UVB-Induced Formation of Intrastrand Cross-Link Products of DNA in MCF-7 Cells Treated with 5-Bromo-2′-deoxyuridine†

Yu Zeng and Yinsheng Wang*

Department of Chemistry-027, University of California, Riverside, California 92521-0403

Received March 3, 2007; Revised Manuscript Received April 26, 2007

ABSTRACT: Nucleoside 5-bromo-2′-deoxyuridine (^{Br}dU), after being incorporated into cellular DNA, is well-known to sensitize cells to ionizing radiation and UV irradiation. We reported here, for the first time, the sequence-dependent formation of intrastrand cross-link products from the UVB irradiation of ^{Br}dU-treated MCF-7 human breast cancer cells. Our results showed that ^{Br}dU replaced more than 30% dT in genomic DNA after the cells were treated with 10 μM ^{Br}dU for 48 h. LC−MS/MS data revealed that more than 50% of the incorporated ^{Br}dU was consumed during UVB irradiation, of which more than half was dehalogenated to yield dU. Low-dose (5.0 kJ/m^2) UVB irradiation of ^{Br}dU-treated cells yielded four intrastrand cross-link products, where the C5 of uracil is covalently bonded to the C8 of its neighboring 5′ or 3′ guanine to give G[8-5]U and U[5-8]G, respectively, and the C5 of uracil could couple with the C2 or C8 of its vicinal 5′ adenine to give A[2-5]U and A[8-5]U, respectively. All the above cross-link products except A[2-5]U could also be induced in ^{Br}dU-treated cells upon UVB irradiation at a dose of 39 kJ/m^2. We further demonstrated, by using LC−MS/MS, that the yield of G[8-5]U was much greater than the total yields of A[2-5]U and A[8-5]U. In addition, our results revealed that ^{Br}dU treatment stimulated considerably the UVB-induced formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) *in vivo*. The formation of these intrastrand cross-link products and 8-oxo-dG *in vivo* underscores the importance of these products in the photosensitizing effect of ^{Br}dU.

It was observed in as early as the 1950s that 5-bromouracil could replace the isosteric thymine and be incorporated into cellular DNA (*1*−*3*). This nucleoside substitution could enhance markedly the lethal effects of exposure from both UV light and ionizing radiation, and it was found that such exposure of 5-bromo-2′-deoxyuridine (^{Br}dU[1])-bearing DNA could result in a considerably increased amount of strand breaks and alkali labile sites (*4*, *5*). Moreover, it has been reported that there exists a linear relationship between the effect of cellular radiosensitization and the percentage of thymidine replacement by ^{Br}dU in cell lines, and similar results were obtained from clinical studies (*5*−*7*). Mechanistic investigations suggested that the UV irradiation- or ionizing radiation-induced formation of the uracil-5-yl radical and the subsequent hydrogen abstraction from the neighboring 5′ 2-deoxyribose by this radical are important for the formation of strand breaks (*4*, *8*, *9*).

Recently there is a renewed interest in the radiosensitizing effect of halopyrimidines, and studies have been extended to other 5-halopyrimidine nucleosides including 5-chloro-2′-deoxycytidine (*10*−*14*) and 5-bromo-2′-deoxycytidine (^{Br}dC) (*15*). In addition, halogenated dC was shown to produce similar degrees of radiosensitization as the corresponding halogenated dU (*6*). In the normal metabolic pathway, a halogenated dC is, however, first converted to halogenated dU by cellular cytidine deaminase and the latter halogenated dU derivative is then incorporated into DNA (*10*, *15*).

Our previous studies showed that UVB irradiation of duplex DNA harboring a ^{Br}dU or ^{Br}dC could result in the facile formation of intrastrand cross-link lesions where the C5 of the pyrimidine base is covalently bonded to a carbon or nitrogen atom of its vicinal purine base (*16*−*18*). In addition, our results revealed that ^{Br}dC is more efficient in inducing intrastrand cross-link lesions in duplex DNA than ^{Br}dU (*16*−*18*). It was also found that exposure of duplex DNA carrying a ^{Br}dU in the bulge region to UV irradiation and ionizing radiation could give rise to the formation of interstrand cross-link lesions (*19*−*21*).

Recent biochemical studies demonstrated that the introduction of intrastrand cross-link lesions of this type into DNA duplex may pose challenges to DNA replication and evoke nucleotide excision repair. In this regard, *in vitro* replication studies revealed that the intrastrand cross-link lesions G[8-5]C and G[8-5m]T, where the C5 of cytosine or the methyl carbon of thymine is covalently bonded to the C8 of its neighboring 5′ guanine, could block DNA synthesis by replicative DNA polymerases (*22*, *23*) and lead to error-prone replication by a translesion synthesis DNA polymerase, yeast pol η (*24*). Furthermore, this and other structurely related intrastrand cross-link lesions could be subjected to repair by nucleotide excision repair with *Escherichia coli* UvrABC nuclease (*25*, *26*).

To gain insights into the implications of these intrastrand cross-link lesions in the photosensitizing effect of ^{Br}dU *in*

† This work was supported by the National Institutes of Health (R01 CA96906 and R01 CA101864).

* To whom correspondence should be addressed. Tel: (951) 827-2700. Fax: (951) 827-4713. E-mail: yinsheng.wang@ucr.edu.

[1] Abbreviations: ^{Br}dU, 5-bromo-2′-deoxyuridine; ^{Br}dC, 5-bromo-2′-deoxycytidine; 8-oxo-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; PBS, phosphate-buffered saline; SICs, selected-ion chromatograms.

10.1021/bi700431q CCC: $37.00
Published on Web 06/13/2007

vivo, it is important to examine whether these cross-link products can also be generated in cells that are treated with ^{Br}dU and UVB irradiation. Herein, we explored the photo-cross-linking chemistry of ^{Br}dU in cellular DNA, and we placed our emphasis on the identification of intrastrand cross-link products, the sequence-dependent formation of these products, and the formation of an oxidative lesion, 8-oxo-7,8-dihydro-2′-deoxyuridine (8-oxo-dG).

EXPERIMENTAL PROCEDURES

Materials. All materials were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. Nuclease P1 was from MP Biomedicals (Aurora, OH), and snake venom phosphodiesterase and calf spleen phosphodiesterase were purchased from US Biological (Swampscott, MA). Uniformly ^{15}N-labeled 8-oxo-dG (8-oxo-dG*) was synthesized according to a previous report (*27*).

Cell Culture and ^{Br}dU Treatment. The MCF-7 human breast cancer cells (ATCC, Manassas, VA) were cultured at 37 °C in minimum essential medium supplemented with 10% fetal bovine serum (ATCC), 0.01 mg/mL bovine insulin, 100 IU/mL penicillin, and 100 μg/mL streptomycin (ATCC). After growing for 72 h, the MCF-7 cells were cultured in the above medium doped with 1.0×10^{-5} M ^{Br}dU for another 24, 48, or 96 h to determine the optimum drug treatment period. It was found that the treatments for 48 and 96 h gave similar levels of ^{Br}dU incorporation, and 48 h treatment was employed for the following UVB irradiation experiments.

UVB Irradiation. After being cultured in ^{Br}dU-containing media for 48 h, 1.0×10^8 cells were harvested by centrifugation. The cells were then washed with phosphate-buffered saline (PBS) and resuspended in 120 mL of PBS. The resulting cell suspension was dispersed into eighteen 13 × 100 mm (O. D. × length) Pyrex tubes (Catalog No. 99447-13, Corning Inc., Corning, NY). The 50% cutoff wavelength for the Pyrex tube was determined to be ~290 nm. Photoirradiation was carried out with a Hanovia 450 W medium-pressure mercury lamp for 9 (dose: 5.0 kJ/m^2) or 70 min (dose: 39 kJ/m^2). During irradiation, the Pyrex tube and lamp were immersed in an ice−water bath. Immediately after irradiation, the cells were harvested by centrifugation and the nuclear DNA was isolated by phenol extraction.

Enzymatic Digestion. Two units of nuclease P1, 0.01 unit of calf spleen phosphodiesterase, and a 3.8 μL solution containing 300 mM sodium acetate (pH 5.0) and 10 mM zinc acetate were added to 200 μg of DNA sample and diluted to 380 μL. The digestion was continued at 37 °C for 6 h. To the digestion mixture were then added 40 units of alkaline phosphatase, 0.1 unit of snake venom phosphodiesterase, and 50 μL of 0.5 M Tris-HCl (pH 8.9). The resulting solution was incubated at 37 °C for 4 h and then passed through a Centricon microcentrifuge filter (Millipore, Billerica, MA) to remove the enzymes. The aqueous layer was dried and redissolved in water for off-line HPLC enrichment.

HPLC. The off-line HPLC removal of unmodified nucleosides from the digestion mixture was performed with a 4.6 × 50 mm Luna reverse-phase column (5 μm in particle size, Phenomenex, Torrance, CA). A 50 min gradient of 0−12% acetonitrile in 10 mM ammonium formate (pH 6.3) was employed, and the flow rate was 0.40 mL/min (Figure 1 gives the HPLC trace for the separation of a mixture composed of standard unmodified nucleosides and cross-link products). The extinction coefficient of ^{Br}dU at 260 nm was determined to be 6.0×10^3 L/mol·cm by using a previously reported method (*18*).

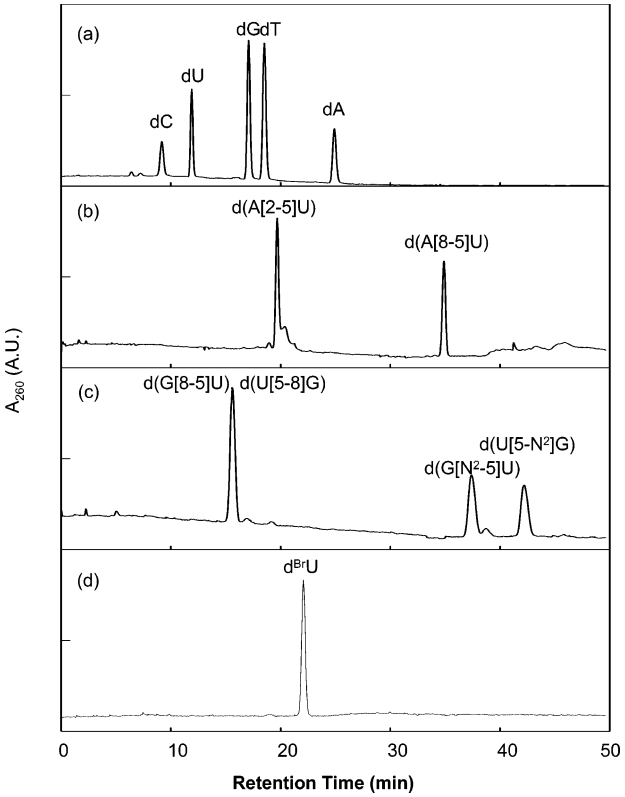


FIGURE 1: HPLC traces for the separation of (a) a nucleoside mixture of dC, dU, dT, dG, and dA; (b) a mixture of two intrastrand cross-link products d(A[2-5]U) and d(A[8-5]U); (c) a mixture of four intrastrand cross-link products d(G[8-5]U), d(U[5-8]G), d(G[N^2-5]U), and d(U[5-N^2]G); (d) ^{Br}dU.

The following procedures were employed to construct the calibration curves for the quantification of intrastrand cross-link lesions: Cross-link standards were mixed with 30 nmol of nucleoside mixture from the enzymatic digestion of DNA isolated from MCF-7 cells without ^{Br}dU treatment, and the resulting samples were subjected to the same off-line HPLC enrichment and on-line LC−MS/MS analysis as for the DNA samples isolated from ^{Br}dU-treated cells. For the quantification of 8-oxo-dG, 30 pmol of uniformly ^{15}N-labeled 8-oxo-dG was added to each digestion sample prior to the off-line HPLC enrichment.

LC−MS/MS. A Zorbax SB-C18 column (0.5 × 150 mm, 5 μm in particle size, Agilent Technologies, Palo Alto, CA) and an Agilent 1100 capillary HPLC pump were employed for the LC−MS/MS experiments. A 60 min gradient of 0−25% acetonitrile in 15 mM ammonium formate was used, and the flow rate was 6.0 μL/min. The effluent from the HPLC column was coupled to an LTQ linear ion-trap mass spectrometer (Thermo Electron, San Jose, CA), which was operated in the positive-ion mode. The mass spectrometer was set up to alternate among MS, MS/MS, MS^3, and MS^4 modes.

RESULTS

1. Formation of Cross-Link Products in Cells Treated with ^{Br}dU and UVB Irradiation. To understand the biological

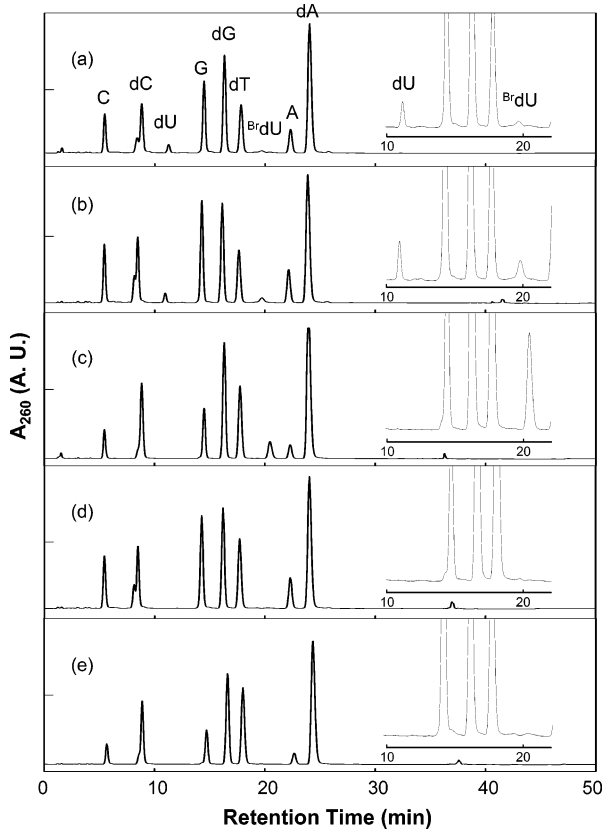


FIGURE 2: The HPLC traces for the separation of the digestion mixtures of DNA samples isolated from MCF-7 cells that were (a) treated with ^{Br}dU and irradiated with UVB light for 70 min (dose: 39 kJ/m^2); (b) treated with ^{Br}dU and irradiated with UVB light for 9 min (dose: 5.0 kJ/m^2); (c) treated with ^{Br}dU but without UVB irradiation; (d) irradiated with UVB light for 70 min but without ^{Br}dU treatment; and (e) irradiated with UVB light for 9 min but with no ^{Br}dU treatment. "A", "C", and "G" represent ribonucleosides adenosine, cytidine, and guanosine, respectively. Shown in the insets are the expanded chromatograms to visualize better the peaks corresponding to dU and ^{Br}dU.

implications of intrastrand cross-link products in the photosensitizing effect of ^{Br}dU, it is important to examine whether these products can also be induced in cells. To this end, we cultured MCF-7 cells in ^{Br}dU-containing medium, irradiated the cells with UVB light, isolated the genomic DNA from the cells, and digested the DNA samples with four enzymes (see Experimental Procedures). We then subjected the digestion mixtures to off-line HPLC enrichment and on-line LC−MS/MS analysis (the HPLC traces for the off-line separation of the nucleoside mixtures are shown in Figure 2).

The selected-ion chromatograms (SICs) from the analysis of the sample isolated from the ^{Br}dU-treated and low-dose (5.0 kJ/m^2) UVB-irradiated cells showed the presence of four cross-link products, d(G[8-5]U), d(U[5-8]G), d(A[8-5]U), and d(A[2-5]U), according to the similar HPLC retention times and tandem mass spectra as those of the standard cross-link products (Figures 3−5 and Figure S3 (in Supporting Information). In this regard, the standard intrastrand cross-link products involving uracil, namely, d(U[5-N^2]G), d(G[N^2-5]U), d(U[5-8]G), d(G[8-5]U), d(A[8-5]U), and d(A[2-5]U), were obtained from the UVB irradiation of the corresponding ^{Br}dU-containing dinucleoside monophosphates (*18*).

Two cross-link products formed between uracil and guanine, i.e., d(G[8-5]U) and d(U[5-8]G), elute at 11.9 and

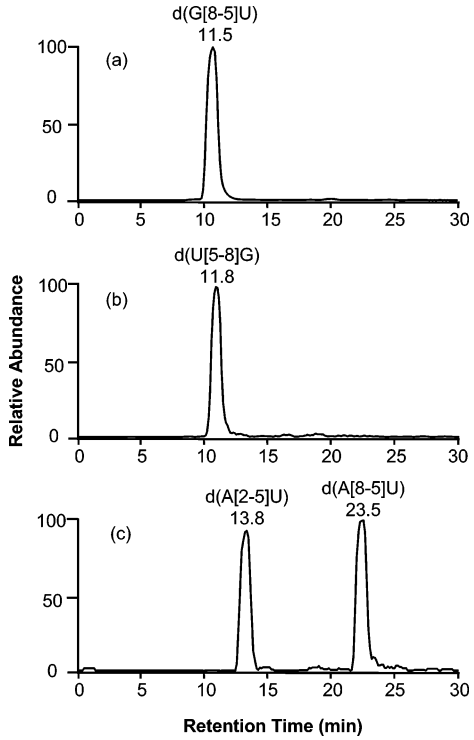


FIGURE 3: LC−MS/MS analysis of standard intrastrand cross-link lesions. Shown are the SICs for the following transitions: (a) *m*/*z* 556 → 458 for d(G[8-5]U), (b) *m*/*z* 556 → 538 for d(U[5-8]G), and (c) *m*/*z* 540 → 246 for d(A[2-5]U) and d(A[8-5]U).

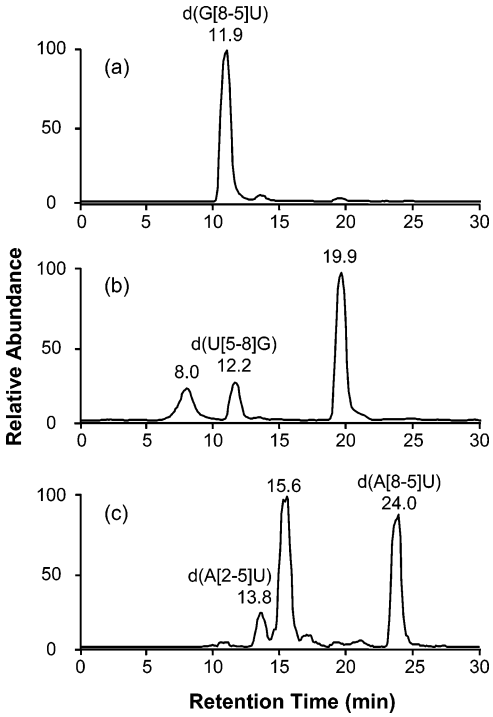


FIGURE 4: LC−MS/MS analysis of the sample enriched from the enzymatic digestion mixture of the DNA isolated from MCF-7 cells that were treated with ^{Br}dU and irradiated with UVB light for 9 min (dose: 5.0 kJ/m^2). Shown are the SICs for monitoring the following transitions: (a) *m*/*z* 556 → 458 for d(G[8-5]U), (b) *m*/*z* 556 → 538 for d(U[5-8]G), and (c) *m*/*z* 540 → 246 for d(A[2-5]U) and d(A[8-5]U).

12.2 min, respectively, which were found in the SICs for monitoring the *m*/*z* 556 → 458 and *m*/*z* 556 → 538 transitions, respectively (Figure 4a,b). Both cross-link prod-

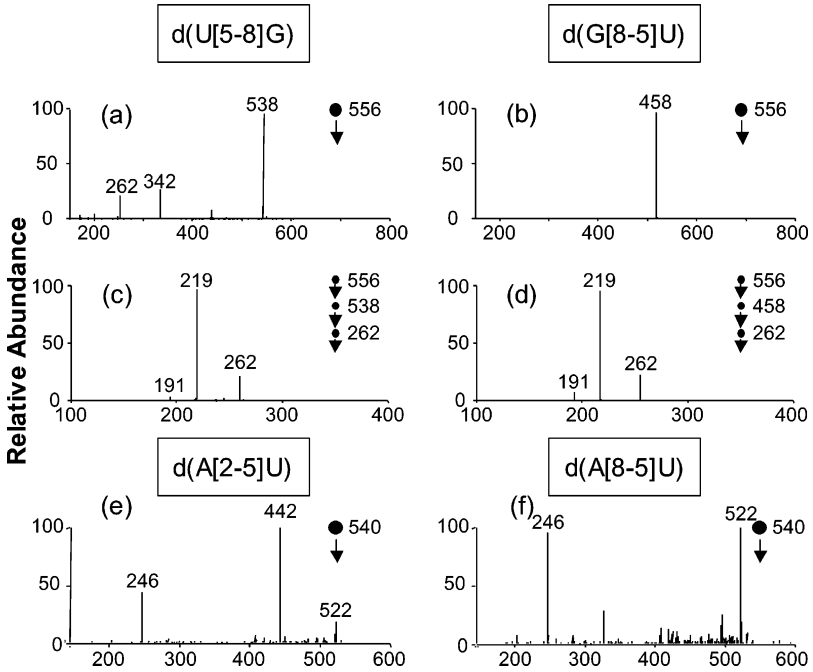
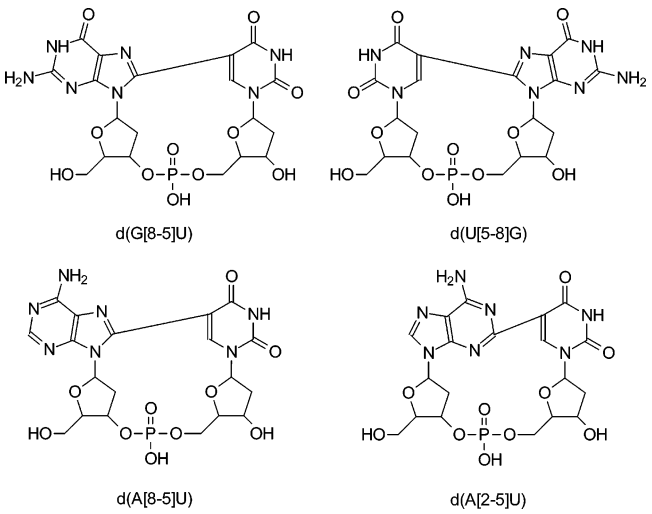


FIGURE 5: Product-ion spectra averaged from peaks in SICs shown in Figure 4. Shown are the product-ion spectra of the $[M + H]^+$ ions of d(U[5-8]G) (a), d(G[8-5]U) (b), d(A[2-5]U) (e), and d(A[8-5]U) (f). Depicted also are the MS^4 which monitor the fragmentation of the $[M + H]^+$ ions of the cross-linked nucleobase portions of d(U[5-8]G) (c) and d(G[8-5]U) (d).

Scheme 1: Structures of Intrastrand Cross-Link Lesions Identified in This Study



ucts have the C5 of uracil and the C8 of its neighboring guanine being covalently bonded, while the difference lies in the orientations of the two nucleobases being cross-linked (Scheme 1). These two isomers can be distinguished by the product-ion spectra of their $[M + H]^+$ ions: The $[M + H]^+$ ion of d(U[5-8]G) can undergo the facile loss of a H_2O molecule to give the ion of *m*/*z* 538, whereas the $[M + H]^+$ ion of d(G[8-5]U) can eliminate readily a neutral $C_5H_6O_2$ moiety (the 2-deoxyribose component) to afford the ion of *m*/*z* 458 (Figures 5 and S3). Further breaking down of the ion of *m*/*z* 538 or *m*/*z* 458 (MS^3) led to the formation of a common fragment ion of *m*/*z* 262, which is attributed to the protonated ion of the cross-linked nucleobase component (Figure S3) (*18*). On the other hand, the MS^4 of the two isomers, which monitor the fragmentation of the ion of *m*/*z* 262, are identical, supporting that the two nucleobases in these two isomers are covalently bonded in the same fashion (Figures 5 and S3). In this respect, we found, in the MS^4, two fragment ions, that is, the ions of *m*/*z* 219 and 191. The ion of *m*/*z* 219 is attributed to the elimination of an HNCO moiety, whereas the ion of *m*/*z* 191 is originated from the further loss of a CO molecule from the ion of *m*/*z* 219 (Figure 5). Similar mass spectrometric results were obtained for d(G[8-5]U) and d(U[5-8]G) induced from the UVB irradiation of ^{Br}dU-carrying synthetic duplex DNA (*18*), which further substantiated the present identification of the cross-link products formed *in vivo*.

It is worth noting that two isomeric intrastrand cross-link products could arise from the UVB irradiation of dinucleoside monophosphate d(^{Br}UG) [i.e., d(U[5-N^2]G) and d(U[5-8]G)] or d(G^{Br}U) [i.e., d(G[N^2-5]U) and d(G[8-5]U)]. Similar irradiation of duplex DNA housing a G^{Br}UG sequence motif can facilitate the formation of all the above lesions except G[N^2-5]U. Moreover, in duplex DNA, the yield of U[5-N^2]G was less than 20% of that of U[5-8]G. Thus, the failure to detect U[5-N^2]G from the enzymatic digestion mixture of cellular DNA is very likely due to the relatively low yield of its formation, though we cannot exclude the possibility of the more efficient decomposition and/or repair of this lesion *in vivo*.

The 13.8 and 24.0 min fractions found in the SIC for monitoring the *m*/*z* 540 → 246 transition correspond to d(A[2-5]U) and d(A[8-5]U), respectively (Scheme 1, Figure 3c, and Figure 4c), and the product-ion spectra of the $[M + H]^+$ ions of these two products are shown in Figure 5. The SICs for the analysis of the high-dose (39 kJ/m^2) irradiation sample showed the presence of three cross-link products, d(G[8-5]U), d(U[5-8]G), and d(A[8-5]U) (Figure S2, Supporting Information). The d(A[2-5]U), however, was undetectable in this sample.

After identifying those intrastrand cross-link products, we further quantified their amounts by LC−MS/MS. In this respect, HPLC enrichment is necessary for the detection of

Table 1: The Yields for the Intrastrand Cross-Link Products Generated from the UVB Irradiation of ^{Br}dU-Treated Cells[a]

irradiation doses (kJ/m^2)	yields (lesions/10^6 nucleosides)			
	U[5-8]G	G[8-5]U	A[8-5]U	A[2-5]U
5.0	57 ± 10	130 ± 20	5 ± 1	1.1 ± 0.1
39	71 ± 10	89 ± 10	7 ± 1	

[a] The values represent the means and standard deviations of results from three independent LC−MS/MS measurements.

intrastrand cross-link products formed *in vivo*. To correct for the loss of lesions during the HPLC enrichment, we constructed calibration curves by mixing the standard intrastrand cross-links with a nucleoside mixture from the digestion of DNA samples isolated from the untreated MCF-7 cells and subjecting these samples to the same HPLC enrichment and LC−MS/MS analysis procedures (see Experimental Procedures, and the calibration curves are shown in Figure S1, Supporting Information).

The quantification results clearly demonstrated that the yield of d(G[8-5]U) is much higher than the total yields of d(A[8-5]U) and d(A[2-5]U) in both the 5.0 kJ/m^2 (130 vs 6 lesions per 10^6 nucleosides, Table 1) and the 39 kJ/m^2 (89 vs 7 lesions per 10^6 nucleosides, Table 1) irradiated samples. The more facile formation of cross-link lesions at GU site than at AU site is in keeping with our previous findings for the formation of these products from the UVB irradiation of ^{Br}dU-containing short duplex DNA (*18*).

On the other hand, when the irradiation dose was increased from 5.0 to 39 kJ/m^2, the yields of d(U[5-8]G) and d(A[8-5]U) increased by 25 and 28%, respectively, whereas the yield of d(G[8-5]U) dropped by more than 30% (Table 1). Moreover, d(A[2-5]U) was not detectable in the sample isolated from cells exposed to UVB light at a dose of 39 kJ/m^2. The decrease in yields of d(G[8-5]U) and d(A[2-5]U) upon UVB exposure at this higher dose might be attributed to the preferential decomposition and/or repair of these two cross-link lesions *in vivo*.

2. Quantification of ^{Br}dU and dU. Dehalogenation represents an important metabolic step of halopyrimidine nucleosides *in vivo* (*13*, *14*, *28*). To assess the percentage of ^{Br}dU incorporation into cellular DNA and the efficiency of consumption of ^{Br}dU during UVB irradiation, we quantified the amounts of ^{Br}dU and its dehalogenation product, dU, by HPLC analysis.

The HPLC traces for the separation of the nucleoside mixtures revealed the presence of ^{Br}dU only in those nucleoside samples from cells treated with ^{Br}dU (eluting at ~20 min) and the existence of dU (eluting at ~12 min) only in those samples from cells that were treated with both ^{Br}dU and UVB irradiation (Figure 2). LC−MS/MS results confirmed that these fractions contained ^{Br}dU and dU (data not shown). The amounts of ^{Br}dU and dU, expressed as ratios over the amount of dT, were then calculated from the ratios of the peak areas of these two nucleosides over the area of dT, together with the consideration of the extinction coefficients of these three nucleosides (Table 2).

The quantification results (Table 2) showed that, after 48 h treatment with 10 μM ^{Br}dU, approximately 30.6% of dT in cellular DNA was replaced with ^{Br}dU, which is comparable with the previous measurements on ^{Br}dU incorporation (*29*). After these cells were exposed to UVB light at a dose of 5.0 kJ/m^2, the amount of intact ^{Br}dU decreased to 14.5% of dT; in the meantime we observed the formation of a significant amount of dU (~10.8% of dT, Table 2 and Figure 2b). Therefore, approximately 53% of the incorporated ^{Br}dU was consumed during the irradiation, and, among the ^{Br}dU consumed, about 67% was dehalogenated to give dU.

Table 2: The Yields of ^{Br}dU, dU, and 8-Oxo-dG in the Enzymatic Digestion Mixtures of DNA Samples Isolated from MCF-7 Cells[a]

treatment conditions	yields		
	8-oxo-dG (lesions/10^6 nucleosides)	^{Br}dU (% dT)	dU (% dT)
no ^{Br}dU, UVB (5.0 kJ/m^2)	67 ± 8	nd[b]	nd
no ^{Br}dU, UVB (39 kJ/m^2)	190 ± 24	nd	nd
with ^{Br}dU, UVB (0 kJ/m^2)	25 ± 3	30.6 ± 0.2	nd
with ^{Br}dU, UVB (5.0 kJ/m^2)	490 ± 43	14.5 ± 0.3	10.8 ± 0.4
with ^{Br}dU, UVB (39 kJ/m^2)	420 ± 68	8.4 ± 0.9	11.3 ± 0.3

[a] The yields of ^{Br}dU and dU are reported as percentages of total dT, which were calculated based on the peak areas for ^{Br}dU, dU, and dT found in the HPLC chromatograms shown in Figure 2 and the molar extinction coefficients of the three pyrimidine nucleosides. For instance, the percentage of dU was calculated by using % dU = $(A_{dU}/A_{dT}) \times (\epsilon_{260,dT}/\epsilon_{260,dU})$, where "$A$" and "$\epsilon_{260}$" represent the HPLC peak area and molar extinction coefficient at 260 nm, respectively. The data represent the means and standard deviations of the results from three independent measurements. [b] Not detectable.

After the cells were exposed to UVB light at a dose of 39 kJ/m^2, the amount of intact ^{Br}dU decreased further to 8.4% of dT, which is accompanied with an increase of the amount of dU to 11.3% of dT (Table 2 and Figure 2a).

3. Quantification of 8-Oxo-dG. It was proposed that UV irradiation can lead to the transfer of an electron from guanine to its vicinal 5-bromouracil, which leads to the formation of a guanine radical cation (*16*, *18*). The guanine radical cation in DNA can be hydrated and give rise to the formation of 8-oxoguanine (*30*). To examine whether the substitution of thymidine with ^{Br}dU in cellular DNA can enhance the UVB-mediated formation of 8-oxo-dG, we further quantified the formation of 8-oxo-dG by LC−MS/MS with the standard isotope dilution method (*27*).

The quantification results showed that ^{Br}dU incorporation stimulated considerably the UVB-induced formation of 8-oxo-dG (Table 2). Without UVB irradiation, the level of 8-oxo-dG in ^{Br}dU-treated cells was ~25 lesions per 10^6 nucleosides. When the ^{Br}dU-treated cells were irradiated with UVB light at doses of 5.0 and 39 kJ/m^2, the yields of 8-oxo-dG were increased to 490 and 420 lesions per 10^6 nucleosides, respectively (Table 2). In stark contrast, the amounts of 8-oxo-dG were 67 and 190 lesions per 10^6 nucleosides while the control ^{Br}dU-free cells were exposed to UVB light at doses of 5.0 and 39 kJ/m^2, respectively (Table 2).

DISCUSSION

Substitution of thymidine in cellular DNA with ^{Br}dU has long been known to increase markedly the lethal effects of exposure from UV light and ionizing radiation, and it was found that such exposure of ^{Br}dU-carrying DNA could result in a considerably increased amount of strand breaks and alkali labile sites (*4*, *5*). Our quantification data revealed that approximately 35% of the incorporated ^{Br}dU was dehalogenated to give dU during photoirradiation. In addition, ^{Br}dU

incorporation stimulated substantially the generation of the oxidative single-base lesion, 8-oxo-dG.

Importantly, we demonstrated, for the first time, that intrastrand cross-link products of DNA could be induced in ^{Br}dU-incorporated cellular DNA upon UVB irradiation. The replacement of thymidine with ^{Br}dU facilitated the generation of intrastrand cross-link lesions between uracil and its neighboring guanine or adenine with total yields of ~190 and 170 lesions per 10^6 nucleosides upon irradiation with UVB light at doses of 5.0 and 39 kJ/m^2, respectively, whereas these intrastrand cross-link lesions were not detectable in DNA samples isolated from the MCF-7 cells that were irradiated with UVB light but not treated with ^{Br}dU. The quantification results demonstrated that the sequence selectivity of 5′-dG > 5′-dA applies to the formation of intrastrand cross-link products from ^{Br}dU-bearing cellular DNA as it did *in vitro* (*18*). It suggests that stacking between 5-bromouracil and its neighboring purine base plays an important role in the formation of the intrastrand cross-link products (*18*).

It is well-known that UVB irradiation induces the formation of bipyrimidine photoproducts, including the cyclobutane pyrimidine dimers and the pyrimidine(6-4)pyrimidone products, and the formation of these photoproducts confers the major cytotoxic effects of UVB irradiation (*31*, *32*). Therefore, it is important to compare the relative occurrences of intrastrand cross-link lesions quantified here and the dimeric photoproducts formed from UVB irradiation. In this context, Cadet et al. (*33*) recently showed that, upon UVB irradiation of THP1 monocyte cells at a dose range of 0−2.6 kJ/m^2, TT cyclobutane dimer was induced with the highest efficiency, with a formation rate of 3.1 lesions/10^4 bases/(kJ/m^2). Here, we showed that the G[8-5]U was formed at a yield of 0.26 lesions/10^4 bases/(kJ/m^2) with a UVB dose of 5.0 kJ/m^2, which is about 10% of the yield of the TT cyclobutane dimer. However, the yield for G[8-5]U is similar as those of other dimeric photoproducts, such as TT (6-4) dimer, CC and CT cyclobutane dimers, which exhibited the yields of 0.245, 0.279, and 0.577 lesions/10^4 bases/(kJ/m^2), respectively (*33*).

The formation of substantial amounts of intrastrand cross-link lesions, together with the fact that the intrastrand cross-link lesions formed between a pyrimidine base and its neighboring purine base may have significant consequences in DNA replication and repair (*22*, *24*−*26*), supports that intrastrand cross-link lesions may play an important role in the photosensitizing effect of ^{Br}dU. In addition, the high yield of the d(G[8-5]U) may render this lesion a suitable biomarker for assessing the efficacy of photosensitization by ^{Br}dU.

SUPPORTING INFORMATION AVAILABLE

HPLC traces for the separation of standard nucleosides and intrastrand cross-link-bearing dinucleoside monophosphates, calibration plots, and LC−MS/MS results. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Zamenhof, S., and Gribiff, G. (1954) E. coli containing 5-bromouracil in its deoxyribonucleic acid, *Nature 174*, 307−308.
2. Darmstadt, R. A., Hinds, H. A., Hudson, P. B., Price, T. D., and Zamenhof, S. (1956) Mechanism of entry of 5-bromouracil and orthophosphate into deoxyribonucleic acid of E. coli, *Nature 178*, 684−686.
3. Szybalski, W., Opara-Kubinska, Z., Lorkiewicz, Z., Ephrati-Elizur, E., and Zamenhof, S. (1960) Transforming activity of deoxyribonucleic acid labelled with 5-bromouracil, *Nature 188*, 743−745.
4. Cadet, J., and Vigny, P. (1990) in *Bioorganic Photochemistry* (Morrison, H., Ed.) pp 1, John Wiley, New York.
5. Kinsella, T. J., Mitchell, J. B., Russo, A., Morstyn, G., and Glatstein, E. (1984) The use of halogenated thymidine analogs as clinical radiosensitizers: rationale, current status, and future prospects: non-hypoxic cell sensitizers, *Int. J. Radiat. Oncol. Biol. Phys. 10*, 1399−1406.
6. Erikson, R. L., and Szybalski, W. (1963) Molecular radiobiology of human cell lines. V. Comparative radiosensitizing properties of 5-halodeoxycytidines and 5-halodeoxyuridines, *Radiat. Res. 20*, 252−262.
7. Mitchell, J. B., Kinsella, T. J., Russo, A., McPherson, S., Rowland, J., Smith, B. H., Kornblith, P. L., and Glatstein, E. (1983) Radiosensitization of hematopoietic precursor cells (CFUc) in glioblastoma patients receiving intermittent intravenous infusions of bromodeoxyuridine (BUdR), *Int. J. Radiat. Oncol. Biol. Phys. 9*, 457−463.
8. Sugiyama, H., Tsutsumi, Y., and Saito, I. (1990) Highly sequence-selective photoreaction of 5-bromouracil-containing deoxyhexanucleotides, *J. Am. Chem. Soc. 112*, 6720−6721.
9. Chen, T., Cook, G. P., Koppisch, A. T., and Greenberg, M. M. (2000) Investigation of the origin of the sequence selectivity for the 5-halo-2′-deoxyuridine sensitization of DNA to damage by UV-irradiation, *J. Am. Chem. Soc. 122*, 3861−3866.
10. Russell, K. J., Rice, G. C., and Brown, J. M. (1986) In vitro and in vivo radiation sensitization by the halogenated pyrimidine 5-chloro-2′-deoxycytidine, *Cancer Res. 46*, 2883−2887.
11. Perez, L. M., Mekras, J. A., Briggle, T. V., and Greer, S. (1984) Marked radiosensitization of cells in culture to X ray by 5-chlorodeoxycytidine coadministered with tetrahydrouridine, and inhibitors of pyrimidine biosynthesis, *Int. J. Radiat. Oncol. Biol. Phys. 10*, 1453−1458.
12. Perez, L. M., and Greer, S. (1986) Sensitization to X ray by 5-chloro-2′-deoxycytidine co-administered with tetrahydrouridine in several mammalian cell lines and studies of 2′-chloro derivatives, *Int. J. Radiat. Oncol. Biol. Phys. 12*, 1523−1527.
13. Santos, O., Perez, L. M., Briggle, T. V., Boothman, D. A., and Greer, S. B. (1990) Radiation, pool size and incorporation studies in mice with 5-chloro-2′-deoxycytidine, *Int. J. Radiat. Oncol. Biol. Phys. 19*, 357−365.
14. Hale, J. T., Bigelow, J. C., Mathews, L. A., and McCormack, J. J. (2002) Analytical and pharmacokinetic studies with 5-chloro-2′-deoxycytidine, *Biochem. Pharmacol. 64*, 1493−1502.
15. Brust, D., Feden, J., Farnsworth, J., Amir, C., Broaddus, W. C., and Valerie, K. (2000) Radiosensitization of rat glioma with bromodeoxycytidine and adenovirus expressing herpes simplex virus-thymidine kinase delivered by slow, rate-controlled positive pressure infusion, *Cancer Gene Ther. 7*, 778−788.
16. Zeng, Y., and Wang, Y. (2004) Facile formation of an intrastrand cross-link lesion between cytosine and guanine upon Pyrex-filtered UV light irradiation of d(^{Br}CG) and duplex DNA containing 5-bromocytosine, *J. Am. Chem. Soc. 126*, 6552−6553.
17. Hong, H., and Wang, Y. (2005) Formation of intrastrand cross-link products between cytosine and adenine from UV irradiation of d(^{Br}CA) and duplex DNA containing a 5-bromocytosine, *J. Am. Chem. Soc. 127*, 13969−13977.
18. Zeng, Y., and Wang, Y. (2006) Sequence-dependent formation of intrastrand crosslink products from the UVB irradiation of duplex DNA containing a 5-bromo-2′-deoxyuridine or 5-bromo-2′-deoxycytidine, *Nucleic Acids Res. 34*, 6521−6529.
19. Cecchini, S., Girouard, S., Huels, M. A., Sanche, L., and Hunting, D. J. (2004) Single-strand-specific radiosensitization of DNA by bromodeoxyuridine, *Radiat. Res. 162*, 604−615.
20. Cecchini, S., Girouard, S., Huels, M. A., Sanche, L., and Hunting, D. J. (2005) Interstrand cross-links: a new type of gamma-ray damage in bromodeoxyuridine-substituted DNA, *Biochemistry 44*, 1932−1940.
21. Cecchini, S., Masson, C., La Madeleine, C., Huels, M. A., Sanche, L., Wagner, J. R., and Hunting, D. J. (2005) Interstrand cross-link induction by UV radiation in bromodeoxyuridine-substituted DNA: Dependence on DNA conformation, *Biochemistry 44*, 16957−16966.

22. Gu, C., and Wang, Y. (2005) Thermodynamic and in-vitro replication studies of an intrastrand crosslink lesion G[8-5]C, *Biochemistry 44*, 8883−8889.
23. Bellon, S., Gasparutto, D., Saint-Pierre, C., and Cadet, J. (2006) Guanine-thymine intrastrand cross-linked lesion containing oligonucleotides: from chemical synthesis to in vitro enzymatic replication, *Org. Biomol. Chem. 4*, 3831−3837.
24. Gu, C., and Wang, Y. (2004) LC-MS/MS identification and yeast polymerase η bypass of a novel γ-irradiation-induced intrastrand cross-link lesion G[8-5]C, *Biochemistry 43*, 6745−6750.
25. Gu, C., Zhang, Q., Yang, Z., Wang, Y., Zou, Y., and Wang, Y. (2006) Recognition and incision of oxidative intrastrand cross-link lesions by UvrABC nuclease, *Biochemistry 45*, 10739−10746.
26. Yang, Z., Colis, L. C., Basu, A. K., and Zou, Y. (2005) Recognition and incision of gamma-radiation-induced cross-linked guanine-thymine tandem lesion G[8,5-Me]T by UvrABC nuclease, *Chem. Res. Toxicol. 18*, 1339−1346.
27. Hong, H., Cao, H., Wang, Y., and Wang, Y. (2006) Identification and quantification of a guanine-thymine intrastrand cross-link lesion induced by Cu(II)/H_2O_2/ascorbate, *Chem. Res. Toxicol. 19*, 614−621.
28. Mitchell, J. B., Russo, A., Cook, J. A., Straus, K. L., and Glatstein, E. (1989) Radiobiology and clinical application of halogenated pyrimidine radiosensitizers, *Int. J. Radiat. Biol. 56*, 827−836.
29. Ling, L. L., and Ward, J. F. (1990) Radiosensitization of Chinese hamster V79 cells by bromodeoxyuridine substitution of thymidine: enhancement of radiation-induced toxicity and DNA strand break production by monofilar and bifilar substitution, *Radiat. Res. 121*, 76−83.
30. Cadet, J., Douki, T., Gasparutto, D., and Ravanat, J.-L. (2003) Oxidative damage to DNA: formation, measurement and biochemical features, *Mutat. Res. 531*, 5−23.
31. Taylor, J.-S. (1995) DNA, sunlight and skin cancer, *Pure Appl. Chem. 67*, 183−190.
32. Cadet, J., Courdavault, S., Ravanat, J.-L., and Douki, T. (2005) UVB and UVA radiation-mediated damage to isolated and cellular DNA, *Pure Appl. Chem. 77*, 947−961.
33. Douki, T., and Cadet, J. (2001) Individual determination of the yield of the main UV-induced dimeric pyrimidine photoproducts in DNA suggests a high mutagenicity of CC photolesions, *Biochemistry 40*, 2495−2501.

BI700431Q