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Photoreaction of iodouracil in DNA duplex; C–I bond is cleaved via two different pathways 'homolysis and heterolysis'

Ryu Tashiro, Kenta Nakamura, Hiroshi Sugiyama *

Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo, Kyoto 606-8502, Japan

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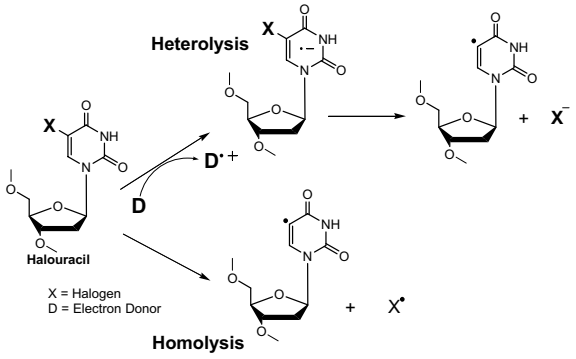
Abstract

We recently found that a selective photoreaction of 5-iodouracil (^{I}U) occurs in 5′-(G/C)AA^{I}U^{I}U-3′ and 5′-(G/C)A^{I}U^{I}U-3′ sequences in ^{I}U-substituted duplex DNA. In this study, the photoreactivity of the 5′-G(A)$_n$^{I}UT-3′ sequence was examined using various ^{I}U-containing oligonucleotides. HPLC analysis revealed that their photoreactivity largely depends on the number of As between G and ^{I}U. The most efficient reactivity was observed when the number of As was two and this decreased with increasing numbers from three to five, as observed for the 5′-G(A)$_n$^{I}UT-3′ sequence. These results indicate that the G located 5′ from ^{I}U acts as an electron donor for ^{I}U, as in the photoreaction of ^{Br}U. In sharp contrast to the ^{Br}U photoreaction, ^{I}U was photoreactive when the number of As was zero or more than five. These results indicate that both homolytic and heterolytic pathways operate in the formation of the uracil-5-yl radical in the photoreaction of ^{I}U in duplex DNA. In addition, the ratio of these pathways is highly dependent on DNA sequence.

Thymine (T) in DNA can be replaced with 5-halouracils such as bromouracil (^{Br}U) and iodouracil (^{I}U), and the resultant photosensitive 5-halouracil-substituted DNA remains functional in vivo. Therefore, these DNAs have been used in various studies in biological, and chemical areas. For example, DNA–protein photocrosslinking[1,2] and DNA photofootprinting[3] have been based on the photoreactivity of 5-halouracil, with the generation of the uracil-5-yl radical in DNA under UV irradiation. Detailed analysis of the photoproducts generated from irradiated ^{Br}U- or ^{I}U-containing oligonucleotides has provided evidence that the uracil-5-yl radical abstracts hydrogen from the sugar moiety of DNA located on the 5′ side of the 5-halouracil.[4] We have examined the photoreactions of 5-halouracil in A-form,[5] B-form,[4,6] Z-form,[7,8] and bent DNA,[9,10] and in G-quadruplexes,[11] and have demonstrated that the hydrogen abstraction by the uracil-5-yl radical is highly dependent on the local DNA conformation. On the basis of these observations, we have proposed that hydrogen abstraction by the uracil-5-yl radical is a useful method for the detection of various DNA structures.[12] Because this assay is highly dependent on the efficiency of the generation of the uracil-5-yl radical from 5-halouracil, it is important to understand the reaction mechanism to improve the detection method.

It is generally accepted that the halogen–carbon bond is cleaved by a homolytic or heterolytic pathway. These two



Scheme 1.

* Corresponding author. Tel.: +81 75 753 4002; fax: +81 75 753 3670.
E-mail address: hs@kuchem.kyoto-u.ac.jp (H. Sugiyama).

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pathways have also been proposed to operate in the generation of the uracil-5-yl radical (Scheme 1).[13,14] Recently, we discovered that the photoirradiation of ^{Br}U-substituted DNA fragments with 302 nm UV light efficiently produced ribonolactone residues in the 5′-(G/C)AA^{Br}U^{Br}U-3′ and 5′-(G/C)A^{Br}U^{Br}U-3′ sequences.[15] Using various oligonucleotides, we demonstrated that sequence-selective electron transfer plays an essential role in the generation of the uracil-5-yl radical in the hotspot sequence.

We concluded that the hotspot sequence consists of an electron-donating G/C base pair, the 5′-^{Br}U^{Br}U-3′ or 5′-^{Br}UT-3′ sequence as the acceptor, and an appropriate number of A/T base pairs as the bridge for the electron-transfer process.[16] Recently, the analogous generation of the uracil-5-yl radical from ^{Br}U by electron transfer has been reported by another group.[17–22]

Although homolytic cleavage was expected to occur in ^{I}U-containing DNA because the photoreactivities of ^{I}U-containing oligonucleotides differed from those of ^{Br}U-containing oligonucleotides,[6] the analogous hotspot sequences 5′-(G/C)AA^{I}U^{I}U-3′ and 5′-(G/C)A^{I}U^{I}U-3′ were identified in the photoirradiation of ^{I}U-substituted duplex DNA.[15] These results clearly suggest that an electron-transfer mechanism plays an important role in this hotspot sequence. In this report, the photoreactivities of various oligonucleotides containing the 5′-G(A)$_n$^{I}UT-3′ sequence were investigated to determine the contributions of the homolytic and heterolytic pathways.

We first investigated the photoreactivity of 5′-dCGAA^{I}UTATC-3′/5′-dGATAATTCG-3′ (GA2). GA2 was photoirradiated at 0 °C for 15 min using a monochromator (302 nm).[23,24] Figure 1 shows the HPLC analysis of the photolysate,[24] and demonstrates that 5′-dCGALUTGC-3′ (L = ribonolactone residue) was a major photoproduct, together with released free adenine. This indicates that 5′-(G/C)AA^{I}UT-3′ retains the high reactivity of the hotspot sequence, which is similar to that of 5′-dCGAA^{Br}UTATC-3′/5′-dGATAATTCG-3′.[15] This result

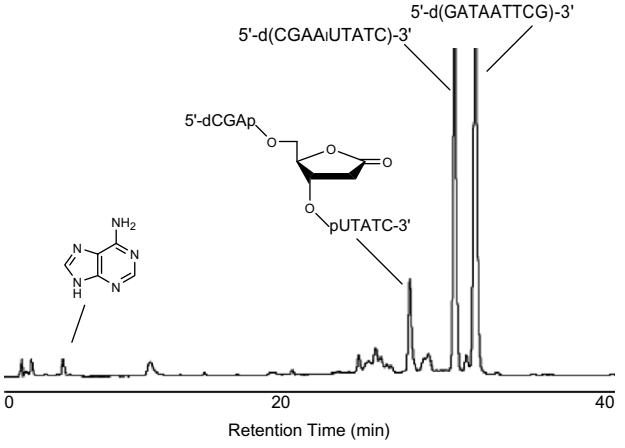
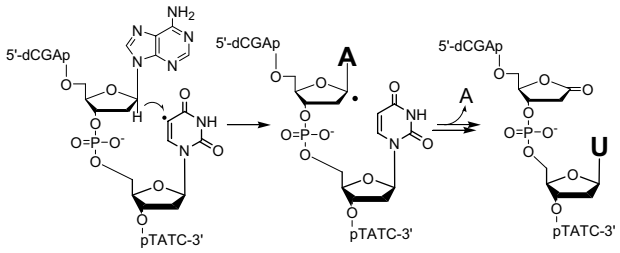


Fig. 1. HPLC profile of photoirradiated GA2. The reaction mixture containing GA2 (100 μM total base concentration) in 50 mM sodium cacodylate buffer (pH 7.0) in the presence of 50 mM NaCl was irradiated for 5 min at 0 °C using a monochromator (302 nm).

is consistent with the previous observation of selective ribonolactone formation in B-DNA (Scheme 2).[4,6] The relationship between charge transfer and donor–acceptor distance along the DNA has been discussed for several years.[25–27] Thus, we next investigated the photoreactivity of ^{I}U when the distance between the putative electron donor (G) and the electron acceptor ^{I}U in the double strand was altered.

Thus, GA0–GA7, which have different numbers of As in the 5′-G(A)$_n$^{I}U-3′ sequence, were synthesized and their photoreactivities compared. The extent of the hydrogen abstraction reaction was estimated from the yield of free adenine released, which is shown in Figure 2. The amount of free adenine increased with increasing numbers of A/Ts from zero to two, and gradually decreased with more than three A/Ts. Analogous to the reaction mechanism for ^{Br}U,[16] the reactivity of ^{I}U can be explained by an electron-transfer mechanism as follows: When the number of A/Ts is zero or one, the back electron transfer is much faster than the release of iodide ions, whereas for $n = 2$, the back electron transfer is slow enough to allow the release of iodide ions from the anion radicals of ^{I}U. These results suggest that the G located 5′ upstream from ^{I}U acts as an electron donor for ^{I}U and the intervening A/T base pairs as a bridge for the electron-transfer process, as in the case of the photoreaction of ^{Br}U.[16]

It is important to note that a significantly different reactivity was observed in the ^{I}U photoreaction relative to that of ^{Br}U when the number of As was 0, 6, or 7 (Fig. 2). With ^{Br}U, almost no reactivity was observed in such sequences.[16] These results suggest the coexistence of a distance-independent hemolytic process in the generation of the uracil-5-yl radical in the ^{I}U photoreaction. To test this hypothesis, photoreactions of IA2–IA5 were performed, in which the electron-donating Gs were replaced with electron-deficient deoxyinosine (I). The substitution of the electron-donating G with I completely abolished their photoreactivity in the ^{I}U photoreaction, because G and I have different ionization potentials: G = 1.2 V and I = 1.4 V.[25] The photoreactivites of IA2–IA5 were significantly lower than those of GA2 and GA3, and approximately the same amounts of free adenine were released from photoirradiated IA2–IA5 as from GA0, GA6, and GA7 (Fig. 3). These results can be explained by the fact that the lack of an electron donor eliminates the heterolytic pathway from the C–I cleavage pathways, and only the homolytic pathway remains



Scheme 2.

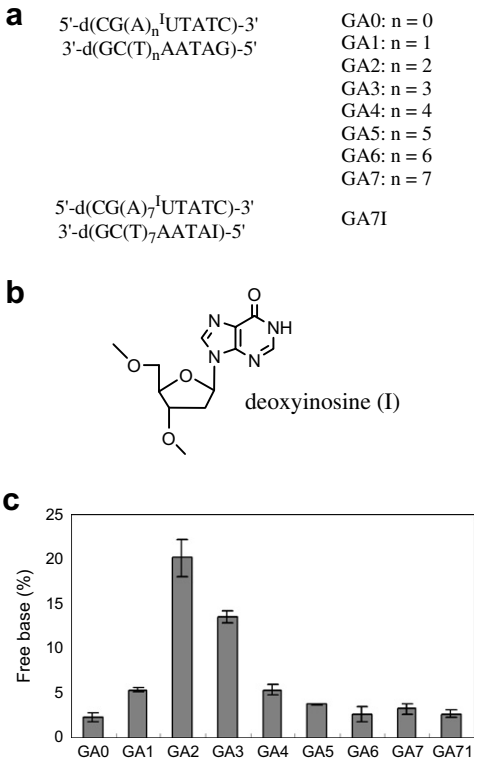


Fig. 2. (a) Series of ^{I}U containing DNA. (b) Structure of deoxyinosine. (c) Amount of free base produced from oligonucleotides with different numbers (*n*) of intervening bridges (A/Ts).

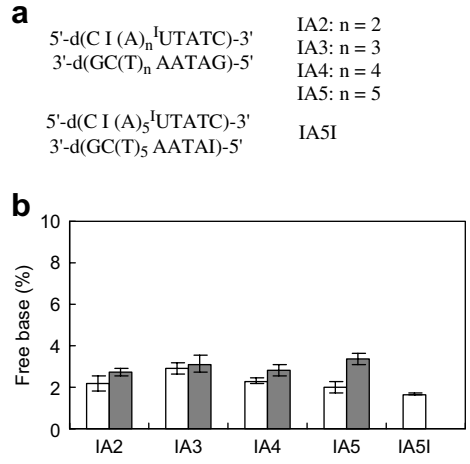
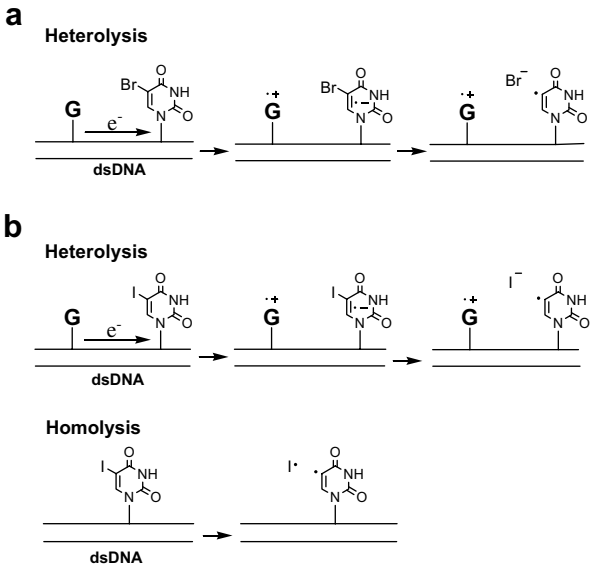


Fig. 3. (a) Series of inosine- and ^{I}U-containing DNA. (b) Amount of free base produced from inosine (I)-containing oligodeoxynucleotides with different numbers (*n*) of intervening bridges (A/Ts). Open bars and black bars indicate double-stranded and single-stranded DNAs, respectively. The experiments using single-stranded DNA were performed with only the ^{I}U-containing strand.

(Scheme 3). The photoreactivities of GA7I and IA5I were almost the same with that of GA7 and IA5, respectively, indicating that the G located at the 5′ end of the complementary strand does not act as an electron donor (Figs. 2 and 3).



Scheme 3.

Because the free As from irradiated GA1–GA5 are derived via the heterolytic and homolytic pathways, the proportion of the reaction contributed by heterolysis can be evaluated by subtracting the background-level homolysis from the average yields. For example, the photoreaction of GA2 via the electron-transfer process is approximately seven times higher than the reaction via homolysis.

We also investigated the photoreactivity of ^{I}U in single-stranded DNA (IA2–IA5) (Fig. 3). The disruption of π-stacking in the single-stranded form is known to reduce the electron transport along the DNA.[25] The fact that the photoreactivities were retained in photoirradiated single-stranded IA2–IA5 further supports the reaction pathway of C–I homolysis. The photoreactivities of ^{I}U in single-stranded DNA were slightly higher than those of ^{I}U in double-stranded DNA. This increase of photoreactivity may be due to different properties of single-stranded DNA, such as increasing incident light absorption or different reactivity of uracil-5yl and sugar radicals. Further investigation is necessary to clarify this point.

The distance dependency of the electron transfer (β) of ^{I}U was estimated as 0.25 Å (Fig. 4). This value is similar to that for ^{Br}U (0.20 Å).[16] The lifetime of the anion radical of ^{Br}U has been estimated as 7 ns and this value is longer than that for the anion radical of ^{I}U, which is 1.5 ns.[28] However, no significant relationship between the β value and the lifetime of the anion radical was observed.

In summary, we clearly demonstrated that both homolytic and heterolytic pathways are involved in the C–I bond cleavage mechanism of ^{I}U in DNA duplexes. In addition, the ratio of these pathways is highly dependent on the sequence of DNA. Previously, we found that ^{I}U and ^{Br}U have different sequence dependencies, in that the photoreaction occurs at the $G^{I}U$ but not at the $G^{Br}U$ sequence.[4,6] Present results imply that the $G^{I}U$ sequence produces hydrogen abstraction products via the homolytic pathway.

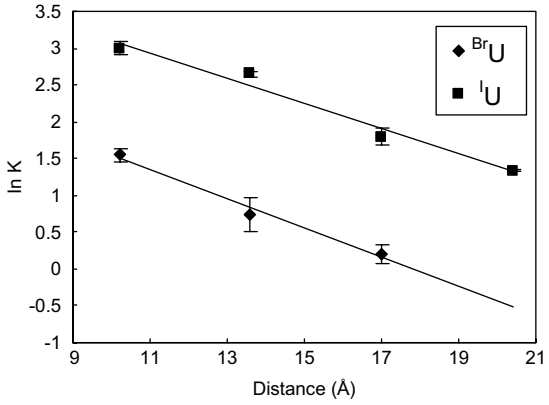


Fig. 4. In (k) plotted against the G–^{X}U distance. In (k) is calculated from yields of free adenine via heterolytic pathway obtained from Figure 2. The amount of adenine via heterolytic pathway in the case of ^{I}U reaction was estimated as the subtraction of the average of adenine via homolytic pathway from GA0, GA6 and GA7 from each value of GA2–GA5 which includes heterolytic and homolytic pathways. Data for ^{Br}U are taken from Ref. 16.

In the case of irradiated G^{Br}U sequence, rapid back electron transfer does not allow the reaction.[4] This also supports the evidence that heterolysis is the main pathway in dehalogenation of ^{Br}U. The coexistence of heterolytic and homolytic pathways in the photoreaction of ^{I}U is consistent with the reactivities at the hotspot sequence and G^{I}U. These fundamental properties of the 5-halouracils provide useful information when choosing a 5-halouracil for photochemical applications, such as photocrosslinking or photofootprinting. For example, in a situation in which no electron donor is located around the 5-halouracil incorporated into the DNA, ^{I}U is more suitable because of the efficient generation of the uracil-5-yl radical via the homolytic pathway.

Acknowledgments

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23. *Synthesis of deoxynucleotides*: Oligonucleotides were prepared by solid-phase DNA synthesis on a controlled-pore glass supports (1 μmol) using a ABI 3400 DNA synthesizer (Applied Biosystems), and 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). The synthesized oligonucleotides were confirmed by enzymatic digestion and electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS). ESI-TOF-MS was performed on a Bruker Bio TOF II (Bruker Daltonics,. Billerica, MA). Enzymatic digestion was performed with endonuclease P1 (0.3 units/mL, Boehringer Mannheim) and alkaline phosphatase (1000 units/mL, Boehringer Mannheim).
24. *Analysis of photoirradiated oligonucleotides containing 5-iodouracil*: The reaction mixture (total volume 100 μL) contained 100 μM 5-iodouracil-containing deoxyoligonucleotide, 50 mM sodium cacodylate (pH 7.0), and 50 mM NaCl. Photoirradiation at 302 nm was performed in Eppendorf tube using HM-5 hypermonochromator (Jasco, Tokyo, Japan). Photolysis tubes were positioned 5 mm from the outlet of glass fiber and were irradiated for 15 min on ice. The photoreactions were carried out under aerobic conditions. After irradiation, the reaction mixtures were analyzed by HPLC with a Chemcobond. 5-ODS-H column (4.6 × 150 mm). Elution was performed with 50 mM ammonium formate (pH 6.5) and a linear gradient of 0–12% acetonitrile for 40 min at a flow rate of 1.0 mL/min at 40 °C. The yields of reaction product were estimated based on starting ODN.
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