Sequence-Selective RNA Scission by Non-Covalent Combination of Acridine-Tethered DNA and Lanthanide(III) Ion

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Binary non-covalent systems for site-selective RNA scission are prepared from Lu(III) ion and a DNA bearing an acridine in the internal position. On the formation of DNA/RNA hetero-duplex, the target phosphodiester linkage is activated and preferentially hydrolyzed.

Interests in sequence-selective RNA hydrolysis have been rapidly growing.[1] The most common strategy is to tether chemical scissors to DNA oligomers which are complementary with a part of the substrate RNA.[2] However, non-covalent systems, which are easily obtainable without complicated organic synthesis, are also important.[3] In a previous paper,[4] ternary non-covalent systems for selective RNA-scission were prepared from two DNAs and lanthanide(III) ion. The selective scission occurred at the ribonucleotides which were not forming Watson–Crick base pairs with the two DNAs. Furthermore, the scission efficiency was promoted by tethering intercalators to the ends of these two DNAs.[5] These findings have spurred us to study on still simpler binary systems. In this paper, sequence-selective RNA scission is achieved by non-covalent combinations of lanthanide(III) ion and one DNA bearing an acridine in its internal position. The target phosphodiester linkage is activated by the modified DNA, and preferentially hydrolyzed.

The substrate RNA and the DNAs used in this study are shown in Figure 1. Acridine residues were incorporated into desired sites in DNAs by using the standard phosphoramidite chemistry. All the products were fully characterized by MALDI-TOF MS. RNA cleavages were performed at pH 8.0 (10 mmol dm^{-3} Tris buffer) and 37 °C, and analyzed by polyacrylamide-gel electrophoresis under denaturing conditions.

As shown in Figure 2 (lane 6), the substrate RNA was site-selectively and efficiently cleaved by the DNA_1–Acr/Lu(III) binary combination. This modified DNA is complementary with the RNA, except that the dA in the opposite side of U-19 is replaced with an acridine-bearing moiety (X). The selective scission-sites are the 3'-sides of C-18 and U-19 (the former is the major one).[6]

With the use of unmodified DNA (DNA_1), however, RNA scission is far weaker (lane 3). Here, U-19 is not base-pairing with DNA_1, and a bulge structure is formed at this site. The scission is also marginal, when a trimethylene spacer is inserted to the corresponding place of DNA_1 (DNA_1–S) (lane 4). These results show that the target phosphodiester linkage in the substrate RNA is notably activated by the acridine residue in the DNA_1–Acr. Thus, site-selective scission is successful, even when the Lu(III) ion is not fixed near them as is the case in the ribozyme mimics previously reported.[2]

The RNA was also site-selectively hydrolyzed by the DNA_2–Acr/DNA_3/Lu(III) ternary system (lane 5), where DNA_1–Acr is divided into two moieties (DNA_2–Acr and DNA_3: see Figure 1).[7,8] However, the DNA_1–Acr/Lu(III) binary system is still more (about 20%) active than this ternary system.[9]

The absorption spectra for the acridine moiety in the DNA_1–Acr was not much changed by the addition of Lu(III) ion (data not shown). Thus, the interaction between Lu(III) ion and the acridine, if any, is marginal. It is presumed that the main role of the acridine for the present selective scission is to perturb the conformation of the RNA at the target site. The 2'-OH is brought closer to the P-atom of the scissile phosphodiester and the nucleophilic attack is facilitated. These sufficiently active, simple, and easily handled tools should be promising for various applications.[10] These attempts are currently under way in our laboratory.

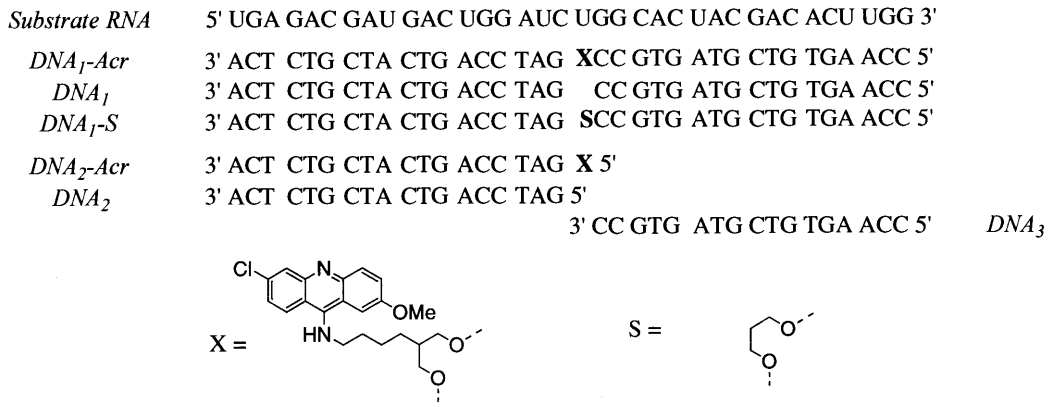

Figure 1. Structures of the substrate RNA and the DNA oligomers used in the present study.

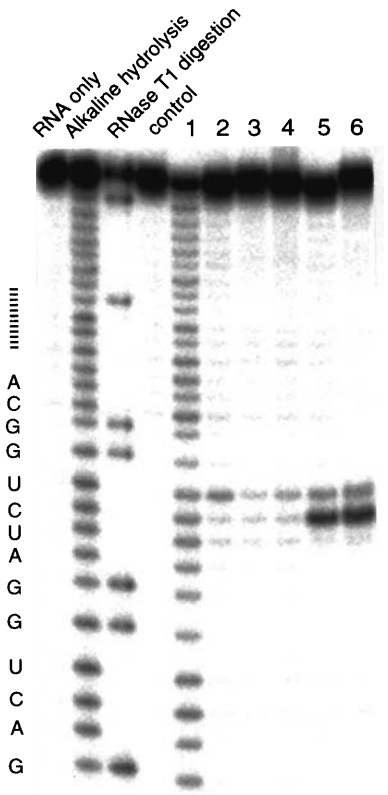

Figure 2. RNA scission by the combinations of various DNAs and Lu(III). Lane 1, Lu(III) only; lane 2, DNA_2/DNA_3/Lu(III); lane 3, DNA_1/Lu(III); lane 4, DNA_1-S/Lu(III); lane 5, DNA_2-Acr/DNA_3/Lu(III); lane 6, DNA_1-Acr/Lu(III). At pH 8.0 and 37 °C for 2 h; $[RNA]_0$ = 0.1, [each of modified or unmodified DNAs]$_0$ = 10 and $[LuCl_3]_0$ = 100 μmol dm^{-3}; $[NaCl]_0$ = 200 mmol dm^{-3}.

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References and Notes

1 a) B. N. Trawick, A. T. Daniher, and J. K. Bashkin, *Chem. Rev.*, **98**, 939 (1998). b) M. Oivanen, S. Kuusela, and H. Lönnberg, *Chem. Rev.*, **98**, 961 (1998). c) M. Komiyama, N. Takeda, and H. Shigekawa, *Chem. Commun.*, **1999**, 1443.

2 a) K. Matsumura, M. Endo, and M. Komiyama, *J. Chem. Soc., Chem. Commun.*, **1994**, 2019. b) M. Komiyama, T. Inokawa, and K. Yoshinari, *J. Chem. Soc., Chem. Commun.*, **1995**, 77. c) J. Hovinen, A. Guzaev, E. Azhayeva, A. Azhayev, and H. Lönnberg, *J. Org. Chem.*, **60**, 2205 (1995). d) M. A. Reynolds, T. A. Beck, P. B. Say, D. A. Schwartz, B. P. Dwyer, W. J. Daily, M. M. Vaghefi, M. D. Metzler, R. E. Klem, and L. J. Arnold, Jr., *Nucleic Acids Res.*, **24**, 760 (1996). e) J. Hall, D. Hüsken, and R. Häner, *Nucleosides Nucleotides*, **16**, 1357 (1997). f) D. Magda, M. Wright, S. Crofts, A. Lin, and J. L. Sessler, *J. Am. Chem. Soc.*, **119**, 6947 (1997). g) S. Matsuda, A. Ishikubo, A. Kuzuya, M. Yashiro, and M. Komiyama, *Angew. Chem., Int. Ed.*, **37**, 3284 (1998). h) W. C. Putnam and J. K. Bashkin, *Chem. Commun.*, **2000**, 767.

3 A pioneering work on non-covalent systems for site-selective RNA hydrolysis was reported: D. Hüsken, G. Goodall, M. J. J. Blommers, W. Jahnke, J. Hall, R. Häner, and H. E. Moser, *Biochemistry*, **35**, 16591 (1996).

4 A. Kuzuya, M. Akai, and M. Komiyama, *Chem. Lett.*, **1999**, 1035.

5 A. Kuzuya and M. Komiyama, *Polymer Preprints, Japan*, **49**, 981 (2000).

6 At the reaction time 2 h, the conversions for the scission at C-18 and U-19 were 12 and 3 mol%, respectively.

7 The scission sites by this ternary system are also C-18 and U-19.

8 When unmodified DNA (DNA_2) was used in place of DNA_2–Acr, the scission was much less efficient (lane 2). An essential role of the acridine is also evident in the binary systems.

9 This system is one of the most active site-selective RNA-cleaving agents that ever reported (ref 5).

10 By promoting the activating effect still more, longer RNA should be able to be hydrolyzed at the target site.