Photoinduced Deoxyribose C2′ Oxidation in DNA. Alkali-Dependent Cleavage of Erythrose-Containing Sites via a Retroaldol Reaction

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Abstract: Photoreactions of various 5-iodouracil- (^{I}U)-containing oligonucleotides have been investigated. It was found that d(GCA^{I}UGC)$_2$ undergoes a competitive C1′ and C2′ oxidation at the 5′ side of the ^{I}U residue to give deoxyribonolactone-containing hexamer **1** and erythrose-containing hexamer **2**, respectively. Upon heating under alkaline conditions, erythrose-containing hexamer **2** was shown to undergo a remarkably facile retroaldol reaction to give two fragments, both having phosphoroglycoaldehyde termini in high yields. On the basis of the chemical reactivity of **2**, a new specific method for detection of the erythrose-containing sites resulting from deoxyribose C2′ oxidation in DNA was devised. Erythrose-containing sites were prepared by photoirradiation of duplex ^{I}U-containing 13 mer 5′-d(CG^{I}UGT^{I}UTA^{I}UAC^{I}UG)-3′/5′-d(CAGTATAAACACG)-3′. After photoirradiation, the reaction mixture was treated with hot alkali and $NaBH_4$ and then subjected to enzymatic digestion. HPLC analysis of the digested mixture revealed the formation of modified mononucleotides **25–28**, allowing the quantification of the erythrose-containing sites being produced at the 5′ side of all four ^{I}U residues of the 13 mer. These results indicate that this method can be used for the detection and quantification of erythrose-containing sites resulting from deoxyribose C2′ oxidation in DNA.

Introduction

Organic molecules that can induce DNA cleavage in a sequence-specific fashion are potent pharmaceuticals and powerful reagents in molecular biology.[1] Most of the DNA-cleaving molecules so far reported, including natural antitumor antibiotics[2] and designed synthetic DNA-cleavers,[1a,c3] are known to cleave DNA by H abstraction from the deoxyribose backbone via a radical process. While the chemistry of DNA damage resulting from H abstraction at C1′,[4] C3′,[5] C4′,[6] and C5′ [7] of the DNA deoxyribose has been investigated, the DNA lesion resulting from C2′ H abstraction has been scarcely studied. One exception is the report on the formation of erythrose-containing sites in γ-radiolysis of calf thymus DNA where silylated erythritol was detected by GC–MS after $NaBH_4$ reduction and followed by enzymatic digestion and silylation.[8] Since erythrose-containing sites arising from C2′ H abstraction are suggested to be one of the major damages induced by γ-radiolysis of DNA, it is very important to know the detailed chemical behaviors of these sites in DNA. Unfortunately, no detailed information is available for the chemistry of DNA lesion resulting from C2′ H abstraction.

The thymine residue in DNA can be replaced by 5-bromo- or 5-iodouracil, and such 5-halouracil-containing DNA still remains functional in vivo even though the frequency of mutation has been shown to be enhanced.[9] The incorporation of 5-halouracils into DNA has long been known to enhance photosensitivity with respect to DNA–protein photo-cross-linking, DNA strand breaks, and creation of alkali-labile sites.[10] Photo-cross-linking between ^{Br}U-containing DNA and DNA-binding proteins has been effectively used for the investigation of their sequence-specific interactions.[11] Recently, we have found that photoexcited d(GCA^{Br}UGC)$_2$ undergoes a facile C1′ oxidation to provide deoxyribonolactone-containing hexamer **1** with release of free adenine.[4d] Photoreactions of various 5-bromouracil-containing

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hexamers indicated that both duplex structure and 5′-A^{Br}U-3′ sequence are essential for this type of reaction. We also observed that the uracilyl-5-yl radical produced by photoexcitation of the 5′-A^{Br}U-3′ sequence selectively abstracts the C1′ H of the deoxyribose backbone of duplex DNA. We proposed that electron transfer from photoexcited adenine at the 5′ side to the adjacent ^{Br}U in the duplex DNA is responsible for the efficient formation of the uracilyl-5-yl radical.[4d]

In the present study, we found that photoirradiation of 5-iodouracil-(^{I}U)-containing oligonucleotides produces a novel C2′ oxidation product together with the C1′ oxidation product at the 5′ side of the ^{I}U residue. This is the first demonstration for the direct isolation of the C2′ oxidation product in deoxyoligonucleotides. Interestingly, such competitive C1′ and C2′ oxidation occurs even in a single-stranded DNA with a smaller sequence preference, in sharp contrast to the case of the 5′-A^{Br}U-3′ sequence. By the use of erythrose-containing hexamer **2**, the detailed chemistry for the degradation of C2′ oxidation sites in DNA under alkaline conditions was investigated. On the basis of the chemical reactivity of **2**, a new method for the detection and quantification of the deoxyribose C2′ oxidation in DNA has been devised.

Results and Discussion

Photoirradiation of d(GCA^{I}UGC)$_2$. Although ^{I}U is known to be incorporated into DNA in place of thymine, the photochemistry of ^{I}U-containing DNA has not attracted much attention.[10] In order to understand the detailed chemistry of the uracilyl-5-yl radical produced in DNA, photoreactions of various ^{I}U-containing oligonucleotides were examined. ^{I}U-containing oligonucleotides were synthesized by the β-cyanoethyl phosphoramidite method.[12] The β-cyanoethyl phosphoramidite of 5-iodo-2′-deoxyuridine was prepared from 5′-*O*-(4,4′-dimethoxytrityl)-5-iodo-2′-deoxyuridine and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite.[13] Melting temperature (T_m) experiments suggested that d(GCA^{I}UGC)$_2$ (T_m 39.8 °C) probably forms a duplex of a stability comparable with that of d(GCATGC)$_2$ (T_m 40.6 °C). Therefore, it is very likely that the ^{I}U moiety forms a base pairing with the complementary adenine site.

We first examined the photoreaction of self-complementary hexamer d(GCA^{I}UGC)$_2$ in order to compare the photoreactivity of the ^{I}U site with that of the ^{Br}U site. Photoirradiation of self-complementary duplex d(GCA^{I}UGC)$_2$ was performed at 0 °C with a transilluminator (302 nm) for 3 h. Figure 1 shows the HPLC profile of the reaction mixture, showing the formation of C1′ oxidation product **1**[4d] and an unknown product **2** as a broad peak together with adenine and a minor amount of dehalogenated product d(GCAUGC). The structure of **2** was characterized by the sequence shown in Scheme I. $NaBH_4$ reduction of **2** gave a reduced hexamer **3**, and subsequent digestion with snake venom phosphodiesterase (s. v. PDE) and alkaline phosphatase (AP) provided 3′-[1-(2*R*,3*S*)-erythritolyl] 2′-deoxycytidylate (**4**) together with dG, dC, and dU in an approximately 2:1:1 ratio. These results indicate that the 3′ phosphodiester bond of **4** is not digested with s. v. PDE. The structure of **4** was confirmed by independent synthesis of the authentic sample. To the best of our knowledge, this is the first demonstration for the direct isolation of a C2′ oxidation product from deoxyoligonucleotides.

Quantitative HPLC analysis of the photolysate revealed that 63% of d(G$_1$C$_2$A$_3$^{I}U$_4$G$_5$C$_6$) was consumed to provide **1** (16%), **2** (13%), and d(GCAUGC) (5%). The formation of **2** together with **1** and d(GCAUGC) indicates that C1′ and C2′ hydrogens of the deoxyribose moiety of (adenine)$_3$ (A$_3$) are competitively abstracted by the uracilyl-5-yl radical.

Photoirradiation of Various 5-Iodouracil-Containing Hexanucleotides. In order to get a general picture for the photoreaction

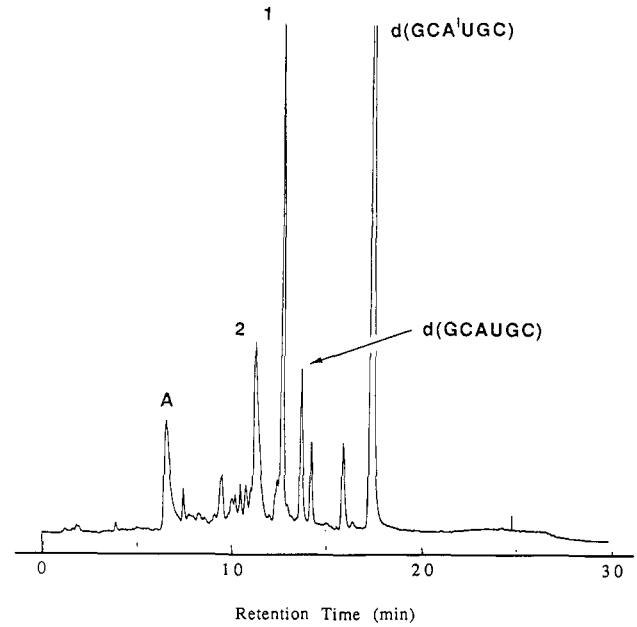
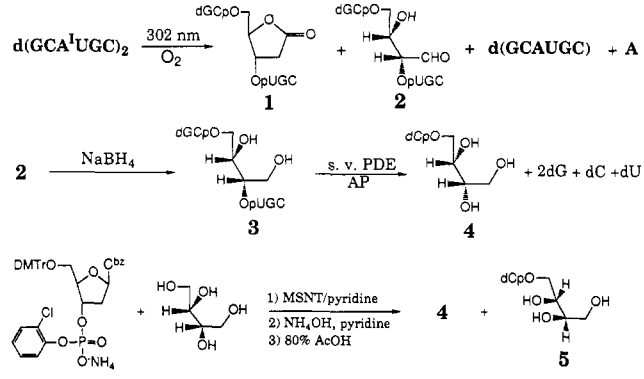
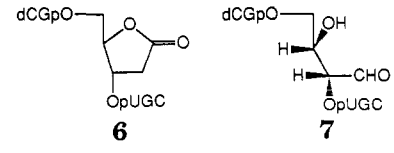


Figure 1. HPLC analysis of UV-irradiated d(GCA^{I}UGC)$_2$. The reaction mixture was analyzed by HPLC on a Cosmosil 5C$_{18}$ column (4.6 × 150 mm), detected at 254 nm, elution was with 0.05 M ammonium formate containing 0–15% acetonitrile, linear gradient (20 min) at a flow rate of 1.5 mL/min. A:adenine.

Scheme I



of ^{I}U-containing DNA, photoirradiation of various ^{I}U-containing hexamers was carried out and the photodegradation products were analyzed by means of HPLC (Table I). In all cases, the formation of C1′ and C2′ oxidation products was observed. In the case of photoirradiation of non-self-complementary d(CGA^{I}UGC), we obtained C1′ oxidation product **6** and C2′ oxidation



product **7** (run 2). These results indicate that the uracilyl-5-yl radical presumably produced by the homolytic cleavage of the C–I bond competitively abstracts C1′ and C2′ hydrogens of the deoxyribose at the 5′ side of ^{I}U. Interestingly, such competitive C1′ and C2′ H abstraction occurs even in a single-stranded ^{I}U-containing hexamer (run 2), and there was a smaller sequence preference (runs 4, 5, and 6), in contrast to the photoreactions of ^{Br}U-containing oligonucleotides previously reported.[4d] These results strongly suggest that the photoreaction of ^{I}U in DNA proceeds via a mechanism different from that of A^{Br}U, which strictly requires duplex structure for the highly selective C1′ H abstraction.

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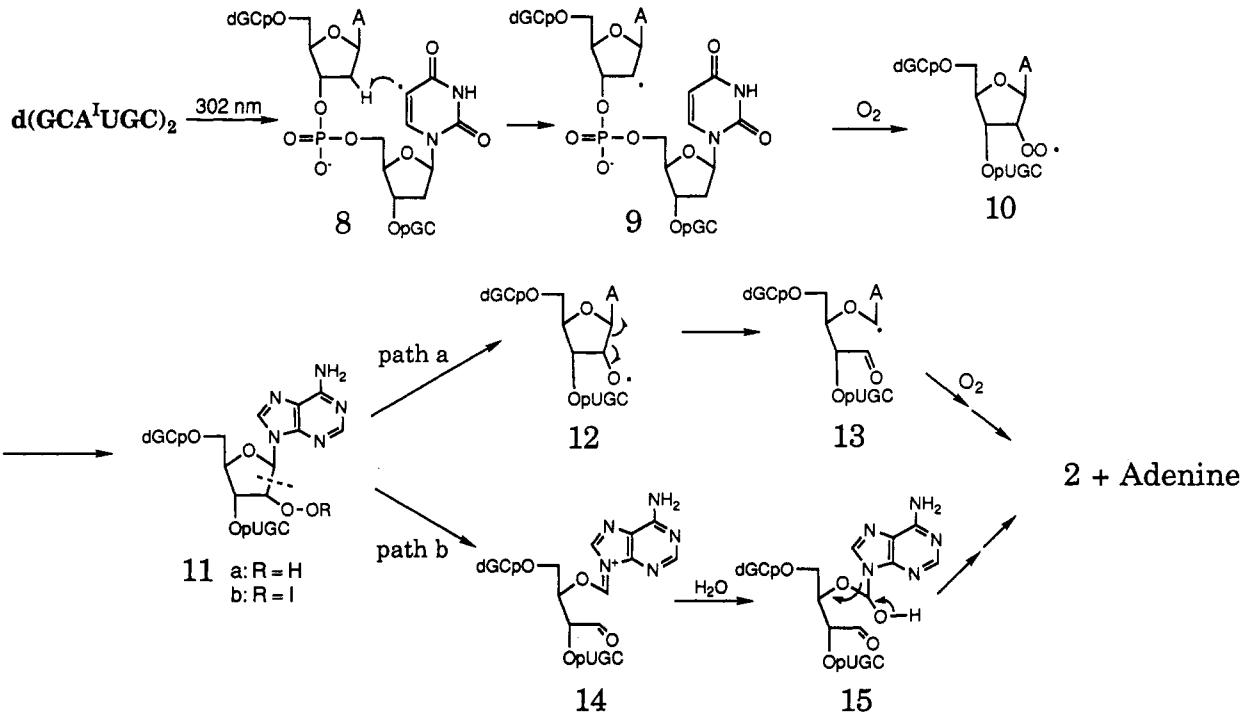
Table I. Product Analysis in Photoirradiation of 5-Iodouracil-Containing Deoxyhexanucleotides[a]

run	hexamer	free base, μM A	T	C	G	consumed hexamer, %	C1′ oxidation, % (product)	C2′ oxidation, % (product)	dehalogenated product, %
1	d(GCA^{I}UGC)$_2$	65	0	0	0	63	16 (**1**)	13 (**2**)	5
2	d(CGA^{I}UGC)	<20	0	0	0	61	6 (**6**)	4 (**7**)	2
3	d(CGA^{I}UGC) d(GCTACG)	28	0	0	0	49	7 (**6**)	14 (**7**)	7
4	d(GCT^{I}UGC) d(CGAACG)	0	11	0	0	55	5 (**1**)	5 (**2**)	2
5	d(GCC^{I}UGC) d(CGGACG)	0	0	19	0	48	6 (**1**)	5 (**2**)	3
6	d(GCG^{I}UGC) d(CGCACG)	0	0	0	7	19	7 (**1**)	7 (**2**)	

[a] Each of the reaction mixtures (30 μL) containing hexaner (1 mM base concentration) and NaCl (1 M) in 50 mM sodium cacodylate buffer (pH 7.0) in a capillary cell was irradiated for 3 h at 0 °C with transilluminator (302 nm) under aerobic conditions.

Scheme II



While there are many examples[14] for electron-transfer-type photoreactions of ^{Br}U derivatives, the photoreaction of ^{I}U is believed to proceed via a homolytic cleavage of the C–I bond.[15] This difference may be interpreted in terms of the difference in the bond energies of the halogen–carbon bonds, *i.e.*, the C–I bond being weaker than the C–Br bond.[16] Taking into account the experimental results, homolytic cleavage of the C–I bond is suggested to be an initial step of the photoreaction, and the resulting uracilyl-5-yl radical **8** competitively abstracts C2′ and C1′ hydrogens of the deoxyribose at the 5′ side of ^{I}U. von Sonntag et al.[8] reported that the formation of erythrose-containing sites in DNA is observed in γ-radiolysis of calf thymus DNA under aerobic conditions. They proposed that deoxyribose C2′ radicals are the precursors of the erythrose-containing sites in DNA on the basis of the model study in which erythrose was formed in pulse radiolysis of 2′-bromo-2′-deoxyuridine under aerobic conditions.[17]

The sequence of the events leading to **2** is illustrated in Scheme II. The proposed mechanism involves hydroperoxide **11a** as an intermediate which is followed by β-scission of the resulting alkoxy radical **12** to produce radical **13**. Further reaction of **13** with molecular oxygen would produce **2** with the release of free adenine (path a). Alkoxy radical **12** may be formed by homolysis of hydroperoxide **11a**, which is produced via H abstraction of **10**. Although a similar type of alkoxy radical formation from a peroxy radical via tetroxide has been precedented in a model system,[18] the formation of such tetroxide in a structurally restricted double-stranded DNA seems to be highly unlikely. It is also possible that recombination of peroxy radical **10** with I• would produce peroxide **11b**. However, homolysis of **11b** also gives rise to the formation of the same alkoxy radical, **12**. Alternatively, **2** may be formed by a facile heterolytic cleavage of the C1′–C2′ bond of unstable β-nitrogen-substituted hydroperoxide **11a** via the formation of **14** (path b). Hydrolytic cleavage of **14** via **15** would produce **2** and 9-formyladenine, which immediately decomposes to adenine under aqueous conditions. In contrast to the competitive C1′ and C2′ H abstraction observed for ^{I}U, highly selective C1′ H abstraction was observed in the photoreaction of the 5′-A^{Br}U-3′ sequence.[4d] These facts indicate that these two photoreactions proceed via an entirely different mechanism. In the case of the photoreaction of the 5′-A^{Br}U-3′ sequence, we proposed that sequence- and structure-dependent intrastrand electron transfer from excited adenine to adjacent ^{Br}U at the 3′ side would facilitate an extremely facile formation of the uracilyl-5-yl radical from ^{Br}U.[4d]

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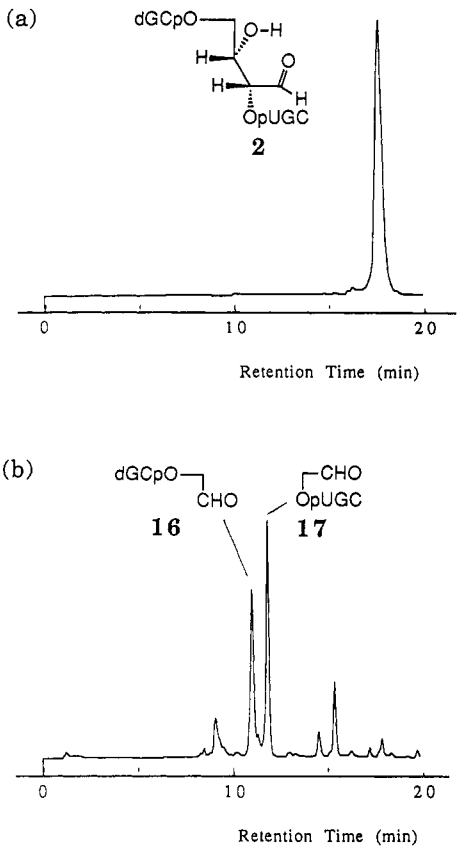
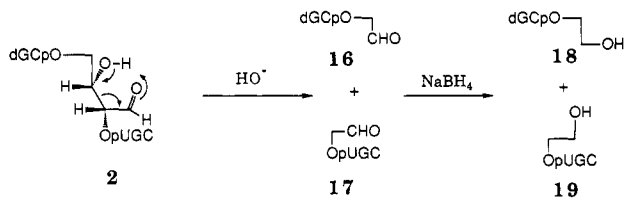


Figure 2. HPLC of (a) purified **2** (top) and of (b) the degradation products of **2** by alkaline treatment (bottom). Analysis was carried out on a Wakosil DNA $5C_{18}$ column (4.6 × 150 mm) detected at 254 nm; elution was with 0.05 M ammonium formate containing 0–8% acetonitrile, linear gradient (20 min) at a flow rate of 1.5 mL/min.

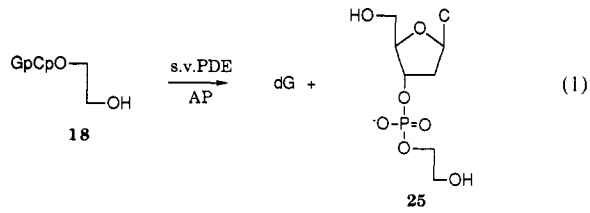
Scheme III



Alkali-Induced Degradation of Erythrose-Containing Sites. Although the alkali instability of erythrose-containing sites in DNA has been pointed out,[8] the mechanism of the strand scission by alkali treatment has not been well understood. Therefore, the degradation of the isolated erythrose-containing hexamer **2** under alkaline conditions was carefully investigated. Upon treatment with hot alkali (0.1 N NaOH, 90 °C, 5 min), **2** was found to decompose to **16** and **17** in a ratio of 1:1 in 70% yield, as shown in Figure 2. The structural confirmation of **16** and **17** was conducted after reduction with $NaBH_4$ to **18** and **19**, respectively (Scheme III). The structures of **18** and **19** were confirmed by comparison with their authentic samples prepared independently. The formation of **16** and **17** from **2** under alkaline conditions indicates that **2** undergoes a retroaldol reaction under the alkaline conditions. The result also indicates that the erythrose-containing site **20** in DNA is cleaved to the fragments containing phosphoglycoaldehyde termini such as **21** and **22**, as illustrated in Scheme IV.

Method for Detection of Erythrose-Containing Site 20 in DNA. Although the degradation products derived from hydrogen abstraction at C1′,[4] C3′,[5] C4′,[6], and C5′ [7] of the deoxyribose in DNA have been studied in recent years, DNA lesion resulting from C2′ H abstraction has not been thoroughly investigated due to the lack of a specific method for detection of these sites in DNA. We have devised a new method of detection for these lesions in longer DNA on the basis of the chemical reaction of erythrose-containing oligonucleotide **2**.

When dinucleotide **18** was incubated with s. v. PDE and AP at 37 °C, both dG and **25** were obtained in high yields (eq 1).



(1)

Further digestion of **25** was not observed even after prolonged incubation, indicating that the phosphodiester bond with ethylene glycol at the 3′ end resists the digestion with s. v. PDE. Thus, four modified mononucleotides **25–28** were prepared and their stabilities against s. v. PDE digestion were examined. Under the standard conditions, **25** and **26** were quite stable, whereas only small amounts of **27** and **28** were hydrolyzed to dT and dA, respectively. These results suggest that s. v. PDE digestion of the DNA fragment like **23** would produce mononucleotides **25–28** almost quantitatively under normal digestion conditions.

DNA fragments possessing erythrose-containing sites were prepared by UV irradiation of ^{1}U-containing 13 mer 5′-d(CG^{1}-UGT^{1}UTA^{1}UAC^{1}UG)-3′/5′-d(CAGTATAAACACG)-3′. Upon UV irradiation at 302 nm, the release of free bases C (10.5 μM), G (3.0 μM), T (3.7 μM), and A (14.4 μM) was observed on HPLC. The photolysate was heated in 0.1 N NaOH at 90 °C and then treated with 0.1 M $NaBH_4$. The mixture was digested with s. v. PDE and AP and subjected to HPLC analysis. Figure 3 demonstrates the HPLC profile of the digestion mixture, where the formation of **25–28** was confirmed by comparison of their retention times under three different HPLC conditions with those of authentic samples. UV absorption spectra of these peaks measured by a diode array detector were identical with those of the authentic samples. These results clearly indicate that erythrose-containing sites are indeed produced at the 5′ side of all four ^{1}U residues of the 13 mer and that this assay method may be effectively used for the detection of erythrose-containing sites formed in DNA.

Finally, it should be pointed out that this type of deoxyribose C2′ oxidation may play an important role not only in the photoreaction of 5-halouracil-containing DNA but also in the oxidative DNA modification induced by various types of DNA-cleaving molecules. Since H abstraction reactions by such DNA-cleaving molecules may directly reflect their interaction sites in DNA,[19] identification of the H abstraction sites on DNA deoxyribose would provide valuable information for the understanding of their interaction sites on DNA. The method of detection for the C2′ oxidation in DNA developed in the present study would also provide a valuable tool for the analysis of other radical-induced oxidative DNA lesions.

Experimental Section

Materials and Methods. Calf intestine alkaline phosphatase (AP, 1000 unit/mL) and snake venom phosphodiesterase (s. v. PDE, 3 unit/mL) were purchased from Boehringer Mannheim. 5-Iodo-2′-deoxyuridine was purchased from Yamasa Shoyu Co. Ltd. 5′-*O*-(Dimethoxytrityl)-2′-deoxyuridine was prepared according to the published procedure.[20] 2-Cyanoethyl *N,N*-disopropylchlorophosphoramidite was purchased from Aldrich. The reagents for the DNA synthesizer such as I_2 solution (I_2/H_2O/pyridine/tetrahydrofuran, 3:2:19:76) A-, G-, C-, and T-β-cyanoethyl phosphoramidites were purchased from Applied Biosystems. (2,4,6-Trimethylbenzenesulfonyl)-3-nitrotriazolide (MSNT) and 1*H*-tetrazole

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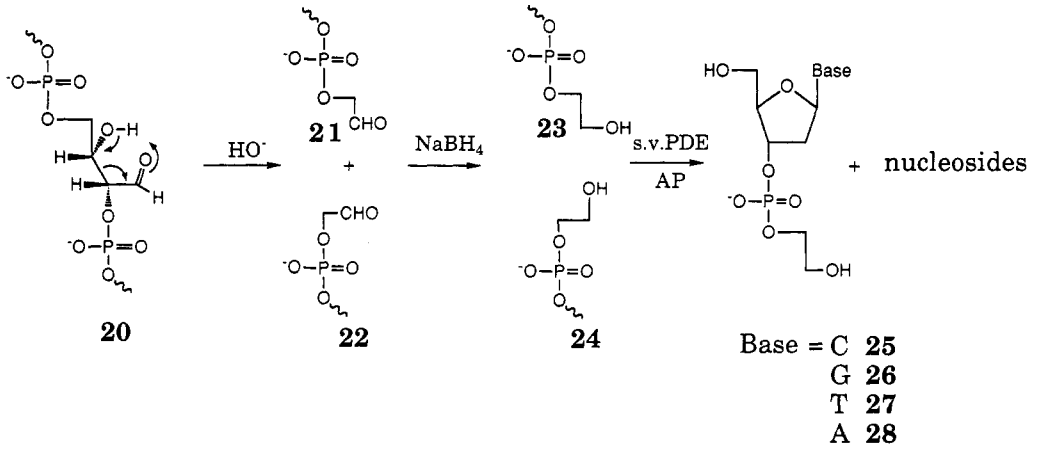
Scheme IV



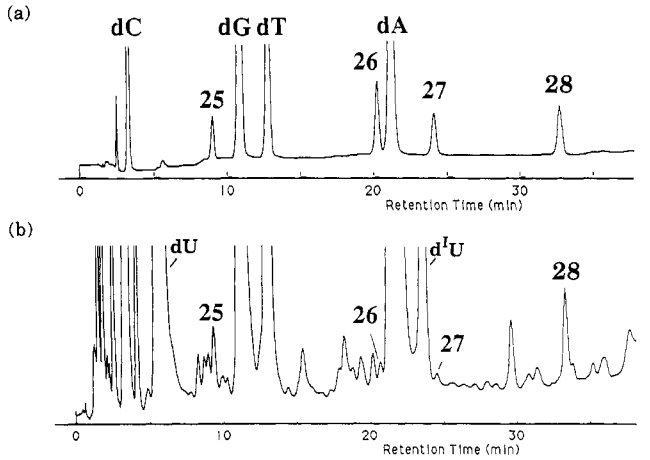


Figure 3. HPLC analysis of (a) the mixture of authentic mononucleotides **25–28** (25 μM each) and nucleosides (150 μM each) (top) and of (b) the products obtained from photoirradiation of 5′-d(CG¹UGT¹UTA¹UAC¹-UG)-3′/5′-d(CAGTATAAACACG)-3′ followed by alkaline treatment, $NaBH_4$ reduction, and subsequent enzymatic digestion (bottom). The assigned peaks comigrated with authentic samples prepared independently. Analysis was carried out on a Wakosil DNA $5C_{18}$ column (4.6 × 150 mm); elution was with 0.05 M triethylammonium acetate (pH 7.0) containing 0–4% acetonitrile, linear gradient (30 min) at a flow rate of 1.5 mL/min.

were purchased from Dojin Laboratories. Silica gel column chromatography was carried out on a Wakogel C-200. Preparative TLC was carried out on Merck silica gel 60 PF_{254} plates. HPLC analysis was carried out using a CCPE II HPLC system (TOSO, Tokyo) equipped with a Wakopak $5C_{18}$ (4.6 × 150 mm) or a Cosmosil $5C_{18}$ column (4.6 × 150 mm). Detection was carried out at 254 nm. ^1H NMR spectra were recorded on a JEOL-JNM-GX400 spectrometer. UV irradiation was carried out with a transilluminator (302 nm, Funakoshi FTI-36M). The ice-cooled reaction mixture in a Pyrex capillary cell (7-mm diameter) was irradiated under aerobic conditions from the distance of 10 cm.

Synthesis of Deoxyoligonucleotides. Oligonucleotides were prepared by the β-cyanoethyl phosphoramidite method[12] on controlled-pore glass supports (1 mmol) by using an ABI 381 A DNA synthesizer. The cyanoethyl phosphoramidite of 5-iodo-2′-deoxyuridine was prepared by the reported procedure.[13] After automated synthesis, the oligomer was detached from the support, deprotected, and purified by HPLC as described previously.[19] After lyophilization, approximately 20 OD of pure oligomer was isolated. Purity and concentrations of all oligonucleotides were determined by complete digestion with s. v. PDE and AP to 2′-deoxymononucleosides. The melting temperature (T_m) of $d(GCA^1UGC)_2$ was 39.8 °C at 0.1 mM base concentration in 50 mM phosphate (pH 7.0)–1 M NaCl, whereas the T_m of hexamer $d(GCATGC)_2$ was 40.6 °C under the same conditions.

Synthesis of 3′-[1-(2*R*,3*S*)-Erythritolyl] 2′-Deoxycytidylate (4) and 3′-[1-(2*S*,3*R*)-Erythritolyl] 2′-Deoxycytidylate (5). A mixture of 5′-*O*-(dimethoxytrityl)-*N*-benzoyl-2′-deoxycytidine 3′-(*o*-chlorophenyl phosphate) triethylammonium salt (100 mg, 0.108 mmol) and *meso*-erythritol (124 mg, 1.02 mmol) was dried by coevaporation with pyridine (20 mL, three times) and dissolved in dry pyridine (20 mL). To this solution was added MSNT (77 mg, 0.26 mmol), and the solution was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was treated with concentrated ammonium hydroxide–pyridine (10:1, 10 mL) at 55 °C for 12 h and then concentrated to dryness. The crude product thus obtained was treated with 80% aqueous acetic acid (50 mL) at room temperature for 1 h, and the solvent was evaporated under reduced pressure. Compounds **4** and **5** were purified by reverse-phase HPLC (Wakopak $5C_{18}$, 0.05 M triethylammonium acetate (pH 7.0) containing 0–4% acetonitrile, linear gradient (10 min) at a flow rate of 1.5 mL/min, retention time of 8.0 min for **4** and 8.4 min for **5**). After lyophilization, **4** (1 mg, 4.6%) and **5** (0.6 mg, 1.4%) were obtained as white solids. Analysis of each of the isolated products by ^1H NMR indicated that these two products (**4** and **5**) are diastereomeric to each other, one of which showed exactly the same HPLC behavior under three different conditions as that of the digestion product **4** obtained from the reduced photoproduct **3**. The purities of **4** and **5** were more than 95%, as determined by HPLC analysis. **4**: ^1H NMR (D_2O, TSP) δ 2.20 (ddd, 1 H, J = 14.2, 6.4, 6.3 Hz, 2′), 2.46 (ddd, 1 H, J = 14.2, 6.2, 3.3 Hz, 2′), 3.49 (dd, 1 H, J = 16.0, 6.3 Hz, 4″), 3.56–3.67 (m, 4 H, 5′, 2″, 3″ and 4″), 3.70 (dd, 1 H, J = 12.7, 3.5 Hz, 5′), 3.79–3.85 (m, 1 H, 1″), 3.88–3.93 (m, 1 H, 1″), 4.08–4.11 (m, 1 H, 4′), 4.60–4.64 (m, overlaped 1 H, 3′), 5.91 (d, 1 H, J = 7.6 Hz, 5), 6.15 (dd, 1 H, J = 6.3, 6.2 Hz, 1′), 7.69 (d, 1 H, J = 7.6 Hz, 6). **5**: ^1H NMR (D_2O, TSP) δ 2.14–2.24 (m, 1 H, 2′), 2.56 (ddd, 1 H, J = 12.9, 6.0, 3.1 Hz, 2′), 3.50 (dd, 1 H, J = 12.0, 6.6 Hz, 4″), 3.59–3.77 (m, 6 H, 1″,2″,3″,4″,5′), 3.90–4.02 (m, 1 H, 1″), 4.08–4.14 (m, 1 H, 4′), 4.60–4.64 (m, overlaped 1 H, 3′), 5.90 (d, 1 H, J = 7.6 Hz, 5), 6.15 (t, 1 H, J = 6.9 Hz, 1′), 7.69 (d, 1 H, J = 7.6 Hz, 6).

Synthesis of 3′-(2-Hydroxyethyl) 2′-Deoxycytidylate (25). A mixture of 5′-*O*-(dimethoxytrityl)-*N*-benzoyl-2′-deoxycytidine β-cyanoethyl phosphoramidite (100 mg, 0.12 mmol) and 2-hydroxyethyl acetate (520 mg, 5 mmol) was evaporated with 2 mL of acetonitrile (three times) and dissolved in dry acetonitrile (1 mL). To this solution was added 0.5 M 1*H*-tetrazole acetonitrile (1 mL) under an argon atmosphere, and the solution was stirred at room temperature overnight. To this reaction was added 2 mL of I_2 solution (I_2/H_2O/pyridine/tetrahydrofuran, 3:2:19:76), and the solution was stirred at room temperature overnight. After evaporation of the solvent, the residue was extracted with ethyl acetate. The organic layer was washed with saturated sodium thiosulfate and dried over anhydrous Na_2SO_4 and concentrated. The residue was treated with a mixture (1 mL) of concentrated ammonium hydroxide–pyridine (1:1) at 55 °C for 12 h and then concentrated. The crude product thus obtained was treated with 80% aqueous acetic acid (400 μL) at room temperature for 1 h, and the solvent was evaporated under reduced pressure. The residue was dissolved in 500 μL of distilled water, and then the solution was subjected to preparative TLC. After lyophilization, **25** was obtained as a white powder, yield 6 mg (14% from phophoramidite). The purity of **25** was more than 95%, as determined by HPLC analysis. **25**: ^1H NMR (D_2O) δ 2.22 (ddd, 1 H, J = 14.3, 6.7, 6.7 Hz, 2′), 2.46 (ddd, 1 H, J = 14.3, 6.7, 3.2 Hz, 2′), 3.60–3.66 (m, 3 H, 5′ and $HOCH_2CH_2$–), 3.70 (dd, 1 H, J = 12.5, 3.5 Hz, 5′), 3.79–3.82 (m, 2 H, $HOCH_2CH_2$–), 4.10 (dt, 1 H, J = 4.8, 3.5 Hz, 4′), 4.65–4.75 (m, overlaped 1 H, 3′), 5.94 (d, 1 H, J = 7.6 Hz, 5), 6.15 (t, 1 H, J = 6.7 Hz, 1′), 7.74

(d, 1 H, J = 7.6 Hz, 6). FABMS (negative ion, triethanolamine) m/z 350 $(M - 1)^-$, triacetyl derivative (positive ion, glycerol) m/z 478 $(M + 1)^+$.

Synthesis of 3′-(2-Hydroxyethyl) 2′-Deoxyguanylate (26). A procedure similar to the synthesis of **25** was followed by using 5′-*O*-(dimethoxytrityl)-*N*-isobutyl-2′-deoxyguanosine β-cyanoethyl phosphoramidite (100 mg, 0.12 mmol). After lyophilization, **26** was obtained as a white powder, yield 4.7 mg (10% from phophoramidite). The purity of **26** was more than 95%, as determined by HPLC analysis. **26**: ^{1}H NMR (D_2O) δ 2.54 (ddd, 1 H, J = 14.1, 6.1, 2.6 Hz, 2′), 2.69 (ddd, 1 H, 14.1, 7.8, 6.4 Hz, 2′), 3.58–3.68 (m, 4 H, 5′ and $HOCH_2CH_2$–), 3.76–3.85 (m, 2 H, $HOCH_2CH_2$–), 4.17 (q, 1 H, $J \approx 3.7$ Hz, 4′), 4.80 (ddd, 1 H, J = 6.4, 3.7, 2.6 Hz, 3′), 6.15 (dd, 1 H, J = 7.8, 6.1 Hz, 1′), 7.82 (s, 1 H, 8).

Synthesis of 3′-(2-Hydroxyethyl) Thymidylate (27). A procedure similar to the synthesis of **25** was followed by using 5′-*O*-(dimethoxytriyl)-thymidine β-cyanoethyl phosphoramidites (100 mg, 0.14 mmol). After lyophilization, **27** was obtained as a white powder, yield 10 mg (20% from phophoramidite). The purity of **27** was more than 95%, as determined by HPLC analysis. **27**: ^{1}H NMR (D_2O) δ 1.72 (s, 3 H, 5-CH_3), 2.25 (ddd, 1 H, J = 14.3, 7.1, 7.0 Hz, 2′), 2.39 (ddd, 1 H, J = 14.3, 6.2, 3.2 Hz, 2′), 3.59–3.70 (m, 4 H, 5′ and $HOCH_2CH_2$–), 3.77–3.81 (m, 2 H, $HOCH_2CH_2$–), 4.04 (q, 1 H, $J \approx 3.7$ Hz, 4′), 4.59–4.67 (m, overlaped 1 H, 3′), 6.14 (dd, 1 H, J = 7.1, 6.2 Hz, 1′), 7.49 (s, 1 H, 6). FABMS (positive ion, glycerol) diacetyl derivative m/z 473 $(M + Na)^+$.

Synthesis of 3′-(2-Hydroxyethyl) 2′-Deoxyadenylate (28). A procedure similar to the synthesis of **25** was followed by using 5′-*O*-(dimethoxytrityl)-*N*-benzoyl-2′-deoxyadenosine β-cyanoethyl phosphoramidites. After lyophilization, **28** was obtained as a white powder, yield 3.0 mg (7% from phophoramidite). The purity of **28** was more than 95%, as determined by HPLC analysis. **28**: ^{1}H NMR (D_2O) δ 2.58 (ddd, 1 H, J = 14.1, 5.9, 2.4 Hz, 2′), 2.74 (ddd, 1 H, J = 14.1, 8.2, 5.9 Hz, 2′), 3.61–3.64 (m, 2 H, $HOCH_2CH_2$–), 3.65–3.73 (m, 2 H, $HOCH_2CH_2$–), 3.83 (t, 1 H, J = 4.0 Hz, 5′), 3.84 (t, 1 H, J = 4.0 Hz, 5′), 4.23 (q, 1 H, J = 4.0 Hz, 4′), 4.81 (ddd, 1 H, J = 5.9, 4.0, 2.4 Hz, 3′), 6.33 (dd, 1 H, J = 8.2, 5.9 Hz, 1′), 8.03 (s, 1 H, 2), 8.04 (s, 1 H, 8). FABMS (positive ion, glycerol) triacetyl derivative m/z 524 $(M + Na)^+$.

Photoirradiation of d(GCA^{1}UGC)$_2$. The reaction mixture (total volume 50 μL) containing 1 mM (base concentration) d(GCA^{1}UGC)$_2$ in 50 mM sodium cacodylate buffer (pH 7.0) in a Pyrex capillary cell was irradiated at 0 °C for 1 h. After irradiation, 10 μL of the aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Cosmosil $5C_{18}$ column; elution was with 0.05 M ammonium formate containing 0–15% acetonitrile, linear gradient (20 min) at a flow rate of 1.5 mL/min. The HPLC profile of the photoirradiated d(GCA^{1}UGC)$_2$ was shown in Figure 1.

Characterization of Photoproduct 2. The reaction mixture (total volume 2 mL) containing 1 mM (base concentration) d(GCA^{1}UGC)$_2$ in 50 mM sodium cacodylate buffer (pH 7.0) in a Pyrex NMR tube (5-mm diameter) was irradiated at 0 °C with a transilluminator (302 nm) for 3 h from the distance of 10 cm. Then 1 mL of the reaction mixture was taken out and treated with 0.1 M $NaBH_4$ for 1 h at room temperature. After quenching of the reaction with addition of 100 μL of 1 N acetic acid, the solution was subjected to HPLC analysis. The HPLC fraction eluted at 12.0 min was collected and concentrated. The residue was dissolved in water, and then the solution was subjected to enzymatic digestion with s. v. PDE (0.3 unit/mL) and calf intestine AP (100 unit/mL) and to HPLC analysis (Cosmosil $5C_{18}$ ODS column, 0.05 M ammonium formate containing 0–6% acetonitrile, linear gradient (20 min) at a flow rate of 1.5 mL/min). Another 1 mL of the reaction mixture was subjected to HPLC purification. The HPLC fraction of **2** eluted at 11.3 min was collected, and the combined fraction was lyophilized. The HPLC profile of **2** was shown in Figure 2(a).

Characterization of Photoproducts 6 and 7. The reaction mixture (total volume 50 μL) containing 1 mM (base concentration) d(GCA^{1}UGC)/d(GCATCG) in 50 mM sodium cacodylate buffer (pH 7.0) in a Pyrex capillary cell was irradiated at 0 °C for 3 h. The HPLC fraction of **6** eluted at 11.1 min was collected and concentrated. The residue was dissolved in water, and then the mixture was heated at 90 °C for 30 min and then subjected to HPLC analysis. Formation of 5-methylene-2-furanone together with d(CG)p and pd(UGC) was observed. The HPLC fraction of **7** (retention time 9.8 min) was collected and concentrated. The residue was dissolved in 20 μL of 0.1 N NaOH, and then the solution was heated at 90 °C for 5 min and then treated with 0.1 M $NaBH_4$ at room temperature for 15 min. After quenching of the reaction with addition of 10 μL of 1 N acetic acid, the solution was subjected to enzymatic digestion with s. v. PDE (0.3 unit/mL) and calf intestine AP (100 unit/mL), and then the mixture was subjected to HPLC analysis (Cosmosil $5C_{18}$ ODS column, 0.05 M ammonium formate containing 0–6% acetonitrile, linear gradient (20 min) at a flow rate of 1.5 mL/min). Formation of modified mononucleotide **26** together with dC, dG, and dU was observed.

Quantitative Analysis of Photoirradiated ^{1}U-Containing Hexanucleotides. The reaction mixture (total volume 50 μL) contained 1 mM (base concentration) hexamer(s) in 50 mM sodium cacodylate buffer (pH 7.0) in a Pyrex capillary cell. After irradiation at 0 °C for 3 h, 10 μL of the aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Cosmosil $5C_{18}$ column; elution was with 0.05 M ammonium formate containing 0–15% acetonitrile, linear gradient (20 min) at a flow rate of 1.5 mL/min. Yields of C1′ and C2′ oxidation products and dehalogenated hexamer were determined by comparison of HPLC peak areas with those of the authentic samples.

Degradation of Erythrose-Containing Oligomer 2 under Alkaline Conditions. A solution (10 μL) of **2** (ca. 0.3 mM) in 0.1 N NaOH was heated at 90 °C for 5 min. The resulting mixture was neutralized with 1 μL of 1 M CH_3COOH and then subjected to HPLC analysis. Analysis was carried out on a Cosmosil $5C_{18}$ column; elution was with 0.05 M ammonium formate containing 0–8% acetonitrile, linear gradient (20 min) at a flow rate of 1.5 mL/min. Formation of **16** (70%), **17** (70%), d(GC) (9%), and d(UGC) (20%) was observed. The HPLC profile was shown in Figure 2.

Structural Determination of 16 and 17. A solution (100 μL) of **2** (0.3 mM) in 0.1 N NaOH was heated at 90 °C for 5 min. The resulting mixture was treated with 1 M $NaBH_4$ at room temperature for 15 min and was neutralized with 1 μL of 1 M CH_3COOH and subjected to HPLC analysis. Analysis was carried out on a Cosmosil $5C_{18}$ column, elution was with 0.05 M ammonium formate containing 0–15% acetonitrile, linear gradient (20 min) at a flow rate of 1.5 mL/min. The HPLC fraction eluted at 9.9 min was collected and concentrated to dryness. Enzymatic digestion of **18** with s. v. PDE and AP provided 2′-deoxyguanosine and 3′-(2-hydroxyethyl) 2′-deoxycytidylate (**25**) in a 1:1 ratio. The HPLC fraction of **19** eluting at 10.0 min was collected and concentrated to dryness. Enzymatic digestion with s. v. PDE and AP provided 2′-deoxyuridine, 2′-deoxyguanosine, and 2′-deoxycytidine. The structure of **19** was determined by independent synthesis according to the β-cyanoethyl phosphoramidite method.[12] The cyanoethyl phosphoramidite of dimethoxytrityl–ethylene glycol was prepared by the reported procedure.[13] The purity of **19** was more than 95%, as determined by HPLC analysis. **19**: ^{1}H NMR (D_2O) δ 2.06–2.28 (m, 3 H), 2.48–2.74 (m, 3 H), 3.49–3.84 (m, 4 H), 3.87–4.08 (m, 6 H), 4.11 (m, 1 H), 4.36–4.54 (m, 2 H), 4.68–4.77 (m, 1 H), 4.81–4.87 (m, 1 H), 5.64 (d, 1 H, J = 7.5 Hz), 5.64–5.75 (m, 1 H), 5.89–6.02 (m, 1 H), 6.05 (t, 1 H, J = 6.8 Hz), 6.11 (t, 1 H, J = 6.8 Hz), 7.34–7.55 (m, 1 H), 7.61 (d, 1 H, J = 7.5 Hz), 7.62–7.68 (m, 1H), 7.81 (s, 1 H).

Quantitative Analysis of Erythrose-Containing Sites in Duplex 13 Mer. The reaction mixture (total volume 100 μL) contained 1 mM (base concentration) 5′-d(CG^{1}UGT^{1}UTA^{1}UAC^{1}UG)-3′/5′-d(CAGTATAAACACG)-3′ in 50 mM sodium cacodylate buffer (pH 7.0). After irradiation (transilluminator, 302 nm) for 4 h at 0 °C from the distance of 10 cm, 10 μL of the aliquot was taken up and subjected to HPLC analysis for spontaneously released free bases. To another 70 μL of the aliquot was added 5 μL of 1 N NaOH, and the solution was heated at 90 °C for 5 min and then treated with 0.1 M $NaBH_4$ at room temperature for 15 min. The resulting mixture was neutralized with 1 N CH_3COOH and then subjected to enzymatic digestion with s. v. PDE (0.3 unit/mL) and calf intestine AP (100 unit/mL) as described before. The reaction mixture was immediately analyzed by reverse-phase HPLC. Analysis was carried out on a Cosmosil $5C_{18}$ column; elution was with 0.05 M ammonium formate containing 0–8% acetonitrile, linear gradient (40 min) at a flow rate of 1.5 mL/min. The HPLC profile on the mixture was shown in Figure 3.

Stability of 25–28 against Enzymatic Digestion. To a solution containg a modified mononucleotide (**25–28**) (50 μM) in 50 mM Tris–HCl buffer (pH 7.2) were added s. v. PDE (0.3 unit/mL) and calf intestine AP (100 unit/mL). After 2 h of incubation at 37 °C, the solution was subjected to HPLC analysis in each case. Analysis was carried out on a Cosmosil $5C_{18}$ column (4.6 × 150 mm), linear gradient (40 min) at a flow rate of 1.5 mL/min. Under the conditions, no digestion of **25** and **26** was observed, whereas **27** and **28** were partially hydrolyzed to thymidine (2.8%) and 2′-deoxyadenosine (2.4%), respectively.

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