Non-Covalent Combinations of Lanthanide(III) Ion and Two DNA Oligomers for Sequence-Selective RNA Scission

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By using non-covalent combinations of Lu(III) ion and two DNA oligomers, each of which is complementary with a part of RNA, the RNA is sequence-selectively hydrolyzed. When all the ribonucleotides, except for three consecutive ones, form base-pairs with the DNA oligomers, the selective scission occurs at the 3'-side of the middle of these three non-pairing ribonucleotides.

Non-enzymatic scission of RNA has been attracting interests due to promising applications *in vivo* and *in vitro*. To date, a number of catalysts for the purpose were reported.[1] The lanthanide ions and their complexes are especially active.[2,3] Furthermore, sequence-selective artificial ribonucleases were obtained by tethering these chemical scissors to DNA oligomers as sequence-recognizing moieties.[4] They are useful for future biotechnology. However, preparation of these artificial enzymes (covalent attachment of the scissors to DNA oligomers) is often time-consuming, and imposes some limitations to the scope of their practical applications. Still simpler tools are desirable.

A few years ago, Hüsken *et al.* reported an interesting non-covalent system for somewhat selective RNA scission.[5] A bulge-structured RNA was formed by using an appropriate DNA oligomer. When this RNA/DNA composite was treated with lanthanide ions, the RNA was preferentially cut around the bulge-site. A new methodology for selective RNA scission was presented. However, the selectivity achieved there was not sufficiently high (several phosphodiester linkages near the bulge were cleaved).

In this communication, we show that RNA is site-selectively hydrolyzed by simply mixing lanthanide(III) ion and two DNA oligomers. These DNAs are complementary with parts of the RNA, so that predetermined ribonucleotides are left free from base-pairing (no bulge-structure is formed). Site-selective scission is successfully accomplished, when the number of non-pairing ribonucleotides is carefully controlled. No specific organic synthesis is required.

The RNA and the DNA oligomers, used in the present study, are shown in Figure 1. In the absence of the DNA oligomers, the RNA hydrolysis by the Lu(III) ion randomly took place throughout the RNA chain (lane 5 in Figure 2). When either of DNA-1 and DNA-2 was added to the solution, however, only the single-stranded portion of the RNA was hydrolyzed (lanes 7 and 8). Apparently, single-stranded RNA is overwhelmingly more reactive than is the RNA in RNA/DNA duplex.[6] Consistently, the Lu(III)-induced RNA scission was almost completely inhibited by the 1:1 mixture of DNA-1 and DNA-2 which forms base-pairs with all the ribonucleotides in the RNA (lane 6).

These findings were extended to the site-selective RNA scission in Figure 3. When the RNA was treated with Lu(III) ion in the presence of the 1:1 mixture of DNA-1 and DNA-5, highly site-selective RNA scission was successfully achieved (lane 9). Here, three consecutive ribonucleotides

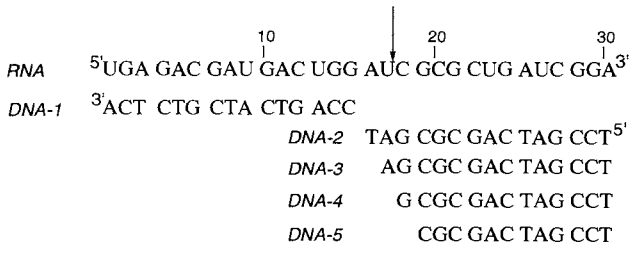

Figure 1. The structures of the substrate RNA and the DNA oligomers used in the present study. The site of selective scission by the Lu(III)/DNA-1/DNA-5 system is shown by the arrow.

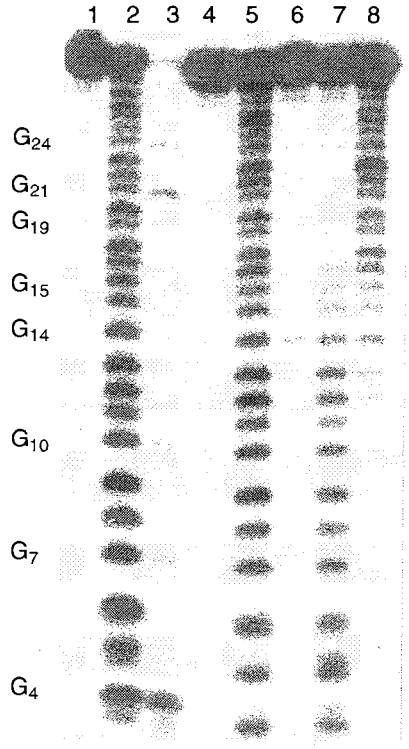

Figure 2. The scission of single-stranded and double-stranded RNA (^{32}P-labelled at the 5'-end) by the Lu(III) ion at pH 8.0 (10 mmol dm^{-3} Tris buffer) and 37 °C for 4 h. Lane 1, RNA only; lane 2, alkaline hydrolysis; lane 3, RNase T1 digestion; lane 4, control (Lu^{3+}-free); lane 5, Lu^{3+}; lane 6, Lu^{3+}/(DNA-1 + DNA-2); lane 7, Lu^{3+}/ DNA-2; lane 8, Lu^{3+}/ DNA-1. [each of DNAs]$_0$ = [Lu^{3+}]$_0$ =10.0 and [RNA]$_0$ = 0.1 μmol dm^{-3}; [NaCl]$_0$ = 150 mmol dm^{-3}.

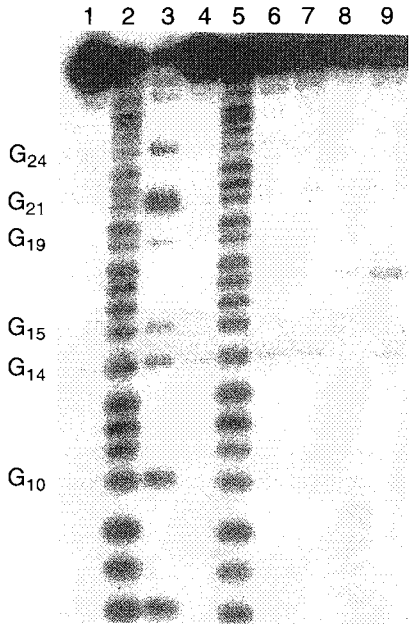

Figure 3. Sequence-selective scission of RNA by the Lu(III) in the presence of two DNA oligomers. Lane 1, RNA only; lane 2, alkaline hydrolysis; lane 3, RNase T1 digestion; lane 4, control (Lu^{3+}-free); lane 5, Lu^{3+}; lane 6, Lu^{3+}/(DNA-1 + DNA-2); lane 7, Lu^{3+}/(DNA-1 + DNA-3); lane 8, Lu^{3+}/(DNA-1 + DNA-4); lane 9, Lu^{3+}/(DNA-1 + DNA-5). The reaction conditions are identical with those described in Figure 2.

in the RNA (from A16 to C18) were free from base-pairing with the DNA oligomers, and the selective scission occurred at the 3'-side of the middle one (U17) of these three ribonucleotides. The conversion of scission monotonically increased with the reaction time, as expected. A similar result was obtained when La(III) ion was used in place of Lu(III). Quite simple artificial ribonuclease for sequence-selective RNA scission is now in hand.

Three consecutive non-pairing ribonucleotides were necessary for the scission. The combination of DNA-1 and DNA-2, which covered the whole of the RNA, totally inhibited the scission (lane 6 in Figure 3).[7] Similarly, the RNA scission was virtually nil, when DNA-3 was used in place of DNA-2 (lane 7: only one ribonucleotide was free from base-pairing). With the combination of DNA-1 and DNA-4, which left two ribonucleotides non-pairing, the RNA was selectively cut at the site of the selective scission by the Lu(III)/DNA-1/DNA-5 system (lane 8).[8] However, the scission efficiency was small.

It is noteworthy that the present non-covalent artificial ribonuclease is almost as active as the covalent artificial ribonuclease (the conjugate of the Lu(III)-iminodiacetate complex and the DNA oligomer).[9] Although the intermolecular catalysis by the Lu(III) ion in the present system is entropically less favorable than the corresponding intramolecular one in the latter, this effect is efficiently compensated by the greater catalytic activity of free Lu(III) ion than that of the Lu(III)-iminodiacetate complex. The present non-covalent systems can be easily prepared without any complicated organic synthesis, and thus be potent for versatile practical applications.

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6 In the double-stranded portion, the negatively-charged transition state is destabilized by the electrostatic repulsion by the phosphate residues. Alternatively, the conformational change of the ribonucleotides, required to proceed from the initial state to the transition state, might be suppressed there.

7 Exactly the same result is presented in the lane 6 in Figure 2.

8 When four bases (A16 to G19) were unpaired, the 3'-side of C18 was additionally cleaved (data not shown).

9 Under the conditions employed in Figure 3, for example, the conversion of RNA hydrolysis by the present system was 5 mol%, whereas that for the covalent conjugate (in Ref. 4a) was 7 mol%.