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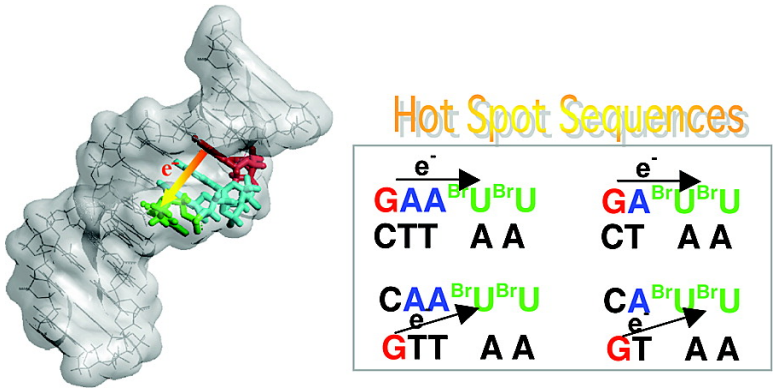

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Photoreaction at 5′-(G/C)AA^{Br}UT-3′ Sequence in Duplex DNA: Efficent Generation of Uracil-5-yl Radical by Charge Transfer

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Abstract: The photoreactivities of 5-halouracil-containing DNA have widely been used for analysis of protein−DNA interactions and have recently been used for probing charge-transfer processes along DNA. Despite such practical usefulness, the detailed mechanisms of the photochemistry of 5-halouracil-containing DNA are not well understood. We recently discovered that photoirradiation of ^{Br}U-substituted DNA efficiently produced 2′-deoxyribonolactone at 5′-(G/C)AA^{Br}U^{Br}U-3′ and 5′-(G/C)A^{Br}U^{Br}U-3′ sequences in duplex DNA. Using synthetic oligonucleotides, we found that similar photoreactivities were maintained at the 5′-(G/C)-AA^{Br}UT-3′ sequence, providing ribonolactone as a major product with concomitant release of adenine base. In this paper, the photoreactivities of various oligonucleotides possessing the 5′-^{Br}UT-3′ sequence were examined to elucidate the essential factors of this photoreaction. HPLC product analysis indicated that the yield of 2′-deoxyribonolactone largely depends on the ionization potential of the purine derivatives located 5′-upstream of 5′-^{Br}UT-3′, as well as the electron-donating ability of their pairing cytosine derivatives. Oligonucleotides that possess G in the complementary strand provided the ribonolactone with almost the same efficiency. These results clearly suggest that the photoinduced charge transfer from the G-5′ upstream of 5′-^{Br}UT-3′ sequence, in the same strand and the complementary strand, initiates the reaction. To examine the role of intervening A/T base pair(s) between the G/C and the 5′-^{Br}UT-3′ sequence, the photoreactivities of a series of oligonucleotides with different numbers of intervening A/T base pairs were examined. The results revealed that the hotspot sequence consists of the electron-donating G/C base pair, the 5′-^{Br}UT-3′ sequence as an acceptor, and an appropriate number of A/T base pairs as a bridge for the charge-transfer process.

Introduction

5-Halouracils such as bromouracil (^{Br}U) and iodouracil (^{I}U) are photoreactive analogues of thymine and are widely used in biological and chemical research. Thymine in DNA can be replaced with a 5-halouracil, and the resulting 5-halouracil-substituted DNA remains functional in vivo.[1] This replacement of thymine in DNA by 5-halouracil enhances the photosensitivity of the cell with respect to DNA−protein cross-linking, DNA strand breakage, and the creation of alkali-labile sites by the formation of uracil-5-yl radical under irradiation conditions.[1] 5-Halouracil-based photocross-linking and photofootprinting methods have been effectively used for the investigation of sequence-specific DNA−protein interactions.[2] For several years, we have examined the photoreactions of 5-halouracil-containing DNA and demonstrated that hydrogen abstraction by uracil-5-yl is atom specific and highly dependent on the local DNA conformation, such as A-form, B-form, Z-form, and bent DNA and G-quadruplexes.[3] We have proposed that the photoreactivity of ^{Br}U is useful as a photochemical method for the detection of various DNA structures.[3]

In addition to hydrogen abstraction by the uracil-5-yl radical, ^{Br}U has been used recently as an electron acceptor in a probe of excess electron-transfer processes, because the anion radical of ^{Br}U rapidly eliminates the bromide ion to generate a uracil

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radical.[4] We have demonstrated electron transfer from protein to DNA by using this property.[5]

As the reactivity of ^{Br}U residues in DNA has been used in much biochemical and chemical research, understanding the detailed mechanisms of the photochemistry of ^{Br}U-containing DNA is important to the development of ^{Br}U-based assays. It has been shown that the photoreactivity of ^{Br}U is highly dependent on the DNA sequence.[2d,3b,3l,6,7] For example, we found that photoirradiation of the self-complementary 5′-(GCA^{Br}UGC)-3′ sequence efficiently produces 5′-(GCLUGC)-3′ (L = ribonolactone), with concomitant release of an adenine base.[3b,3l] Because efficient photoreaction of ^{Br}U was observed when the A was located at a site on the 5′ side of the ^{Br}U in the duplex DNA, we initially proposed sequence-specific electron transfer from the 5′-A to the ^{Br}U. Because the oxidation potential of G is much lower than that of A, the 5′-G^{Br}U-3′ sequence was expected to show more efficient photoreactivity. However, almost no reaction was observed in this sequence. Therefore, we assume that the lack of photoreactivity at 5′-G^{Br}U-3′ is due to a rapid back electron-transfer mechanism.[3b,3l]

Recently, we found that the flanking sequence of the 5′-A^{Br}U-3′ significantly affects the photoreactivity of ^{Br}U.[6] We demonstrated that photoirradiation of ^{Br}U-substituted DNA fragments efficiently generates ribonolactone residues in 5′-(G/C)AA^{Br}U-^{Br}U-3′ and 5′-(G/C)A^{Br}U^{Br}U-3′ sequences.[6] Using synthetic oligonucleotides, we found that the same photoreactivities were maintained in the 5′-(G/C)AA^{Br}UT-3′ sequence, giving ribonolactone as a major product. Using synthetic oligonucleotides, we found that not only A residues but also G residues are important for this photoreaction. This observation led us to propose a new mechanism in which the G residue is an electron donor for ^{Br}U, with the A or AA residues between the donor and acceptor preventing back electron transfer. In this study, detailed analysis of the photoreaction of ^{Br}U at these hotspot sequences was performed using various types of modified oligonucleotides containing a 5′-^{Br}UT-3′ sequence to elucidate the essential factors in this photoreaction.

Results and Discussion

Role of Purine Derivatives on the Photoreactivity of the Hotspot Sequences. Our research group and others have proposed that a charge-transfer process is essential for the photoreactions of ^{Br}U.[3b,3l,7] Because the oxidation potential of G is the lowest among those of the four DNA bases (G, A, T, C = 1.24, 1.69, 1.9, 1.9 V, respectively),[8] we anticipated that G was the putative electron donor in the hotspot sequences. To test this hypothesis, the photoreactivity of various oligonucleotides in which the putative electron donor G was replaced with various modified purine bases such as hypoxanthine (I), 8-bromoguanine (^{Br}G), 8-methoxyguanine (^{MeO}G), 7-deazaguanine (Z) with different oxidation potentials (I = 1.40 V, ^{MeO}G = 1.08 V, Z = 0.95 V).[8,9] Because we have already shown that

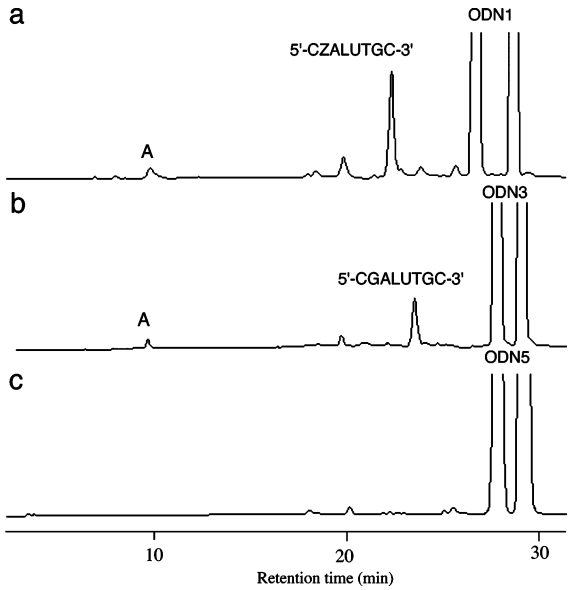
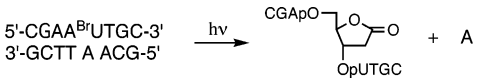


Figure 1. HPLC analysis of UV-irradiated 5′-CZAABrUTGC-3′ (ODN1), 5′-CGAABrUTGC-3′ (ODN3), and 5′-CIAABrUTGC-3′ (ODN5). L indicates the 2′-deoxyribonolactone. The reaction mixture containing BrU-modified ODNs (0.1 mM total base concentration) in 50 mM sodium cacodylate buffer (pH 7.0) in the presence of 50 mM NaCl was irradiated at 0 °C using a monochromator (302 nm) for 10 min.

Scheme 1



5′-CGAA^{Br}UTGC-3′/5′-GCAATTCG-3′ (ODN3) provides a ribonolactone-containing product, 5′-CGALUTGC-3′ (L = ribonolactone residue) as a major product with an efficiency similar to that of d(CGAA^{Br}U^{Br}UCG)$_2$ (Scheme 1),[6] we introduced a series of purine derivatives at N of 5′-CNAA^{Br}-UTGC-3′ and examined their photoreactivities. HPLC profiles of photoirradiated ODN1,3,5 are shown in Figure 1, the rest of HPLC profiles are shown in Figure S1, and the results of quantitative analysis of the photoreactions are summarized in Table 1.

It was found that the yield of ribonolactone-containing products increased with decreasing ionization potentials (IP) of the purine bases in ODN1−4, ^{Br}G < G < ^{MeO}G < Z; almost no ribonolactone was observed in the case of A (ODN5) and I (ODN6) (Figure 2). These results clearly indicate that the G in the 5′-GAA^{Br}U^{Br}U-3′ sequence acts as an electron donor, even though it is not oxidized itself.

Recently, Kawai and Majima demonstrated that charge transfer from G is controlled by the introduction of a methyl or bromo group at the C5 position of paired C's through hydrogen bonding of the G/C base pair.[10] That is, electron-donating 5-methylcytosine (^{Me}C) enhances the charge-transfer process, whereas electron-withdrawing 5-bromocytosine (^{Br}C) reduces the process. Thus, ^{Me}C or ^{Br}C was introduced into the complementary strand (ODN7 and ODN8), and the resultant photoreactivities were compared with that of ODN3 (Figure S1). Quantitative analyses of the photoirradiated ODNs are summarized in Table 1 and Figure 2. Significant enhancement and

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Table 1. Photoreactivity[d] of ^{Br}U-Containing Various Oligonucleotides with Various Base Pairs

ODN	Sequence	Adenine (%)	Ribonolactone (%)	Conversion (%)	E_{OX}(V)	IP(eV)[c]
1	5'-CZAA^{Br}UTGC-3' 3'-GCTT A ACG-5'	16±0.5	14±0.5	25±0.5	0.95[a]	5.12
2	5'-C^{MeO}GAA^{Br}UTGC-3' 3'-G C TT A ACG-5'	13±1.0	12±0.5	19±0.5	1.08[b]	5.20
3	5'-CGAA^{Br}UTGC-3' 3'-GCTT A ACG-5'	6.9±0.2	5.6±0.3	15±0.5	1.24[a]	5.50
4	5'-C^{Br}GAA^{Br}UTGC-3' 3'-G C TT A ACG-5'	3.1±0.1	2.6±0.2	8.9±0.3	nd	5.69
5	5'-CIAA^{Br}UTGC-3' 3'-GCTT A ACG-5'	<0.1	<0.1	1.2±0.4	1.40[a]	6.13
6	5'-CAAA^{Br}UTGC-3' 3'-GTTT A ACG-5'	<0.1	<0.1	0.5±0.3	1.69[a]	6.42
7	5'-C G AA^{Br}UTGC-3' 3'-G^{Me}CTT A ACG-5'	9.7±0.3	10±1.0	17±1.0	nd	5.30
8	5'-C G AA^{Br}UTGC-3' 3'-G^{Br}CTT A ACG-5'	4.6±0.4	3.8±0.2	9.3±0.3	nd	5.59

[a] Obtained from ref 8. [b] Obtained from ref 9. [c] Adiabatic IP values of the various base pairs were calculated at HF/6-31G* levels using Gaussian 03 and Spartan 04. [d] Reaction conditions were the same as in Figure 1. The yields of reaction products and conversions of ^{Br}U-containing DNA were determined based on 10 μM of starting ODN by comparing their HPLC peak areas.

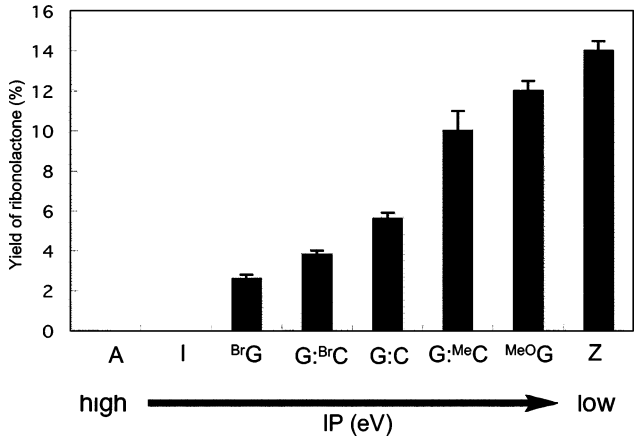

Figure 2. Plot of the oxidation potential of various base pairs vs yield of ribonolactone.

reduction were observed with ODN7 and ODN8, respectively. These results clearly indicate that the electron-donating ability of guanine determines the efficiency of the ribonolactone formation and gives further support that G acts as an electron donor for the photoreaction at the hotspot sequences.

Influence of Guanine in the Complementary Strand. Polyacrylamide gel electrophoresis (PAGE) analysis of the photoirradiated 5-halouracil-containing DNA fragments indicated that 5′-CA^{Br}U^{Br}U-3′ and 5′-CAA^{Br}U^{Br}U-3′ sequences have photoreactivities similar to those of the 5′-GA^{Br}U^{Br}U-3′ and 5′-GAA^{Br}U^{Br}U-3′ sequences.[6] In fact, photoirradiation of 5′-CCAA^{Br}UTGC-3′/5′-CGAATTGG-3′ (ODN10) provided the ribonolactone-containing octamer with the same efficiency as that of ODN3. These results clearly suggest that the G in the complementary strand can also act as an electron donor for ^{Br}U. To test this hypothesis, two oligonucleotides possessing ^{MeO}G (ODN9) and I (ODN11) were synthesized and their photoreactivities compared with that of 5′-CCAA^{Br}UTGC-3′ (ODN10). HPLC analysis of the photolyates of ODN10−12 is shown in Figure S2. Quantitative analyses of the photoproducts, summarized in Table 2, indicated that the amounts of ribonolactone from photoirradiated ODN9, ODN10, and ODN11 correlate well

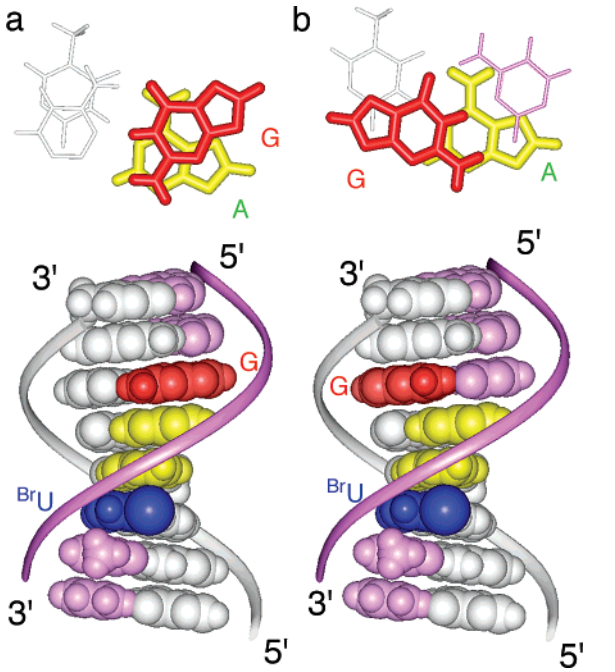

Figure 3. Schematic presentation of base stacking of (a) 5′-ACGAA^{Br}-UTC-3′/5′-GAATTCGT-3′ and (b) 5′-ACCAA^{Br}UTC-3′/5′-GAATTGGT-3′ (lower). Base stacking of 5′-GA-3′/5′-TC-3′ and 5′-CA-3′/5′-TG-3′ (top). G, ^{Br}U, and A are shown in red, blue and white, respectively.

Table 2. Photoreactivities[a] of Various ^{Br}U-Containing Oligonucleotides with Guanine Derivatives in the Complementary Strand

ODN	Sequence	Adenine (%)	Ribonolactone (%)	Conversion (%)
9	5'-C C AA^{Br}UTGC-3' 3'-G^{MeO}GT TAACG-5'	11±0.1	9.7±0.3	21±0.5
10	5'-CCAA^{Br}UTGC-3' 3'-GGTT A ACG-5'	6.6±0.1	4.6±0.1	14±0.1
11	5'-CCAA^{Br}UTGC-3' 3'-G I TTA A CG-5'	<0.1	<0.1	<0.1
12	5'-C^{Me}CAA^{Br}UTGC-3' 3'-G G TT A ACG-5'	8.2±0.4	7.4±0.2	17±1.0
13	5'-C^{Br}CAA^{Br}UTGC-3' 3'-G G TT A ACG-5'	4.3±0.3	3.2±0.2	12±1.0

[a] Reaction conditions are the same as in Figure 1.

with the electron-donating ability of C. These results clearly indicate that the electron-donating properties of the G derivative in the complementary strand also directly affect the reactivity of the octanucleotides. These results strongly suggest that the G in the complementary strand of the 5′-CAA^{Br}UT-3′ sequence acts as an electron donor.

Similarly, we examined the effects of the electron-donating properties of cytosine derivatives on the complementary strand (ODN12, 13). As shown in Table 2, the amounts of ribonolactone correlate well with the electron-donating ability of C, which are similar to the results shown in Table 1. The results clearly indicate that the introduction of an electron-donating group to C enhances the photoreactivity and further supports that the G in the complementary strand is a source of electrons.

In order to investigate why the G on the complementary strand acts as the electron donor for ^{Br}U, the stacking interactions of the G's on the same or complementary strand and those of A were examined. As shown in Figure 3, there is a considerable

Table 3. Photoreactivities of Various ^{Br}U-Containing Oligonucleotides with Different Numbers of A/T Bridges between the Donor and Acceptor[a]

5'-CX(A)$_n$^{Br}UTATC-3 X = G or Z
3'-GC(T)$_n$ A ATAG-5' n = 0 - 6

		X = G				X = Z		
n	ODN	adenine (%)	ribonolactone (%)	conversion (%)	ODN	adenine (%)	ribonolactone (%)	conversion (%)
0	**14**	<0.1	<0.1	<0.1	**21**	<0.1	<0.1	0.5 ± 0.3
1	**15**	1.7 ± 0.4	1.1 ± 0.2	2.7 ± 0.5	**22**	2.8 ± 0.3	2.1 ± 0.3	4.7 ± 0.2
2	**16**	0.2	4.7 ± 0.4	12 ± 0.5	**23**	8.5 ± 0.5	7.9 ± 0.1	15 ± 0.7
3	**17**	2.7 ± 0.3	2.1 ± 0.4	5.6 ± 0.1	**24**	15 ± 0.5	14 ± 0.7	26 ± 1.7
4	**18**	1.5 ± 0.1	1.0 ± 0.2	2.7 ± 0.1	**25**	3.3 ± 0.3	2.3 ± 0.2	5.8 ± 0.1
5	**19**	<0.1	<0.1	<0.1	**26**	<0.1	<0.1	0.9 ± 0.1
6	**20**	<0.1	<0.1	<0.1	**27**	<0.1	<0.1	0.4 ± 0.2

[a] The reaction conditions are the same as those for Figure 1, except for the irradiation period of 5 min.

stacking interaction between G and A in the 5′-GAA^{Br}UT-3′/5′-AATTC-3′ and 5′-CAA^{Br}UT-3′/5′-AATTG-3′ sequences, which may explain the similar reactivities of these sequences. These results suggest that a base-stacking overlap is important for charge transfer: for efficient charge transfer the two purines, G and A, or A and A, are not necessarily on the same strand but the A and ^{Br}U should be on the same strand.

Effect of Number of Adenines between the Electron Donor and Acceptor in the Hotspot Sequences. The above examination suggests that in the hotspot sequences the G-5′ upstream of ^{Br}U acts as an electron donor and the ^{Br}UT sequence acts as an electron acceptor. The charge transfer between donor and acceptor along DNA is known to depend on the sequence and distance.[11−14] Therefore, the efficiency of charge transfer between the electron donor (G or Z) and the acceptor of ^{Br}UT separated by various numbers of $(A/T)_n$ bridges in double strands was examined. ODNs 14−20 were synthesized and their photoreactivities compared. The results of HPLC product analysis of the photoirradiated ODNs are listed in Table 3. Consistent with the previous results of photoirradiation of ^{Br}U-containing oligonucleotides and DNA fragments, no formation of ribonolactone at 5′-CG^{Br}UTATC-3′/5′-GATAACG-3′ (ODN14) without an A/T bridge was observed. The amount of ribonolactone increased with the number of A/T bridges increasing from one to two, and gradually decreased with more than three, as shown in Table 3. The yield of ribonolactone from the oligonucleotides with different numbers of A/T bridges (n) can be explained by the different rates of back electron transfer, which are distance dependent (see Figure 4). When $n = 0$ or 1, back electron transfer is much faster than the release of bromide ions, whereas for $n \geq 2$, the back electron transfer is slow enough to allow release of bromide ions from anion radicals of ^{Br}U, the lifetime of which was estimated as 7 ns.[15] Under such conditions, the charge-transfer process depends on the distance. The β value was evaluated as 0.20 Å^{-1} (Figure 5).

Similarly, the effects on photoreactivity of the number of A/T bridges between the donor Z and acceptor ^{Br}UT were investigated. Quantitative analyses of the products from photoirradiated ODN21−27 are summarized in Table 3. HPLC profiles of the photolysates are shown in Figures S9−11. Analogous to the reaction of ODN17, no formation of ribonolactone at 5′-CZ^{Br}-UTATC-3′/5′-GATAACG-3′ (ODN21, without an A/T bridge) was observed. The amount of ribonolactone increased as the number of A/T bridges increased from one to three, and gradually decreased with more than four, as shown in Figure 4. These results clearly indicate that the optimal numbers of A/T bridges for G and Z as electron donors are two and three, respectively. The reason for the different numbers of A/T bridges for G and Z is presumably due to the different rates of back electron transfer. The β-values were evaluated as 0.20 and 0.53 Å^{-1}, respectively (Figure 5). Analogous β-values for G and Z have been reported previously.[11]

Electron Density of the A/T Bridges. Using the modified oligonucleotides, we demonstrated that the $(A/T)_2$ bridge gives the most efficient reaction, with charge separation between G (electron donor) and ^{Br}U (electron acceptor). However, the sequencing experiments clearly demonstrated that the cleavage at the 5′-(G/C)AA^{Br}U^{Br}U-3′ site is almost the same as that at the 5′-(G/C)A^{Br}U^{Br}U-3′ site. In the PCR-amplified DNA fragments used, A always pairs with ^{Br}U, and we thought that ^{Br}U might change the oxidation potential of A to alter the efficiency of the charge separation. To assess this hypothesis, we next examined the effect of a base opposite the A bridge by using uracil (U) and 5-bromouracil (^{Br}U). As shown in Table 3, the photoirradiation of ODN15 gave ribonolactone with a 1.1% yield, which was significantly lower than that of ODN16. When the T was replaced with uracil (ODN28) or bromouracil (ODN29), acceleration of the photoreactivity was observed (Figure S12). The yields of 5′-d(CGLUTAC)-3′ from ODN28 and ODN29 were 2.0% and 3.5%, respectively (Table 4). These results strongly suggest that the electronic effect of pairing thymine can be transmitted to the adenine through hydrogen bonding. The bromo substituent on C5 of thymine acts as an electron-accepting group to retard the charge-transport process. These results clearly explain the discrepancy between the oligomer and sequencing experiments, in that the $(A/T)_2$ bridge is optimal in the oligonucleotide experiments and that 5′-(G/C)AA^{Br}U^{Br}U-3′ and 5′-(G/C)A^{Br}U^{Br}U-3′ are the hotspot sequences in ^{Br}U-containing DNA fragments.

Proposed Mechanism for the Photoreaction at the Hotspot Sequence. Previous investigations demonstrated that an efficient photoreaction at the hotspot sequence occurred with three elements: G as the electron donor, the stacking ^{Br}UT as the electron acceptor, and A/T as a bridge between the electron

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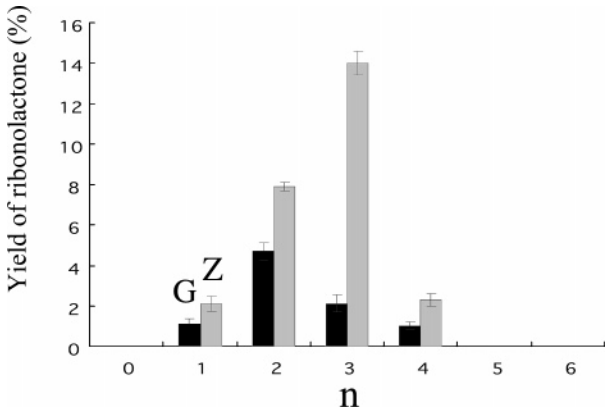


Figure 4. Amount of ribonolactone produced with different numbers (*n*) of intervening bridge bases (A/T)s.

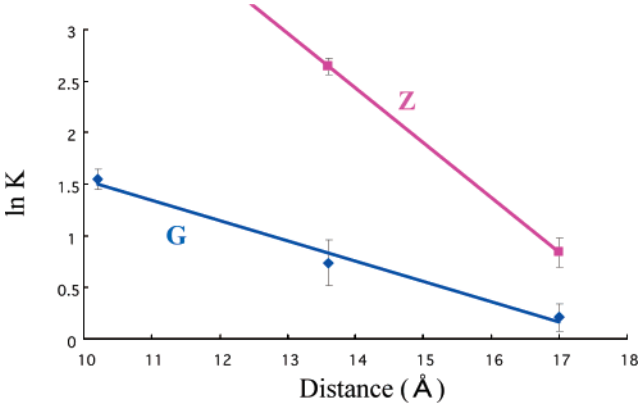


Figure 5. ln(*k*) plotted against the G-^{Br}U (blue) and Z-^{Br}U (pink) distance. The linear fit with data points gives $\beta = 0.20$ Å^{-1} for G and $\beta = 0.53$ Å^{-1} for Z. The value of donor−acceptor distance is obtained from ref 16. The yield of ribonolactone (*k*) is obtained from Table 3.

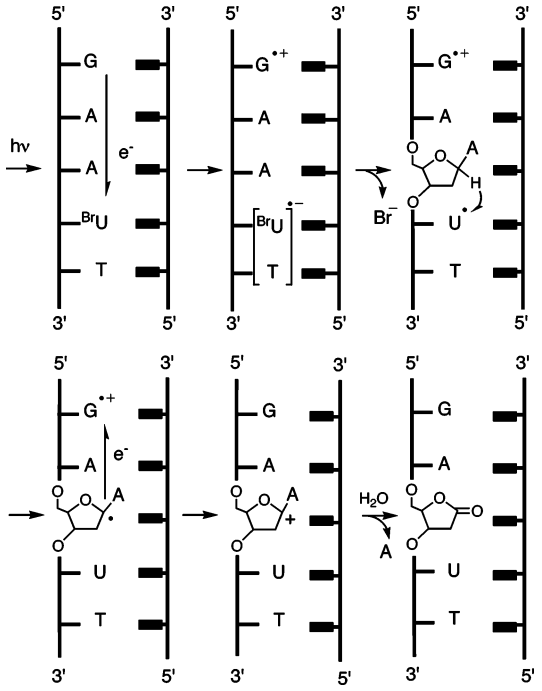
Table 4. Photoreactivities[a] of Various ^{Br}U-Containing Oligonucleotides with Different Bridges between the Donor and Acceptor

ODN	Sequence	Adenine (%)	Ribonolactone (%)	Conversion (%)
28	5'-CGA^{Br}UTATC-3' 3'-GCU A ATAG-5'	3.0±0.1	2.0±0.1	4.6±0.5
29	5'-CG A ^{Br}UTATC-3' 3'-GC^{Br}U A ATAG-5'	4.7±0.2	3.5±0.1	7.4±0.1
30	5'-CG A A ^{Br}UTATC-3' 3'-GC^{Br}U^{Br}U A ATAG-5'	4.1±0.8	3.0±0.1	7.9±0.2

[a] The reaction conditions are the same as those for Figure 1, except for the irradiation period of 5 min.

donor and acceptor.[6] From these observations, the proposed mechanism for the efficient photoreaction at 5′-GAA^{Br}U^{Br}U-3′ sequences is as shown in Scheme 2. Initial charge transfer occurs from G to electron-deficient stacked ^{Br}UT to provide the anion radical. Release of the bromide ion generates a uracil radical, which abstracts the C1′ hydrogen from the adjacent 2′-deoxyadenosine at the 5′-side. Oxidation of the C1′ radical by the guanine cation radical gives rise to a C1′ cation and regeneration of the guanine. It has been suggested that trapping of the guanine radical cation by oxygen is very slow (in milliseconds).[17] This may explain the reason why the reduction

Scheme 2



of the guanine cation radical by back electron transfer from C1′ radical is more favorable than oxidation of guanine after charge separation in the hotspot sequence.

The ESI-Mass analysis demonstrated the incorporation of ^{18}O atoms into the ribonolactone when 5′-CGAAA^{Br}U^{Br}UCG-3′ was photoirradiated in $H_2{}^{18}O$.[6] These results clearly demonstrated that H_2O is the source of the C1′ oxygen of the ribonolactone. The reaction of the ribose C1′ cation with H_2O generates ribonolactone with concomitant release of free adenine.[6] Previously, we found specific charge transfer within a four-base π-stack in Z-form DNA.[3j] Similar activation of charge transfer was observed with ^{MeO}G substitution. However, ^{MeO}G is effectively oxidized to imidazolone within the four-base π-stack. The different reactivities in these two cases is presumably due to the different distances between and reactivities of the forming radicals and cation radical of G.

Conclusion

In this study, using various modified oligonucleotides, the mechanism of ribonolactone formation in 5′-(G/C)AA^{Br}UT-3′ and 5′-(G/C)A^{Br}UT-3′ was extensively investigated. The results clearly demonstrated that hotspot sequences consist of a G donor, a ^{Br}UT acceptor, and an A/T bridge. The photoreactivity was influenced by the base stacking of the ^{Br}U-T,[6] the oxidation potential of the donor, and the number of A/T bridges. These results suggest that the photoreactivity may be controlled using nucleic acid derivatives with different oxidation potentials and by adjusting the distance between the electron donor and acceptor. Moreover, at the hotspot sequence, the photoreaction was very efficient compared to that at other sequences under irradiation conditions. Therefore, these results suggest an important finding that can lead to a basic understanding of photocross-linking between 5-halouracil-containing DNA and -DNA-binding proteins. Moreover, as the guanine is an electron

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donor at the hotspot sequence and the structure of 5-halouracil is similar to thymine, substantial DNA conformational change does not result. Therefore, the hotspot sequence found in these studies is useful for understanding the nature of charge transfer along DNA. These results indicate that in duplex structures, uracil-5-yl is not generated in a sequence-independent manner, but is selectively generated at 5′-(G/C)AATT-3′ or 5′-(G/C)-ATT-3′ in ^{Br}U-substituted DNA under UV-irradiation conditions. Information on such sequence dependencies of ^{Br}U-containing DNA is important information in discussing the hotspot sequences for photoinduced damage of ^{Br}U-substituted DNA as well as for ^{Br}U-containing DNA−protein photocross-linking. The activation mechanism of ^{Br}U through charge transfer from guanine derivatives is also important for the application of the photoreactivities of ^{Br}U. The present results also suggest that the incorporation of Z at a specific sequence enhances the efficiency of DNA−protein photocross-linking.

The predominant formation of uracil-5-yl at 5′-(G/C)AATT-3′ or 5′-(G/C)ATT-3′ sequences in ^{Br}U-substituted DNA might have significant biological impact. Recently, we have demonstrated that a tryptophan residue of a protein acts as an electron donor for ^{Br}U. The modulation of the sequence specificity of ^{Br}U in the presence of protein needs to be addressed in the future.

Experimental Section

General Methods. Deoxyoligonucleotides were purified by high performance liquid chromatography (HPLC) with a Jasco PU-980 HPLC pump, a UV-975 HPLC UV/vis detector, and a Chemcobond 5-ODS-H column (4.6 × 150 mm) (Chemco Scientific, Osaka, Japan). A-, G-, C-, and T-3-cyanoethyl phosphoramidites were purchased from Applied Biosystems (Forster City, CA). Cyanoethyl phosphoramidites of modified nucleotides such as 5-bromo-2′-deoxyuridine, 5-iodo-2′-deoxyuridine, 7-deaza-2′-deoxyguanosine, 8-methoxy-2′-deoxyguanosine, 2′-deoxyinosine, 8-bromo-2′-deoxyguanosine, 5-methyl-2′-deoxycytidine, and 5-bromo-2′-deoxycytidine were purchased from Glen Research (Sterling, VA). Syntheses of deoxyoligonucleotides were performed on an ABI 3400 DNA synthesizer (Applied Biosystems). Concentrations of DNA were determined by Beckman DU650 spectrophotometer (Fullerton, CA) of UV absorption at 260 nm. Electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) was performed on a Bruker Bio TOF II (Bruker Daltonics, Billerica, MA).

Photoirradiation at 302 nm was performed in Eppendorf tubes using an HM-5 hypermonochromator (Jasco, Tokyo, Japan).[3m] Photolysis tubes were positioned 5 mm from the outlet of the glass fiber and irradiated for 5 or 10 min (1.11×10^{16} photons/s). The number of incident photons was measured using a 75III multimeter (Fluke Corporation, Everett, WA). The photoreactions were carried out under aerobic conditions.

Synthesis of Deoxynucleotide. Oligonicleotides were prepared by solid-phase DNA synthesis on a controlled-pore glass support (1 μmol) by using DNA synthesizer and were purified by reverse-phase HPLC. Synthesized oligonucleotides were confirmed by enzymatic digestion and by ESI-MS. Enzymatic digestion is performed with endonuclease P1 (0.3 units/mL, Boehringer Mannheim, Germany) and alkaline phosphatase (1000 units/mL, Boehringer Mannheim).

Analysis of Photoirradiated Oigonucleotides Containing 5-Bromouracil. The reaction mixture (total volume 100 μL) contained 100 μM 5-bromouracil-containing deoxyoligonucleotide, 50 mM sodium cacodylate (pH 7.0), and 50 mM NaCl. Irradiation was performed with 302 nm monochromatic UV light at 0 °C for 10 min. After irradiation, reaction mixtures were analyzed by HPLC equipped with a 5-ODS-H column. Elution was performed with 50 mM ammonium formate (pH 6.5) containing 0−12% acetonitrile over a linear gradient for 40 min at a flow rate of 1.0 mL/min at 40 °C. The yields of reaction products and conversions of ^{Br}U-containing DNA were determined based on 10 μM solution of starting ODN by comparing their HPLC peak areas.

Characterization of Ribonolactone-Containing Oligonucleotides. The HPLC fractions of putative ribonolactone-containing oligonuleotides were collected and concentrated. The residue was dissolved in 50 μL of 10 mM sodium cacodylate buffer (pH 7.0), and then the mixture was heated at 90 °C for 20 min; 10 μL aliquots of each, before and after heating, were analyzed by HPLC. Elution was performed with 50 mM TEAA (pH 7.0) containing 0−9% acetonitrile over a linear gradient for 50 min at a flow rate of 1.0 mL/min at 40 °C. Formation of 5-methylenefuranone from each sample was observed. Authentic 5-methylenefuranone was generated from ribonolactone containing the hexamer, d(GCLUGC) (L = 2′-deoxyribonolactone). ESI-TOF MS (negative).[3b,6] ESI-MS (negative) for 5′-CZALUTGC-3′ (ODN1) calcd 2274.38, found 2274.59; for 5′-C^{MeO}GALUTGC-3′ (ODN2) calcd 2303.58, found 2305.57; for 5′-CGALUTGC-3′ (ODN3) calcd 2275.37, found 2275.55; for 5′-C^{Br}GALUTGC-3′ (ODN4, -7, -8) calcd 2353.28, found 2353.60; for 5′-CCALUTGC-3′ (ODN9, -10) calcd 2236.43, found 2236.55; for 5′-C^{Me}CALUTGC-3′ (ODN12) calcd 2249.38, found 2249.42; for 5′-C^{Br}CALUTGC-3′ (ODN13) calcd 2313.28, found 2313.35; for 5′-CGLUTATC-3′ (ODN15, -28, -29) calcd 2250.24, found 2250.61; for 5′-CGALUTATC-3′ (ODN16, -30) calcd 2563.59, found 2563.72; for 5′-CGAALUTATC-3′ (ODN17) calcd 2876.77, found 2876.91; for 5′-CGAAALUTATC-3′ (ODN18) calcd 3189.54, found 3189.11; for 5′-CZLUTATC-3′ (ODN22) calcd 2249.37, found 2249.22; for 5′-CZALUTATC-3′ (ODN23) calcd 2562.43, found 2562.76; for 5′-CZAALUTATC-3′ (ODN24) calcd 2875.49, found 2875.88; for 5′-CZAAALUTATC-3′ (ODN25) calcd 3188.55, found 3188.72.

Method of Calculations. The DNA models were built up using Insight II (Accelrys, San Diego, CA) program with standard B form helical parameters (pitch, 3.38 Å; twist, 36°; tilt, 1°). Molecular orbital calculations were performed at HF/6-31G* levels utilizing Gaussian 03 and Spartan 04. The adiabatic IPs were calculated by computing the difference in energies between the neutral base pair system and the cation radical with the energy minimized structures.

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Supporting Information Available: HPLC analysis of photoirradiated ODNs. This material is available free of charge via the Internet at http://pubs.acs.org.

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