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Lanthanide ions as versatile catalyst in biochemistry: Efficient site-selective scission of RNA by free lanthanide ions

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Abstract

Novel systems for site-selective RNA scission are prepared by combining lanthanide(III) ions with oligonucleotides bearing an acridine. The modified oligonucleotide forms a heteroduplex with the substrate RNA, and selectively activates the phosphodiester linkages in front of the acridine. As a result, these linkages are preferentially hydrolyzed over the others, even though the lanthanide(III) ions are not fixed anywhere. The scission is efficient under physiological conditions, irrespective of the sequence at the target site. Effect of the kind of the lanthanide on the scission is emphasized.

Keywords: Polymers

1. Introduction

Ribonucleic acid (RNA), which mediates genetic information encoded in DNA, is one of the most important components in life. If only one RNA can be chosen from many other RNAs in cells and selectively cleaved at a desired site, it opens the way to new RNA science (regulation of expression of a specific gene, advanced therapy, RNA manipulation, and others). However, none of naturally occurring ribonucleases shows such high sequence selectivity. Accordingly, development of site-selective artificial ribonucleases is necessary [1].

In 1992, we have discovered that lanthanide(III) ions, especially the last three of the series (Tm(III), Yb(III), and Lu(III)), are enormously active for RNA scission [2]. Under physiological conditions, they cleave dinucleotides in less than 30 min. According to the dependence of scission rate on pH and metal concentration, the active species for Lu(III) ion is the dinuclear $[Lu_2(OH)_2]^{4+}$ [3]. Although lanthanide(III) ions are the best candidate for catalytic center of artificial ribonuclease, the ions themselves have no sequence selectivity. Thus, these ions randomly cleave RNA unless they are aided by some sequence-recognizing moieties [4].

In almost all the artificial ribonucleases hitherto reported, the catalyst for RNA hydrolysis was tethered to oligonucleotide which bind the substrate RNA near the target site (Fig. 1a). For example, an artificial ribonuclease employing Lu(III) as catalytic center was constructed by tethering its iminodiacetate complex to DNA [5]. Their site-selectivity originates primarily from favorable activation–entropy term for the hydrolysis of target phosphodiester linkage.

In this paper, we report an entirely new strategy, which involves no covalent fixation of lanthanide(III) ion to oligonucleotide (Fig. 1b). There, the target phosphodiester linkage in substrate RNA is activated through non-covalent interactions with oligonucleotide bearing an acridine, and differentiated from the other linkages in terms of reactivity. Thus, this linkage is selectively hydrolyzed even when lanthanide(III) ions are not fixed anywhere. The site-selective scission is highly efficient, mainly because these metal ions are free from complexation with strong ligands.

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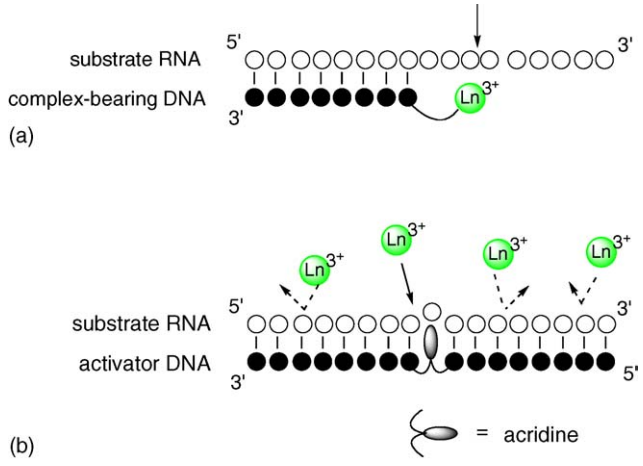

Fig. 1. Schematic representations of the two strategies for site-selective RNA scission: (a) fixation of lanthanide(III) complex to oligonucleotides; (b) site-selective activation of RNA by oligonucleotide bearing acridine. The selective-scission sites are indicated by arrows.

2. Experimental procedures

2.1. Preparation of oligonucleotides

All the oligonucleotides used in this study were synthesized on an automated DNA synthesizer using standard phosphoramidite chemistry. After removal from the support and deprotection, crude oligonucleotides were purified by 20% denaturing polyacrylamide gel electrophoresis (PAGE), and then by a reversed-phase HPLC equipped with an RP-C18 column. All the oligonucleotides were completely characterized by MALDI/TOF-MS.

2.2. RNA cleavage assay

The substrate RNA (5′-end ^{32}P-labeled) and the corresponding complementary oligonucleotide bearing an acridine (1 and 10 μM final concentrations, respectively) were dissolved in 10 mM Tris–HCl buffer (pH 8.0) containing NaCl (200 mM). The mixture was heated to 90 °C (for 1 min), and slowly cooled to room temperature. Then 1/10 volume of aqueous solution of lanthanide(III) chloride was added to the mixture (100 μM final concentration). The lanthanide(III) chlorides were commercially obtained. After 2 h at 37 °C under dark, the reaction was quenched by 100 mM EDTA–2Na solution and analyzed on 20% denaturing PAGE. Imaging and quantification of RNA cleavage were carried out on a Fuji Film FLA-3000G fluorescent imaging analyzer.

3. Results

3.1. Molecular design of novel artificial ribonuclease

The novel sequence-selective artificial ribonucleases reported here are composed of an RNA activator (chemically modified oligonucleotide) and lanthanide(III) ions for RNA scission. The RNA activator is an oligonucleotide which has an acridine moiety in the middle (DNA-Acr in Fig. 2). When the substrate RNA forms a heteroduplex with the oligonucleotide in DNA-Acr, most of the ribonucleotides in the RNA form Watson–Crick base pairs with the counterpart nucleotides. However, only the ribonucleotide U-19, which is located in front of the acridine, remains unpaired. As a result, the phosphodiester linkages adjacent to this unpaired ribonucleotide are selectively activated, and preferentially hydrolyzed by lanthanide(III) ions over the other linkages.

3.2. Site-selective cleavage of RNA by La(III) and Lu(III) ions

Fig. 3 shows a typical PAGE pattern of the present site-selective RNA scission. When $LaCl_3$ and $LuCl_3$ are added to the system, site-selective RNA scission is successfully accomplished (lanes 3 and 6). The scission occurs overwhelmingly at the 5′-side of the unpaired ribonucleotide U-19 or its 3′-side (Fig. 2). After 2 h at pH 8.0 and 37 °C, the total conversions of the selective scission are 23 and 17% for La(III) and Lu(III), respectively ([$LnCl_3$] = 100 μM). The site-selective scission is also effective at pH 7.0, and the conversion of RNA cleavage by Lu(III) is 29%. With the use of DNA-S, in which simple 1,3-propanediol linker

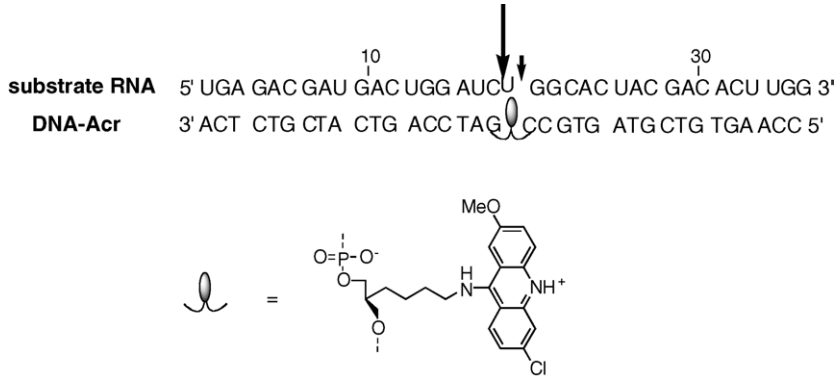

Fig. 2. Structures of the oligonucleotides used in this study. The selective-scission sites are indicated by arrows.

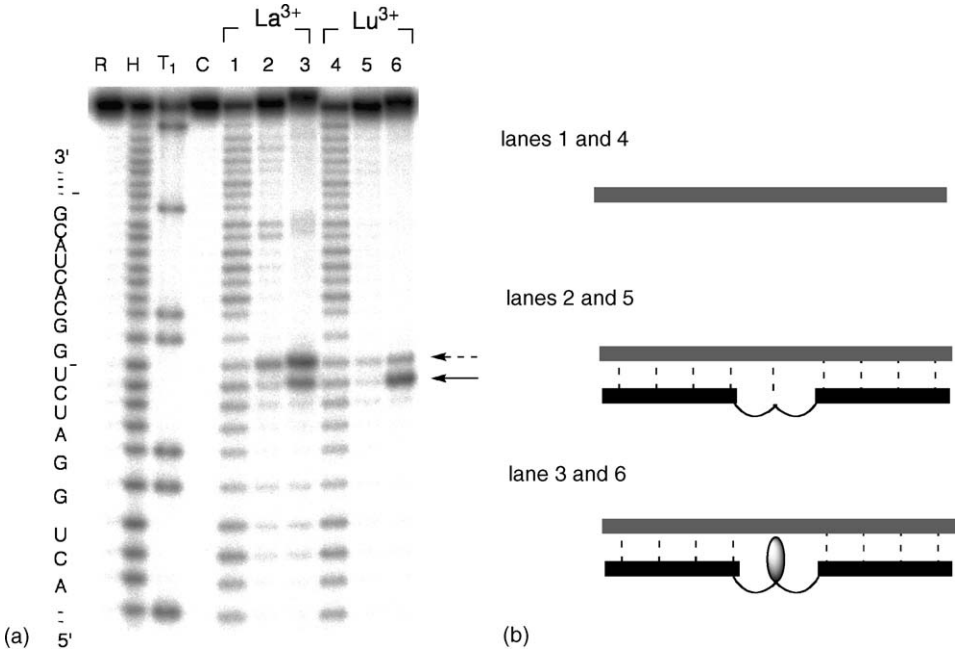

Fig. 3. Site-selective RNA scission by combinations of RNA activators and lanthanide(III) ion. (a) A typical PAGE pattern of the scission. Lane 1, La(III) only; lane 2, DNA-S/La(III); lane 3, DNA-Acr/La(III); lane 4, Lu(III) only; lane 5, DNA-S/Lu(III); lane 6, DNA-Acr/Lu(III). At pH 8.0 and 37 °C for 2 h; $[RNA]_0 = 1$, [DNA-S] = [DNA-Acr] = 10, and $[LnCl_3] = 100\ \mu M$; [NaCl] = 200 mM. R, RNA only; H, alkaline hydrolysis; T_1, RNase T_1 digestion; C, control reaction in buffer solution. (b) Schematic representations of the oligonucleotides used in part a.

is introduced and has no acridine, however, the scission is much slower (lanes 2 and 5). The ratio of the scission rate by DNA-Acr to the rate by DNA-S, which represents the magnitude of acceleration by the acridine, is far greater than 1. For example, the scission at the 5′-side by the DNA-Acr/Lu(III) system is 14 times as fast as that by the DNA-S/Lu(III) system. Apparently, the target phosphodiester linkages are selectively and efficiently activated by the acridine in DNA-Acr. In contrast, the other phosphodiester linkages are deactivated when the heteroduplex is formed. Because of the cooperation of these two factors, the discrimination of the target linkage from the others is sufficiently clear-cut. In the absence of oligonucleotide, both the ions cut the RNA almost randomly (lanes 1 and 4).

When the position of acridine in the DNA/RNA heteroduplex is shifted by using appropriate oligonucleotide, the selective-scission site is accordingly altered (data not shown). The scission efficiency is not much different from each other. Thus, the present site-selective scission is successful irrespective of the sequence at the scission-site. This is one of the most important advantages of the present non-covalent systems.

3.3. Sequence-selective RNA scission by other lanthanide(III) ions

Other lanthanide(III) ions of the series are also applicable to the catalyst. Interestingly, whether the scission dominantly occurs at the 5′-side of the unpaired ribonucleotide or its 3′-side is strongly dependent on the kind of lanthanide ion used (Fig. 4). As the atomic number in the lanthanide series

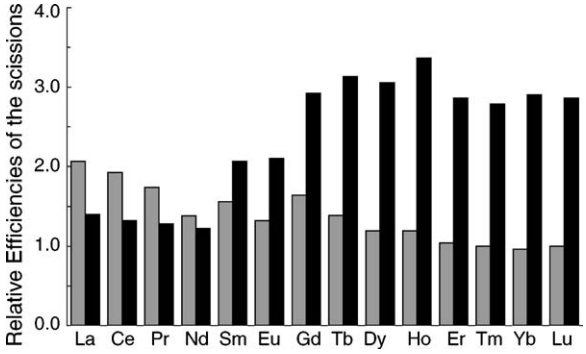

Fig. 4. Relative efficiencies of the site-selective scission by the combinations of DNA-Acr and various lanthanide ions. Shaded bars are for the 3′-side scission and filled ones are for the 5′-side scission. The efficiency for the total scission by Lu(III) is taken as unity.

increases, the scission at the 5′-side is gradually promoted (the filled bars). On the other hand, the scission at the 3′-side is suppressed (the shaded ones). As the result, the 5′/3′ ratio for each of the lanthanide ions monotonously increases with increasing atomic number. Heavy rare-earth metal ions preferentially hydrolyze the 5′-side, whereas light rare-earth metals choose the 3′-side. Thus, the scission site can be freely modulated by using appropriate lanthanide ion.

4. Conclusions

Novel sequence-selective artificial ribonucleases are constructed by combining modified oligonucleotide with

lanthanide(III) ions as catalyst. The target phosphodiester linkages are selectively activated by non-covalent interactions with the oligonucleotide, whereas the others are deactivated on the formation of the heteroduplex. Thus, the site-selective scission is successfully accomplished, although the catalysts are never bound to any sequence-recognizing moiety. When necessary, various chemical and physiological functions can be provided by appropriate chemical modification. Both the scission rates and selectivities can be further improved, leading to versatile applications in various research fields.

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