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Unique Hole-Trapping Property of the Degenerate Base, 2-Amino-7-deazaadenine

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Abstract—An artificial degenerate nucleobase, 2-amino-7-deazaadenine (**1**) showed remarkably high hole-trapping efficiency, similar to that of ^{Z}G and superior to that of the GGG triplet. The hole-trapping efficiency varied with the counterbase that base-pairs with **1**.

Long-range hole migration in duplex DNA has recently become a topic of interest at the interface of chemistry and biology. Long-range hole migration in DNA has been extensively investigated, especially the kinetics of hole generation and migration.[1–6] A conventional method with which to investigate the efficiency of hole migration in DNA involves the analysis of the band intensities of the products of oxidative DNA cleavage at multiple G nucleotides (i.e., GG and GGG steps).[1,4–8] However, with this detection method, the oligonucleotides used are limited to those especially designed sequences containing GG and GGG steps as hole traps. As an alternative base for efficient hole trapping at multiple G nucleotides, the synthetic G analogue 7-deazaguanine (^{Z}G) has often been used.[9,10] This nucleobase can act as a surrogate for G but not for AT base pairs. Development of an efficient hole-trapping nucleobase that can be incorporated into any DNA sequence, regardless of the presence of AT or GC base pairs, is very important to the study of long-range hole migration in DNA containing diverse sequences.

We have already reported a highly effective degenerate nucleobase, 2-amino-7-deazaadenine (**1**, Fig. 1), which can form stable base pairs with both T and C.[11] If **1** acts as an effective hole trap like ^{Z}G, it would be a useful indicator for hole migration studies of DNA.

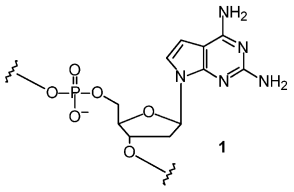

Figure 1. 2-Amino-7-deazaadenine (**1**) used in this study.

We herein report that **1** is the first hole-trapping degenerate base to be identified. The hole-trapping efficiency of **1** in duplex DNA is superior to that of the GGG step and similar to that of ^{Z}G. We also demonstrate that the hole-trapping efficiency of **1** varies with the base-pairing pyrimidine base.

First, hole-trapping was evaluated by means of remote oxidative DNA damage induced by photoirradiation of a cyanobenzophenone (**S**)-tethered oligodeoxynucleotide (ODN). ODNs containing **1** were prepared according to the method reported earlier.[12] The ^{32}P-labeled ODN, containing an **X**–**Y** base pair (**X** = A, G, ^{Z}A, ^{Z}G or **1**; **Y** = T or C) and two GGG steps (*prox*-G_3 and *dist*-G_3), was annealed with an ODN containing **S** as an electron-accepting photosensitizer (Fig. 2a). The duplexes were irradiated at 312 nm for 15 min at 0 °C, then treated with hot piperidine (90 °C, 20 min). DNA cleavage with alkaline treatment was assayed by PAGE (Fig. 2b). The values calculated by quantifying the intensities of the cleavage band are shown in Table 1. When **X** was G or 7-deazaadenine (^{Z}A), the cleavage bands were observed almost exclusively at both *prox*-G_3 and *dist*-G_3 (lanes 5

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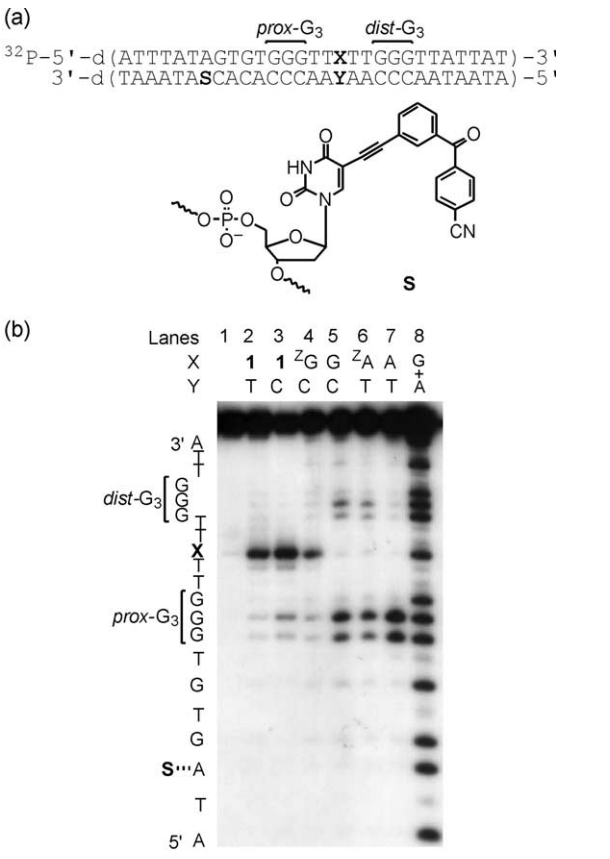

Figure 2. Remote oxidation of DNA containing **1:** (a) DNA sequence in this experiment. **S** represents cyanobenzophenone-tethered uridine ($d^{CNBP}U$); (b) autoradiogram of a denaturing gel electrophoresis of ^{32}P-5′-end-labeled DNA after photooxidation of the duplexes. The DNA duplexes were photoirradiated at 312 nm in 10 mM sodium cacodylate (pH 7.0) at 0 °C for 15 min, then treated with hot piperidine (90 °C, 20 min). Lane 1, control (**X** = **1**, **Y** = T) without irradiation; lane 2, **X** = **1**, **Y** = T; lane 3, **X** = **1**, **Y** = C; lane 4, **X** = ^{Z}G, **Y** = C; lane 5, **X** = G, **Y** = C; lane 6, **X** = ^{Z}A, **Y** = T; lane 7, **X** = A, **Y** = T; lane 8, Maxam-Gilbert G + A sequencing lane. ^{Z}G and ^{Z}A denote 7-deazaguanine and 7-deazaadenine, respectively.

and 6), indicating that G and ^{Z}A act as bridging bases for hole migration from *prox*-G_3 to *dist*-G_3. In contrast, strong cleavage bands at **X** were observed when **X** = **1** (lanes 2 and 3) or ^{Z}G (lane 4). In both cases, cleavage at *prox*-G_3 and *dist*-G_3 was strongly suppressed. The ODNs containing **1** were cleaved effectively at **1** regardless of the pyrimidine base with which it was base-paired. Cleavage at **1** did not occur without light

Table 1. Efficiency of oxidative cleavage of DNA containing **1**[a]

X–Y		Cleavage band intensities (%)		
		prox-G_3	**X**	*dist*-G_3
1–T	(Lane 2)	9	84	7
1–C	(Lane 3)	20	76	4
^{Z}G–C	(Lane 4)	25	66	9
G–C	(Lane 5)	73	2	25
^{Z}A–T	(Lane 6)	71	~0	29
A–T	(Lane 7)	>99	~0	~0

[a]The cleavage band intensities were calculated by densitometry, as shown in Figure 2b. The numbers in the table represent the percentage strand cleavage at a given site relative to the total strand cleavage. ^{Z}G and ^{Z}A denote 7-deazaguanine and 7-deazaadenine, respectively.

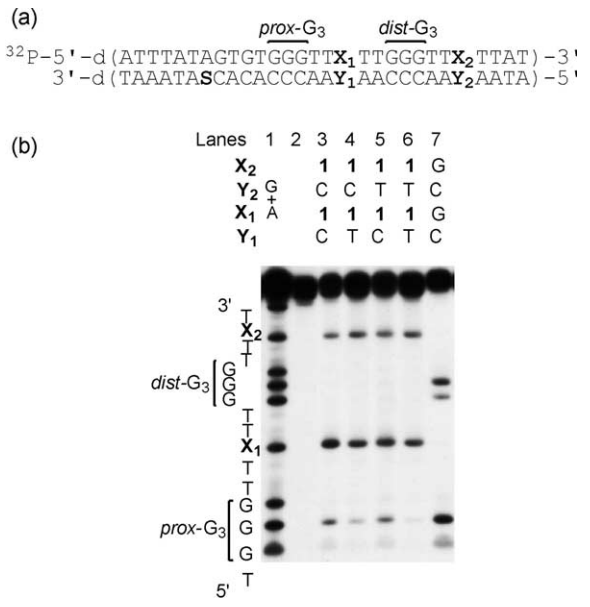

Figure 3. Remote oxidation of DNA containing two **1**'s. (a) DNA sequence in this experiment. **S** represents cyanobenzophenone-tethered uridine ($d^{CNBP}U$). (b) Autoradiogram of denaturing gel electrophoresis of ^{32}P-5′-end-labeled DNA after photooxidation of the duplexes. The DNA duplexes were photoirradiated at 312 nm in 10 mM sodium cacodylate (pH 7.0) at 0 °C for 45 min, then treated with hot piperidine treatment (90 °C, 20 min). Lane 1, Maxam–Gilbert G + A sequencing lane; lane 2, control (**X_1** = **1**, **Y_1** = T, **X_2** = **1**, **Y_2** = T); lane 3, **X_1** = **1**, **Y_1** = C, **X_2** = **1**, **Y_2** = C; lane 4, **X_1** = **1**, **Y_1** = T, **X_2** = **1**, **Y_2** = C; lane 5, **X_1** = **1**, **Y_1** = C, **X_2** = **1**, **Y_2** = T; lane 6, **X_1** = **1**, **Y_1** = T, **X_2** = **1**, **Y_2** = T; lane 7, **X_1** = G, **Y_1** = C, **X_2** = G, **Y_2** = C.

or in the absence of a photosensitizer, indicating that the cleavage was caused by remote oxidation through hole migration in a duplex ODN.

Having established the hole-trapping property of **1**, we electrochemically estimated the ability of **1** to act as an electron-donating nucleobase. The oxidation potential of **1** was measured by cyclic voltammetry (CV), and compared with those of ^{Z}G and G. The peak potential (E_p) for the irreversible oxidation of **1** occurred at 0.79 V, whereas those of G and ^{Z}G were at 1.15 and 0.79 V *versus* SCE, respectively, indicating that **1** is a nucleobase more readily oxidized than G, and oxidized to a similar degree as ^{Z}G. Furthermore, the irreversiblity of the scanning wave of **1** suggests that the lifetime of **1** oxidized at the electrode surface is very short in aqueous medium. Its ready oxidation and subsequent rapid decomposition would be an important factor in the good hole-trapping properties of **1**.

To better evaluate the effect of the pyrimidine base opposite **1** on the efficiency of hole migration, we prepared ODNs containing two **1**'s (**X_1** and **X_2**) located on either side of *dist*-G_3 as shown in Figure 3a. Photooxidation and PAGE analysis was carried out using the methods described above. The efficiency of cleavage at each **1** site is shown in Figure 3b and Table 2. The results can be divided into two categories according to the nature of the pyrimidine base (**Y_1**) base-paired with **X_1**. In the duplex ODN containing a **1**–T base pair at **X_1**–**Y_1**, we observed strong cleavage bands at both **X_1** and **X_2**, indicating that a hole can migrate effectively

Table 2. Efficiency of oxidative cleavage of DNA containing two **1**'s[a]

Sequences		Cleavage band intensities (%)			
		prox-G_3	**1** on $\mathbf{X_1}$	*Dist*-G_3	**1** on $\mathbf{X_2}$
$Y_1=C$, $Y_2=C$	(Lane 3)	24	57	~0	19
$Y_1=T$, $Y_2=C$	(Lane 4)	8	47	~0	44
$Y_1=C$, $Y_2=T$	(Lane 5)	28	49	~0	23
$Y_1=T$, $Y_2=T$	(Lane 6)	3	48	~0	48

[a]The cleavage band intensities were calculated by densitometry, as shown in Figure 3b. The numbers in the table represent the percentage strand cleavage at a given site relative to the total strand cleavage.

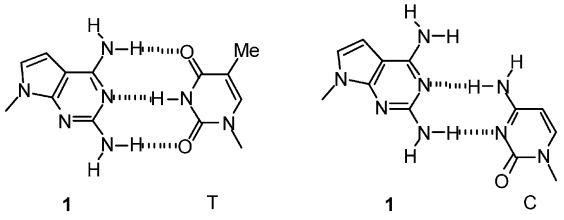

Figure 4. Watson–Crick **1**–T base pair (left) and wobble **1**–C base pair (right).

from $\mathbf{X_1}$ to $\mathbf{X_2}$ (lanes 4 and 6). Cleavage was negligible at *dist*-G_3, which acts as a bridge for the hole migration between $\mathbf{X_1}$ and $\mathbf{X_2}$, although strong cleavage bands at GGG sites are observed in natural DNA (lane 7). This result suggests that the **1**–T base pair is a shallow hole trap and acts, not only as an effective hole trap suppressing the oxidation of G bases, but also as a good hole carrier. In contrast, when $\mathbf{X_1}$ formed a base pair with C, a strong cleavage band was observed at $\mathbf{X_1}$, whereas the cleavage band observed at $\mathbf{X_2}$ was 2–3 times weaker than that observed at $\mathbf{X_1}$ (lanes 3 and 5). This result shows that hole migration to the $\mathbf{X_2}$ site beyond the **1**–C base pair was not as effective as that beyond the **1**–T base pair, and that the **1**–C base pair suppresses hole migration.

The reason of the suppression of hole migration by the **1**–C base pair probably involves the disruption of π-stacking by the formation of a non-Watson–Crick base pair.[13] Nucleobase **1** should form a wobble base pair with C at pH 7, as observed for the base pair formed between 2-aminopurine and C[14–16] (Fig. 4). If so, the formation of the **1**–C base pair in duplex DNA would cause a decrease in π-stacking with the flanking base on the 3′ side, because of the sliding of **1** in the base pair into the major groove of the duplex. Systematic π-stacking in duplex DNA is an important factor in hole migration in DNA.[9] Therefore, disruption of π-stacking by the formation of a **1**–C base pair is unfavorable for effective hole migration in DNA, and leads to the suppression of hole migration.

In conclusion, we have identified an artificial degenerate nucleobase **1** that can control long-range hole migration through an ODN by its unique hole-trapping capacity. The hole-trapping efficiency of **1** is superior to that of the GGG step and similar to that of ^{Z}G, regardless of the pyrimidine base that base-pairs with **1**. We also found that a hole can effectively migrate via a **1**–T base pair without decomposition of the GGG bridge, whereas the **1**–C base pair suppressed hole migration from $\mathbf{X_1}$ to $\mathbf{X_2}$. Consequently, **1** is a hole-trapping degenerate base, which can be incorporated at any site regardless of the AT or GC content, and can be used as a very effective tool for studying long-range hole migration in DNA containing diverse sequences.

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13. We also considered the electronic effect of the formation of a base pair with T or C on the oxidation of **1**. The HOMO energies of a Watson-Crick **1**–T base pair and a wobble **1**–C base pair, as drawn in Figure 4, were estimated as 4.54 and 4.57 eV, respectively, in the B3LYP/6-31G(d) calculation. The gap between **1**–T and **1**–C in HOMO energy was not wide enough to discuss any electronic effect of base-pairing on hole-trapping by **1**.
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