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Non-covalent ternary systems (DNA-acridine hybrid/DNA/lanthanide(III)) for efficient and site-selective RNA scission†

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The target phosphodiester linkage in RNA is activated by the combination of acridine-attached DNA and unmodified DNA, so that the RNA is site-selectively hydrolysed at this linkage by free Lu^{III} ion.

Interests in sequence-selective RNA hydrolysis have been rapidly growing.[1] The most common strategy is to tether chemical scissors (either organic or inorganic) to DNA oligomers.[2] However, non-covalent systems, which involve no tethering and are obtainable without complicated organic synthesis, are also valuable for practical applications.[3] Here we show that non-covalent systems, composed of (i) DNA oligomer bearing an acridine residue, (ii) unmodified DNA, and (iii) free lanthanide(III) ion, selectively and efficiently hydrolyse RNA at the target site. The corresponding phosphodiester linkage is notably activated by non-covalent interactions with the DNAs ((i) and (ii)), and thus the site-selective scission by the metal ion promptly proceeds under physiological conditions. The predominant role of the acridine residue is evidenced.

The substrate RNA (36-mer: ^{32}P-labeled at its 5′-end) and the DNA oligomers are presented in Fig. 1a. When the DNA bears an acridine at the 5′-end (DNA_1-Acr), another DNA (DNA_2), and $LuCl_3$ are combined, the RNA is efficiently and site-selectively hydrolysed (lane 3 in Fig. 2).[4] Here, all the ribonucleotides in the RNA, except for U-19, are forming Watson–Crick base-pairs with either DNA_1-Acr or DNA_2. The selective scission occurs at the 3′-sides of C-18 and U-19 (Fig. 1b). The C-18/U-19 ratio is around 3. When the position of unpaired ribonucleotide in the RNA is moved from U-19 to C-18 by using appropriate DNAs, the site of selective scission accordingly shifts to the 3′-sides of U-17 and C-18 (Electronic Supplementary Information (ESI) Fig. 1a). In the absence of these DNA oligomers, however, RNA scission by Lu^{III} (and by other lanthanide(III) ions also) takes place almost randomly (lane 1 in Fig. 2).

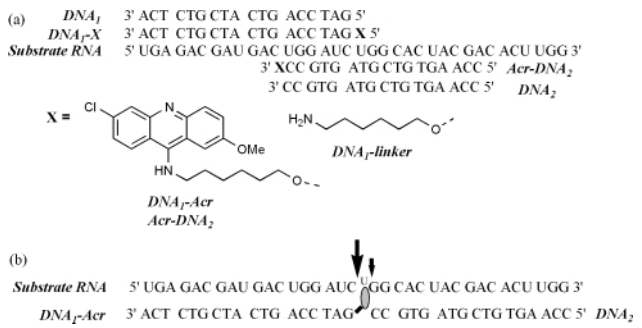

Fig. 1 Structures of the substrate RNA and the DNA oligomers used in the present study (a), and the scission pattern by the DNA_1-Acr/DNA_2/Lu^{III} system (b). The hatched ellipse shows the acridine residue, and the length of arrow corresponds to the scission efficiency.

† Electronic supplementary information (ESI) is available: supplementary Figs. 1–3 and Scheme 1. See http://www.rsc.org/suppdata/cc/b0/b006772p/

Quite significantly, the site-selective RNA scission is far more (> 30 fold)[5] efficient than that by the DNA_1/DNA_2/Lu^{III} system (compare lane 3 in Fig. 2 with lane 2). Furthermore, this selective scission is faster than the scission (at the corresponding site) in the absence of the DNAs (lane 3 *vs.* lane 1). Apparently, the target phosphodiester linkage is significantly activated by the acridine moiety of DNA_1-Acr. A similar site-selective scission is achieved, when Acr-DNA_2 bearing an acridine at the 3′-end is combined with DNA_1 (lane 4). The scission by the DNA_1-Acr/Acr-DNA_2/Lu^{III} system is also selective, but less efficient (lane 5).

Both DNA_1-Acr and DNA_2 (as well as the relevant combinations) are essential for prompt and site-selective scission. When only one of them is used, the whole single-stranded portion in RNA is hydrolysed without any specific selectivity (lanes 2 and 3 in Fig. 3). The DNA bearing only the linker moiety (DNA_1-linker) is also poor in the activity (lane 5), further substantiating the necessary role of the acridine. In the absence of the lanthanide ions, no scission occurs. Site-selective scission is also successful, when two ribonucleotides in the RNA are unpaired (lane 3 in ESI Fig. 1b). Without any unpaired ribonucleotide, however, no scission takes place (lane 1).[6]

One of the most important characteristics of the present non-covalent ternary systems is that the RNA hydrolysis comes up near to completion under physiological conditions. At pH 8.0 and 37 °C ($[LuCl_3]_0$ = 100 μmol dm^{-3}), more than half of the

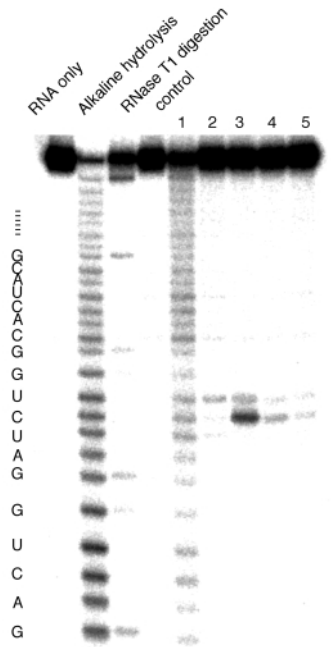

Fig. 2 RNA scission by the ternary systems composed of two DNAs and Lu^{III}. Lane 1, Lu^{III} only; lane 2, DNA_1/DNA_2/Lu^{III}; lane 3, DNA_1-Acr/DNA_2/Lu^{III}; lane 4, DNA_1-Acr/DNA_2/Lu^{III}; lane 5, DNA_1-Acr/Acr-DNA_2/Lu^{III}. At pH 8.0 and 37 °C for 2 h; $[RNA]_0$ = 1, [each of modified or unmodified DNAs]$_0$ = 10, and $[LuCl_3]_0$ = 100 μmol dm^{-3}; $[NaCl]_0$ = 200 mmol dm^{-3}.

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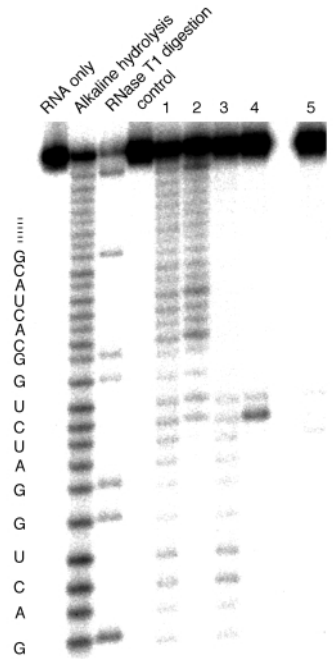

Fig. 3 RNA scission by various non-covalent systems. Lane 1, Lu^{III} only; lane 2, DNA_1-Acr/Lu^{III}; lane 3, DNA_2/Lu^{III}; lane 4, DNA_1-Acr/DNA_2/Lu^{III}; lane 5, DNA_1-linker/DNA_2/Lu^{III}. The reaction conditions are presented in the legend for Fig. 2.

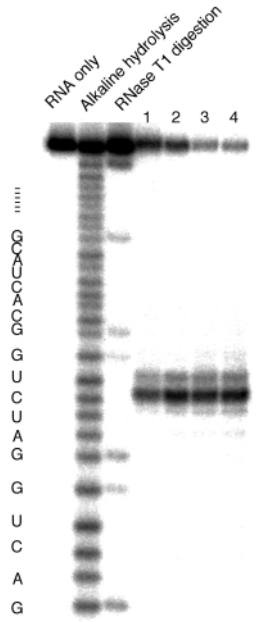

Fig. 4 Time-course of the sequence-selective RNA scission by the DNA_1-Acr/DNA_2/Lu^{III} system: lane 1, 13 h; lane 2, 23 h; lane 3, 40 h; lane 4, 48 h.

RNA is hydrolysed in 13 h by the DNA_1-Acr/DNA_2/Lu^{III} system (lane 1 in Fig. 4). The conversion is nearly 90 mol% at 48 h (lane 4).[7]

In the DNA_1-Acr/DNA_2/RNA system, the UV-visible absorption band of acridine (380–480 nm) shows a clear-cut hypochromicity. In the DNA_1-Acr/RNA system, however, hypochromicity is marginal. These results indicate that the acridine (bound to the 5′-end of DNA_1) is sandwiched by the DNA–RNA base-pair (G–C) at the 5′-terminus of DNA_1 and the C–G base-pair at the 3′-terminus of DNA_2 (see Fig. 1b). Consistently, the fluorescence from the acridine is significantly quenched (ESI Fig. 3).[8] Assumedly, the conformation of the RNA near the sandwiching site is perturbed, and the attacking 2′-OH is brought closer to the P-atom for facile nucleophilic attack. These arguments fairly agree with the fact that neither DNA_1-Acr nor DNA_2 alone activates the RNA (note that the scission efficiencies at C-18 and U-19 in lanes 2 and 3 in Fig. 3 are almost the same as those in lane 1). Only by combining both of them is the target phosphodiester linkage efficiently activated (lane 4). Applications of the present findings are currently under way.

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4 The RNA cleavage was achieved at pH 8.0 (10 mmol dm^{-3} Tris buffer: [NaCl] = 200 mmol dm^{-3}) and 37 °C, and analysed by denaturing polyacrylamide gel electrophoresis. The melting temperatures of the RNA–DNA hetero-duplexes are significantly higher than the reaction temperature, so that they are almost completely formed in the reaction mixtures. The phosphoramidite monomer bearing an acridine was synthesized according to ESI Scheme 1.

5 This value is evaluated from the ratio of band intensities at C-18 and U-19 in lane 3 to those in lane 2. The weak scission in lane 2 is mainly at U-19.

6 When bulge structures were formed in the RNA by using appropriate DNAs (without an acridine residue), the RNA was cleaved at several positions around near the bulge (see ESI Fig. 2).

7 The RNA is hydrolysed mostly at C-18 and U-19, although a minor scission occurs at U-17.

8 The G-20 and G-21 of RNA and the 5′-terminal G of DNA_1-Acr are fixed near the acridine residue, and their guanine residues quench the fluorescence.