Highly Sequence Selective Photoreaction of 5-Bromouracil-Containing Deoxyhexanucleotides

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Replacement of thymine in DNA by 5-bromouracil (BrU) has long been known to enhance photosensitivity with respect to DNA–protein photo-cross-linking,[1] single- and double-strand breaks, and creation of alkali-labile sites.[2] While hydrogen abstraction from the deoxyribose moiety at the 5′ position by a uracilyl-5-yl radical has been proposed for the photochemical DNA strand cleavage,[2a] the detailed chemistry for the radical-induced DNA degradation has not been clarified. We now report herein that deoxyoligonucleotides containing a $^{5'}A^{Br}U$ site in the middle of the duplex structure undergo extremely facile photoreaction to produce a 2-deoxyribonolactone residue with release of free adenine (Scheme I).

A typical reaction mixture containing self-complementary duplex $d(GCA^{Br}UGC)_2$ in sodium cacodylate buffer (pH 7.0) in a Pyrex capillary cell was irradiated at 0 °C with a transilluminator (302 nm) for 30 min under anaerobic conditions. HPLC analysis of the mixture revealed the formation of one major photoproduct together with adenine and a minor amount of the normal reduction product d(GCAUGC) (Figure 1). The structure of the phototransformed oligonucleotide **1** was characterized as follows. Treatment of the photoproduct, separated by HPLC, with hot alkali (0.1 N NaOH, 90 °C, 5 min) produced GCp and pUGC quantitatively, whereas partial digestion with snake venom phosphodiesterase (s.v. PDE) followed by alkaline phosphatase (AP) treatment provided dG, dC, and dU together with dGC containing a modified ribose residue dGCX.[3] Subsequent digestion of dGCX (**2**) with calf spleen phosphodiesterase and AP gave dG and 2-deoxyribonolactone **3**,[4,5] which was further hydrolyzed to **4** with 0.1 N aqueous NH_3. The structure of **4** was confirmed by independent synthesis.[11]

Efficient formation of a 2-deoxyribonolactone residue with concomitant release of free base has been observed only when duplex hexamers containing a $^{5'}A^{Br}U$ site in the middle were irradiated.[12] Irradiation of heteroduplex $d(GCG^{Br}UGC)/d(CGCACG)$ (run 2) or $d(GCC^{Br}UGC)/d(CGGACG)$ (run 3) never produced free base under the irradiation conditions (Table I). Self-complementary duplex $d(CG^{Br}UACG)_2$ provided only a small amount of free guanine but no free adenine or 2-deoxyribonolactone residue (run 6). Of particular interest is the irradiation of non-self-complementary $d(GCA^{Br}UCG)$. Irradiation of this hexamer alone gave only a small amount of 2-deoxyribonolactone-containing oligomer **5**[13] with trace adenine together

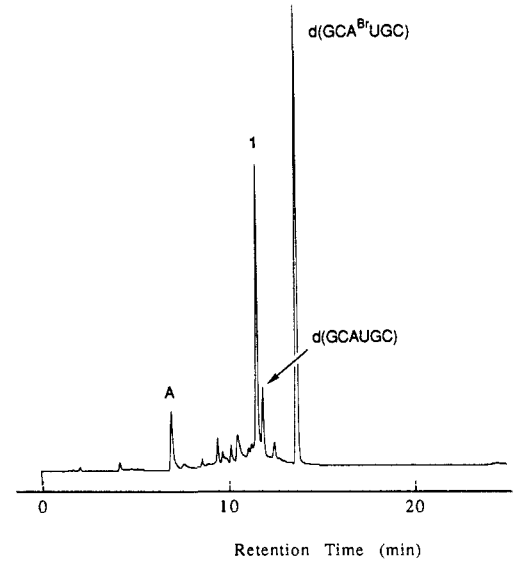
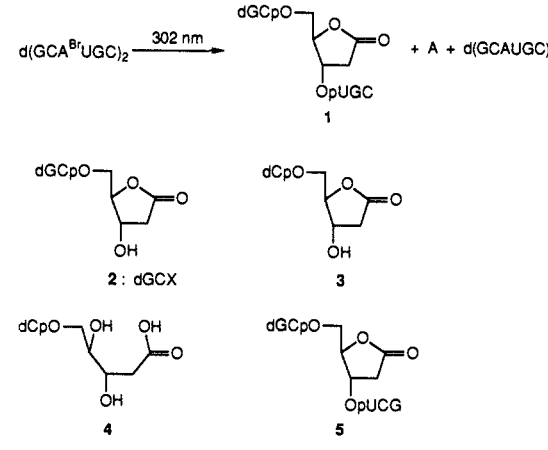
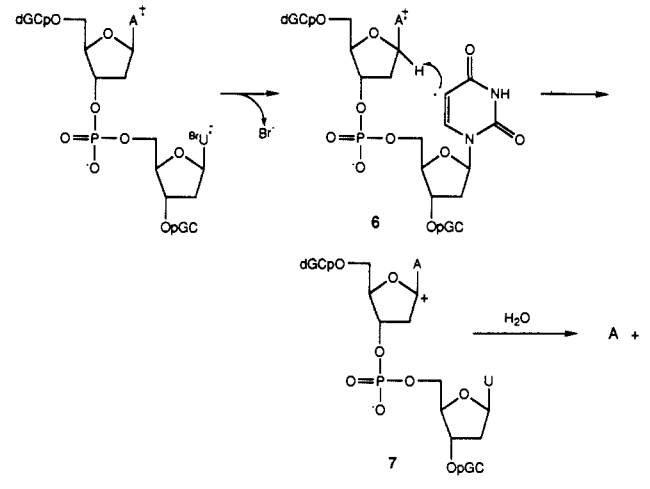


Figure 1. HPLC analysis of UV-irradiated hexanucleotide d-($GCA^{Br}UGC$). Photoirradiation was conducted under the conditions shown in Table I and the mixture was analyzed by HPLC on a Cosmosil $5C_{18}$ column (4.6 × 150 mm), detected at 254 nm; elution was with 0.05 M ammonium formate, 0–15% acetonitrile, linear gradient, 20 min, at a flow rate of 1.5 mL/min.

Scheme I



Scheme II



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(3) Extensive digestion with s.v. PDE and AP gave dG, dC, and dU in a 2:2:1 ratio.

(4) ^1H NMR (D_2O, TSP) δ 2.35 (dd, 1 H, J = 6.8, 7.1 Hz, $H_{2'}$), 2.54–2.60 (m, 2 H, $H_{2'}$, $H_{2''}$), 3.11 (dd, 1 H, J = 6.7, 19.2 Hz, $H_{2''}$), 3.77 (dd, 1 H, J = 4.9, 12.5 Hz, $H_{5'}$), 3.84 (dd, 1 H, J = 3.6, 12.5 Hz, $H_{5''}$), 4.04–4.20 (m, 2 H, $H_{5''}$), 4.20–4.23 (m, 1 H, $H_{4'}$), 4.64–4.85 (m, 3 H, $H_{3'}$, $H_{3''}$, $H_{4''}$), 6.07 (d, 1 H, J = 7.6 Hz, H_5), 6.30 (t, 1 H, J = 6.8 Hz, $H_{1'}$), 7.84 (d, 1 H, J = 7.6 Hz, H_6).

(5) 2-Deoxyribonolactone residues have been reported to be produced by γ-irradiation,[6] neocarzinostatin[7] and 1,10-phenanthroline–copper complex[8] and UV irradiation,[9] and this lesion was shown to be highly mutagenic.[10]

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(12) For example, irradiation of self-complementary duplex d-($CGA^{Br}UCG)_2$ gave a similar 2-deoxyribonolactone residue with release of adenine.

(13) The peak corresponding to **5** was isolated by HPLC and subjected to enzymatic digestion as described for **1**.

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Table I. Product Analysis from Irradiation of BrU-Containing Deoxyhexanucleotides[a]

run	hexamer	free base, μM (%[b]) C	G	T	A	consumed hexamer, %	debrominated hexamer,[b] %	2-deoxyribonolactone-containing hexamer,[b] %
1	d(GCA^{Br}UGC)	0	0	0	39 (61)	38	10	47 (1)
2	d(GCG^{Br}UGC)	0	0	0	0	0	0	0
	d(CGCACG)					0		
3	d(GCC^{Br}UGC)	0	0	0	0	20	0	0
	d(CGGACG)					11		
4	d(GCA^{Br}UCG)	0	0	0	0.6 (1.3)	27[c]	5	4[d] (5)
5	d(GCA^{Br}UCG)	0	0	0	21 (41)	61	8	29 (5)
	d(CGTAGC)					0		
6	d(CG^{Br}UACG)	0	4 (24)	0	0	10	0	0

[a] Each of the reaction mixtures (30 μL) containing hexamer (1 mM base concentration) and NaCl (1 M) in 50 mM sodium cacodylate buffer (pH 7.0) in a capillary cell was irradiated for 30 min at 0 °C with a transilluminator (302 nm) under otherwise identical conditions. The reaction mixture was analyzed by HPLC under the conditions as described in Figure 1. [b] Yields based on consumed BrU-containing hexamer as determined by HPLC. [c] Considerable amounts of unknown photoproducts were detected. [d] Due to the overlapping of the peak of **5** with unknown products, the value was somewhat inaccurate.

with other unidentified products (run 4). By contrast, addition of complementary strand d(CGTAGC) to the reaction system resulted in more than 7-fold enhancement of the photoreactivity to cleanly produce **5** with efficient release of adenine (run 5). These results indicate that both the duplex structure and the 5′A^{Br}U sequence are essential for the efficient formation of the 2-deoxyribonolactone residue and free base release.[14,15]

The formation of **1** and adenine from d($G_1C_2A_3{}^{Br}U_4G_5C_6$) apparently indicates that the ribose C-1′ hydrogen at A_3 is abstracted by an adjacent uracilyl-5-yl radical formed from BrU in the same strand of the duplex. The quantum yield ($\phi = 1.4 \times 10^{-2}$ at 0 °C) for the formation of 2-deoxyribonolactone-containing oligomer **1** from duplex d(GCA^{Br}UGC)$_2$ is remarkably higher than that for the photoreduction of monomeric BrU in water containing 0.1 M 2-propanol ($\phi = 1.8 \times 10^{-3}$)[16] or the photoreduction in the presence of a mixture of dG, dC, and dA in a 2:2:1 ratio under the same conditions ($\phi = 1.7 \times 10^{-3}$).[17,18] While the reason for the specific and highly efficient photoreaction of the 5′A^{Br}U sequence is unclear, an attractive mechanism appears to involve an intramolecular electron transfer from adenine at the 5′-side to an adjacent BrU in a specially oriented complex formed in the duplex (Scheme II).[1b,c,19,20] The resulting BrU anion radical would release Br anion to produce uracilyl-5-yl radical **6**, which can immediately abstract the adjacent C-1′ hydrogen of the adenosine radical cation to give rise to cationic species **7**. Hydrolytic cleavage of the *N*-glycosidic bond of **7** would provide **1** with release of adenine.[21]

The present results strongly suggest that such C-1′ hydrogen abstraction giving the 2-deoxyribonolactone residue may play an important role in the formation of the alkaline-labile lesion in UV-irradiated BrU-containing DNA.[1,2,5] Further studies on the mechanistic aspect of this novel photoreaction are in progress in our laboratory.

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(14) In fact, photoreaction of d(GCA^{Br}UGC)$_2$ is temperature dependent. Photoreaction of d(GCA^{Br}UGC)$_2$ proceeded much more slowly at 50 °C to give only 40% of the photoproducts obtained at 0 °C. Melting temperature (T_m) of d(GCA^{Br}UGC)$_2$ at 1.85×10^{-5} M was 33 °C.

(15) Neither 5′^{Br}UA (run 6) nor 5′T^{Br}U sequence in the middle of double stranded hexanucleotide induced free base release.

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(17) Quantum yield measurements were carried out at 0 °C in a merry-go-round apparatus by using 5-bromouracil as an actinometer.[15]

(18) For a similar enhanced photoreactivity of BrU in DNA, see; Wacker, A.; Dellweg, H.; Trager, L.; Kornhauser, A.; Lodemann, E.; Turck, G.; Selzer, R.; Chandra, P.; Ishimoto, M. *Photochem. Photobiol.* **1964**, *4*, 477.

(19) Examples for electron-transfer reactions of BrU by n,π* excitation (308 nm) via the triplet manifold; see also: (a) Swanson, B. J.; Kutzer, J. C.; Koch, J. H. *J. Am. Chem. Soc.* **1981**, *103*, 1274. (b) Ito, S.; Saito, I.; Matsuura, T. *Ibid.* **1980**, *102*, 7535. (c) Dietz, T. M.; von Trebra, B. J.; Swanson, B. J.; Koch, T. H. *Ibid.* **1987**, *109*, 1793, and references therein.

(20) The reason why a 5′G^{Br}U sequence containing a more easily oxidizable guanine does not undergo this reaction (runs 2 and 6) is unclear. However, even if electron transfer from 5′G to BrU is faster, the back-electron-transfer rate and the subsequent reaction from G$^{\bullet+}$ would be quite different from those observed with 5′ABrU sequence. Electron transfer from photoexcited A to BrU in the complex would also be feasible.

(21) Since ribonolactone formation and adenine release occur under rigorously degassed conditions, oxygenation of ribose C1′ radial is not involved.

Synthesis of Poly[bis(trifluoroethoxy)phosphazene] under Mild Conditions Using a Fluoride Initiator

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Polyphosphazenes are inorganic macromolecules with backbones consisting of alternating phosphorus and nitrogen atoms. A large range of polymer properties can be controlled by varying the structure of the substituents attached to the phosphorus atoms of the chain. It is possible to provide materials with such interesting properties as flame resistance, low-temperature flexibility, and biocompatibility.[1]

At the present time, there are two main synthetic routes to the polyphosphazenes: ring-opening polymerization of halogenated cyclotriphosphazenes followed by replacement of the halogens by hydrolytically stable groups,[2-4] and the condensation of substituted phosphoranimines.[5-7]

The latter method provides a route to a variety of poly(alkylphosphazenes) and poly(arylphosphazenes), thus expanding the field from the poly(alkoxyphosphazenes), poly(aryloxyphosphazenes), and poly(aminophosphazenes) of earlier efforts.[8,9] This approach, however, involves multistep synthesis of various substituted monomers and usually requires 2-12 days and high temperatures (160-220 °C) to produce polymer.[10]

One of the most important polyphosphazenes, poly[bis(2,2,2-trifluoroethoxy)phosphazene], can be prepared either by the ring-opening polymerization/halogen substitution method de-

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