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Efficient C2′α-Hydroxylation of Deoxyribose in Protein-Induced Z-Form DNA

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Abstract: DNA local conformations are thought to play an important biological role in processes such as gene expression by altering DNA−protein interactions. Although left-handed Z-form DNA is one of the best-characterized and significant local structures of DNA, having been extensively investigated for more than two decades, the biological relevance of Z-form DNA remains unclear. This is presumably due to the lack of a versatile detection method in a living cell. Previously, we demonstrated that the incorporation of a methyl group at the guanine C8 position (m^8G) dramatically stabilizes the Z-form of short oligonucleotides in a variety of sequences. To develop a photochemical method to detect Z-form DNA, we examined the photoreaction of 5-iodouracil-containing Z-form d(CGCG^{I}UGCG)(ODN **1**)/d(Cm^{8}GCAm^{8}GCG)(ODN **2**) in 2 M NaCl and found stereospecific C2′α-hydroxylation occurred at G_4 to provide d(CGCrGUGCG), **5**. Recently, Rich and co-workers [Schwartz et al. *Science* **1999**, *284*, 1841. Schwartz et al. *Nat. Struct. Biol.* **2001**, *8*, 761] found that an ubiquitous RNA editing enzyme, adenosine deaminase 1 (ADAR1), and tumor-associated protein DML-1 specifically bind to Z-form DNA. In the present study, we investigate the photoreactivity of octanucleotide ODN **1**−**2** in Z-form induced by Zα, which is the NH_2-terminal domain of ADAR1 responsible for tight binding of ADAR1. Detailed product analysis revealed that the C2′α-hydroxylated products **5** and **6** produced significantly higher yields in Z-form ODN **1**−**2** induced by Zα compared with that in 2 M NaCl. Upon treatment with ribonuclease T1, **5** and **6** were quantitatively hydrolyzed at the 3′-phosphodiester bond of the rG residue to provide d(UGCG) as a common hydrolyzed fragment on the 3′ side. Quantitative analysis demonstrated that the amount of photochemically formed **5** and **6** from ODN **1**−**2** directly correlated with the proportion of Z-form induced by Zα or NaCl. These results suggest that this photochemical and enzymatic procedure can be used as a specific probe for the existence of local Z-form structure in cellular DNA.

Introduction

DNA is polymorphic and exists in a variety of distinct conformations.[1] Duplex DNA can adopt a variety of sequence-dependent secondary structures that range from the canonical right-handed B-form through the left-handed Z-form.[2] Triplex and tetraplex structures are also known to exist.[2] All of these unique conformations may play important functional roles in gene expression by altering DNA−protein interactions.[1,2] For several years, the photoreaction of 5-halouracil-containing DNA duplexes has been investigated[3] and we have demonstrated that hydrogen abstraction by deoxyuridin-5-yl is atom-specific and highly conformation-dependent.[4] For example, competitive C1′- and C2′α-hydrogen abstractions are observed in B-DNA,[4a,b] whereas predominantly C1′-hydrogen abstraction occurs in the A-like structure of DNA−RNA hybrids.[4c] In Z-form DNA, stereospecific C2′β-hydrogen abstraction gives rise to C2′α-hydroxylation.[4d,e] We recently demonstrated that hydrogen abstraction occurs at the methyl group of an adjacent T of the sharp kink in Sso7d protein and 5-iodouracil- (^{I}U-) containing DNA complex.[4e] Although Z-form DNA is one of the characteristic and significant local structures of DNA and it has been extensively investigated in relation to transcription,[5] methylation of cytosine,[6] and the level of DNA supercoiling,[7] the biological

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function of Z-form DNA has not been well established. However, Rich and colleagues recently discovered that double-stranded RNA adenosine deaminase (ADAR1)[8] and tumor-associated protein DLM-1[9] specifically bind to Z-form DNA, using a common winged-helix motif. This biological role for Z-DNA has drawn much interest.[8c,9] We deduced from this interest that an appropriate detection method for Z-form DNA in a living cell system would be a powerful tool to reveal its true biological functions. Because the Z-form region might exist for only a very short period in a living cell, a photochemical reaction that directly reflects the local DNA conformation would be infinitely useful. In the present study, we examined the photoreaction of the ^{I}U in Z-form DNA induced by the binding of Zα, which is the NH_2-terminus of ADAR1 and is responsible for high-affinity binding to Z-form DNA,[8f] and compared the results with that induced under 2 M NaCl. We found that the stereospecific C2′α-hydroxylation efficiently occurred at the 5′ side of the ^{I}U in Z-form DNA induced by Zα.[4d,f]

Experimental Section

Synthesis of Oligonucleotides. ODN **1** and ODN **2** were prepared by the (β-cyanoethyl)phosphoramidite method on controlled pore glass supports (1 μmol) by use of an ABI 381 A DNA synthesizer. The 5′-dimethoxytrityl-(β-cyanoethyl)phosphoramidites of 8-methyl-2′-deoxyguanosine (m^8G) were prepared according to a previously reported procedure.[10] After automated synthesis, the oligomers were detached from the support, deprotected, and purified by HPLC as described previously. After lyophilization, approximately 20 OD of pure oligomer was isolated. ODN **1** and ODN **2** were identified by electrospray ionization mass spectra (ESI-MASS) on a PE Sciex API 165 mass spectrometer (negative mode). ESI-MASS for ODN **1**, 5′-d(CGCG^{I}UGCG)-3′: calcd 2554.5, found 2554.4. ESI-MASS for ODN **2**, 5′-d(Cm^8-GCACm^8GCG)-3′: calcd 2423.6, found 2423.5. Purity and concentrations of ODN **1** and ODN **2** were determined by complete digestion of the oligomers with snake venom phosphodiesterase and alkaline phosphatase to 2′-deoxymononucleosides.

Peptide Preparation. The Zα domain of human ADAR1 protein was expressed as a six-histidine tag fusion peptide cloned into a pET28a plasmid containing a thrombin cleavage site.[11] Novable DE3 cells were induced at 0.6 OD (after 5−6 h) with 1 mM isopropyl thiogalactoside (IPTG). Cells were harvested after 3 h and centrifuged at 6400 *g* for 5 min. Pellets were resuspended in 20 mM Tris-HCl (pH 8.0) containing 150 mM NaCl. Cell were lysed with an ultrasonic cell disrupter (Microson, Misonix) over ice and centrifuged for 30 min at 16200*g* at 5 °C. The supernatant was loaded on a HiTrap chelating HP column containing a 1 mL volume of Zn-charged His-bind resin (Amersham Pharmacia Biotech). Eluted peptide was collected in aliquots into microcentrifuge tubes containing 2 μL of 0.5 M ethylenediaminetetraacetic acid (EDTA). The buffer solution of the elution was exchanged to 50 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) containing 20 mM NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT) by HiTrap Desalting (Amersham Pharmacia Biotech). After being allowed to stand overnight at 4 °C, the peptide was purified on an anion-exchange column, HS column, on a Vision Workstation (Applied Biosystems). Elution was carried out with 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES)/HEPES acetate (pH 7.0) containing 0−1 M NaCl, over a linear gradient at a flow rate of 10 mL/min for 6 min. HPLC fractions eluted at 4.6−5.4 min were collected. Stock solutions of peptide sample were prepared in a 20 mM sodium phosphate buffer (pH 7.0), containing 20 mM NaF and 0.5 mM DTT at 4 °C. The activity of the overexpressed Zα was confirmed by titration of a hairpin with the sequence $d(CG)_3T_4(CG)_3$, which showed the reported isodichroic point at 285 nm.[11]

Circular Dichroic (CD) Spectroscopy. CD spectra were recorded on an Aviv CD spectrometer model 202SF equipped with a Peltier temperature controller. CD spectra of ODN **1** and **2** (0.5 mM base concentration) and (a) NaCl (2, 1.5, 1, 0.75, 0.5, 0.25, 0.1, or 0.02 M) or (b) Zα [2, 1.5, 1, 0.75, 0.5, 0.25, or 0 equiv to double-stranded DNA (Zα/dsDNA)] in 20 mM sodium phosphate buffer (pH 7.0) were recorded in a 0.2 cm path length cell at 5 °C. The results are shown in Figure 1.

Quantitative HPLC Analysis of Photoirradiated ^{I}U-Containing Deoxyoctanucleotides. The reaction mixture (total volume 100 μL) contained deoxyoctanucleotide d(CGCG^{I}UGCG) (ODN **1**)/d(Cm^8-GCACm^8GCG) (ODN **2**) (0.5 mM total base concentration) in 20 mM sodium phosphate buffer (pH 7.0) in the presence of 2 equiv of Zα or 2 M NaCl in a microcentrifuge tube (0.6 mL). After irradiation with monochromatic 302 nm UV light (HM-5 hypermonochromator, Jasco) at 0 °C, 10 mL of the aliquot was analyzed by HPLC. HPLC analysis was carried out on a Cosmosil 5C18-MS column; elution was with 0.05 M ammonium formate containing 2−7% acetonitrile, in a linear gradient at a flow rate of 1.0 mL/min for 50 min, at 30 °C. Yields of products **3**−**7** were determined by comparison of their HPLC peak areas. HPLC profiles of the reaction mixture are shown in Figure 2, panels a−c, respectively. The result of the quantitative analysis is shown in Table 1. The photoenergy of this photoreaction at 302 nm was 2.3×10^{-3} J/s. Each reaction mixture (100 mL) was monitored by UV absorption at 302 nm assuming an extinction coefficient of $A_{302} = 776$ (2 M NaCl) and 880 M^{-1} cm^{-1} (2 equiv of Zα). After the reaction mixture (total volume 100 μL) had been irradiated at 302 nm under 0 °C for 2 h (2 M NaCl) or 1 h (2 equiv of Zα) by monochromator, 10 mL of the aliquot was analyzed by HPLC.

Characterization of Photoproducts 3, 4, and 7. Photoproducts **3**, **4**, and **7** were characterized by enzymatic digestion according to a published procedure.[4a,b,f] Structure of photoproducts **3**, **4**, and **7** were further confirmed by ESI-MASS. ESI-MASS (negative) for **3**, calcd 2277.5, found 2277.1; for **4**, calcd 2265.5, found 2264.8; for **7**, calcd 2447.1, found 2446.2.

Characterization of Photoproducts 5 and 6. After irradiation of ODN **1**−**2** (0.5 mM total base concentration) at 0 °C for 1 h in 20 mM sodium phosphate buffer (pH 7.0) with 2 equiv of Zα or 2 M NaCl, the photoproducts **5** and **6** were collected by HPLC and each fraction was concentrated. The residues were subjected to enzymatic digestion with P1 (0.3 unit/mL, Boehringer Mannheim) and calf intestine alkaline phosphatase (1000 units/mL, Boehringer Mannheim) at 37 °C for 2 h in 50 mM sodium cacodylate buffer (pH 7.0) in 50 μL of water, and the mixture was analyzed by HPLC. HPLC conditions: Cosmosil 5C18-MS column with 0.05 M ammonium formate containing 0−15% acetonitrile, eluted over a linear gradient at a flow rate of 1.0 mL/min for 20 min. In the case of photoproduct **5**, formation of dG, dC, rG, and dU in a ratio of 3:3:1:1 was observed. The HPLC profile of product **5** digested by ribonuclease T1 (10 units/μL, Roche Applied Science) at 37 °C for 4 h in 50 mM sodium cacodylate buffer (pH 7.0) in 20 μL of water showed the two peaks of d(CGC)rG> (**8**) (where > represents cyclic phosphate) and d(UGCG) **9** as previously reported.[4d] Products

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8 and **9** were characterized by enzymatic digestion and further confirmed by ESI-MASS. ESI-MASS (negative) for **8**, calcd 1254.8, found 1254.6; for **9**, calcd 1175.8, found 1175.6.

In the case of photoproduct **6**, formation of dG, dC, and dU in a ratio of 3:3:1 with formation of rX was observed. Ribonuclease T1 treatment of product **6** showed the two peaks for d(CGC)rX> and **9**. d(CGC)rX> was subjected to enzymatic digestion with P1 and calf intestine alkaline phosphatase (AP), and the mixture was analyzed by HPLC as described above. Formation of dC and dG is in a ratio of 1:2. Each oligomer was further confirmed by ESI-MASS. ESI-MASS (negative) for **5**, calcd 2428.6, found 2428.2; for **6**, calcd 2444.6, found 2444.2.

Formation of d(UGCG) from Photoirradiated ODN 1−2 in the Presence of Various Amounts of Zα after Ribonuclease T1 Treatment. The reaction mixture (total volume 100 μL) contained deoxyoligonucleotide ODN **1**−**2** (0.5 mM total base concentration) in 20 mM sodium phosphate buffer (pH 7.0) and 20 mM NaCl with various amounts of Zα (2, 1.5, 1, 0.75, 0.5, 0.25, and 0 equiv of Zα) in a microfuge tube (0.6 mL). The CD spectrum of the reaction mixture was measured before irradiation. The solutions were irradiated at 302 nm for 1 h at 0 °C. An aliquot (10 μL) was taken up and 0.5 unit/mL ribonuclease T1 was added. After 4 h of incubation at 37 °C, the solution was analyzed by HPLC. Analysis was carried out on a Cosmosil 5C18-MS column; HPLC analysis was performed with 0.05 M ammonium formate containing 2−8% acetonitrile, eluted over a linear gradient at a flow rate of 1.0 mL/min for 40 min, at 40 °C. HPLC profiles of these reaction mixtures are shown in Figure 4

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Results and Discussion

To date, only a few chemical or photochemical reactions that are specific to Z-form DNA have been developed.[12] Most of the available experimental data are limited to investigations on poly(dG-dC), which exists in a Z-form only under high salt conditions.[8a,6] Previously, we demonstrated that the incorporation of a methyl group at the guanine C8 position (m^8G) dramatically stabilizes the Z-form of short oligonucleotides in a variety of sequences.[10a] As a result, concentration of NaCl to induce B−Z transition is significantly reduced, with the midpoint NaCl concentrations for $d(CGCGCG)_2$ and $d(CGCm^8GCG)_2$ being 2.6 M and 30 mM, respectively. ^{1}H NMR analysis demonstrated that the solution structure of $d(CGCm^8GCG)_2$ is very similar to the Z-form crystal structure of $d(CGCGCG)_2$, and the hydrophobic C8 methyl group exposed to the solvent does not distort the global structure of Z-form DNA.[10a] We also have demonstrated that ^{I}U-containing deoxyoctanucleotide, d(CGCG^{I}UGCG) (ODN **1**)/d(Cm^{8}GCACm^{8}GCG) (ODN **2**), adopts a Z-form at 2 M NaCl, and under UV irradiation conditions an unprecedented stereospecific C2′α-hydroxylation occurs at G_4 of ODN **1**.[4d]

Because Rich and colleagues recently found that the NH_2-terminus domain of ADAR1 stabilizes the Z-form, we first examined the conformation of ODN **1**−**2** in the presence of Zα by circular dichroic (CD) spectroscopy. Figure 1a shows CD spectra of ODN **1**−**2** in the presence of different concentrations of Zα, showing that the maximal amplitude at 270 nm was reached at 2 equiv of Zα (Zα/dsDNA) against dsDNA. Further addition of Zα did not change the CD spectrum. Similar CD spectral change was observed after addition of NaCl (Figure 1b).[4d] These results indicate that the addition of Zα or NaCl induces the positive peak around 270 nm and the negative peak around 300 nm in the CD spectrum, which is a typical CD spectral change due to B−Z transition. The results clearly indicate that Zα and NaCl stabilize ODN **1**−**2** to the Z-form even if DNA substrates contain an A-^{I}U base pair.[4d] There are, however, several differences in the CD spectra. The maximum of the CD spectrum shifted from 266 nm (2M NaCl) to 270 nm (2 equiv of Zα), and the negative peak at 294 nm (2 M NaCl) was less pronounced and shifted to 296 nm (2 equiv of Zα). These differences of CD spectrum were previously reported out in the case of Z-form poly(dG-dC) and poly(dG-dm^{5}C) induced by Zα and NaCl.[13,14] The difference suggests a slight conformational change in Z-form DNA under the two separate conditions. A relatively large negative Cotton effect exists below 250 nm in the case of Zα due to the α-helical structure of Zα. Importantly, a corresponding duplex without m^8G ODN **1**/d(CGCACGCG) was not converted into Z-form DNA at all in the

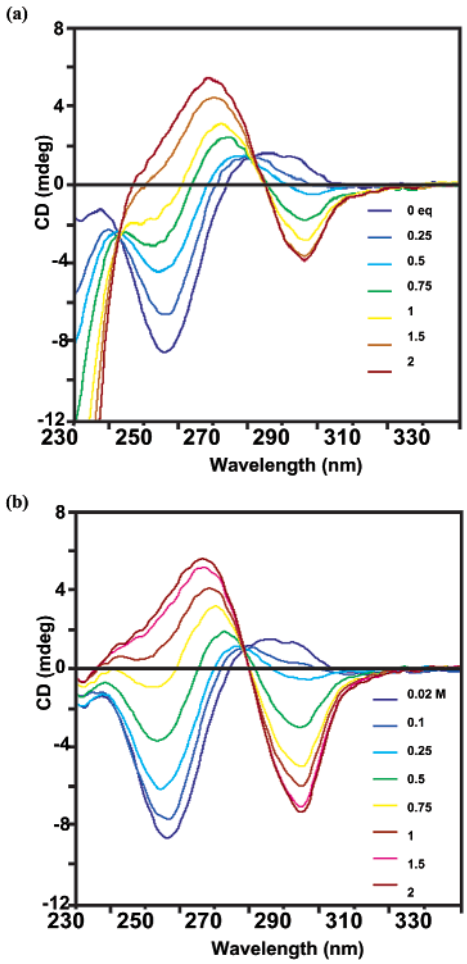

Figure 1. Circular dichroism (CD) of octanucleotide in the presence of various amounts of Zα or NaCl. (a) Titration of d(CGCG^{I}UGCG)(ODN **1**)/d(Cm^{8}GCACm^{8}GCG)(ODN **2**) (a) with Zα (2, 1.5, 1, 0.75, 0.5, 0.25, or 0 equiv of Zα) or (b) in NaCl (2, 1.5, 1, 0.75, 0.5, 0.25, 0.1, or 0.02 M) in 20 mM NaCl and 20 mM sodium phosphate buffer (pH 7.0) at 5 °C. The concentration of octanucleotide was 0.5 mM base concentration.

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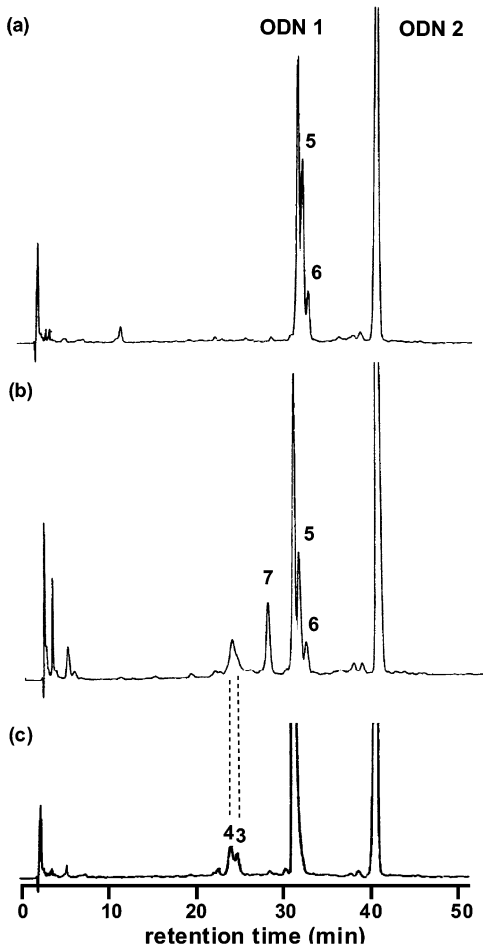


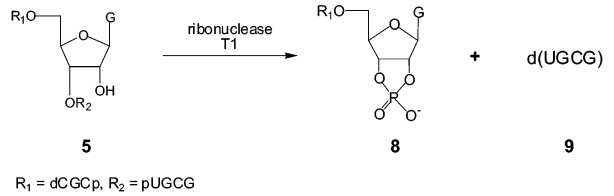
Figure 2. HPLC profiles of UV-irradiated d(CGCG^{I}UGCG)(ODN **1**)/d(Cm^{8}GCACm^{8}GCG)(ODN **2**) (a) in the presence of Zα (2 equiv/dsDNA), (b) in high salt (2 M), and (c) in the absence of Zα. Each of the reaction mixtures (100 μL) contained octamer (0.5 mM total base concentration) in a microfuge tube (0.6 mL) and was irradiated for (a) 1.5 h, (b) 2 h, or (c) 3 h at 0 °C with a monochromator (302 nm).

presence of 2 equiv of Zα or 2 M NaCl, indicating that the incorporation of m^{8}G effectively stabilizes the Z-form DNA by favoring the syn glycosyl conformation.[11]

We next examined the photoreaction of Z-form DNA induced by Zα. A reaction mixture containing ODN **1**−**2** and 2 equiv of Zα in 20 mM sodium phosphate buffer (pH 7.0) was irradiated with a monochromator (302 nm) at 0 °C. The HPLC profile of the photoirradiated reaction mixture (Figure 2a) shows the consumption of ODN **1** with the clear formation of **5** and **6**, which were also produced in the photoreaction of Z-form ODN **1**−**2** in the presence of 2 M NaCl (Figure 2b). Enzymatic digestion of **5** produced dG, dC, rG, and dU in a ratio of 3:3:1:1, indicating that this product is the rG-containing octamer. The mass of product **5** was confirmed by ESI-MASS analysis to be 2428.2, and enzymatic digestion with ribonuclease T1 provided d(CGC)rG> (**8**) (where > represents cyclic phosphate) and d(UGCG) (**9**) (Scheme 1).[4d] A detailed HPLC analysis indicated that 2′-arabinosylguanosine was not detected in the enzymatic digestion of the photoirradiated reaction mixture. These results indicate that stereospecific C2′α-hydroxylation occurs at the G_4 by the deoxyuridin-5-yl.

Enzymatic digestion of product **6** produced dG, dC, and dU in a ratio of 3:3:1 with formation of an unknown product. Interestingly, ribonuclease T1 treatment of **6** showed the formation of **9** with the formation of tetranucleotide d(CGC)rX>, which was hydrolyzed to dG and dC in a ratio of 1:2 and rX by P1 nuclease and AP. These results indicate that the G_4 residue of ODN **1** possesses a 2′α-hydroxy group group. ESI-MASS analysis of photoproduct **6** indicates that the molecular weight of **6** was 2444.2. The results clearly indicate that the base region of rG residue is further oxidized with an increase of molecular weight of +16 relative to **5**. HPLC analysis indicates that rX is not 8-oxoguanosine by comparison with the authentic material. Further characterization was unsuccessful due to the small quantity of rX. Earlier we have found that addition of NaI during the photoreaction of Z-form ODN **1**−**2** in the presence of 2 M NaCl enhances the formation of **5**, presumably by the rapid reduction of the intermediate C2′α-hydroperoxide.[4d] Similarly, in the presence of 50 mM NaI, enhanced formation of **5** in the photoirradiation of Z-form ODN **1**−**2** induced by Zα was observed. Interestingly, formation of **6** was almost completely suppressed under these conditions, suggesting that the oxidation of the base region of rG is caused by the intermediate C2′α-hydroperoxide.

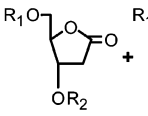
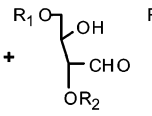
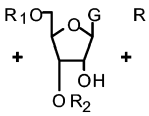
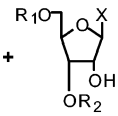
Scheme 1



In accord with the previous observation, photoirradiation (302 nm) of B-form ODN **1**−**2** under low-salt conditions provided C1′- and C2′-oxidation products **3** and **4**, respectively (Figure 2c). The formation of **3** and **4** resulted from conformationally dependent C2′α-hydrogen abstraction by the deoxyuridin-5-yl in B-DNA.[4a] Importantly, in the Z-form DNA in 2 M NaCl, photoproducts **3** and **4** were still formed, whereas in the Z-form DNA induced by Zα, **3** and **4** were not formed. The almost complete disappearance of C1′- and C2′-oxidation in Z-form ODN **1**−**2** induced by Zα is presumably due to the tight packing of Zα−DNA complex to retain the G_4 residue in a C3′-endo conformation, which prevents Criegie-type rearrangement of C2′-hydroperoxide. Slight differences in the CD spectra might indicate such structural differences. In Z-form ODN **1**−**2** in the presence of 2 M NaCl, halogen exchange product **7** was also observed (Figure 2b). The results clearly indicate that, in Z-form DNA induced by Zα, C2′α-hydroxylation by deoxyuridin-5-yl was greatly enhanced by the absence of C1′- and C2′-oxidations and the halogen exchange reaction. The yield of photoproducts **3**−**7** in B-form and Z-form are summarized in Table 1. The quantum yields of C2′α-hydroxylation in the presence of 2 M NaCl and 2 equiv of Zα at 0 °C (302 nm) were estimated as 9.1×10^{-5} and 3.6×10^{-4}, respectively.

Inspection of the X-ray structure of the Zα∠d(CGCGCG)$_2$ complex revealed that Thr^{191} and Arg^{174} bind to the furanose oxygens of G_2 and G_6, and the face of the aromatic ring of Tyr^{177} makes a close van der Waals contact with C8 in the syn conformation of G_4.[8f] The crystal structure suggests that the ribose C2′β hydrogen of the G at the 5′ side is very close to deoxyuridin-5-yl, whereas C1′- and C2′α-hydrogen are very far from the C5 of uracil (Figure 3). The results suggest that Zα tightly packs with C3′-endo sugar puckering of G in ODN **1**−**2** and promotes specific C2′β-hydrogen abstraction by the de-

Table 1. Product Analysis in the Photoreaction of 5-Iodouracil-Containing B- and Z-Form d(CGCG^{I}UGCG)(ODN **1**)/d(Cm^{8}GCAm^{8}GCG)(ODN **2**)[a]

Conformation	reaction period	conversion (%)	Products (%)				
			R_1 = dCGCp, R_2 = pUGCG				
			 **3**	 **4**	 **5**	 **6**	d(CGCG^{Cl}UGCG) **7**
B-Form	2 h	81	14	24	nd	nd	nd
Z-Form (NaCl)	2 h	74	2.8	13	16	3.0	15
Z-Form (Zα)	1 h	77	nd	nd	40	8.1	nd
Z-Form (Zα)[(b)]	1 h	77	nd	nd	62	nd	nd

[a] Each reaction mixture (100 μL) containing ODN **1**/ODN **2** (0.5 mM base concentration) in 20 mM sodium phosphate buffer (pH 7.0) was irradiated at 0 °C with a monochromator (302 nm). [b] NaI (50 mM) was added in the reaction mixture.

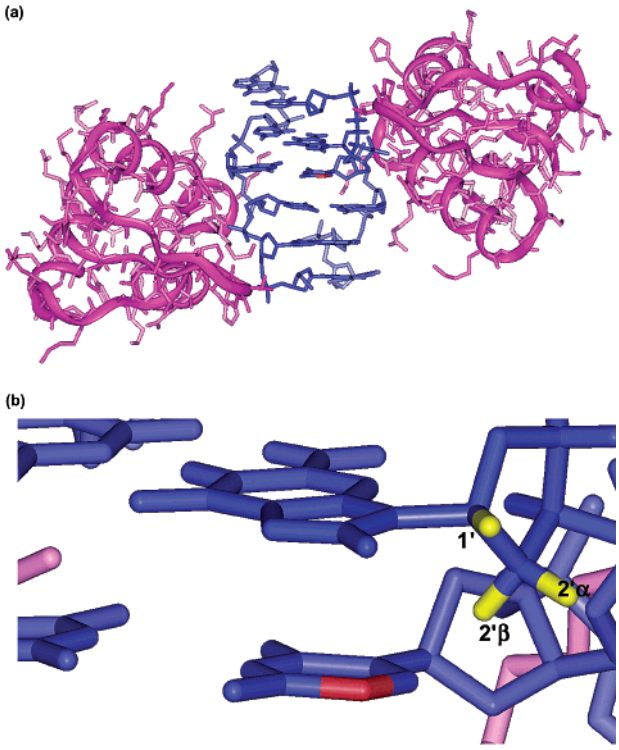
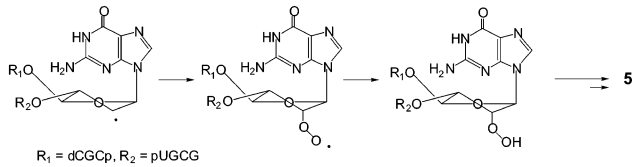


Figure 3. Structure of Zα-d(CGCGCG)$_2$ complex based on the X-ray crystal structure (a) and a close-up view of one 5′-GC-3′ region (b). In panel a, Zα is drawn in purple in a ribbon presentation, and DNA is in blue. In panel b, C5 of C is in red and Hs of G are in yellow.

oxyuridin-5-yl[9f] to cause the suppression of C1′- and C2′-oxidation. Analogous enhancement of reaction by protein binding has been reported.[15] Previously, we examined the photoreaction of d(CGCG(2′β-D)^{I}UGCG)/d(Cm^{8}GCACm^{8}GCG) whose G$_4$ residue was deuterated to identify the site of hydrogen abstraction by the deoxyuridin-5-yl in Z-form DNA.[8f] We found that C2′α-hydroxylation specifically results from the

Scheme 2



abstraction of C2′β-hydrogen atom by the deoxyuridine-5-yl in Z-form.[8f] Photoreaction of ODN **1**−**2** under an $^{18}O_2$ atmosphere clearly demonstrated that the source of C2′-oxygen of the rG of **5** was derived from O_2.[8f] These results indicate that O_2 is the source of the C2′-hydroxy group of G$_4$ (Scheme 2).

We have already demonstrated that C2′α-hydroxylation was promoted proportionally with increasing the ratio of Z-form DNA by increasing NaCl concentration, and this suggests the possibility of monitoring the content of Z-form DNA (Figure 5b).[8f] To test this possibility on Z-form DNA induced by Zα, the photoreaction of ODN **1**−**2** with different concentrations of Zα was investigated. The total amount of C2′α-hydroxylated product was quantitated in terms of **9** from **5** and **6** by ribonuclease T1 treatment (Scheme 2). Figure 4 shows the typical HPLC profiles of ODN **1**−**2** at different concentration of Zα after ribonuclease T1 treatment, indicating that the amount of **9** increased proportionally with increasing amounts of Zα. The results clearly demonstrated that the amount of **5** and **6** reflects the proportion of Z-form DNA (Figure 5b), confirming that this photochemical and enzymatic procedure can be used to quantitate the content of Z-form DNA.

In the present study, we found that C2′α-hydroxylated product was exclusively produced at the 5′ side of the 5-halouracil in the Zα-DNA complex more than under high-salt conditions. Efficient formation of C2′α-hydroxylated product in the Zα-DNA complex compared with high-salt conditions can be explained by three factors upon protein binding. The molecular coefficient of ODN **1**−**2** is twice as large at 302 nm in the complex. Efficient C2′β-hydrogen abstraction occurs at G$_4$, which is tightly packed with the C3′-end sugar puckering. Moreover, favoring C3′-endo conformation in the complex retards the Criegie-type rearrangement of C2′α-hydroperoxide.

(15) (a) Wagenknecht, H.-A.; Rajski, A. R.; Pascaly, M.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **2001**, *123*, 4400. (b) Rajski, S. R.; Barton, J. K. *Biochemistry* **2001**, *40*, 5556.

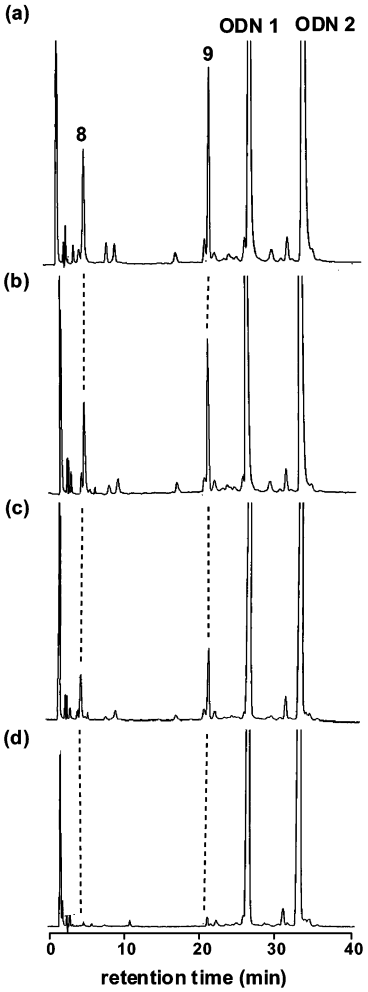


Figure 4. HPLC profiles of photoirradiated d(CGCG^{I}UGCG)(ODN **1**)/d(Cm^{8}GCACm^{8}GCG)(ODN **2**) after ribonuclease T1 in the presence of 2 equiv (a), 1 equiv (b), or 0.5 equiv (c) of Zα (Zα/dsDNA). Each of the reaction mixtures (100 mL) contained octamer (0.5 mM total base concentration) in 20 mM sodium phosphate buffer (pH 7.0) in a microfuge tube (0.6 mL), and was irradiated for 1 h at 0 °C with a monochromator (302 nm). To a 10 mL aliquot was added 0.5 mL of ribonuclease T1 (10 unit/mL), and the solution was incubated at 37 °C for 4 h. Under these conditions, all the hydroxylated product **5** was hydrolyzed to (**6**) d(CGC)rG> and (**7**) d(UGCG), as judged by HPLC analysis.

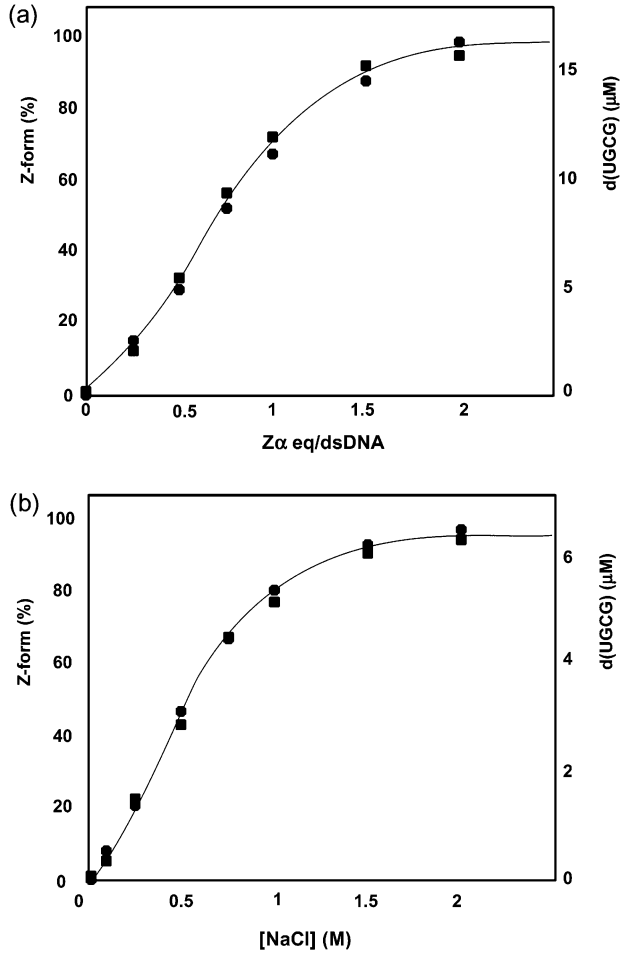
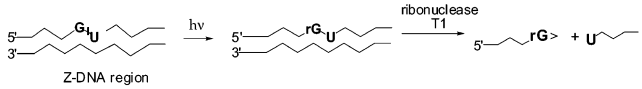


Figure 5. Formation of d(UGCG) and proportion of Z-form of octanucleotide d(CGCG^{I}UGCG)(ODN **1**)/d(Cm^{8}GCACm^{8}GCG)(ODN **2**) as a function of (a) [Zα] and (b) [NaCl]. The proportion of Z-form was estimated by CD spectroscopy shown in Figure 1. Circles represent the ratio of Z-form and squares represent the concentration of d(UGCG).

Scheme 3



Z-form DNA can be formed in longer DNA with Zα under certain physical conditions.[11] In the living cell, DNA often exists as a DNA−protein complex and in some cases much negative supercoiling occurs during transcription. Since the DNA local structures are assumed to appear in a very short period of time, utilization of a photoreaction as a DNA conformational probe would provide important structural information on a short-lived DNA local structure. The present results indicate that this method may be useful to detect Z-form DNA in such circumstances. 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 5-bromouracil (^{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. The efficacy of this method in detecting local Z-form s in longer DNA is currently under investigation.

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