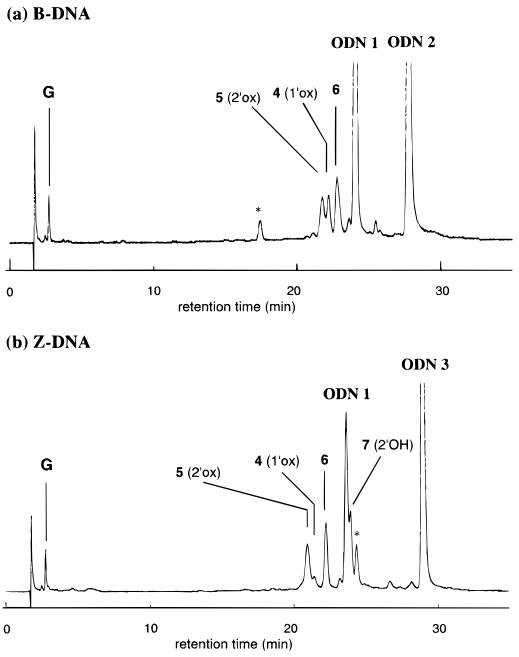

Figure 1. HPLC profiles of UV-irradiated (a) d(CGCG^{I}UGCG) (ODN **1**)/d(CGCACGCG) (ODN **2**) and (b) ODN**1**/d(Cm^{8}GCACm^{8}GCG) (ODN **3**). The reaction mixture was analyzed by HPLC on Cosmosil 5C18 MS column (4.6 × 150 mm) detected at 254 nm; elution was with 0.05 M ammonium formate (pH 6.5) containing 2−8% acetonitrile, linear gradient, 30 min, at a flow rate of 1.0 mL/min, at 30 °C. Identification of each peak (**4**−**7**) was confirmed by ESMS and enzymatic digestion. The peaks with asterisks were found to be thermally unstable; therefore, further characterizations of the products were unsuccessful.

Figure 1a shows a HPLC profile of the photoirradiated B-form ODN **1**−**2**, showing the formation of **4** and **5** as the C1′ and C2′ oxidation products, respectively.[8] The peak eluted at 22.7 min was found to be the halogen-exchanged product **6** which was produced from the photoreaction of ^{I}U with NaCl.[9] In the photoirradiation of the Z-form ODN **1**−**3**, the product **4** resulting from C1′ oxidation was significantly suppressed and the formation of a new product eluted at 24.0 min was observed (Figure 1b). Enzymatic digestion of the new product showed the formation of dG, dC, rG, and dU in a ratio of 3:3:1:1, indicating that this product is the ribonucleotide containing octamer **7** (Scheme 1).[10] The careful HPLC analysis indicated that 2′-arabinosylguanosine was not detected in the enzymatic digestion of the photoirradiated reaction mixture, indicating that stereospecific α-hydroxylation occurs at C2′ carbon of deoxyribose.

Of special importance is that the C2′α-hydroxylated product **7** was obtained preferentially from the Z-form ODN **1**−**3**, whereas

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(8) Products **4** and **5** were characterized according to the reported procedure.[5a] Formation of these photoproducts was further confirmed by ESMS. ESMS (negative); **4**: calcd 2277.5, found 2277.1. **5**: calcd 2265.5, found 2264.8.

(9) Enzymatic digestion of the product showed the formation of dG, dC and d^{Cl}U in a ratio of 4:3:1. ESMS (negative); **6**: calcd 2447.1, found 2446.2. The mechanism of the photochemical halogen-exchange reaction of ^{I}U with NaCl will be reported elsewhere.

(10) ESMS (negative); **7**: calcd 2428.6, found 2428.2.

(11) For example, absorbance at 308 nm is approximately two times greater in Z-form ODN **1**−**3** than in B-form ODN **1**−**2**.

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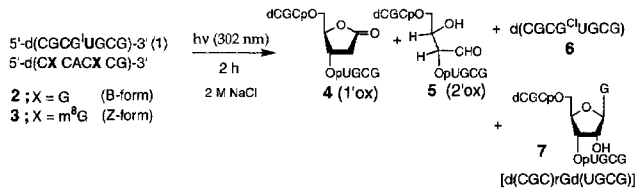
Table 1. Product Analysis in the Photoirradiation of 5-Iodouracil-Containing B- and Z-Form Deoxyoctanucleotides[a]

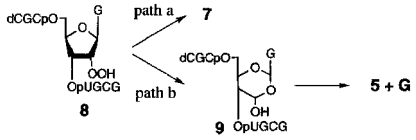
						products (%)[b]			
entry	ODN	condition	NaI (mM)	conversion (%)	G (μM)	**4** (1′ox)	**5** (2′ox)	**6**	**7** (2′OH)
1	**1**−**2**		0	43	7.8	11	14	17	
2	**1**−**2**	O_2-limiting	0	52	7.8	13	5.8	18	
3	**1**−**2**		50	36	10	16	33	19	6.7
4	**1**−**3**		0	70	11	3.1	19	16	14
5	**1**−**3**	O_2-limiting	0	71	2.9	1.1	0.8	17	1.1
6	**1**−**3**		50	62	1.8		1.0	11	68

[a] The reaction mixture (total volume, 100 μL) contained deoxyoctanucleotide (0.5 mM total base concentration), 50 mM sodium cacodylate (pH 7.0), 2.0 M NaCl, and NaI as indicated. Irradiation was performed with a transilluminator (302 nm) for 2 h at 0 °C from a distance of 5 cm. A 10-μL aliquot was analyzed by HPLC as described in Figure 1. For quantitation of **7**, the 10-μL aliquot was directly subjected to enzymatic digestion with snake venom PDE (0.3 unit/mL) and calf intestine alkaline phosphatase (100 unit/mL) and was analyzed by reverse phase HPLC. [b] Yields were determined by a comparison of the peak areas of the authentic material after enzymatic digestion which were calculated based on the consumed ODNs.

Scheme 1



Scheme 2



Scheme 3

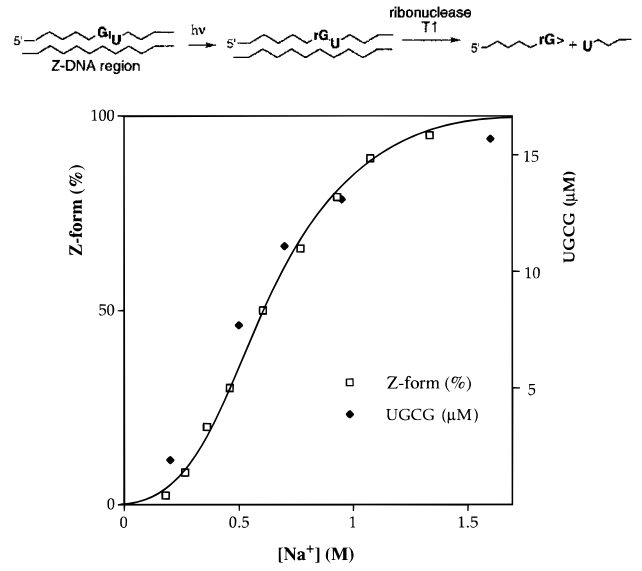


Figure 2. Formation of d(UGCG) and proportion of Z-conformation of ODN **1**−**3** as a function of [Na^+]. The proportion of Z-conformation was estimated by CD and UV spectroscopy as previously described.[7,14]

the C1′ oxidation product **4** decreased considerably in the Z-form. These results indicate that the partitioning between C1′ and C2′ product pathways is different for the two conformations as expected. As shown in Table 1, higher conversions in the photoreaction under identical conditions were always observed in the Z-form compared with B-form. This result can be interpreted by the increased absorption at a longer wavelength by changing from the B- to Z-form DNA.[3,11] Similar Z-form dependent stereospecific C2′α-hydroxylation was also observed in ^{Br}U-containing ODNs (data not shown).

Under O_2-limiting conditions, the yields of both **5** and **7** decreased in the Z-form implying that the reactions giving **5** and **7** are O_2-dependent processes (Table 1). When the photoreaction was carried out under $^{18}O_2$ atomosphere, the electrospray MS (ESMS) demonstrated incorporation of ^{18}O atom into the C2′-hydroxylated product **7** (Supporting Information).[12] These results indicate that O_2 is the source of the C2′ hydroxy group. Upon addition of NaI, which is known as a reductant for hydroperoxides, a dramatic increase of **7** was observed with a concomitant decrease of **5**. These results indicate that both **5** and **7** are produced via a common hydroperoxide intermediate **8** (Scheme 2). However, in the case of the photoreaction of the B-form ODN **1**−**2**, the formation of **5** was preferred over **7** even in the presence of 50 mM NaI. Therefore, the preferential reduction of **8** to **7** (path a) compared to the Criegie-type rearrangement leading to **5** via **9** (path b) would partly contribute to the specific C2′α-hydroxylation observed in the Z-form DNA.[13]

Interestingly, upon treatment with ribonuclease T1, the C2′-hydroxylated product **7** was quantitatively hydrolyzed to d(CGC)-rG> (> = cyclic phosphate) and d(UGCG), which were identified by comparison with authentic samples after enzymatic digestion. Figure 3 in Supporting Information shows the HPLC profiles of photoirradiated ODN **1**−**3** in the presence of NaI at different concentrations of NaCl after ribonuclease T1 treatment, indicating that the amount of d(UGCG) increased proportionally with increasing Z-DNA ratio (Figure 2). These results suggest that this photochemical and enzymatic procedure can be used as a specific detection method for the Z-form region in longer DNA.

In the present study, we found that the stereospecific C2′α-hydroxylation efficiently occurred at the 5′ side of the 5-halouracil in Z-form DNA. 5-Halouracil-substituted DNA is known to be functional in vivo. For instance, all thymine residues in *Escherichia coli* genomic DNA can be substituted with ^{Br}U. Since the C2′α-hydroxylation sites in DNA can be easily detected by ribonuclease T1, this photochemical and enzymatic method would be useful to detect the Z-form region in DNA as shown in Scheme 3. Efficacy of this method in detecting local Z-form region in longer DNA is currently under investigation.

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Supporting Information Available: CD-spectrum of 5-iodouracil-containing oligonucleotides **1**−**2** and **1**−**3** and HPLC profile of UV-irradiated **1**−**3** in the presence of 50 mM NaI and enzymatic digestion profiles of the photoproduct **4**, **6**, and **7** and ESMS of **4**−**7** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) When the photoreaction of Z-form ODN **1**−**3** was carried out in $H_2{}^{18}O$, ESMS of the isolated product **7** showed no incorporation of the ^{18}O atom into the C2′-hydroxylation product.

(13) The molecular basis for the preferential hydroxylation in the Z-form DNA is not fully understood; however, the structural characteristics of Z-form DNA such as C3 endo sugar puckering and alternative syn−anti conformation might effect the partitioning of the decomposition paths of hydroperoxide intermediate **8**.

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