New Ribozyme-Mimics Employing Mg(II) Ion As Catalytic Center

Akinori Kuzuya, Ryo Mizoguchi, and Makoto Komiyama*
Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904

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By using two oligonucleotides as cofactors, Mg(II) ion selectively and efficiently hydrolyzed RNA at the target site under physiological conditions. The site-selective hydrolysis was further accelerated by introducing an acridine to oligonucleotides.

RNA enzymes (ribozymes) were discovered by Cech et al. and have opened a new era in RNA chemistry.[1] Many studies towards practical applications are currently under way. In addition to these "natural" ribozymes, artificial ribonucleases for site-selective scission were synthesized by tethering a catalyst to oligonucleotide which is complementary with a part of substrate RNA.[2] They can be easily modified and provided with desired functions. However, there still remains a big gap between "natural" ribozymes and their mimics. One of the greatest differences is the fact that the mimics require highly active catalysts (e.g., lanthanide(III) ions[3] and Cu(II) ion[4]) whereas ribozymes usually employ Mg(II) ion as catalytic center. Preparation of the mimics involving Mg(II) ion has been hardly successful, mainly because this metal ion is intrinsically poor in the activity and furthermore few Mg(II) complexes are satisfactorily stable in water. For versatile applications either in vitro or in vivo, Mg(II) ion, which is abundant in cells, is undoubtedly preferable to other metal ions.

Recently,[5] the authors proposed a new type of ribozyme-mimic, which is composed of lanthanide ions and two oligonucleotides. The oligonucleotides form hetero-duplexes with substrate RNA, but only one ribonucleotide in the RNA is left free from base-pairing. Under these conditions, the scission by lanthanide ions selectively occurs at the non-pairing ribonucleotide, even though the metal ions are never covalently bound anywhere. This finding prompted us to study on the possibility of using Mg(II) ion in this non-covalent ribozyme-mimic, since the metal ion remains free from strong ligands and thus can be sufficiently active without notable loss of its activity. In this paper, we present Mg(II)-dependent ribozyme-mimics which efficiently hydrolyze RNA at the desired site under physiological conditions.

The substrate RNA and the oligonucleotides in Figure 1 were prepared by using standard phosphoramidite chemistry.[6] The RNA scission was analyzed by polyacrylamide gel electrophoresis under denaturing conditions. The typical results are presented in Figure 2. The concentration of $Mg(NO_3)_2$ is 0.32 mol dm^{-3} at 37 °C and pH 8.0 (the reaction time is 16 h).[7] As shown in lane 3, site-selective scission has been successfully achieved by using two oligonucleotides (DNA_1 and DNA_2). Here, all the ribonucleotides of the RNA, except for U-19 (the underlined one), are forming Watson–Crick base-pairs with either of these two oligonucleotides (see Figure 1). The scission selectively occurs at the 3'-side of this unpaired ribonucleotide.[8] The conversion for the scission is around 10 mol%. Thus, the present site-selective scission is reasonably efficient. As expected, the scission fragment co-migrates with the fragment obtained by alkaline hydrolysis, confirming the hydrolytic nature of the scission. To our best knowledge, this is the first Mg(II)-dependent ribozyme-mimic which satisfactorily works under physiological conditions.[9]

The site-selective RNA scission by Mg(II) is also successful by using DNA_3-S, in which DNA_1 and DNA_2 are connected by a trimethylene linker (lane 4). The scission rate is almost the same as that with the DNA_1/DNA_2/Mg(II) system. In the absence of the DNAs, however, the RNA is hydrolyzed without any specificity (lane 2). The scission at U-19 is virtually nil. Apparently, both the DNA_1/DNA_2 system and DNA_3-S activate the target phosphodiester linkage in the RNA, and promote its

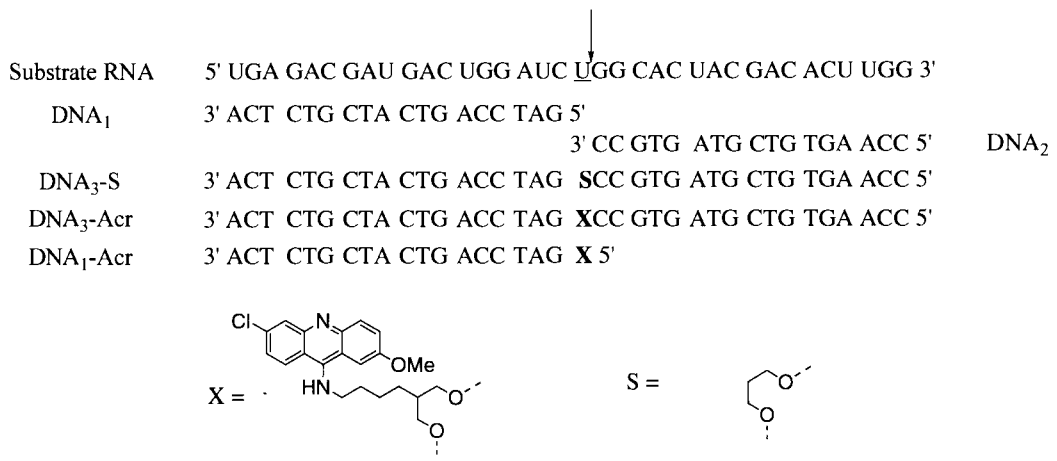

Figure 1. Structures of the substrate RNA and the oligonucleotides used in the present study. The selective scission-site is shown by the arrow.

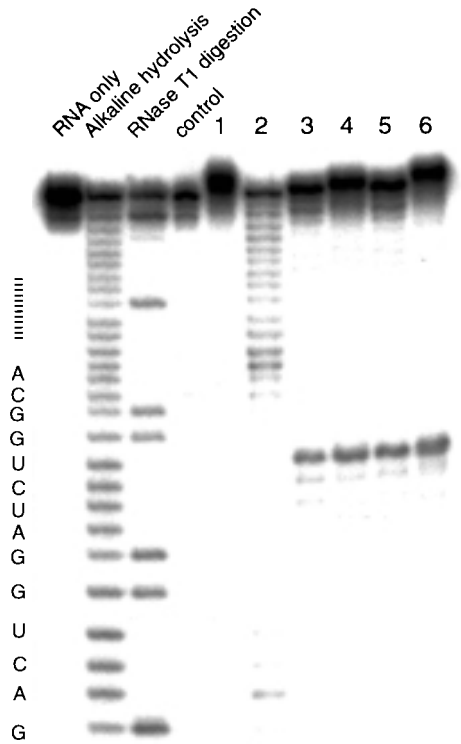

Figure 2. RNA scission by the combinations of various DNAs and Mg(II). Lane 1, DNA_3-Acr only; lane 2, Mg(II) only; lane 3, DNA_1/DNA_2/Mg(II); lane 4, DNA_3-S/Mg(II); lane 5, DNA_1-Acr/DNA_2/Mg(II); lane 6, DNA_3-Acr/Mg(II). At pH 8.0 and 37 °C for 16 h; $[RNA]_0$ = 1 and [each of modified or unmodified DNAs]$_0$ = 10 μmol dm^{-3}; $[Mg(NO_3)_2]_0$ = 0.32 mol dm^{-3}.

hydrolysis by Mg(II).

The site-selective scission has been further promoted by modifying the oligonucleotides. For example, an acridine residue is introduced to the middle of the trimethylene linker in DNA_3-S (DNA_3-Acr in Figure 1).[10] The site-selective scission by this modified DNA with Mg(II) (lane 6) is about 1.5 times as fast as that by the DNA_3-S/Mg(II) system (also that by the DNA_1/DNA_2/Mg(II) system), according to the densitometric analysis. The selectivity remains satisfactorily high. When an acridine is bound to the 5'-end of DNA_1 and this modified oligonucleotide (DNA_1-Acr) is combined with DNA_2, however, the scission is almost as fast as that by the DNA_1/DNA_2 combination (compare lane 5 with lane 3). Restraint of the molecular movement of the acridine is necessary for the rate acceleration.

In conclusion, Mg(II)-dependent ribozyme-mimics have been prepared by using modified or unmodified oligonucleotide(s) as cofactor. The activity should be improved still more by appropriate modification. These attempts are currently under way in our laboratory.

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6 All the products were purified by reversed-phase HPLC, and fully characterized by MALDI-TOF MS.
7 $MgCl_2$ is also applicable, but $Mg(NO_3)_2$ is 1.7 times as active as $MgCl_2$.
8 When neighboring C-18 is made unpaired by appropriate DNAs, the 3'-side of C-18 is cleaved. Scission rate increases with increase in $[Mg(II)]_0$ (data not shown).
9 RNA scission by Mg(II) ion at bulge-site was previously reported: D. Hüsken, G. Goodall, M. J. J. Blommers, W. Jahnke, J. Hall, R. Häner and H. E. Moser, *Biochemistry*, **35**, 16591 (1996). However, the reaction temperature was 60 ˚C, which was far higher than that (37 °C) employed here. No data on the scission rate were presented there.
10 The combination of this modified DNA with Lu(III) ion site-selectively hydrolyzed RNA: A. Kuzuya, and M. Komiyama, *Chem. Lett.*, **2000**, 1378.