# Noncovalent Combination of Oligoamine and Oligonucleotide as Totally Organic Site-selective RNA Cutter

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(Received May 6, 2004; CL-040508)

When a one-base gap was formed in substrate RNA by using two oligonucleotides, the phosphodiester linkage at the gap-site was activated and selectively hydrolyzed by ethylenediamine. This site-selective hydrolysis was further promoted by connecting two gap-forming oligonucleotides with 1,3-propanediol linker.

Development of artificial ribonucleases, which hydrolyze RNA site-selectively, has been a subject of growing interest.[1] In the past two decades, many approaches using metal ions and their complexes as catalysts have been proposed.[2] However, totally organic artificial ribonucleases are also attractive because of their biocompatibility and potential for gene-specific therapeutics.[3] In many cases, organic scissors were tethered to oligonucleotides to provide sequence-selectivity.[4] Alternatively, so-called "hot-spots" in tertiary structure of RNA were used as the target site.[5] However, the former strategy requires tedious organic synthesis, whereas the latter is only applicable to tRNA and other limited RNAs. This study presents a simple and convenient strategy for site-selective hydrolysis of versatile RNA substrates. By using two oligonucleotides, one-base gap is formed at the target site, and this designed "hot-spot" is selectively hydrolyzed by free ethylenediamine. The site-selective scission is further promoted by connecting these two oligonucleotide additives with covalent linkage.

The substrate $RNA_1$ and DNA additives in Figure 1 were prepared on an automated DNA synthesizer, purified by denaturing polyacrylamide gel electrophoresis (PAGE) and reversed-phase HPLC. The characterization was made by MALDI-TOF MS analysis and by HPLC determination of base composition on their digests by snake venom phosphodiesterase and alkaline phosphatase. Site-selective RNA hydrolysis by ethylenediamine was performed at pH 8.0 and 50 °C for 16 h, and analyzed by PAGE under denaturing conditions.

In the absence of DNA additives, $RNA_1$ (labeled with 6-carboxyfluorescein at 5′-end) was randomly hydrolyzed by ethylenediamine (lane 1 in Figure 2). The conversion for the cleavage of each phosphodiester linkage was ca. 0.9%. When 1:1 mixture of $DNA_{1a}$ and $DNA_{1b}$ was added to the system, however, the phosphodiester linkage in the 3′-side of U-19 was selectively and efficiently hydrolyzed (lane 3: the conversion = 4.8%). This scission is 5 times as fast as the scission in the absence of $DNA_{1a}/DNA_{1b}$ (lane 1). In the heteroduplex $RNA_1/DNA_{1a}/DNA_{1b}$, only U-19 remains unpaired and this one-base gap is the "hot-spot" for the scission. Consistently, the 5′-side of U-19 was also hydrolyzed to some extent.[6] On the other hand, no site-selective scission occurred when $DNA_{1b}$ in $DNA_{1a}/DNA_{1b}$ was replaced by $DNA_{1c}$ and a nick was introduced to the 5′-side of U-19 (lane 4). Also no scission was observed when $DNA_{1a}$ and $DNA_{1c}$ were directly connected. Nonpairing nucleotide in RNA is essential for the present site-selective hydrolysis.

Significantly, the site-selective RNA scission was 1.5-fold more efficient when $DNA_{4s}$ was used as a cofactor (lane 2).

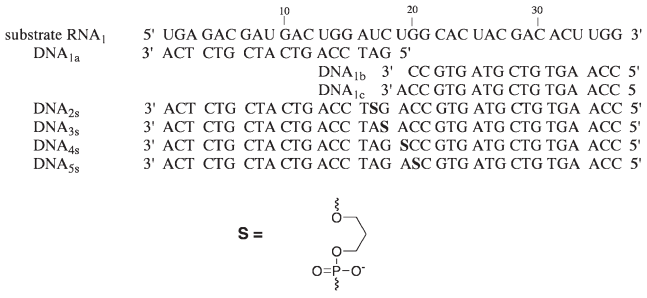


**Figure 1.** Substrate RNA and DNA oligomers used in the present study.

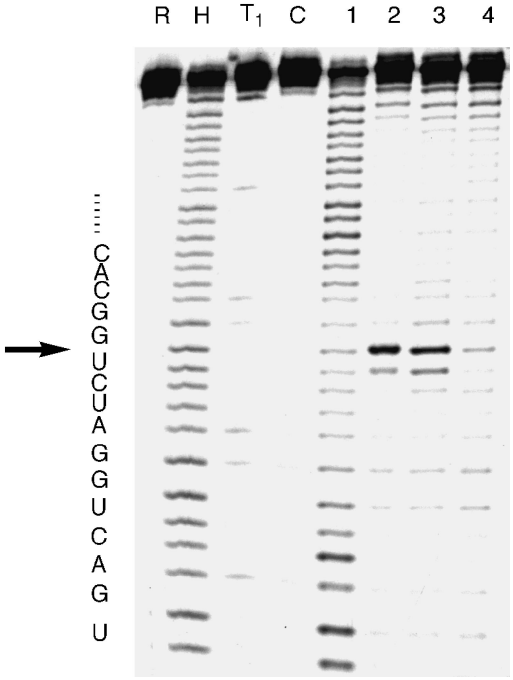


**Figure 2.** Site-selective RNA scission by combination of various DNA additives and free ethylenediamine. Lane 1, ethylenediamine only; lane 2, $DNA_{4s}$/ethylenediamine; lane 3, $DNA_{1a}/DNA_{1b}$/ethylenediamine; lane 4, $DNA_{1a}/DNA_{1c}$/ethylenediamine. At pH 8.0 and 50 °C for 16 h. $[RNA_1] = 5\,\mu mol\,dm^{-3}$; $[DNA] = 10\,\mu mol\,dm^{-3}$; $[ethylenediamine] = 100\,mmol\,dm^{-3}$; $[NaCl] = 200\,mmol\,dm^{-3}$. R, $RNA_1$ only; H, alkaline hydrolysis; $T_1$, RNase $T_1$ digestion; C, $DNA_{4s}$ without ethylenediamine. The fluorescence of 6-carboxyfluorescein (>520 nm, excited at 473 nm) was detected.

The pseudo-first-order rate constant for the scission was $3.0 \times 10^{-3}\,h^{-1}$. In this DNA additive, $DNA_{1a}$ and $DNA_{1b}$ are connected by 1,3-propanediol linker, and thus U-19 is located in front of this linker in the heteroduplex. The RNA scission selectively occurred at this site. Advantageously, non-selective scission is significantly suppressed in this one-additive system, compared with the $DNA_{1a}/DNA_{1b}$ system in lane 3. This promotion in selectivity is ascribed to the increased stability of the heteroduplex which minimizes the amount of single-stranded $RNA_1$ and prevents its undesired nonselective scission (the $T_m$ of the $RNA_1/DNA_{4s}$ is 72 °C, whereas that of the $RNA_1/DNA_{1a}/DNA_{1b}$ is 62 °C).[7]

In Figure 3, $DNA_{2s}$, $DNA_{3s}$, and $DNA_{5s}$ were used in place of $DNA_{4s}$, and the position of 1,3-propanediol linker was systematically changed. In all cases, the $RNA_1$ was selectively hydrolyzed at the 3′-side of the nucleotide which is located just in front of the linker. Accordingly, the scission site of the present system can be freely modulated by using appropriate DNA additive. The scission efficiency was not much dependent on the sequence, although ribozymes crucially require specific sequence at the target site and show the scission only there.

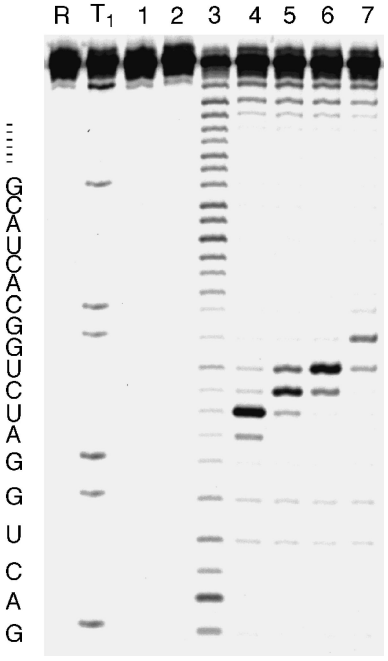


**Figure 3.** Site-selective RNA scission by ethylenediamine at various sites. Lane 1, control reaction in buffer solution; lane 2, $DNA_{4s}$ without ethylenediamine; lane 3, ethylenediamine only; lane 4, $DNA_{2s}$/ethylenediamine; lane 5, $DNA_{3s}$/ethylenediamine; lane 6, $DNA_{4s}$/ethylenediamine; lane 7, $DNA_{5s}$/ethylenediamine; R, $RNA_1$ only; $T_1$, RNase $T_1$ digestion. The reaction conditions are the same as in Figure 2.

In contrast with the remarkable catalysis by ethylenediamine, lanthanide ions were very poor for the present site-selective scission, although they are far more active for the hydrolysis of single-stranded RNA.[8] Assumedly, the rigid structure of dinuclear lanthanide cluster (the active species for RNA hydrolysis)[1c] is unfavorable for intramolecular acid–base cooperation when the RNA hydrolysis occurs in sterically restricted reaction field (the gap-site). Thus, use of more flexible ethylenediamine is essential. As the scissors, 1,2-diaminopropane and diethylenetriamine are also applicable, but they are less effective than ethylenediamine. The possibility that contaminating metal ions, if any, are responsible for the present scission is ruled out, since EDTA ($\leq$10 mmol $dm^{-3}$) caused no deterioration of hydrolysis (data not shown).

In conclusion, site-selective RNA scission is achieved by forming one-base gap at the target site and using ethylenediamine as catalyst. This site-selective scission is further promoted by connecting the gap-forming oligonucleotides with the use of 1,3-propanediol linker. These "totally organic" ribozyme mimics should be useful for various applications.

This work was supported by Bio-oriented Technology Research Advancement Institution. A Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, is also acknowledged.

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6 The linkages at U-13 and G-14 are intrinsically more susceptible to hydrolysis than the other linkages in DNA/RNA duplex probably because of slight difference in duplex structure there.

7 In both systems, only one melting point was observed.

8 Site-selective RNA hydrolysis by lanthanide ions is efficient, only when acridine is attached to DNA additive and placed in front of the gap-site (see Ref. 2d).

Published on the web (Advance View) July 12, 2004; DOI 10.1246/cl.2004.1012