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Molecular Mechanism of the DNA Sequence Selectivity of 5-Halo-2′-Deoxyuridines as Potential Radiosensitizers

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Abstract: The 5-halo-2′-deoxyuridines bromodeoxyuridine (BrdU) and iododeoxyuridine (IdU) are well-known photosensitizers for inducing DNA/RNA−protein cross-linking and potential radiosensitizers for radiotherapy of cancer. The dependence of the photosensitivity of BrdU and IdU on the DNA sequence has been well-observed, but it is unknown whether there is a similar DNA sequence selectivity in their radiosensitivity. Here we show a new ultrafast electron transfer (UET) mechanism for the likely DNA sequence dependence of the radiosensitivity of BrdU and IdU. Our femtosecond time-resolved transient laser absorption spectroscopic measurements provide the first real-time observation of the UET reactions of BrdU/IdU with the anion states of adenine and guanine. It is shown that the UET between BrdU and dA^{*-} (dA^-) is more effective than that between BrdU and dG^{*-}. This is related to the recent observation that dG^{*-} is highly destructive while dA^- is long-lived. This mechanistic understanding may lead to the improvement of BrdU and IdU to achieve sufficient radiosensitizing efficacy and the development of more effective radiosensitizers for clinical uses.

Real-time observation of molecular reactions is of great interest in the studies of chemical and biological systems.[1,2] Ultrafast electron transfer (UET) underlies many chemical, biological, and environmental reactions.[3−7] Applications of ultrafast laser techniques to address biological processes with close relevance to diseases and their treatments may lead to a new transdisciplinary frontier called *femtomedicine*, which, for instance, holds the promise of advances in cancer therapy.[3] Here we show a new UET mechanism for the DNA sequence selectivity of halopyrimidines as potential radiosensitizers for cancer therapy.

Since replacement of thymidine in DNA by 5-bromouracil (BrU) or 5-iodoracil (IU) has long been known to enhance DNA damage and cell death induced by ionizing radiolysis[8,9] and UV photolysis,[10−12] 5-halo-2′-deoxyuridines, especially bromodeoxyuridine (BrdU) and iododeoxyuridine (IdU), have been explored as potential sensitizers for radiotherapy of cancer. In addition, BrdU and IdU can be used as photosensitizers to induce DNA/RNA−protein cross-linking and as probes of protein−nucleic acid interactions.[13] Because of their biological importance, BrdU (BrU) and IdU (IU) have been intensely studied.[8−17] For radiosensitization of BrU (IU)-incorporated DNA, the key initial step was once thought to be the transfer of a *hydrated electron* (e^-_{hyd}) generated from the radiolysis of water to BrU, which then dissociates: $e^-_{hyd} + BrU \rightarrow BrU^{*-} \rightarrow Br^- + U^{\bullet}$.[15] The resultant uracilyl radical $U^{\bullet}$ then attacks DNA, causing DNA strand cleavage. However, using femtosecond time-resolved laser spectroscopy (fs-TRLS), we observed that the $U^{\bullet}$ radical results from the UET reaction of CldU/BrdU/IdU with a precursor electron (e^-_{pre}) weakly bound at about −1.5 eV rather than with a long-lived e^-_{hyd} well-bound at −3.2 eV in the aqueous phase.[17]

One of the most important properties of BrdU/IdU is the dependence of its photosensitivity on the DNA sequence. In the 1990s, Saito, Sugiyama, and co-workers[10] observed that duplex DNA containing the sequence 5′-dABrdU exhibits a significantly larger amount of UV-induced strand damage than analogous duplexes containing either dG, dT, or dC. They postulated a mechanism involving photoinduced single-electron transfer (PSET) from neutral dA to BrdU. The preferential damage observed in 5′-dABrdU sequences via PSET relative to those containing the 5′-dGBrdU was puzzling since electron transfer from dG is more favorable thermodynamically. To account for this, Greenberg and co-workers[11] proposed that the contrathermodynamic sequence selectivity results from the confluence of at least three factors: photoinduced forward electron transfer, charge recombination, and electron migration within the DNA duplex. More recently, Sugiyama and co-workers[12] further showed efficient enhancements in photoinduced damage of BrU-substituted DNA at 5′-(G/C)AABrUBrU-3′ and 5′-(G/C)ABrU-BrU-3′ sequences under UV irradiation at 302 nm, and they proposed that the A/T base pair(s) plays a role as a bridge for the charge transfer between the electron-donating G/C base pair and the 5′-BrUT- 3′ sequence as an acceptor. In those experiments, however, it was not clear whether the prehydrated electrons were produced from two-photon ionization of water, which might occur with the UV light power used (several mW),[10−12] and subsequently captured by DNA bases. Recently, Bowen and co-workers[18] observed the stable anionic state A^- (as well as C^- and T^-) in their photoelectron spectroscopic studies of DNA bases. Using fs-TRLS, we directly observed the dissociative electron transfer (DET) reactions leading to bond dissociations of G and T and the formation of all four stable anions (A^-, G^-, C^-, and T^-) in aqueous nucleotides under UV irradiation.[19] We found that among the four DNA bases, the weakly bound e^-_{pre} (<0 eV) can be most effectively trapped at adenine to form the stable anion A^-, while G is most vulnerable to dissociative capture of e^-_{pre}, leading to bond breakage. These results have provided a molecular mechanism for radiation-induced damage to DNA in an aqueous environment.[20] Since e^-_{pre} is a major species produced by ionizing radiation of biological systems,[3] it is important to know whether the UET reaction involving e^-_{pre} leads to a radiosensitivity dependence of BrdU/IdU on DNA sequence.

In this study, we employed fs-TRLS to demonstrate the likely DNA sequence dependence of the radiosensitivity of BrdU and IdU. We report direct observations of UET from nucleotide anions $dAMP^{*-}$/$dGMP^{*-}$ to BrdU/IdU that leads to the forma-

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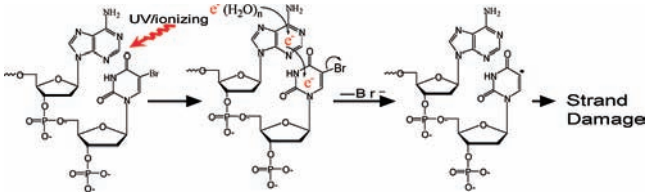
Scheme 1. Ultrafast Electron Transfer (UET) to BrdU/IdU from a Long-Lived Anion A^- Formed by Capture of a Prehydrated Electron Generated by Radiolysis of Water; The Resultant Transient Anion $BrdU^{*-}/IdU^{*-}$ Dissociates To Produce the Uracilyl Radical, Which Causes DNA Damage



tion of the transient anion $BrdU^{*-}/IdU^{*-}$, which dissociates to produce the reactive uracilyl radical. As shown in Scheme 1, this UET mechanism can well explain the sequence selectivity.

The standard methodology for real-time fs-TRLS transient absorption measurements of weakly bound prehydrated electrons e^-_{pre} produced by two-photon excitation of water and of the intermediate state $BrdU^{*-}/IdU^{*-}$ ($dXMP^{*-}$) formed by UET of e^-_{pre} to BrdU/IdU (dXMP) has been described previously.[17,19] In the present experiments, we measured the formation of $BrdU^{*-}/IdU^{*-}$ from UET of e^-_{pre} or a nucleotide anion $dXMP^{*-}$ (X = A, G) in BrdU/IdU only and BrdU/IdU−dXMP complexes. A pump beam (120 fs, 40 μW) at 322 nm was focused to a diameter of ∼0.5 mm to produce prehydrated electrons in water, while a probe beam at 333 nm detected the formation and dissociation of $BrdU^{*-}/IdU^{*-}$ directly.[17] Free BrdU/IdU molecules may exist in BrdU/IdU + dXMP mixtures, depending on the molecular ratio of dXMP to BrdU/IdU, but only UET in formed BrdU/IdU−dXMP heterodimers can be measured in picosecond dynamics because ET reactions from diffusive free-molecule encounters would need much longer time scales (μs). Since the bases A and G are much more efficient at capturing e^-_{pre} than C and T[19] and are frequently responsible for the sequence selectivity of BrdU/IdU, only the results on the effect of dAMP and dGMP are shown in order to demonstrate the UET mechanism.

The kinetic traces of $BrdU^{*-}$ formed from BrdU only and from BrdU−dXMP complexes are shown in Figure 1. To explain the results, we need to mention the following processes. In the present experiments, the first step was to produce weakly bound e^-_{pre} in aqueous BrdU−dAMP/dGMP complexes (eq 1a). We previously demonstrated the direct UET between BrdU and e^-_{pre}, leading to the formation of $BrdU^{*-}$ (eq 1b);[17] e^-_{pre} can also be effectively transferred to dAMP or dGMP to form $dAMP^{*-}$or $dGMP^{*-}$ (eq 1c).[19] However, most of the formed $dGMP^{*-}$ quickly dissociates within the first 5 ps after its formation, while $dAMP^{*-}$ does not dissociate but instead forms a long-lived anion, $dAMP^-$, that exhibits a flat kinetic trace.[19] Thus, the ET between $dGMP^{*-}$ and BrdU can occur effectively only within the shorter lifetime of $dGMP^{*-}$, as observed in Figure 1. For the BrdU−dAMP complex, the effective ET from $dAMP^{*-}$ occurs on much longer time scales (up to ∼30 ps), leading to a stronger enhancement in the total yield of $BrdU^{*-}$ (eq 1d). The enhancement of the yield of $BrdU^{*-}$ (integration of the signal over time) leads to an increased yield of the $dU^\bullet$ radical (eq 1e), which ultimately causes more DNA strand breakage. A small percentage of $BrdU^{*-}$ becomes stable $BrdU^-$ (eq 1f), resulting in the long-lived tails in Figure 1.[17]

$$H_2O + 2h\nu(UV) \rightarrow H_2O^* \rightarrow H_2O^+ + e^-_{pre} \qquad (1a)$$

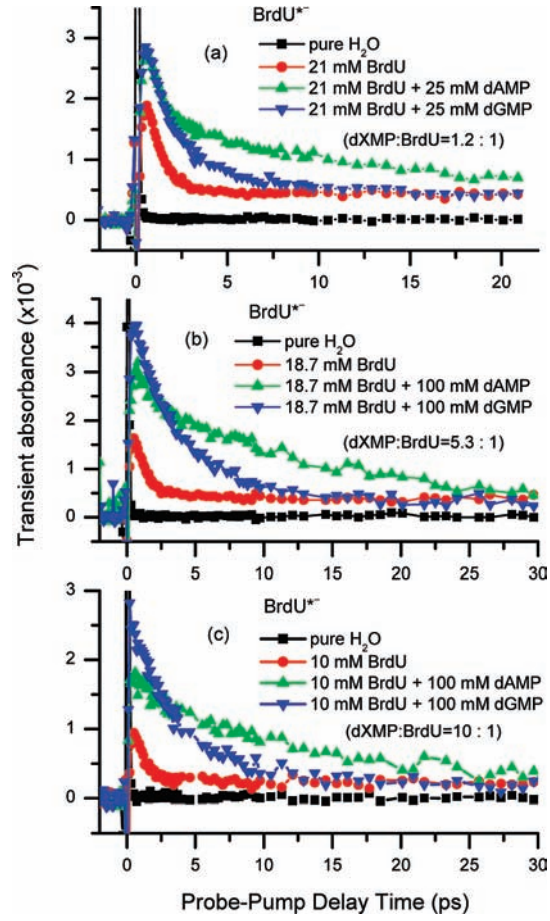


Figure 1. Femtosecond transient absorption kinetic traces of $BrdU^{*-}$ generated by UET to BrdU/IdU from $dAMP^{*-}$ and $dGMP^{*-}$ formed by capture of e^-_{pre}: (a) pure water, 21 mM BrdU, 21 mM BrdU + 25 mM dAMP/dGMP mixture (dXMP/BrdU molecular ratio = 1.2:1); (b) pure water, 18.7 mM BrdU, 18.7 mM BrdU + 100 mM dAMP/dGMP mixture (dXMP/BrdU molecular ratio = 5.3:1); (c) pure water, 10 mM BrdU, 10 mM BrdU + 100 mM dAMP/dGMP mixture (dXMP/BrdU molecular ratio = 10:1). The pump and probe wavelengths were 322 and 333 nm, respectively. The sharp peak at time zero is the coherence "spike" of the pump and probe pulses. The kinetic trace for BrdU was subtracted from that for the solvent (H_2O), while the kinetic traces for the BrdU + dAMP/dGMP mixtures were subtracted from that of pure dAMP/dGMP alone.

$$BrdU + e^-_{pre} \rightarrow BrdU^{*-} \qquad (1b)$$

$$dAMP/dGMP + e^-_{pre} \rightarrow dAMP^{*-}/dGMP^{*-} \qquad (1c)$$

$$dAMP^{*-}/dGMP^{*-} + BrdU \rightarrow dAMP/dGMP + BrdU^{*-} \qquad (1d)$$

$$BrdU^{*-} \rightarrow Br^- + dU^\bullet \qquad (1e)$$

$$BrdU^{*-} \rightarrow BrdU^- \qquad (1f)$$

Although the yield of $dXMP^{*-}$ formed by UET of e^-_{pre} (eq 1c) contributes to the original signal detected at the probe wavelength of 333 nm,[19] this contribution was removed from the kinetic traces of $BrdU^{*-}$ for BrdU−dAMP/dGMP complexes shown in Figure 1, where the measured kinetic traces were subtracted from that of pure dAMP/dGMP. In this processing, a simplified assumption is made: the presence of BrdU/IdU would not cause any decrease in the yield of $dXMP^{*-}$. Certainly, the real situation is that the presence of BrdU should somewhat reduce the probability of $dXMP^{*-}$ formation relative to the case for pure dXMP only because

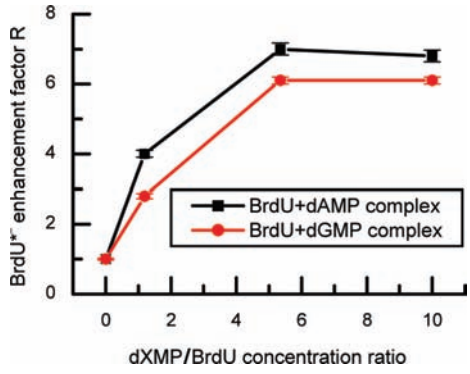


Figure 2. Enhancement factor of the BrdU*$^-$ yield (R) for BrdU + dXMP mixtures as a function of the dXMP/BrdU molecular ratio (see the text).

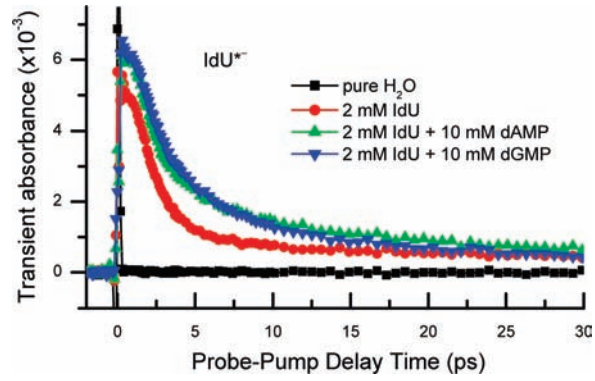


Figure 3. Femtosecond transient absorption kinetic traces of IdU*$^-$ generated by UET to IdU from dAMP*$^-$ and dGMP*$^-$ formed by capture of e^-_{pre}. Traces for pure water, pure IdU, and 2 mM IdU + 10 mM dAMP/dGMP mixtures are shown. The pump and probe wavelengths were 322 and 333 nm, respectively. The kinetic trace for IdU was subtracted from that for the solvent (H_2O), while the kinetic traces for the IdU + dAMP/dGMP mixtures were subtracted from that of pure dAMP/dGMP alone.

of the competition between dXMP and BrdU in capturing e^-_{pre} (eqs 1b and 1c). In regard to this point, one might argue for a very unlikely case that instead of the ET from dXMP*$^-$ to BrdU, the ET from BrdU*$^-$ to dXMP might occur and lead to a larger dXMP*$^-$ yield for BrdU−dXMP complexes than for pure dXMP. This argument, however, cannot stand for many well-known reasons. First, BrdU and IdU are well-known to be strong electron capturers (much stronger than any dXMP), which is why the replacement of dXMP by BrdU/IdU has been tested as a potential source of radiosensitizers and photosensitizers. Second, it has been well-observed that the lifetimes of BrdU*$^-$ and IdU*$^-$ are less than 2 ps.[17a−c] Thus, any ET from BrdU*$^-$ to dXMP must occur within 2 ps, and any enhancement of the dXMP*$^-$ yield must be observed within only 2 ps (it should also be noted that the kinetic trace of dAMP*$^-$ once formed would exhibit only a flat line[19]). This drastically differs from the observed results in Figure 1. Thus, the kinetic traces of BrdU*$^-$ shown in Figure 1 are actually the lower limit to the real yields of the BrdU*$^-$ formed from the BrdU−dXMP complexes. That is, the true enhancement of the BrdU*$^-$ yield due to the UET from dXMP*$^-$ should be slightly larger than that shown in Figure 1. Nevertheless, the results in Figure 1 clearly demonstrate that the UET from dXMP*$^-$ to BrdU in BrdU−dXMP complexes leads to a significant enhancement in the yield of BrdU*$^-$ relative to the yield for pure BrdU and that the enhancement is stronger for BrdU−dAMP complexes than for BrdU−dGMP complexes.

The total yields (Y) of dissociated BrdU*$^-$ can be obtained by integrating the decaying signal over the time window from 0.5 to 30 ps for the BrdU and BrdU + dXMP samples. The yield enhancement factor R for the BrdU + dXMP mixture, defined as $R = Y(\text{BrdU + dXMP})/Y(\text{BrdU})$, can then be found. The R value is expected to rise as the fraction of BrdU in BrdU−dXMP dimers increases and to reach a maximum when all BrdU form dimers with dXMP. The latter can be accomplished using mixtures with dXMP in far excess. To examine this possibility, the kinetic traces of BrdU*$^-$ for BrdU + dXMP mixtures with various dXMP/BrdU molecular ratios were measured. To avoid the formation of dXMP clusters and other measurement difficulties (e.g., a sudden large noise), the highest concentration of dXMP was kept below 100 mM. The obtained R values with various dXMP/BrdU molecular ratios (up to 10:1) from the kinetic traces shown in Figure 1 are plotted in Figure 2. Interestingly, R indeed rose with increasing dXMP/BrdU ratio and reached saturation (7.0 ± 0.2 for BrdU + dAMP and 6.1 ± 0.1 for dGMP + BrdU) when the molecular ratio became larger than ∼5. These results indicate that for the mixtures with low dXMP/BrdU ratios, there are some free BrdU molecules in the mixtures; with higher ratios (≥5), all of the BrdU molecules form heterodimers with dXMP.

When BrdU was replaced by IdU to form IdU−dXMP complexes, similar UET reactions were observed. The kinetic traces of IdU*$^-$ formed from IdU only and from IdU−dXMP complexes are shown in Figure 3. Nearly identical kinetic behavior with a much lower maximum enhancement factor for dissociated IdU*$^-$ ($R \approx 1.6$) was observed using the 2 mM IdU + 10 mM dAMP/dGMP mixtures. This is reasonable because the direct UET reaction of IdU with e^-_{pre} is much stronger than that of BrdU.[17] Thus, the value of R for UET from dAMP*$^-$/dGMP*$^-$ is smaller for IdU than for BrdU. This result is also consistent with the observation that much smaller sequence selectivity of photosensitivity was observed for similar duplex DNA containing IdU than for DNA containing BrdU.[11]

It is also interesting to compare the present results with those of previous radiolysis studies by Nese et al.[15b] using submicrosecond electron pulses of aqueous BrdU and nucleobase complexes. In those studies, the dU$^\bullet$ yield was attributed to ET from nucleobase electron adducts to BrU under γ irradiation, and it was proposed that ET from T and A electron adducts and their protonated forms to BrU occurs but that no ET to BrU occurs from the electron adduct of G, which was thought to serve as an ultimate electron sink in irradiated DNA. In contrast, our real-time fs-TRLS observations[19] and the present results provide *direct* evidence that (1) the weakly bound e^-_{pre} can first be trapped at both A and G and then transferred to BrdU/IdU and (2) *the base A is not only the main electron sink but also an effective promoter for ET reactions, while G is the major damaging site*.

Finally, it should be noted that the present results may not be exactly identical to those of photosensitivity experiments using UV light at 302 nm.[12] There may exist two major differences. First, ET from the excited state G* to BrU/IU might occur in those experiments, as the base G has a UV absorption tail extending to 302 nm (i.e., G* might be generated). Second, it is unclear from the given experimental conditions whether or not prehydrated electrons were produced by two-photon excitation of water under the irradiation using conventional (non-laser) UV light sources. However, there is an agreement between those photosensitivity results and the present results that adenine is an effective promoter/bridge for ET leading to the formation of BrdU*$^-$/IdU*$^-$.

In summary, our results present the first *real-time* observation of UET from the anions of dAMP and dGMP to BrdU/IdU in aqueous BrdU−dAMP/dGMP and IdU−dAMP/dGMP complexes under ionizing radiation. The results provide a molecular mechanism

for the likely sequence selectivity of BrdU and IdU in radiosensitization. The capability of IdU for dissociative electron transfer of weakly bound e^-_{pre} is stronger than that of BrdU.[17] Correspondingly, BrdU shows a more significant DNA sequence selectivity through UET from $dXMP^{*-}$ formed by attachment of e^-_{pre}. This mechanistic understanding may lead to the improvement of BrdU and IdU to achieve sufficient radiosensitizing efficacy and the development of more effective radiosensitizers for clinical uses.

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