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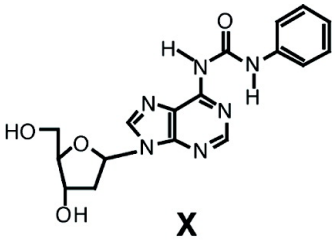

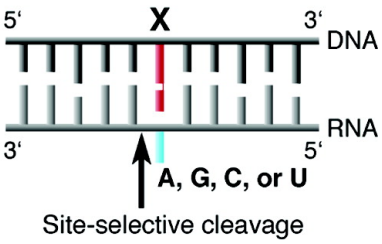

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Site-Selective RNA Cleavage by DNA Bearing a Base Pair-Mimic Nucleoside

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Site-selective RNA cleavage is important for biotechnology and therapy as well as biology. Natural ribozymes catalyze the site-selective RNA cleavage reaction by utilizing metal ions and nucleotide bases as catalysts, in which the nucleotide subject to be cleaved generally is located in an unpaired region involving the tertiary interactions.[1] However, the facts that formation of a correct tertiary structure is absolutely required for the activity and the sequence near the active site is strictly restricted limit the application of ribozymes and deoxyribozymes. It is suggested that RNA hydrolysis proceeds via an in-line attack mechanism, in which the hydrolysis is favored if the arrangement of the attacking 2′-hydroxyl group and the 5′-leaving oxygen atom adopt an apical orientation during the transesterification reaction, and it has also been indicated that a duplex backbone geometry prohibits formation of the in-line attack arrangement while the unpaired sites are preferentially hydrolyzed.[2] Moreover, although RNA cleavage can be achieved using artificial scissors, such as ion macrocycles, cationic amines, imidazole derivatives, and acridine derivatives,[3] it is also difficult to restrict the nucleotide to be cleaved because of a lack of rigidity in the nucleotide conformation and nonspecific hydrolysis by the scissors.

We have synthesized a novel phenylurea derivative of deoxyadenosine that tethers a phenyl group at N6 of deoxyadenosine by an amide linker (**X**) in Figure 1, which mimics the Watson−Crick A/T base pair. Our previous study revealed that the single **X** nucleoside at the 5′-dangling ends stabilized a DNA duplex as well as or better than the formation of an A/T Watson−Crick base pair.[4] Furthermore, when the **X** nucleoside was incorporated in the middle of a DNA sequence, **X** induced a conformational change of its opposite nucleotide.[5] Therefore, it is expected that the ribonucleotide opposite to the **X** nucleoside is prone to be cleaved if the conformation of the ribonucleotide is perturbed by **X**. Here, we show that the RNA strand cleavage exclusively occurs on the 3′-side of the ribonucleotide opposite to **X**, which can be used as a new tool for the site-selective RNA strand cleavage.

We prepared the RNA oligomers labeled by 6-carboxyl fluorescein (6-FAM) at the 5′-end. Each could hybridize with a DNA strand, forming RNA/DNA hybrid duplexes containing the A/W_2 pair, **X**/W_2 pair, or a single W_2 bulge (W_2 = A, G, C, or U) (Figure 1).[6] The melting temperature (T_m) value substantially differs depending on the types of the A/W_2 pair (63.0−50.1 °C) and the W_2 bulge (50.9−45.4 °C) (see Supporting Information Table S1).[7] In contrast, the T_m values of the duplexes containing **X** are relatively high and are similar to each other (53.2−52.4 °C). This observation suggests that the interaction in the duplexes containing the **X**/W_2 pair is similar regardless of the W_2 nucleotide.

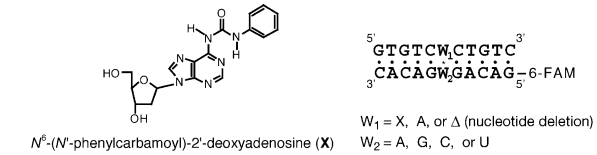

Figure 1. Chemical structure of X (left) and the nucleotide sequences used in this study (right).

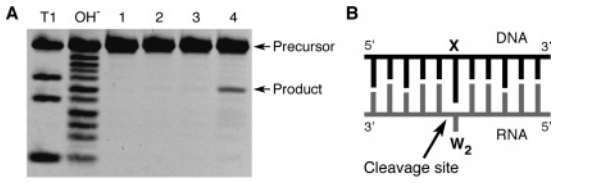

Figure 2. (A) Image of the electrophoresis of r(GACAGUGACAC)/d(GTGTC**X**CTGTC) in the 20% PAGE containing 7 M urea. T1, RNase T1 digestion; OH^-, alkaline hydrolysis; lane 1, no metal ion added; lane 2, 1 M NaCl; lane 3, 10 mM $Co(NH_3)_6Cl_3$; lane 4, 10 mM $MgCl_2$. The hydrolysis was carried out for 12 h at pH 8.0 at 37 °C. (B) Schematic representation of the site-specific cleavage of the RNA (gray) by the DNA strand containing **X** (black).

The electrophoresis in denaturing polyacrylamide gel (PAGE) was carried out to monitor RNA hydrolysis.[8] Figure 2A shows the PAGE image of RNA strand (W_2 = U) hybridized with the DNA strand bearing **X** in the absence and presence of a metal ion (1 M NaCl, 10 mM $Co(NH_3)_6Cl_3$, or 10 mM $MgCl_2$). A single product band in addition to the precursor band was observed in the presence of $MgCl_2$, while no product band was seen under other conditions. A comparison of the bands provided by the RNase T1 digestion and the alkaline hydrolysis confirmed that the product generated in the presence of $MgCl_2$ had the six-nucleotide length and corresponds to the scission on the 3′-side of the uracil opposite to **X** (Figure 2B). The observation that NaCl and $Co(NH_3)_6Cl_3$ did not promote the hydrolysis implies that the transesterification reaction is catalyzed by a metal hydroxide.[1,9]

Figure 3 demonstrates the sequence specificity of the RNA strand scission. Regardless of the W_2 nucleotide (A, G, C, or U), all the duplexes containing **X** demonstrated a single product band, corresponding to the scission on the 3′-side of the W_2, while the duplexes containing the A/W_2 pair showed no product band (Figure 3A). Importantly, the amounts of their cleaved products are similar (the difference was less than 2-fold),[8] suggesting that all the **X**/W_2 pairs adopt a similar conformation, consistent with the T_m data. Figure 3B indicates the influences of the adjacent base pairs of the **X**/U pair (T**X**T/AUA, C**X**C/GUG, G**X**G/CUC, and A**X**A/UUU as a trinucleotide representation). Although the efficiency of the hydrolysis of the duplex containing G**X**G/C**U**C was slightly greater (∼2-fold) than those of the other duplexes, all the duplexes showed the cleaved product. Intriguingly, the duplex containing T**X**T/AUA also generated the three-nucleotide-length product, which is another

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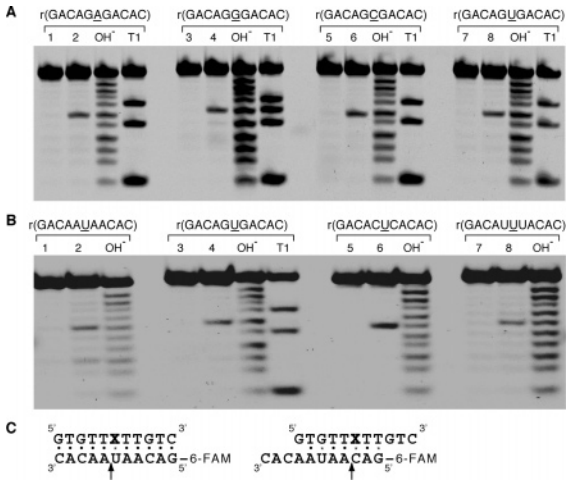

Figure 3. (A, B) PAGE images of the RNA strand hydrolysis in the presence of 10 mM $MgCl_2$. The reaction conditions were the same as those described in Figure 2. (A) r(GACAGAGACAC) (lanes 1 and 2), r(GACAGGGACAC) (lanes 3 and 4), r(GACAGCGACAC) (lanes 5 and 6), and r(GACAGUGACAC) (lanes 7 and 8) hybridized with d(GTGTCACTGTC) (lanes 1, 3, 5, and 7) or d(GTGTC**X**CTGTC) (lanes 2, 4, 6, and 8). (B) r(GACAAUAACAC) (lanes 1 and 2), r(GACAGUGACAC) (lanes 3 and 4), r(GACACUCACAC) (lanes 5 and 6), and r(GACAUUUACAC) (lanes 7 and 8) hybridized with the complementary DNA sequence containing A (lanes 1, 3, 5, and 7) or **X** (lanes 2, 4, 6, and 8) at the 6-position of the DNA strand. (C) Two patterns of the r(GACAAUAACAC) hydrolysis hybridized with d(GTGTT**X**TTGTC). The arrow indicates the cleavage site indicated in Figure 3B.

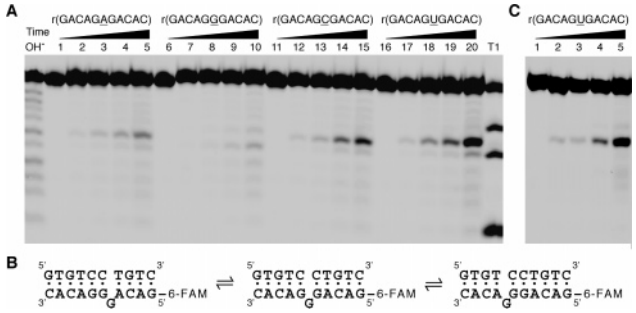

Figure 4. (A) PAGE images of the RNA hydrolysis hybridized with d(GTGTCCTGTC) forming the single W_2 bulge. The reaction was carried out at 37 °C in the presence of 10 mM $MgCl_2$ for 0, 12, 24, 48, and 72 h from left to right. (B) Possible migration of the G-bulge in the duplex of r(GACAGGGACAC)/d(GTGTCCTGTC). (C) PAGE images of the RNA hydrolysis hybridized with d(GTGTC**X**CTGTC) forming the **X**/U pair.

site able to be hybridized with the DNA strand (Figure 3C). This is consistent with the lower specificity of the ribonucleotide opposite **X** on the hydrolysis if the DNA strand can bind to an RNA.

On the other hand, the RNA/DNA duplexes containing a single W_2 bulge showed a lower amount of the six-nucleotide-length product and less site-selective cleavage (Figure 4A), as has been reported.[10] The main product of the G-bulge duplex was five nucleotides long, probably because the bulge nucleotide was the same as the neighboring nucleotides (Figure 4B). This is consistent with a slightly higher T_m (50.9 °C) than the other bulge duplexes (48.7−45.4 °C).[11] In contrast with the duplexes containing a single bulge, the exclusive scission on the 3′-side of the nucleotide opposite to **X** (Figure 4C) is suggestive of a high rigidity in the conformation.[12]

In conclusion, the adenosine derivative **X** incorporated in a DNA strand promoted the site-selective hydrolysis of the complementary RNA in the presence of $MgCl_2$. This observation suggests that the DNA strand containing **X** changes the conformation of the ribonucleotide opposite to **X**, probably because of the ability of the phenyl group stacking inside of the duplex accompanied by the W_2 base flipped in an extrahelical position (Figure 2B). The lack of pairing selectivity and the high T_m have the advantage of the site-selective base flipping in the target sequence and the site-specific RNA cleavage. Because the RNA cleavage, in which the sequence to be cleaved is not restricted, can be achieved with a short DNA sequence under mild conditions, the DNA strand incorporating the base-pair mimic nucleoside might be useful for the development of a "universal deoxyribozyme" to exclusively cleave a target RNA when the DNA strand can hybridize with the RNA sequence. Modifications at the aromatic hydrocarbon group and the amide linker may further expand the application of the base pair-mimic nucleosides, such as for probing the RNA hydrolysis mechanism, molecular biology, and antisense drugs.

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Supporting Information Available: UV melting curves of the duplexes, the PAGE image of the product band before and after the acid treatment, and the T_m values of the duplexes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) Preparations of the **X** and the oligonucleotides were previously described.[4]

(7) The T_m values were determined from the UV melting curve (Figure S1) as already described (e.g., Nakano, S.; Kanzaki, T.; Sugimoto, N. *J. Am. Chem. Soc.* **2004**, *126*, 1088−1095). The results that the RNA/DNA duplex containing the single A/G mismatch showed a greater stability than those containing the A/A and A/C mismatches (see Table S1) have also been reported in our previous article (Sugimoto, N.; Nakano, M.; Nakano, S. *Biochemistry* **2000**, *39*, 11270−11281).

(8) The mixture of RNA (10 μM) and DNA (20 μM) in a buffer containing 20 mM HEPES (pH 8.0) and 0.13 mM Na_2EDTA was incubated at 60 °C for 2 min prior to use. The reaction was initiated by adding the metal ion solution at 37 °C and stopped by adding three equivalent volumes of a solution containing 20 mM Na_2EDTA and 7 M urea. The electrophoresis was performed in a 20% polyacrylamide gel containing 7 M urea in a 1× TBE buffer. The fluorescence from 6-FAM was visualized by FUJIFILM FLA-5100. The amount of the product was the average of 2−3 independent trials.

(9) The acid treatment of the product confirmed the product containing a 2′,3′-cyclic phosphate group (Figure S2).

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(12) Because the **X** nucleoside is chemically inert to the hydrolysis reaction in contrast to the ion macrocycles, cationic amines, imidazole derivatives, and acridine derivatives,[3] nonspecific hydrolysis by the DNA strand bearing **X** is negligible.

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