

## The Distance Between Donor and Acceptor Affects the Proportion of C1' and C2' Oxidation Products of DNA in a <sup>Br</sup>U-Containing Excess Electron Transfer System

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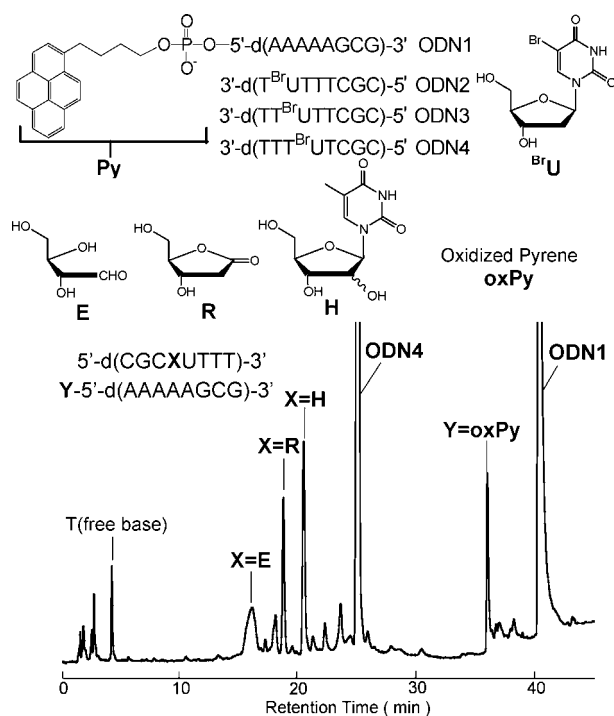
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**Abstract:** We have investigated the products of <sup>Br</sup>U in excess electron transfer and have demonstrated that in DNA the proportion of products changes with the distance between the donor and acceptor. On the basis of a labeling experiment using H<sub>2</sub><sup>18</sup>O, we have shown that hole migration from Py<sup>++</sup> formed after charge separation is involved in the reaction.

DNA has the potential to mediate positive or negative charge transfer through  $\pi$  stacks of DNA bases.<sup>1,2</sup> Photoinduced negative charge transfer, called excess electron transfer (EET), from one-electron reduction of 5-bromouracil (<sup>Br</sup>U) and reductive splitting of cyclobutane derivatives has been demonstrated.<sup>1d,e,2</sup> The efficiency of EET through DNA has also been evaluated using <sup>Br</sup>U as the electron acceptor and a naphthalene derivative as the electron donor.<sup>2a</sup> Because naphthalene derivatives can be selectively excited by UV ( $\lambda > 335$  nm) as a result of their longer-wavelength UV absorbance relative to DNA, this system is useful for mechanistic investigation of EET. In this system, photoinduced electron transfer from the donor produces bromouracil anion radical (<sup>Br</sup>U<sup>•-</sup>), which then generates the uracil-5-yl radical with release of bromide ion. The formed uracil-5-yl radical abstracts H from deoxyribose on the backbone of DNA to create alkaline-labile sites that are analyzed as DNA cleavage after hot alkaline treatment.<sup>3-6</sup> Although <sup>Br</sup>U has been employed in numerous EET studies,<sup>2</sup> the nature of the products is not well understood. In this study, the products in an EET system were directly analyzed by using HPLC without any follow-up treatment. Here we show that the distribution of products generated by the uracil-5-yl radical is largely dependent on the distance between the donor and acceptor.

Because pyrene (Py) ( $E_{ox}^* = -2.17$  V vs SCE)<sup>2g</sup> has been used as a donor for DNA-mediated EET studies,<sup>2b-d</sup> we utilized 5'-Py-modified oligodeoxynucleotide ODN1 with complementary ODNs ODN2-4 containing <sup>Br</sup>U ( $E_{red} \approx -2.0$  V vs SCE)<sup>2g</sup> (Figure 1). In this system, Py and <sup>Br</sup>U are separated by different numbers of intervening AT base pairs, which are known to be efficient mediators for EET. Polyacrylamide gel electrophoresis (PAGE) analysis of the photoirradiated Py-conjugated ODN and <sup>Br</sup>U-containing ODNs clearly showed the formation of alkaline-labile sites and indicated that EET occurred from Py to <sup>Br</sup>U under these conditions (Figure S1 in the Supporting Information). These results are consistent with previous reports.<sup>2a</sup>

The HPLC profile of photoirradiated ODN1/ODN4 conjugates (denoted as ODN1/4) at 365 nm is shown in Figure 1. Formation



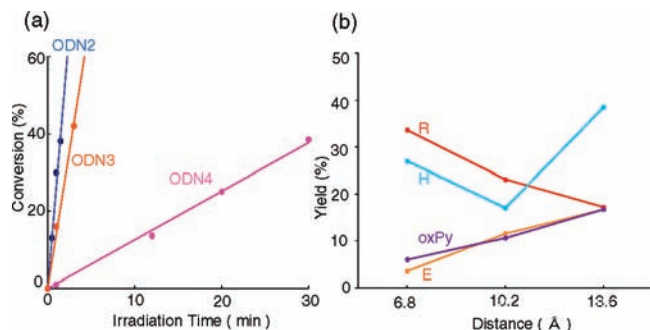
**Figure 1.** Sequences of ODNs used in this study and HPLC analysis of UV-irradiated ODN1/4. Photoirradiation was conducted for 12 min at 0 °C with a monochromator (365 nm). The reaction mixture was analyzed by HPLC on a Chemcobond 5C18 column (4.6 × 150 mm) with detection at 254 nm. Elution was performed using 3–10% 0.05 M ammonium formate (0–30 min) followed by 10–40% acetonitrile (30–45 min) at a flow rate of 1.5 mL/min.

of DNA strands containing ribonolactone (**R** in Figure 1) and erythrose (**E** in Figure 1) was observed. On the basis of previous investigations, **R** and **E** are assumed to be the oxidative degradation products derived from C1' and C2' H abstraction, respectively, by the uracil-5-yl radical.<sup>4,5</sup> The structures of **R** and **E** were confirmed using previously reported methods and by negative-ion electrospray ionization–time of flight mass spectrometry (ESI–TOF MS), as shown in Figure 3 and Figure S2.<sup>3b</sup> In addition to these products, the C2'-hydroxylated product (**H** in Figure 1) was also formed. Enzymatic digestion of product **H** with P1 endonuclease and alkaline phosphatase (AP) provided dG, dC, dT, and a mixture of rT and arabinoT in an approximately 1:2.5:1 ratio. Negative-ion ESI–TOF MS spectra of H-containing ODNs are shown in Figure S3. On the basis of the enzymatic digestion results, oxidized Py

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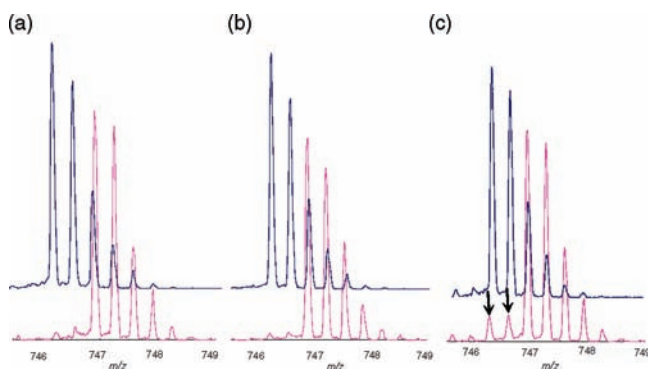
<sup>§</sup> iCeMS, Kyoto University.



**Figure 2.** (left) Conversions of the  $^{Br}U$ -containing ODNs at different irradiation times. (right) Relationships between the photoproduct yields and the distance between the donor (Py) and acceptor ( $^{Br}U$ ). Yields are based on consumed  $^{Br}U$ -containing octamer, as determined by HPLC. To evaluate the yields at almost the same conversion rate (13–16%), photoirradiation was performed at 365 nm for 0.5 min for ODN1/2, 1 min for ODN1/3, and 12 min for ODN1/4.

(oxPy)-containing ODN1 was also formed under these conditions.<sup>6</sup> The UV spectrum of oxPy-containing ODN1 is shown in Figure S5.

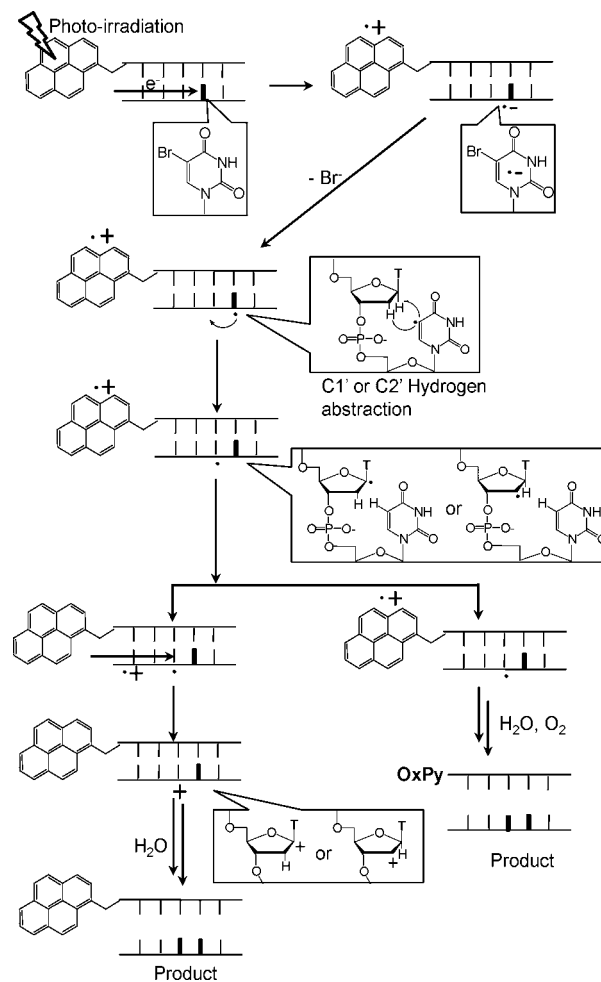
Similarly, the photoreactions of ODN1/2–4 produced **E**–oxPy-containing ODNs. The conversions of ODN2–4 after different irradiation periods are shown in Figure 2a. The yields of products at similar conversion of  $^{Br}U$  are summarized in Figure 2b. Surprisingly, the yield of **E** increased with increasing distance between the donor and acceptor, whereas the yield of **R** decreased with increasing distance, as shown in Figure 2b. These results indicate that the distance dependence ( $\beta$  value) of the charge transfer cannot be estimated simply from the yields of either **E** or **R** in this system.<sup>7</sup> Similar to **E**, the amount of oxPy increased with increasing donor–acceptor distance.<sup>8</sup> We noticed this distance dependence of oxPy formation because, in the absence of a reduction process, the pyrenyl cation radical ( $Py^{+\bullet}$ ) formed after charge separation should be oxidized to oxPy.



**Figure 3.** Negative-ion ESI–TOF MS spectra of **R**-containing ODNs isolated from irradiated (a) ODN1/2, (b) ODN1/3, (c) and ODN1/4 in  $H_2^{16}O$  (blue) and  $H_2^{18}O$  (pink).

We assumed that  $Py^{+\bullet}$  is reduced by a radical at the reactive site in a distance-dependent manner via a hole migration process. If the hole migration is related to the reaction, the reaction mechanism should be modulated by the distance. To test this hypothesis, the reaction mechanism for the formation of **R** was explored. ESI–TOF MS spectra of isolated **R**-containing ODNs obtained from irradiated ODN1/2–4 in  $H_2O$  and  $H_2^{18}O$  are shown in Figure 3. The results clearly indicate that O1 of **R** is mainly derived from  $H_2O$ . Interestingly, in the case of ODN1/4 in  $H_2^{18}O$ , a significant amount of  $^{16}O$  incorporation ( $\sim 10\%$ ) in comparison with ODN1/2 and

**Scheme 1**

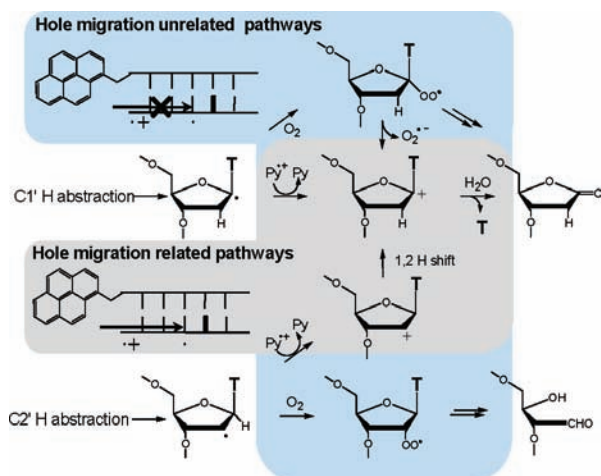


ODN1/3 was observed, as indicated by the arrows (Figure 3c). The results imply that the reaction mechanism is altered by the distance between the donor and acceptor and suggest the participation of molecular oxygen in the formation of **R** in the case of ODN1/4.

On the basis of the results, we propose the mechanism for the EET-mediated  $^{Br}U$  reaction shown in Scheme 1. Under irradiation conditions, charge separation states such as  $Py^{+\bullet}$  and  $^{Br}U^{\bullet-}$  are formed. The formed  $^{Br}U^{\bullet-}$  releases  $Br^-$ , and the resultant uracil-5-yl radical abstracts deoxyribose C1' H or C2' H at the 5' site to generate the C1' or C2' radical, respectively (Scheme 1). When the donor–acceptor distance is short, the C1' radical is rapidly oxidized to the corresponding cation by  $Py^{+\bullet}$ , whereas when the distance is long, some of the C1' radicals react with  $O_2$  before oxidation by  $Py^{+\bullet}$  (Scheme 1). It is noteworthy that the C1' cation is assumed to form after the reaction between molecular oxygen and C1' radical with the release of the superoxide from the peroxy radical ( $-\bullet OO^{\bullet}$ ) at C1' (Scheme 2).<sup>9</sup> Therefore,  $-\bullet OO^{\bullet}$  formation at C1' via reaction of the C1' radical and molecular oxygen might occur to a greater extent than indicated by the labeling experiment. In the case of product **E** formation, the molecular ion was unchanged whether it was irradiated in  $H_2^{18}O$  or in  $H_2^{16}O$  (Scheme S1). In addition, under  $O_2$ -limiting conditions, the **E** formation was significantly suppressed (data not shown). These results indicate that the oxygen atom of erythrose is exclusively derived from molecular oxygen (Scheme 2).

Taking into account all of the data, we assumed that the results can be explained by the existence of distance-dependent competitive pathways between **R** and **E** formation from C2' H abstraction. From

Scheme 2



the results shown in Figure S2, formation of **E** is derived from the incorporation of oxygen into the C2' radical. When this radical is oxidized to a cation, hole migration from the formed  $\text{Py}^{++}$  cation may be moved from C2' to C1' via a 1,2-hydride process that results in formation of **R**. This process has an effect on the content of **E** and **R** in a distance-dependent manner (Scheme 2).

The mechanism for the formation of **H** is not clear; however, photoreaction in  $\text{H}_2^{18}\text{O}$  showed that the source of oxygen at C2' OH is derived from  $\text{O}_2$  (Figure S3).<sup>10</sup> The yield of **H** obtained from irradiated ODN1/2 is slightly higher. This might indicate that the reaction pathway for formation of **H** is not simple and that several factors are involved in the formation of **H**. For example, the reaction mechanism of **H** might be related to that of **E**. However, further investigation is required to elucidate the mechanism.

In conclusion, we have investigated the products of  $^{\text{Br}}\text{U}$  in the EET system and demonstrated that in DNA the proportion of **E** and **R** changes with the distance between the donor and acceptor. Both **E** and **R** are cleavable under hot alkaline conditions. Therefore, the contents of these photoproducts are indistinguishable by PAGE analysis. The case that the electron donor participates in the reaction after donation of the electron has not been well-documented. On the basis of the labeling experiment using  $\text{H}_2^{18}\text{O}$ , we have shown that hole migration from  $\text{Py}^{++}$  formed after charge separation is involved in the reaction. On this basis, we have proposed a reaction mechanism that is dependent on the donor–acceptor distance. Several factors, such as DNA conformation, DNA sequence, and pH, have been reported to modulate the proportion of DNA damage products.<sup>3,4,11</sup> The present results indicate that hole migration has

the potential to change the distribution of DNA damage products. These results provide important information for the understanding of the molecular mechanism of radical-induced DNA damage, including photosensitizer-induced damage and X-ray irradiation.

**Supporting Information Available:** Experimental section, PAGE analysis, ESI–TOF MS spectra, and UV spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (6) Enzymatic digestion of product **oxPy** with P1 endonuclease and AP provided dG, dC, and dA in an approximately 2:1:5 ratio. ESI–TOF MS spectra of **oxPy** revealed that two O atoms are incorporated into **oxPy** formation and two H atoms are eliminated (Figure S4). An irradiation experiment in  $\text{H}_2^{18}\text{O}$  revealed that one O is derived from molecular oxygen and the other from water, as shown in Figure S4.
- (7) The  $\beta$  value estimated from consumed  $^{\text{Br}}\text{U}$ -containing ODNs after irradiation for 1 min is  $0.19 \text{ \AA}^{-1}$ .
- (8) Without  $^{\text{Br}}\text{U}$ -containing ODN, formation of **oxPy** was not observed from irradiated ODN1.
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