

## Unique Charge Transfer Properties of the Four-Base $\pi$ -Stacks in Z-DNA

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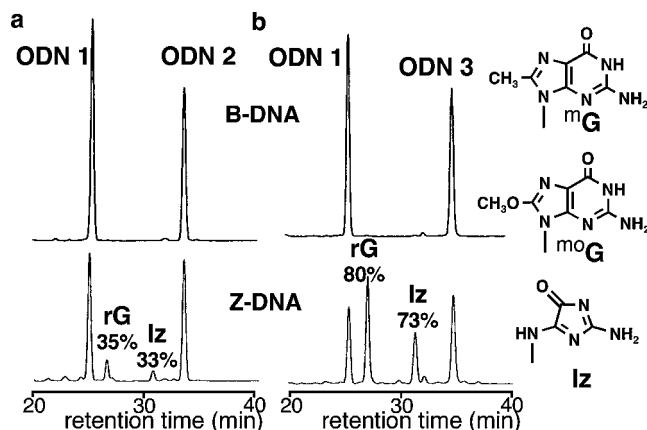
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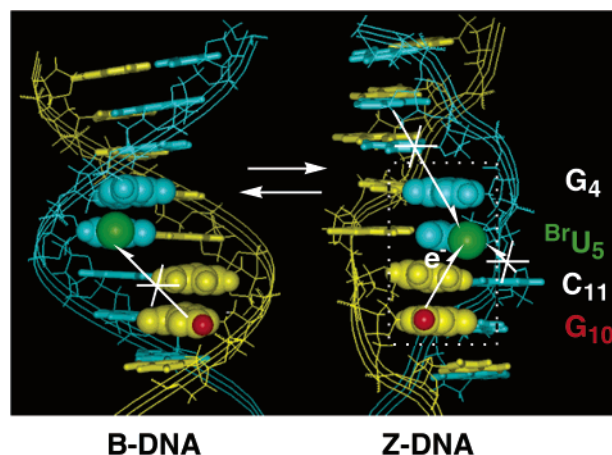
Charge transfer through DNA has been extensively investigated in recent years.<sup>1</sup> Efficiency of charge transfer through DNA is sensitive to the orientation of their  $\pi$ -stackings.<sup>2</sup> Modulation of the charge transfer through DNA, controlled by the change of  $\pi$ -stackings is attractive for designing novel nanoelectronic devices. Because the overall base  $\pi$ -stackings of B- and Z-DNA are different, the electronic property of these two duplexes is also assumed to be different. Schuster and colleagues examined charge transfer in Z-DNA by comparing the oxidation in the 5'-GG sequence of the hairpin loop regions by injection of cation radicals generated from the excitation of anthraquinone at the end of the hairpin.<sup>3</sup> However, no clear difference in the charge transfer attributed to the structure of Z- and B-DNA was observed. For several years, the photo-reaction of 5-halouracil-containing DNA duplexes has been investigated, and we have demonstrated that hydrogen abstraction by deoxyuridin-5-yl is highly conformation dependent.<sup>4</sup> We also found that the photoreaction of 5-bromouracil (<sup>B</sup>rU)-containing DNA is highly sequence specific. Competitive C1'- and C2' $\beta$ -hydrogen abstractions are observed at the 5'-A<sup>B</sup>rU sequence in B-DNA, whereas the 5'-A<sup>B</sup>rU sequence (in single strand) and other sequences such as 5'-G<sup>B</sup>rU in B-DNA do not show such reactivity.<sup>4a,5</sup> Our research group, and others, have proposed that electron transfer from 5'A to <sup>B</sup>rU is essential for this photoreaction, and the lack of reactivity at the 5'-G<sup>B</sup>rU sequence can be explained by a rapid back electron-transfer mechanism.<sup>4a,5c</sup> We hypothesized that if a charge-transfer process is involved in this reaction, then the photoreactivity of <sup>B</sup>rU may be different in Z-DNA. Here we show that Z-DNA has a unique electronic property and charge transfer from G to <sup>B</sup>rU occurs efficiently within the four-base  $\pi$ -stacks in Z-DNA.

To examine the photoreactions of <sup>B</sup>rU in Z-DNA, we first investigated the photoirradiation of 5'-d(C<sub>1</sub>G<sub>2</sub>C<sub>3</sub>G<sub>4</sub><sup>B</sup>rU<sub>5</sub>G<sub>6</sub>C<sub>7</sub>G<sub>8</sub>)-3'/5'-d(C<sub>9</sub><sup>m</sup>G<sub>10</sub>C<sub>11</sub>A<sub>12</sub>C<sub>13</sub><sup>m</sup>G<sub>14</sub>C<sub>15</sub>G<sub>16</sub>)-3' (ODN 1-2).<sup>6</sup> In accord with previous observations, B-form ODN 1-2 with the 5'-G<sup>B</sup>rU sequence showed no photoreactivity (Figure 1a, top).<sup>5</sup> However, Z-form ODN 1-2 in 2 M NaCl underwent efficient photoreaction to afford 5'-d(CG<sup>m</sup>rGd(UGCG)-3' in 35% yield, based on ODN 1 consumed (Figure 1a, bottom). The results clearly indicate that structural changes caused by the B-Z transition dramatically increased the photoreactivity of ODN 1-2. The structural changes under these conditions were confirmed by the circular dichroism (CD) spectrum (Figure 1S).

Inspection of the molecular structures suggests that the stacking between G and <sup>B</sup>rU in the 5'-G<sup>B</sup>rU sequence in B- and Z-DNA is almost the same, indicating that the 5'G may not be responsible for the reactivity. However, it was found that there are unique clusters of four-base  $\pi$ -stacks in Z-DNA, i.e. in ODN 1-2 the four-base  $\pi$ -stacks are formed at the G<sub>4</sub>-<sup>B</sup>rU<sub>5</sub>-C<sub>11</sub>-<sup>m</sup>G<sub>10</sub> sequence (Figures 2 and 2S).



**Figure 1.** Photoreaction of bromouracil containing octanucleotides. (a) HPLC profiles of UV-irradiated ODN 1-2 (left) and ODN 1-3 (right) in the B- or Z-conformation. The rG and Iz represent rG- and Iz<sub>10</sub>-containing octanucleotides, respectively. The reaction mixture (total volume 100  $\mu$ L) contained deoxyoctanucleotide (36  $\mu$ M total base concentration), 20 mM phosphate buffer (pH 7.0), NaCl 20 mM (B-DNA) or 2.0 M (Z-DNA). Irradiation was performed with a monochromatic 302 nm UV light for 30 min at 0 °C. The reaction mixture was analyzed by HPLC on a Chemcobond 50DS-H column (4.6 mm  $\times$  150 mm) detected at 254 nm; elution was with 0.05 M ammonium formate (pH 6.5) containing 2–7% acetonitrile, linear gradient, 40 min, at 40 °C, at a flow rate of 1 mL/min. The structures of rG and Iz were confirmed by enzymatic digestion and electrospray ionization mass spectra (ESI-MS, negative): 5'-d(CG)rGd(UGCG)-3': calcd 2428.6, found 2428.2; 5'-d(CIzCAC<sup>m</sup>GCG)-3': calcd 2369.4, found 2369.8.

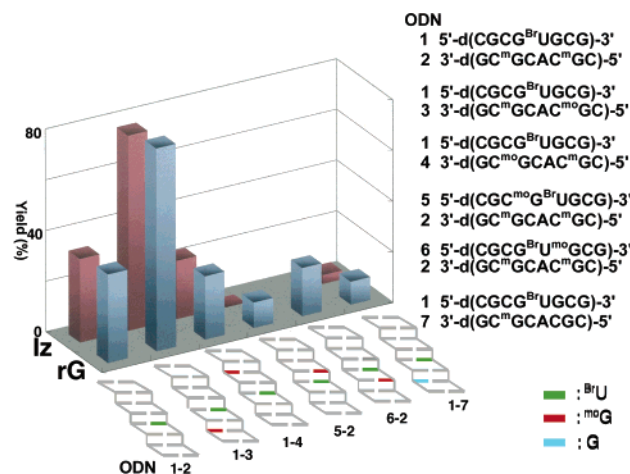


**Figure 2.** Molecular models of B-form (left) and Z-form DNA (right). Two strands are colored in yellow and blue. The bromine atom of <sup>B</sup>rU and the methyl group of <sup>m</sup>G are drawn in green and red, respectively. The four-base  $\pi$ -stacks in which charge transfer occurred is indicated by a dotted line.

Interestingly, a small peak eluted at 30.5 min appeared to be identified as imidazolone (Iz)<sup>8</sup> contained 5'-d(CIzCAC<sup>m</sup>GCG)-3'

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**Figure 3.** The yields of photoproduct **rG** and **Iz** from photoirradiated Z-form octamers. The blue and red bars represent the yield of **rG** and **Iz**, respectively. The **rG** and **Iz** represent rG<sub>4</sub> or r<sup>mG</sup>G<sub>4</sub>- and Iz<sub>10</sub>-containing octanucleotides, respectively.

(Figure 1a, bottom), suggesting the intriguing possibility that the <sup>m</sup>G<sub>10</sub> in a complementary strand located at the end of the four-base  $\pi$ -stacks may act as an electron donor (Figure 2).

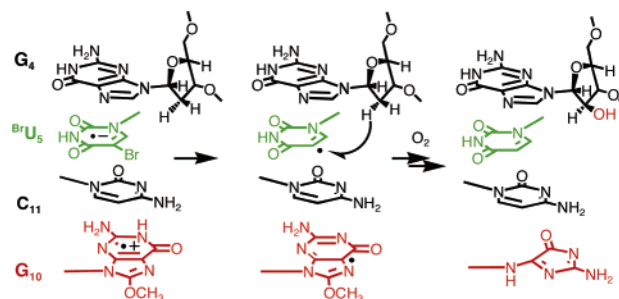
To test the hypothesis of interstrand charge transfer from <sup>m</sup>G<sub>10</sub> to <sup>Br</sup>U<sub>5</sub> within the four-base  $\pi$ -stacks in Z-DNA, ODN 1-3 samples in which the putative donor G<sub>10</sub> residue was replaced with 8-methoxyguanine (<sup>m</sup>G) were prepared, since <sup>m</sup>G is known to trap cation radicals to yield Iz moieties in DNA.<sup>9</sup> As shown in Figure 1b (bottom) photoirradiation of ODN 1-3 efficiently produced 5'-d(CGCG)rGd(UGCG)-3' together with the formation of 5'-d(CIzCAC<sup>m</sup>GCG)-3'. The yields of 5'-d(CGCG)rGd(UGCG)-3' and 5'-d(CIzCAC<sup>m</sup>GCG)-3' were 80 and 73%, respectively, based on the consumed hexamers. These results clearly indicate that the interstrand charge transfer from <sup>m</sup>G<sub>10</sub> to <sup>Br</sup>U<sub>5</sub> initiates the photoreaction. In clear contrast, other replacements of G with <sup>m</sup>G, such as ODN 1-4, ODN 5-2, and ODN 6-2 did not enhance the photoreactivity.

The yields of the oxidation products are summarized in Figure 3. It is important to note that <sup>m</sup>G located in different clusters of four-base  $\pi$ -stacks (ODN 6-2, ODN 1-4) does not cause any enhancement of the photoreaction. The lack of enhancement in the case of ODN 5-2 could be explained by a rapid back electron transfer analogous to that in 5'-G<sup>Br</sup>U in B-DNA.<sup>5</sup>

Since the ionization potential of <sup>m</sup>G is lower than that of G,<sup>3,10</sup> there is a possibility that this type of photoactivation only occurs at <sup>m</sup>G<sub>10</sub> in Z-DNA. Photoirradiation of ODN 1-7 was carried out to exclude this possibility. The CD spectrum indicated that 50% of ODN 1-7 adopted the Z-conformation under the experimental conditions of 2 M NaCl, due to the lack of one <sup>m</sup>G in ODN 7 relative to ODN 2. Under these conditions, a reasonable amount of 5'-d(CGCG)rGd(UGCG)-3' (9.0%) and 5'-d(CIzCAC<sup>m</sup>GCG)-3' (3.5%) was obtained, indicating that G in the four-base  $\pi$ -stacks activates <sup>Br</sup>U and that this type of electron transfer generally occurs in <sup>Br</sup>U-containing Z-DNA.

These results clearly indicate that electron transfer only occurs efficiently within the four-base  $\pi$ -stacks in Z-DNA compared with that between two four-base  $\pi$ -stacks without evident overlapping. Analogous slow interstrand charge transfer relative to the intrastrand

**Scheme 1**



process in B-DNA has been pointed out.<sup>11</sup> The proposed mechanism of electron transfer from G<sub>10</sub> to <sup>Br</sup>U<sub>5</sub> and the following hydroxylation to rG<sub>4</sub> and  $\pi$ -stacks in Z-DNA is shown in Scheme 1. Photoexcitation initiates charge transfer from <sup>m</sup>G<sub>10</sub> to <sup>Br</sup>U<sub>5</sub>. This causes formation of the ribonucleotide due to C2' $\beta$  hydrogen abstraction, and photooxidation of <sup>m</sup>G<sub>10</sub> giving dlZ.

In conclusion, the present study revealed that the presence of unique four-base  $\pi$ -stacks in Z-DNA and photoirradiation of <sup>Br</sup>U in Z-DNA causes efficient electron transfer from G within this cluster. Since it has been shown that the photoreaction of <sup>Br</sup>U also largely depends on the DNA conformations, <sup>Br</sup>U could be a dual probe of DNA local structure and pathway of charge transfer through DNA.

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**Supporting Information Available:** CD spectrum of ODNs 1-2 and 1-3, and schematic presentation of the four-base  $\pi$ -stack in Z-DNA (PDF). This material is available free of charge via Internet at <http://pubs.acs.org>.

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- We have demonstrated that oligonucleotides that contain 8-methylguanine (<sup>m</sup>G) can form the Z-conformation efficiently.<sup>7</sup> Using this unit, we have demonstrated that the photoirradiation of 5'-d(CGCG<sup>BrU</sup>UGCG)-3'/5'-d(C<sup>m</sup>G<sup>m</sup>GCAC<sup>m</sup>GCG)-3' in Z-form DNA caused C2' $\alpha$ -hydroxylation, and yielded 5'-d(CGCG)rG(UGCG)-3'.<sup>4d,e</sup>
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