# Investigation of the Origin of the Sequence Selectivity for the 5-Halo-2′-deoxyuridine Sensitization of DNA to Damage by UV-Irradiation

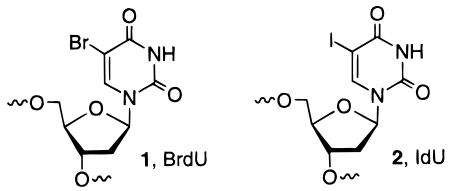
**Tongqian Chen, Gary P. Cook, Andrew T. Koppisch, and Marc M. Greenberg***

*Contribution from the Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523*

*Received December 13, 1999*

**Abstract:** A contrathermodynamic sequence selectivity (5′-deoxyadenosine > 5′-deoxyguanosine) for UV-irradiation-induced strand damage in duplex DNA containing 5-bromo-2′-deoxyuridine was reported several years ago (Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **1990**, *112*, 6720.). In contrast, much smaller sequence selectivity was observed for similar duplexes containing 5-iodo-2′-deoxyuridine. We investigated the mechanism of UV-irradiation-induced cleavage of duplex DNA containing 5-bromo-2′-deoxyuridine (**1**, BrdU) and 5-iodo-2′-deoxyuridine (**2**, IdU) under anaerobic conditions using a variety of structural probes. The preference for UV-induced cleavage in 5′-dABrdU sequences is a confluence of at least three factors, photoinduced forward electron transfer, charge recombination, and electron migration within the DNA duplex. Our results also indicate that UV-irradiation of duplexes (32 nucleotides long) containing 5-iodo-2′-deoxyuridine results in strand scission involving initial photoinduced single electron transfer. The selectivity for 5′-dAIdU sequences is smaller than that in the analogous 5-bromo-2′-deoxyuridine duplexes and may be the result of faster dehalogenation of the initially formed 5-halopyrimidine radical anion and/or competitive direct carbon−iodine bond homolysis.

5-Bromo-2′-deoxyuridine (**1**, BrdU) and 5-iodo-2′-deoxyuridine (**2**, IdU) exhibit a number of interesting and potentially useful chemical properties.[1] For instance, incorporation of these molecules in nucleic acids sensitizes the biopolymers to $\gamma$-radiolysis.[2] In addition, the 5-halopyrimidine nucleosides' sensitivity to UV-irradiation has been exploited in the application of these molecules as structural probes of protein−nucleic acid interactions and nucleic acid structure.[3,4] The enhancement of DNA damage caused by UV-irradiation of duplexes containing **1** and **2** has received considerable attention.[5−8] Approximately 10 years ago Saito and Sugiyama reported that UV-induced strand damage in duplex DNA containing **1** was highly dependent on the identity of the nucleotide bonded to the 5′-phosphate of 5-bromo-2′-deoxyuridine.[5] In contrast to previous studies, it was observed that UV-irradiated duplexes containing the sequence 5′-dABrdU exhibited significantly greater amounts of alkali-labile lesion formation than analogous molecules containing either a 2′-deoxyguanosine (dG) or 2′-deoxycytidine (dC) in place of the adjacent 2′-deoxyadenosine (dA).[9] These researchers postulated a novel mechanism involving initial photoinduced single electron transfer (PSET) from a 2′-deoxyadenosine bonded to the 5′-phosphate of **1** (Scheme 1). Preferential damage observed in 5′-dABrdU (**3**) sequences via PSET compared to those containing a 2′-deoxyguanosine nucleotide bonded to the 5′-phosphate of the halopyrimidine was surprising, given that electron transfer from deoxyguanosine is more favorable thermodynamically.[10] We now wish to report on studies that support this proposal, and demonstrate that the origin of this unexpected sequence selectivity for DNA damage in biopolymers containing BrdU and IdU is the result of several physical properties of the molecules.




The chemistry that occurs following the excitation process which results ultimately in the formation of direct strand breaks

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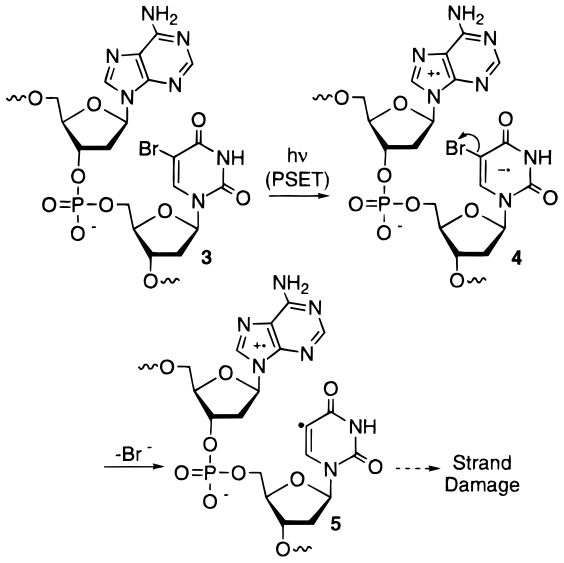
10.1021/ja994357i CCC: $19.00 
Published on Web 04/07/2000

**Scheme 1**




and alkali-labile lesions has been examined.[5−8] The $\sigma$ radical (2′-deoxyuridin-5-yl, **5**) is believed to be formed by loss of bromide from the originally formed radical ion pair (**4**). A series of product studies, kinetic (product) isotope effect experiments, and isotopic labeling studies have implicated **5** as a common reactive intermediate in these processes. Under anaerobic conditions, the $\sigma$ radical (**5**) is believed to damage the 2′-deoxyribonucleotide bonded to its 5′-phosphate by abstracting hydrogen atoms from its C1′- and C2′-positions.[6,7a] The proportionation of the $\sigma$ radical's reactivity is dependent upon the structural status (e.g. single stranded, double stranded, B-form, Z-form) of the oligonucleotide containing the 5-halopyrimidine nucleotide.

Considerably less is known about the mechanism by which the 2′-deoxyuridin-5-yl radical (**3**) is formed in duplex DNA. The enhancement of the efficiency for the UV-induced photodehalogenation of BrdU in biopolymers has been known for more than a quarter of a century.[1] In studies on monomeric 5-bromo-2′-deoxyuridine (**1**), the participation of the $^3$n,$\pi$* state of **1** in PSET processes has been rigorously established.[11] In contrast, 5-iodo-2′-deoxyuridine (**2**) has been shown to undergo photoreduction via direct carbon−iodine bond homolysis from its singlet excited state.[12] These studies are consistent with those carried out on hexameric oligonucleotide duplexes containing **2**.[7b,13] Oligonucleotides of this length containing **2** exhibit enhanced sensitivity to damage initiated by UV-irradiation, but in contrast to similar BrdU-containing substrates, they showed little sequence selectivity, suggesting that carbon−iodine bond homolysis occurs directly from an excited state of IdU.[14]

The PSET decomposition of 5-bromo-2′-deoxyuridine (**1**) in oligonucleotides has not been fully explored. Fluorescence emission studies on duplexes containing **1** were interpreted to support preferential interaction between the adjacent nucleotides in a 5′-dABrdU sequence in the excited state compared to an oligodeoxyribonucleotide containing the sequence 5′-dGBrdU.[7c] However, it is not apparent why emission is observed in

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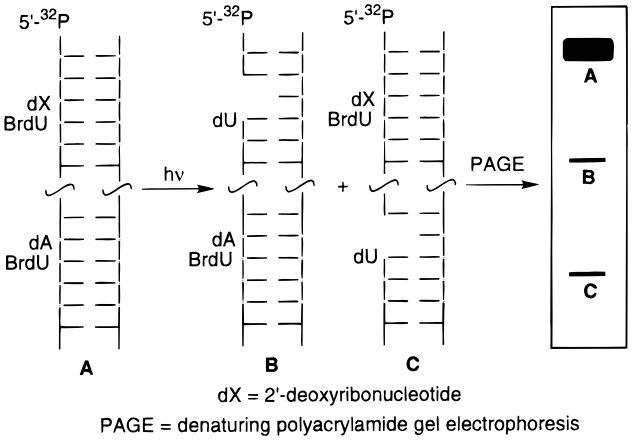
(13) Saito, I. *Pure Appl. Chem.* **1992**, *64*, 1305.

(14) A recent report suggests that 5-iodo-2′-deoxyuridine may participate in PSET processes in the involved halide ions: Kawai, K.; Saito, I.; Sugiyama, H. *Tetrahedron Lett.* **1999**, *40*, 5721.

**Scheme 2**




dX = 2′-deoxyribonucleotide

PAGE = denaturing polyacrylamide gel electrophoresis

oligonucleotides containing 5′-dABrdU and not 5′-dGBrdU sequences. In the original report of this selective photoreduction and accompanying strand damage, it was indicated that there could be several possible explanations as to why electron transfer from 2′-deoxyadenosine to **1** could be more effective than the thermodynamically more favorable transfer of an electron from 2′-deoxyguanosine to **1**.[5] The physical factors in support of this sequence selectivity include differences in back electron-transfer rates in the respective ion pairs and photoexcitation of 2′-deoxyadenosine instead of **1**. We probed the effects of these, as well as other, physical parameters on the formation of direct strand breaks and have determined that there are competing processes which collectively result in preferential UV-irradiation-induced damage of duplex DNA containing 5′-dABrdU sequences. In addition, we observed a similar, but more modest sequence selectivity for UV-irradiation damage in duplexes containing 5-iodo-2′-deoxyuridine (**2**). Using the mechanistic probes employed for studying UV-induced strand damage in BrdU-containing duplexes, we propose that **2** also participates in PSET upon irradiation.

## Results and Discussion

Quantitative determination of direct DNA strand scission was used as a means for probing the source of the sequence selectivity observed upon UV-irradiation of duplexes containing BrdU or IdU (Scheme 2). Each duplex (32 base pairs long) contained two of the identical halopyrimidines separated by 10 bp, which were positioned 10−12 bp from the respective 5′- or 3′-termini.[6] In probing the effects of a given variable on the cleavage selectivity, a 5′-dABrdU site was employed in all duplexes as an internal standard. Experiments were carried out on duplexes in which the halopyrimidine-containing oligonucleotide was enzymatically labeled at its 5′-terminus with $^{32}$P. UV-irradiation experiments were carried out to less than 5% total cleavage to maximize the probability that only a single cleavage event occurred per duplex ("single hit kinetics"). In a typical experiment, strand scission in a given duplex was determined for 4 to 5 samples. All oligonucleotides were synthesized via automated DNA synthesis and those containing **1** or **2** were deprotected using concentrated aqueous ammonia at room temperature for 48 h to prevent decomposition of the 5-halopyrimidine nucleotides. 2-Amino-2′-deoxyadenosine was incorporated using a commercially available phosphoramidite containing *N*,*N*′-diisobutylformamidine protecting groups which are cleaved under the above deprotection conditions.[15]

(15) The phosphoramidite is commercially available from Glen Research, Sterling, VA.

**Table 1.** Sequence Selectivity for UV-Irradition-Induced Direct Strand Scission in Oligonucleotide Duplexes Containing Two 5-Bromo-2′-deoxyuridine Sites

11 21
5′-d(CGCATATGGCX1GCTATAGCA1GCCGCATATG)
d(GCGTATACCGYACGATATCGTACGGCGTATAC)
6-11

| | | cleavage ratio: $A_{21}/X_{11}$ | |
|---|---|---|---|
| duplex | X·Y | observed[a] | normalized[b] |
| **6** | A·T | 1.7 ± 0.1 | |
| **7** | G·C | 36.4 ± 1.6 | 21.4 |
| **8** | C·G | 5.8 ± 0.1 | 3.6 |
| **9** | U·A | 2.4 ± 0.2 | 1.4 |
| **10** | I·C | 4.3 ± 1.4 | 2.5 |
| **11** | AA·T | 4.7 ± 0.9 | 2.8 |

[a] The observed value is the average of two or more experiments, each consisting of 4 or 5 samples $\pm\sigma_{n-1}$ of these values. [b] Observed cleavage ratio is divided by the observed cleavage ratio in **6**.

The original strong preference for cleavage at 5′-dABrdU versus 5′-dGBrdU sequences (**7**) was confirmed using a series of duplexes designed as described above (Table 1).[5,6] Preferential cleavage at 2′-deoxyadenosine sites compared to those containing a 2′-deoxycytidine stacked above BrdU (**8**) was not as great as originally reported, but still substantial.[5] No selectivity for cleavage at dA compared to dG was observed in the respective single strand oligonucleotide substrates. Subsequently, we examined the effects of single nucleotide substitutions on cleavage selectivity to investigate the origin of this preference for BrdU's sensitization of duplex DNA to UV-irradiation.

**Does Duplex Structure Determine Sequence Selectivity?** Various studies on DNA suggest that base pairing schemes affect duplex structure.[16] One model of DNA bending attributes this macroscopic deformation to the junctions between disimilar base pairs.[17] Consequently, we investigated the possibility that the preferential cleavage at 5′-dABrdU sequences compared to 5′-dGBrdU sequences was due solely to better base stacking and/or overall shape in the former, which lead to more efficient electron transfer from 2′-deoxyadenosine to 5-bromo-2′-deoxyuridine. 2′-Deoxyinosine·2′-deoxycytidine (dI·dC) and 2-amino-2′-deoxyadenosine·thymidine (dAA·T) base pairs have been employed as probes of duplex DNA structure.[16,17] Consequently, these nucleotides were substituted for the 2′-deoxyadenosine·thymidine and 2′-deoxyguanosine·2′-deoxycytidine base pairs (Scheme 3) respectively at position 11 in duplexes **10** and **11**.[18] Substitution of 2-amino-2′-deoxyadenosine (diaminopurine) for 2′-deoxyadenosine is known to widen the minor groove and change the hydrophilicity of the minor groove, much as 2′-deoxyguanosine does.[16a] If base stacking and/or groove width were the controlling factors in the observed sequence preference for strand scission, then the ratio of strand scission at 5′-dIBrdU and 5′-dABrdU sequences should have been approximately equal, as should those for 5′-dAABrdU and 5′-dGBrdU.[18] After correcting for the natural bias for cleavage at the nucleotide at position 21 in the BrdU-containing oligonucleotide template sequence (**6**), there was only a slight preference for cleavage at the 5′-dABrdU site compared to the duplex containing a 2′-deoxyinosine nucleotide (Table 1). However, cleavage at the 2-amino-2′-deoxyadenosine site was still significantly greater (7.6) compared to the sequence 5′-dGBrdU, suggesting that base stacking effects are not the sole determinant for sequence selectivity.

**Scheme 3**

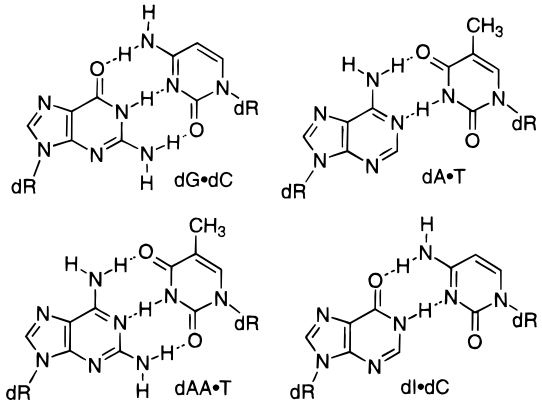



**Table 2.** Prediction of $\Delta\Delta G°$ for Photoinduced Single Electron Transfer from Various 2′-Deoxynucleotides to 5-Bromo-2′-deoxyuridine Compared to 2′-Deoxyadenosine Using the Rehm−Weller Equation

| 2′-dN | $E_S$ (kcal/mol)[a] | $E^{ox}$ (V)[b] | $\Delta\Delta G°$ (kcal/mol) |
|---|---|---|---|
| A | 101 | 1.42 | |
| G | 96.5 | 1.29 | 1.5 |
| U | 100 | 1.7[c] | 7.5 |
| C | 97.2 | 1.6 | 8.0 |

[a] Excited-state energies are for nucleosides. Taken from ref 1c. [b] $E_{Ox}$ are for nucleosides. Taken from ref 20. [c] The $E_{Ox}$ is estimated from that for thymidine. Based upon comparisons of $E_{Ox}$ of the free bases, this should represent a reasonable approximation of the $E_{Ox}$ for 2′-deoxyuridine (see ref 18).

**Does the Rate of Forward Electron-Transfer from an Excited Deoxyribonucleotide Adjacent to 5-Bromo-2′-deoxyuridine Determine Sequence Selectivity?** In their original report Saito and Sugiyama acknowledge that preferential strand damage may occur at duplexes containing 5′-dABrdU sequences due to more favorable forward electron transfer from the singlet excited state of this purine deoxyribonucleotide compared to that of 2′-deoxyguanosine.[5] Recently reported fluorescence experiments are consistent with this scenario.[7c] If forward electron transfer is the sole determining factor for the efficiency of UV-induced strand damage in oligonucleotides containing 5-bromo-2′-deoxyuridine (**1**), then application of the Rehm−Weller equation leads to the prediction that the order of sequence selectivity should be dA > dG > dU > dC (Table 2).[19] One should note that the $\Delta\Delta G°$ values calculated utilized excitation energies and oxidation potentials for the respective nucleosides. Hence, we would be remiss not to point out that the absolute energies and possibly even the order could be different if the respective data were available for the molecules within duplex DNA. In addition, the encounter distance was assumed to be the same for each dinucleotide. Given these caveats, we note

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(18) The oxidation potentials of 2′-deoxyinosine (dI) and 2′-deoxy-2,6-diaminopurine (dAA) at pH 7 are not known. However, extrapolation of $E_{ox}$ measurements for the respective nucleobases suggests that electron transfer should be slightly more favorable thermodynamically from dAA than from dG. Electron transfer from dI should be more favorable thermodynamically than from dA. $E_{ox}$ (V versus NHE): adenine, 1.32; guanine, 1.04; hypoxanthine, 1.16; cytosine, 1.44; uracil, 1.34; thymine, 1.29 (Faraggi, M.; Broitman, F.; Trent, J. B.; Klapper, M. H. *J. Phys. Chem.* **1996**, *100*, 14751.). $E_{ox}$ (V versus NHE) in an independently determined set of measurements: adenine, 1.24; guanine, 0.96; 2,6-diaminopurine, 0.89 (Yao, T.; Musha, S. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 2307.).

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that with the exception of 2′-deoxyguanosine, which is the least efficient nucleotide at enhancing strand damage, the Rehm−Weller equation does predict the correct order for sequence preference (Table 1). However, because it incorrectly predicts the effectiveness of 2′-deoxyguanosine at assisting in the sensitization of DNA to UV-irradiation, this physical phenomenon also cannot be the sole determinant for the observed sequence selectivity.

**Does the Rate of Back Electron Transfer Determine Sequence Selectivity?** The preferential strand damage in oligonucleotide duplexes containing 5′-dABrdU sequences compared to analogous sequences containing deoxyguanosines adjacent to **1** was surprising because electron transfer involving the latter is more favorable thermodynamically.[10] Less efficient strand damage involving deoxyguanosine could result from faster back electron transfer in ion pairs containing this nucleotide. Faster back electron transfer from $dG^{+\bullet}$ $BrdU^{-\bullet}$ ion pairs compared to the respective ion pairs containing $dA^{+\bullet}$ would occur if charge recombination lies in the Marcus-inverted region. This possibility is supported by recent time-resolved experiments involving ion pairs between deoxyadenosine/deoxyguanosine and a nonnucleotidyl electron acceptor.[21] More favorable back electron transfer in radical ion pairs obtained from 2′-deoxyguanosine is consistent with selective cleavage at 5′-dABrdU sites. However, if charge recombination were the sole determining factor in DNA strand damage resulting from UV-irradiation of duplex DNA containing **1**, then the pyrimidine nucleotides should provide the most effective 5′-nucleotide partners, and this was not observed.[20]

**Does Hole Migration Affect DNA Strand Scission?** A number of reports have appeared recently in which it has been shown that electrons are capable of migrating over long distances in duplex DNA.[22−26] Some of these studies have taken advantage of the demonstration that sequences containing contiguous 2′-deoxyguanosines have lower ionization potentials than other DNA sequences of comparable length.[10] In addition, analogous sequences containing a 7,8-dihydro-8-oxo-2′-deoxyguanosine (oxoG) provide an even more favorable driving force for hole migration and/or oxidation.[10d,25,27] The effects of contiguous guanines and oxoG on electron transfer are so broadly agreed upon, that we believed that it could be used as a mechanistic probe in our system as a marker for electron transfer.

Evidence in favor of hole migration, and therefore electron transfer in DNA strand damage produced upon UV-irradiation of duplexes containing BrdU, can be gleaned from observation of preferential strand damage at the 2′-deoxyadenosine distal ($A_{21}$) from the 5′-dGG sequence in **6** (Tables 1 and 3). Support that this almost 1.7:1 preference reflects electron hole migration from the initially oxidized 2′-deoxyadenosine(s) was extracted from the observation that replacement of the 5′-dGG sequence

**Table 3.** Effect of Remote Sequence on UV-Irradiation-Induced Cleavage in Oligonucleotide Duplexes Containing Two 5′-dABrdU Sites


| duplex | W·V | X·Y | obsd cleavage ratio:[a] $A_{21}/X_{11}$ |
|---|---|---|---|
| **6** | G·C | A·T | 1.7 ± 0.1 |
| **12** | A·T | A·T | 1.0 ± 0.04 |
| **8** | G·C | C·G | 5.8 ± 0.1 |
| **13** | A·T | C·G | 5.3 ± 0.6 |

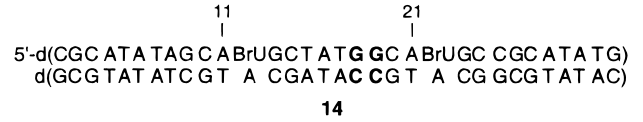
[a] The observed value is the average of two or more experiments, each consisting of 4 or 5 samples $\pm\sigma_{n-1}$ of these values.

**Table 4.** Effect of 5′-dGG and 5′-dGoxoG Sequences on UV-Irradiation-Induced Cleavage in Oligonucleotide Duplexes Containing 5-Halopyrimidines


| duplex | X | Y | obsd cleavage ratio:[a] $A_{22}/A_{12}$ |
|---|---|---|---|
| **15** | BrdU (**1**) | G | 1.5 ± 0.2 |
| **16** | BrdU (**1**) | oxoG | 2.6 ± 0.1 |
| **17** | IdU (**2**) | G | 2.1 ± 0.2 |
| **18** | IdU (**2**) | oxoG | 3.7 ± 0.2 |

[a] The observed value is the average of two or more experiments, each consisting of 4 or 5 samples $\pm\sigma_{n-1}$ of these values.

two nucleotides removed from the 5′-dABrdU sequence in **6** with a 5′-dAG sequence results in no preference for strand damage at either BrdU site in **12** (Table 3). Negligible selectivity was observed in **14**, indicating that the hole migration was bi-




directional.[2c,23c] In addition, the influence of the remote 5′-dGG sequence on UV-irradiation-induced strand scission selectivity was probed using duplexes containing 5′-dCBrdU sites (Table 3). The reduction in preference for cleavage at $dA_{21}$ in **13** was qualitatively consistent with the involvement of hole migration in the DNA damage process.

Further support for electron transfer followed by hole migration was obtained using 8-oxo-2′-deoxyguanosine (oxoG) as a probe for such a process. To make internal comparisons and to facilitate oligonucleotide synthesis, duplexes containing 5′-dGG and 5′-dGoxoG sites were incorporated into the complementary stand of the oligonucleotide containing the two BrdU nucleotides which were separated by 10 base pairs (Table 4). As expected, strand scission occurred preferentially in **15** at the deoxyadenosine distal ($A_{22}$) to the 5′-dGG sequence. The selectivity (1.5 ± 0.2) was slightly lower than that observed in **6**. This slight differential in selectivity could be due to a number of physical phenomena, but one possibility could be the presence of an intervening dA·T base pair between the 5′-$dA_{12}$BrdU and 5′-dGG sequences. Such base pairs have been proposed to be less efficient conductors of electrons in duplex DNA, and this hypothesis is supported by measurement of electron-transfer rates in dinucleotides.[23a,28] Incorporation of a 5′-dGoxoG

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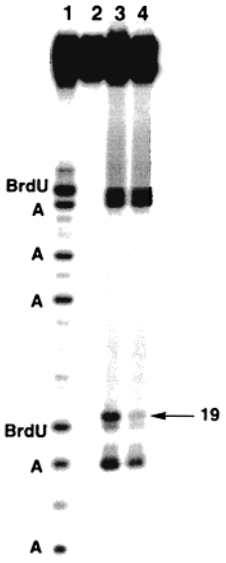
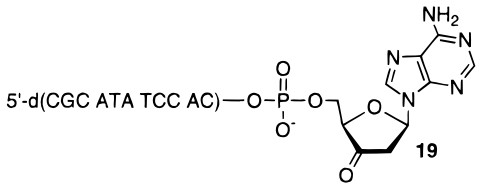



**Figure 1.** Autoradiogram of the effect of the presence of 5′-dGoxoG in the complementary strand of a duplex (**16**) containing 5-bromo-2′-deoxyuridine on direct strand scission incurred upon UV-irradiation. The strand containing **1** in **15** (lanes 1−3) and **16** (lane 4) is labeled with $^{32}$P at its 5′-terminus. Lane 1, 2′-deoxyadenosine selective sequencing reaction; Lane 2, unphotolyzed; Lane 3, **15** photolyzed for 10 min; Lane 4, **16** photolyzed for 10 min.

sequence (**16**) in the complementary strand also had a significant effect on UV-induced strand scission (Table 4). Despite being incorporated in its less than optimal orientation (5′-doxoGG is more readily oxidized than 5′-dGoxoG),[10d] preference for cleavage at the deoxyadenosine ($A_{22}$) remote to the 5′-dGoxoG sequence (**16**, Table 4) was increased to 2.6 from that measured in the analogous duplex containing the sequence 5′-dGG (1.5, **15**, Table 4). In addition, cleavage studies on **16** yielded mechanistic information beyond the effects of oxoG on sequence selectivity. The overall efficiency for strand scission in **16** was reduced to almost one-half of that in the duplex containing a 5′-dGG sequence (**15**). The reduction in strand scission is consistent with previous studies on the mechanism of strand scission following the PSET process, which indicate that direct strand breaks are formed more efficiently when hydrogen atom abstraction occurs from the one-electron oxidized 2′-deoxyadenosine.[6a] Furthermore, the presence of oxoG in **16** had a significant impact on the amount of strand scission product containing the labile 3′-keto-2′-deoxyadenosine at its 3′-terminus (**19**) analogous to **4** in Scheme 2 (Figure 1). Hole migration




(including from the distal site) to the oxoG nucleotide prior to hydrogen atom abstraction by the 2′-deoxyuridin-5-yl radical should result in a reduction in the amount of ketone product and direct strand breaks overall. These results clearly demonstrate that the sequence of a duplex remote from the site of cleavage affects the overall efficiency of the strand scission process. This long-range effect is most readily explained by a PSET process, followed by hole migration.

**Duplex DNA Sequence As a Probe for the Mechanism of UV-Induced Strand Damage in 5-Iodo-2′-deoxyuridine-Containing Oligonucleotides.** Using the experiments involving 5-bromo-2′-deoxyuridine described above as precedent, we explored whether the oligonucleotides containing 5-iodo-2′-deoxyuridine enhanced DNA damage upon UV-irradiation via a PSET process. Using **6** and **7** as templates on which to base comparisons, we prepared the analogous IdU-containing duplexes (**20**, **21**). Although the selectivity was less in each case when compared to the respective BrdU containing duplex (**20** versus **6** and **21** versus **7**), a preference for cleavage at 5′-dAIdU sequences over 5′-dGIdU was clearly observed (Table 5). Furthermore, there was a modest preference for cleavage at 5′-dAIdU sites remote from contiguous 2′-deoxyguanosines (**20**, Table 5). Additional support for direct strand scission resulting from PSET upon UV-irradiation of duplex DNA containing IdU was gleaned from experiments involving duplexes containing oxoG in the complementary strand (Table 4). Selectivity for cleavage at a 5′-dAIdU site remote from the 5′-dGoxoG sequence (**18**) was increased approximately 2-fold compared to the respective duplex containing a 5′-dGG sequence (**17**) in the complementary strand. We believe that these results indicate that at least some of the direct strand breaks produced upon UV-irradiation of the DNA duplexes containing 5-iodo-2′-deoxyuridine examined in this study are initiated by PSET.

**Table 5.** Sequence Selectivity for UV-Irradition-Induced Direct Strand Scission in Oligonucleotide Duplexes Containing Two 5-Iodo-2′-deoxyuridine Sites

11 21
5′-d(CGC ATA TGG C**X** 2GCT ATA GC A 2GC CGC ATA TG)
d(GCG TAT ACC G **Y** ACGA TAT CG T ACG GCG TAT AC)
**20,21**

| | | cleavage ratio: $A_{21}/X_{11}$ | |
|---|---|---|---|
| duplex | X·Y | observed[a] | normalized[b] |
| **20** | A·T | 1.2 ± 0.03 | - |
| **21** | G·C | 6.1 ± 0.1 | 5.1 |

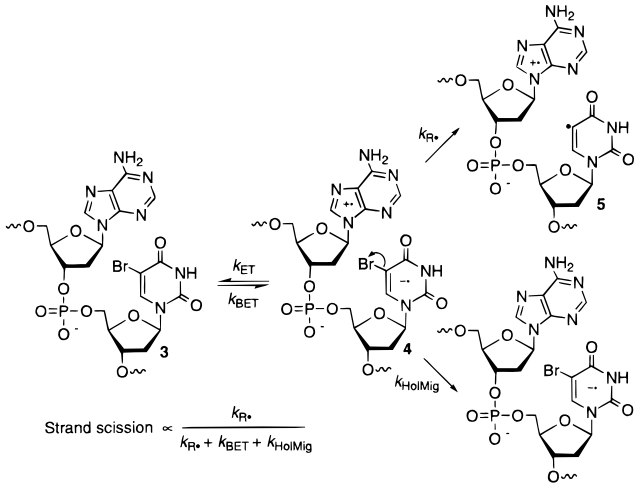
[a] The observed value is the average of two or more experiments, each consisting of 4 or 5 samples $\pm\sigma_{n-1}$ of these values. [b] Observed cleavage ratio is divided by the observed cleavage ratio in **20**.

## Summary

The product studies (where the product is defined as DNA strand scission) described above support the original proposal that the sensitization of DNA containing 5-bromo-2′-deoxyuridine to UV-irradiation is initiated via a photoinduced single electron transfer (PSET) process.[5,6a] Experiments designed to identify the physical phenomena responsible for the contrathermodynamic preference for direct strand scission from 5′-dABrdU sequences compared to 5′-dGBrdU sequences indicate that several factors including forward and back electron transfer rates, hole migration, and possibly even base stacking play a role in strand scission resulting from UV-irradiation.

Forward electron transfer and charge recombination are expected to affect the efficiency of strand scission, and could explain why stacking 2′-deoxyadenosine above the 5-halopyrimidines results in more efficient DNA strand scission upon UV-irradiation compared to sequences containing 2′-deoxyguanosine. However, these two chemical events are insufficient to explain the preference for strand scission of 5′-dABrdU sequences compared to those containing pyrimidine nucleotides bonded to the 5′-phosphate of **1**. This issue was addressed by experiments utilizing contiguous 2′-deoxyguanosines and 7,8-dihydro-8-oxo-2′-deoxyguanosines as markers, which clearly show that hole migration also plays a role in the efficiency of strand scission produced upon UV-irradiation. Hole migration

**Scheme 4**




should be more rapid from radical ion pairs produced from 5′-pyrimidine-containing BrdU sequences than from 5′-dABrdU or 5′-dGBrdU sequences. Hence, while charge recombination in the radical ion pairs formed in 5′-pyrimidineBrdU sequences should be slower than in those formed from 5′-dABrdU sequences, the expected more rapid hole migration (based upon thermodynamic considerations) in duplexes containing a pyrimidine bonded to the 5′-phosphate of BrdU may result in less efficient overall strand scission resulting from UV-irradiation. We suggest that the more efficient strand scission produced in DNA duplexes containing 5′-dABrdU sequences compared to sequences containing other nucleotides bonded to the 5′-phosphate of 5-bromo-2′-deoxyuridine is the result of a balance between these three chemical processes (Scheme 4).

These experiments also suggest that strand scission produced upon UV-irradiation of the duplexes studied here which contain 5-iodo-2′-deoxyuridine is affected by the same chemical processes, albeit to a lesser extent, as evidenced by the smaller preference for cleavage at 5′-dAIdU versus 5′-dGIdU sites in **21**. The smaller sequence selectivity in IdU-containing oligonucleotides compared to those containing BrdU may be the result of one or more independent parameters, including faster dehalogenation of the IdU anion radical and competing direct bond homolysis.[7b,12,29] The former would decrease the consequences of differences in rates of charge recombination in the respective radical ion pairs. The latter would provide an explanation as to why UV-irradiation of the IdU-containing duplexes studied here supports a PSET mechanism, but earlier reports employing shorter oligonucleotides are less definitive.[7b] We suggest that in the shorter oligonucleotides, a greater proportion of the photochemical events result from direct excitation of the IdU molecule. Direct carbon−iodine bond homolysis would result in a manner that is independent of sequence. In comparison to BrdU-containing oligonucleotides of like length, this is consistent with the longer wavelength absorption of the IdU molecule.[12] Direct homolysis accounts for a smaller amount of UV-irradiation induced strand scission in the longer oligonucleotides studied here, because the IdU-molecule absorbs a proportionally smaller amount of the incident photons.

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## Experimental Section

**General Procedures.** Oligonucleotides were synthesized on an Applied Biosystems Inc. 380B DNA synthesizer using standard protocols. All $H_2O$ used was obtained from a Nanopure Barnstead still. DNA synthesis reagents were obtained from Glen Research Inc. Oligonucleotides containing BrdU (**1**) and IdU (**2**) were deprotected in 28% $NH_4OH$ at room temperature for 48 h. All other oligonucleotides were deprotected at 55 °C overnight. Oligonucleotides were purified and analyzed via 20% denaturing polyacrylamide gel electrophoresis (PAGE). Oligonucleotides were radiolabeled and subsequently handled using standard protocols.[30] Oligonucleotides were sequenced using a reaction specific for adenine.[31] Radionuclides were obtained from Amersham. T4 polynucleotide kinase was obtained from New England Biolabs. Radioactive samples were counted via Cerenkov counting, using a Packard Tri-Carb 1500 scintillation counter. Electrospray mass spectrometry was carried out on a VG Fisons Quattro. Samples were prepared by precipitating from $NH_4OAc$.[32] Photolyses at 302 nm were carried out in Pyrex tubes using a UV Photoproducts dual wavelength transilluminator containing six 8 W lamps. Photolysis tubes were positioned 8 cm from the source and were irradiated for 10 min (2.9 mW/cm$^2$). Photon flux was measured using a UVX Radiometer from UV Photoproducts Inc. Phosphorimaging analysis was carried out using a Molecular Dynamics Phosphorimager equipped with Imagequant software (Version 3.3).

**Standard Photolysis Conditions.** Photolyses were carried out in Pyrex tubes containing 50 $\mu$L of a 10 mM phosphate (pH 7.4), 10 mM NaCl buffered solution of DNA. All photolyses were carried out in sealed tubes that were degassed by three freeze, pump (3 min), thaw cycles, prior to flame sealing under dynamic vacuum. The photolyzate was transferred to autoclaved eppendorf tubes (0.6 mL) using a plastic draw bulb pipet. Each photolysis tube was washed with $H_2O$ (50 $\mu$L). The photolyzate was precipitated by adding 3 N NaOAc (pH 5.2, 20 $\mu$L), calf thymus DNA (1.3 mM in base pairs, 5 $\mu$L), and EtOH (400 $\mu$L). The samples were frozen in a dry ice−EtOH bath for 12 min, followed by centrifugation at 14 000 rpm for 12 min. The supernatant was carefully removed by pipet, and the remaining DNA pellet was dried in a speed-vac. The dried DNA pellet was resuspended in formamide loading buffer (8 $\mu$L) by heating at 55 °C for 20 min. The resuspended DNA was quantitated by scintillation counting. Denaturing 20% PAGE analysis was carried out using 30 000 cpm from each photolysis.

**Acknowledgment.** This research was supported by the National Institutes of Health (GM-54996). M.M.G. is grateful for an Alfred P. Sloan Foundation fellowship. Mass spectra were obtained on instruments supported by the NIH shared instrumentation grant GM-49631.

**Supporting Information Available:** Tabulated data of cleavage ratios in individual reactions for all 5-halopyrimidine-containing duplexes; calculation of $\Delta\Delta G°$ for PSET from various 2′-deoxyribonucleotides to **1** compared to 2′-deoxyadenosine (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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