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Sequence Dependent Photoreduction of 5-Bromouracil-Containing Oligonucleotides via Electron Transfer

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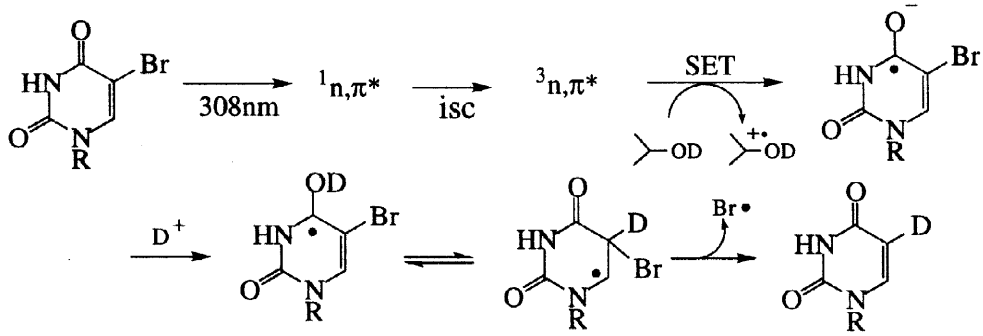
Abstract: In order to investigate photoactivation mechanism of 5-bromouracil (^{Br}U)-containing oligonucleotides, deuterium incorporation experiments and fluorescence emission were examined. Photoreduction of ^{Br}U contained in a duplex oligomer in aqueous solution containing $(CD_3)_2CDOH$ gave 5-deuterated uracil in high yield. It was found that highly photoreactive oligomers possessing 5′-A^{Br}U-3′ sequence show a strong fluorescence emission at 370 nm by excitation at 308 nm. These experimental results support a photoactivation mechanism that involves one electron transfer from adjacent adenine at 5′-side to n,π* excited state of ^{Br}U in DNA duplex.

Photoreactions of 5-bromouracil (^{Br}U)-containing polynucleotides have been exploited for mapping protein-nucleic acid interactions,[1] and the mechanism of the photoreaction of monomeric ^{Br}U was thoroughly investigated.[2] For example, Koch *et al.*, demonstrated a single electron transfer (SET) mechanism for the photoreduction of monomeric ^{Br}U in $(CH_3)_2CHOD$ on the basis of deuterium incorporation into uracil C-5 position (Scheme 1).[2a] However, the detail mechanism of the photoreduction of ^{Br}U contained in duplex oligonucleotides has not

Scheme 1



been fully understood. We previously reported sequence selective photoreaction of ^{Br}U-containing duplex oligomers ultimately leading to the formation of alkaline labile sites.[3] This remarkable 5′-A^{Br}U-3′sequence selective photoreactivity has been explained by assuming SET

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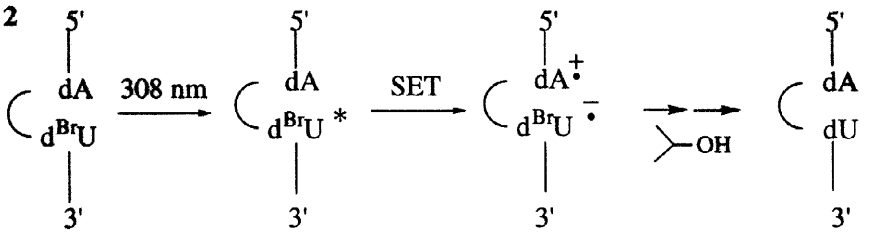
from adjacent adenine moiety at the 5'-side to photoexcited ^{Br}U (Scheme 2).[3] We now report that photoexcitation of ^{Br}U-containing oligomer results in a sequence dependent exciplex formation leading to the photoreduction of ^{Br}U residue via SET.

Scheme 2



In order to investigate the mechanism of photoreduction of ^{Br}U contained in duplex oligonucleotides, irradiation of duplex d(CGA^{Br}UGC)/d(GCATCG) **(1)** in aqueous solution containing various deuterated 2-propanol was examined. After enzymatic digestion of the photoreduced hexamer d(CGAUGC), the photoreduction product, deoxyuridine (dU), was separated by HPLC and subjected to ^{1}H NMR analysis.[4] In the photoreaction in the presence of 100 mM $(CD_3)_2CDOH$, efficient incorporation of deuterium from $(CD_3)_2CDOH$ into C5 position of dU has been observed (D content 93 %)(Figure 1a). However, in the presence of 100 mM $(CH_3)_2CHOD$ in D_2O, only small amount of deuterium was incorporated into dU (D content 7 %) (Figure 1b, Scheme 3).

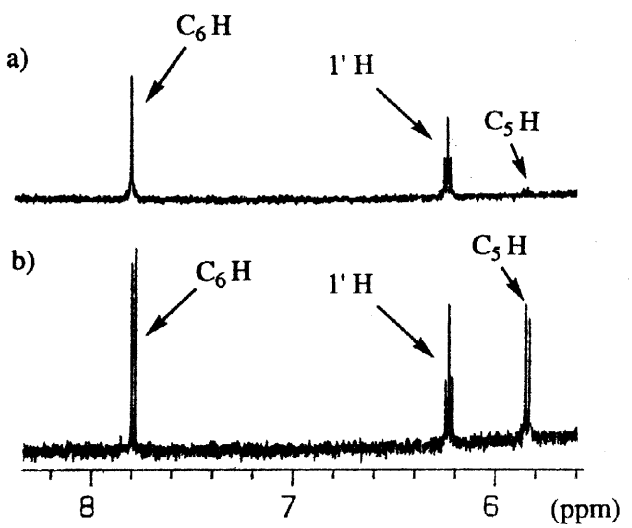
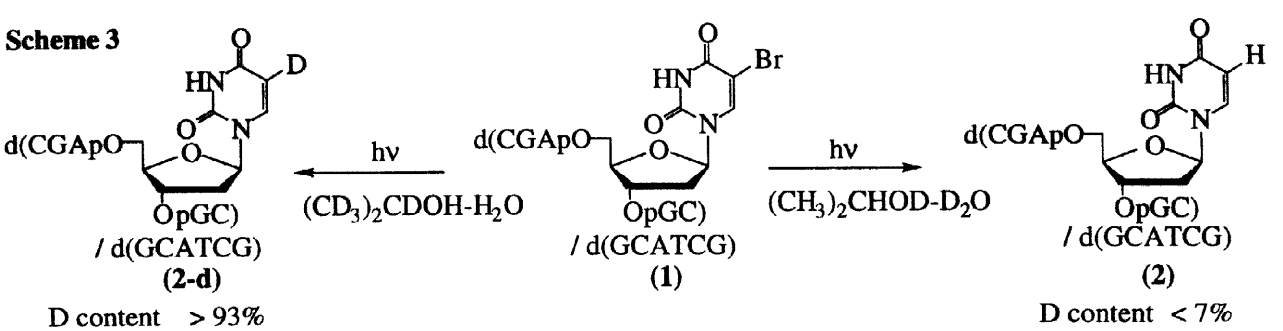


Figure 1. ^{1}H NMR of 2'-deoxyuridine isolated from enzymatic digestion of d(CGAUGC) obtained from photoirradiation of **1** in a buffer containing, a) $(CD_3)_2CDOH$-H_2O and b) $(CH_3)_2CHOD$-D_2O

Scheme 3



In order to gain further insight into the mechanism of the photoreaction of ^{Br}U in oligonucleotides, fluorescence emission spectra of ^{Br}U-containing hexamers were measured (Figure 2). Highly photoreactive duplex oligomers containing 5'-A^{Br}U-3' sequence such as **1** and **3** exhibited a strong emission at 370 nm by excitation at 308 nm. This strong emission is only observable in a duplex form. Thus, it is highly likely that the emission is derived from an exciplex formation between adenine and photoexcited ^{Br}U residues of 5'-A^{Br}U-3' sequence in a duplex structure. It should be emphasized that only strongly emissive duplex oligomers like **1** and **3** can undergo highly efficient photoreduction as indicated in Table 1.[5] In fact, almost nonreactive oligomers such as single stranded **5** or duplex **4** containing 5'-G^{Br}U-3' sequence have only weak emission. This implies that the conformation around adenine and adjacent ^{Br}U is very important for the exciplex emission as well as for the effective photoactivation of ^{Br}U in duplex DNA.

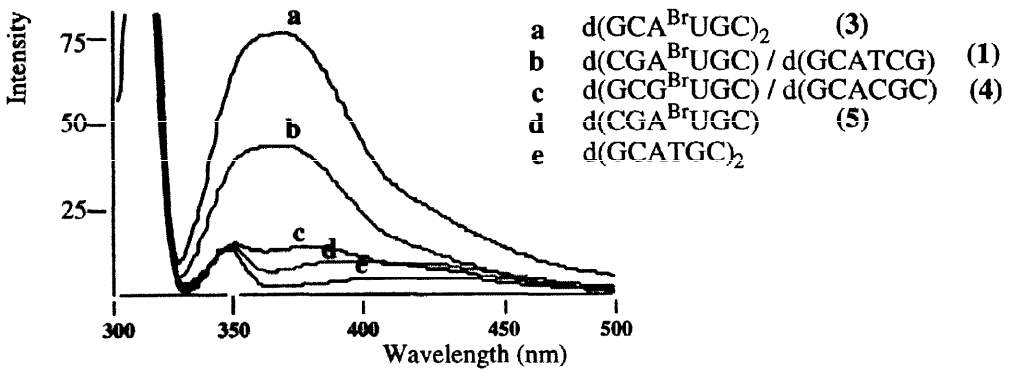


Figure 2. Fluorescence emission of ^{Br}U-containing oligomers. Excitation was at 308 nm at oligomer concentration of 5×10^{-4} M (base concentration) at 4 °C in aqueous 2-propanol (100 mM).

Table 1. Quantum Yields for the Photoreduction of ^{Br}U-Containing Deoxyhexanucleotides

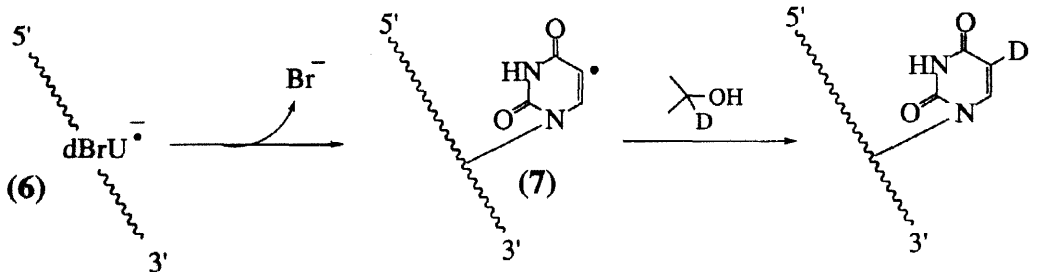
run	hexamer	Φ (photoreduction)[a]
1	d(GCA^{Br}UGC)$_2$ (3)	3.0×10^{-3}
2	d(CGA^{Br}UGC)/ d(GCATCG) (1)	1.0×10^{-3}
3	d(GCG^{Br}UGC)/ d(GCACGC) (4)	$\leq 1.0 \times 10^{-4}$
4	d(CGA^{Br}UGC) (5)	$\leq 1.0 \times 10^{-4}$

[a]Quantum yield measurements were carried out at 0 °C under irradiation at 308 nm. [Oligomer] = 5×10^{-4} M (base conc) in aqueous 2-propanol (100 mM).

The specific exciplex formation between adenine and ^{Br}U in 5'-A^{Br}U-3' sequence being produced by n,π^* excitation of ^{Br}U was very important for the efficient photoreduction of ^{Br}U in oligonucleotides. One electron transfer from adenine to n,π^* ^{Br}U would then occur within a photoexcited complex, and the resulting ^{Br}U anion radical **6** would immediately release Br anion to produce uracilyl-5-yl radical **7**, which abstracts deuterium from $(CD_3)_2CDOH$ to

produce 5-deuterated hexamer **2-d** (Scheme 4). The result is in marked contrast to the photoreduction of monomer ^{Br}U, in which the deuterium was incorporated into uracil C-5 position from $(CH_3)_2CHOD$.[2a] This difference is probably due to the presence of strong hydrogen bond between ^{Br}U C-4 carbonyl and complementary base in a duplex structure. Further work is in progress to elucidate the mechanism of sequence dependent electron transfer reactions.

Scheme 4



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(4) The reaction mixture containing 0.5 m M (base concentration) of oligonucleotide and 100 m M of $(CD_3)_2CDOH$ in 50 m M sodium cacodylate buffer (pH 7.0) in a pyrex tube was irradiated at 0 °C for 2 h. After irradiation, the reaction mixture was subjected to HPLC analysis, and the photoreduced product (retention time, 12.2 min) was collected. The residue was dissolved in water and then subjected to enzymatic digestion with s.v. PDE (0.3 unit/mL) and calf intestine AP (100 unit/mL), and the mixture was subjected to HPLC. Deoxyuridine (retention time, 10 min) was collected, lyophilized, and subjected to ^1H NMR analysis. HPLC conditions; Cosmosil $5C_{18}$ MS ODS column; 0.05 M ammonium formate containing 0-9 % acetonitrile, linear gradient, 20 min; flow rate of 1.0 mL/min.

(5) Quantum yield measurements were carried out at 0 °C on a monochrometer (308 nm) using bromouracil[6] as an actinometer.

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