Lanthanide Complex–Oligo-DNA Hybrid for Sequence-selective Hydrolysis of RNA

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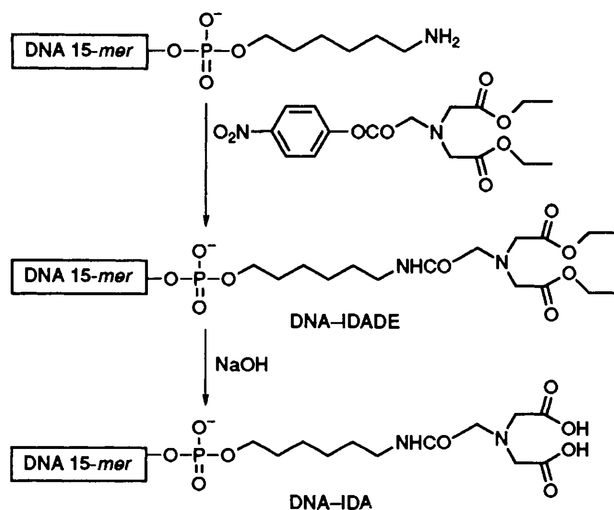
Iminodiacetate complexes of lanthanide(III) ions (lutetium, europium, thulium and lanthanum), attached to the 5′-end of a 15-*mer* DNA, hydrolyse a 39-*mer* RNA selectively at the 3′-side of its 15-*mer* sequence, which is complementary with the DNA.

Sequence-selective scission of RNA has been attracting increasing interest,[1–5] and was achieved by RNA enzymes[1] and nuclease–DNA hybrids.[4,6] However, a totally man-made sequence-selective ribonuclease has not yet been prepared.[7] Recently, it was shown that lanthanide ions and their complexes are very active for the hydrolysis of RNAs.[8–12] Here we report that lanthanide complexes bound to a DNA oligomer as a sequence-recognizing moiety selectively hydrolyse RNA at the target site.

Iminodiacetate-attached DNA oligomer (DNA–IDA) was synthesized as depicted in Scheme 1. A 15-*mer* DNA, which is complementary with the C4–G18 portion of the substrate 39-*mer* RNA (Fig. 1) and has an amino residue at the 5′-end, was reacted with *N,N*-bis(ethoxycarbonylmethyl)glycine *p*-nitrophenyl ester in a 1:1 mixture of DMF and a pH 9 borate buffer. The diethyl ester moieties in the resultant diethyl ester (DNA–IDADE) were hydrolysed at pH 12 at room temperature for 2 h.† Hydrolysis of the substrate RNA (^{32}P-labelled at the 5′-end)‡ at 37 °C in a pH 8 tris buffer (10 mmol dm^{-3}) was initiated by the addition of the RNA to the mixture of DNA–IDA and lanthanide(III) chloride (purity >99.9%). Highly purified water (specific resistance >18.3 MΩ cm) was used throughout the experiments, and great care was taken to avoid contamination by metal ions and natural enzymes.

Typical electrophoresis patterns are presented in Fig. 2. Significantly, a 1:1 mixture of Lu^{III} and DNA–IDA selectively hydrolyses the RNA at the 3′-sides of C20 and U21 (lanes 3 and 4). The ratio of the scission efficiencies at C20 and at U21, analysed by densitometry, is 3:1 (Fig. 1). The total conversion of the RNA hydrolysis is 7.3 mol% at 4 h and 17 mol% at 8 h. A highly selective artificial ribonuclease has been obtained.

The selective scission is definitely ascribed to the catalysis of the Lu^{III} ion complexing with the iminodiacetate of DNA–IDA, on the basis of the following results: (*i*) in the absence of DNA–IDA, Lu^{III} shows no specific selectivity (lane 8); (*ii*) the selectivity disappears when DNA–IDADE which is unable to bind Lu^{III} efficiently, is used in place of DNA–IDA (lane 7); (*iii*) the selectivity is virtually nil when the Lu^{III}:DNA–IDA ratio is 3:1 and a considerable amount of free Lu^{III} ion exists in the reaction mixtures (lanes 5 and 6; non-selective catalysis by the free metal ions is dominant here); (*iv*) DNA–IDA alone does not hydrolyse the RNA (lane 9). More than 95% of



Scheme 1 Synthesis of DNA–IDA

Fig. 1 Structure of the duplex between the 39-*mer* RNA and the Lu^{III}–DNA–IDA complex. The selective scission sites are indicated by the arrows.

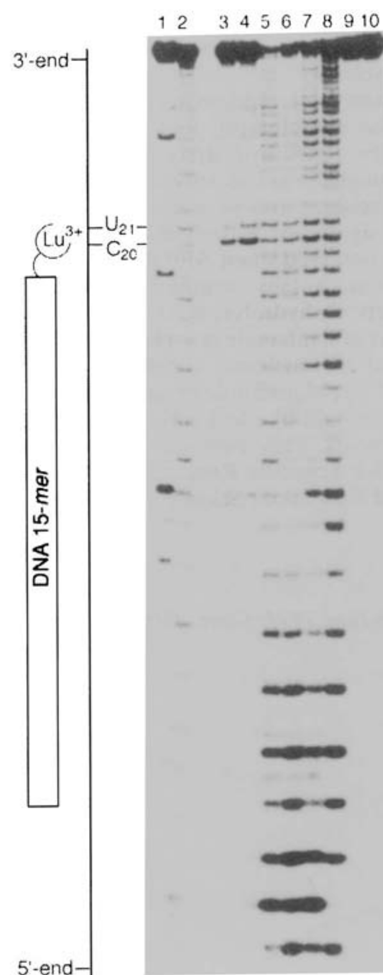


Fig. 2 Autoradiographs of a 20% denaturing polyacrylamide gel for the scission of the 39-*mer* RNA (^{32}P-labelled at the 5′-end) at pH 8 and 37 °C: lanes 3 and 4, Lu^{III}:DNA–IDA = 1:1; lanes 5 and 6, Lu^{III}:DNA–IDA = 3:1; lane 7, Lu^{III}:DNA–IDADE = 1:1; lane 8, Lu^{III} only; lane 9, DNA–IDA only; lane 10, control. The reaction periods are 4 h for lanes 3 and 5, and 8 h for lanes 4, 6, 8–10. Lanes 1 and 2 refer to digestion by ribonuclease T_1 and alkaline hydrolysis, respectively. $[RNA]_0 = 3 \times 10^{-7}$, $[\text{DNA and its derivative}]_0 = 10^{-5}$ mol dm^{-3}.

the Lu^{III} ions in the mixture are complexing with the iminodiacetate of DNA–IDA under the reaction conditions, as estimated from the formation constant of Lu^{III}–iminodiacetate complex ($10^{7.61}$ dm^3 mol^{-1}).[13] When the complementary 15-*mer* sequences in DNA–IDA and in the RNA form a double helix, the Lu^{III} ion is fixed in the vicinity of the hydrolysed phosphodiester linkages (Fig. 1). Formation of the duplex is confirmed by the fact that ribonuclease H, which is specific for the hydrolysis of the RNA in RNA–DNA heteroduplexes, hydrolyses the 39-*mer* RNA at the complementary portion under the reaction conditions. The minor cleavages observed in lanes 3 and 4 occur at the linkages which are intrinsically more susceptible to hydrolysis than the others and are cleaved upon incubation of the RNA at 80 °C for a long time. These scissions are probably associated with catalysis by a small amount of free Lu^{III} ion in the mixtures.

A reversed-phase HPLC showed that only adenosine and its 2′- and 3′-phosphates were formed when adenylyl(3′-5′)adenosine was treated with the 1:1 mixture of $LuCl_3$ and iminodiacetate ion (10 mmol dm^{-3}) of both. Neither release of adenine nor formation of other byproducts, expected for the oxidative cleavage of the ribose residues, was observed. The hydrolytic character of the RNA scission has been proven. Consistently, dithiothreitol (1 mmol dm^{-3}), a well-known promoter of metal-mediated redox reactions, had no measurable effect on the selective hydrolysis of the 39-*mer* RNA. Furthermore, the hydrolysis efficiently proceeded even when molecular oxygen was thoroughly removed by repeated freeze–thaw cycles.§

In the presence of equimolar amounts of DNA–IDA, thulium(III) and europium(III) ions showed similar selective catalysis for the RNA hydrolysis. Lanthanum(III) was also effective, although it was less active (about 50% of the activity of Lu^{III}). The scission sites were identical with those by Lu^{III}. However, no significant hydrolysis (either selective or nonselective) was observed when Al^{III} and Mg^{II} ions were used.

In conclusion, totally synthetic ribonucleases, which sequence-selectively hydrolyse RNAs, have been prepared by the attachment of lanthanide complexes to a DNA oligomer. These artificial ribonucleases should be valuable tools for molecular biology, biotechnology and therapy.

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Footnotes

† Purification of DNA–IDA was achieved by a reversed-phase HPLC; a Merck 18(e) column, an acetonitrile–water mixture (a linear gradient of acetonitrile concentration from 2.5 to 20% at 20 min).

‡ The substrate 39-*mer* RNA of an arbitrary sequence was synthesized on an automated synthesizer.

§ Recently it was proposed for $CeCl_3$-induced DNA hydrolysis that hydrogen peroxide and Ce^{IV} are formed *in situ* from Ce^{III} and molecular oxygen, and that the hydrogen peroxide attacks the DNA with the aid of the Ce^{IV} [ref. 11(*d*)]. However, the present RNA scission does not involve hydrogen peroxide as a cofactor, since hydrogen peroxide (10 mmol dm^{-3}) showed no acceleration. The argument was further substantiated by the fact that the scission proceeds even in the absence of molecular oxygen (see text).

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