Ethylenediamine–oligo DNA Hybrid as Sequence-selective Artificial Ribonuclease

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A hybrid of ethylenediamine and a DNA oligomer selectively hydrolyses linear RNA at the phosphodiester linkage which is adjacent to the sequence complementary to the DNA.

The importance of RNA hydrolysis has been widely accepted,[1–6] and many catalysts have been reported.[7–15] Site-selective scission of RNA can be achieved by the hybrid of natural enzyme and DNA oligomers,[16] by the hybrids of metal complexes and DNA oligomers[17–19] and by RNA enzymes.[3] However, information on totally organic and man-made ribonucleases for the sequence-selective scission has been scarce.

In a previous paper,[12] oligoamines efficiently hydrolysed RNAs, due to intramolecular acid-base cooperation. Here we report that the hybrid of oligoamine and DNA oligomer as sequence-recognizing moiety selectively hydrolyses RNA at the target site.

Ethylenediamine-attached DNA oligomer [N_2-DNA hybrid: Fig. 1(*a*)] was prepared by reacting a 19-mer DNA (bound to the CPG column used for an automated DNA synthesis) with 1,1′-carbonyldiimidazole (0.3 mol dm^{-3}) in 1,4-dioxane at 30 °C. then with diethylenetriamine (0.2 mol dm^{-3}) in 1,4-dioxane at 50 °C.[20] This resulted in an ethylenediamine residue connected to the 5′-end of the 19-mer DNA *via* a urethane linkage.† After being released from the CPG column, the hybrid was purified by reversed-phase HPLC. A substrate 30-mer RNA, which has a 19-mer sequence complementary with the 19-mer DNA [prepared on a DNA synthesizer: see Fig. 1(*b*)], was ^{32}P-labelled either at the 3′-end (by 5′-^{32}P cytidine 3′,5′-bisphosphate) or at the 5′-end (by adenosine 5′-[γ-^{32}P-]triphosphate). The RNA hydrolysis by the N_2-DNA hybrid was carried out at pH 8 (1 mmol dm^{-3} Tris buffer) and 50 °C in the presence of EDTA (1 mmol dm^{-3}),‡ and was analysed by 20% denaturing polyacrylamide gel electrophoresis. Great care was taken to avoid contamination by metal ions and natural enzymes: highly purified water (specific resistance > 18.3 MΩ cm) was used throughout.

Typical electrophoresis patterns for the scission of the 3′-end labelled 30-mer RNA are presented in Fig. 2(*a*). With the N_2-DNA hybrid, the scission takes place only at the 3′-side of C-22 [lane 4, Fig. 2(*b*)]. No other scission is observed. The total conversion for the RNA hydrolysis, estimated by densitometry, is *ca.* 10 mol% after 4 h. The selective scission at the sole phosphodiester linkage has been further confirmed by use of the 5′-end labelled RNA [lane 4, Fig. 2(*b*)].

In contrast, the scission is nil when the 19-mer DNA without ethylenediamine residue is used [lanes 5, Fig. 2(*a*) and (*b*)]. The 19-mer DNA of the same sequence, which has a 6-aminohexyl residue at the 5′-end, is ineffective for the scission (data not presented). Thus the selective scission is ascribed to the catalysis by the ethylenediamine residue, in which an ammonium cation and a neutral amine show an intramolecular acid–base cooperation.[12] The scission is consistently most effective at *ca.* pH 8 where the ethylenediamine residue exists mostly as a monocation (the pK_a values are 9.2 and 6.5). A hybrid obtained from triethylenetetramine and the 19-mer DNA also selectively hydrolysed the RNA at C-22.

Both the 3′-end labelled and the 5′-end labelled fragments comigrate with the corresponding fragments obtained by alkaline hydrolysis [compare lanes 4 and 2; Fig. 2(*a*) and (*b*)]. Clear evidence for the hydrolytic character of this RNA scission has been provided. If the reaction involved the oxidative cleavage of the ribose, 5′-phosphate termini should be formed (in most cases)[21] and thus the mobility of the 3′-labelled fragment should be different from that of the fragment of the alkaline hydrolysis. Apparently, this is not the case. Reversed-phase HPLC

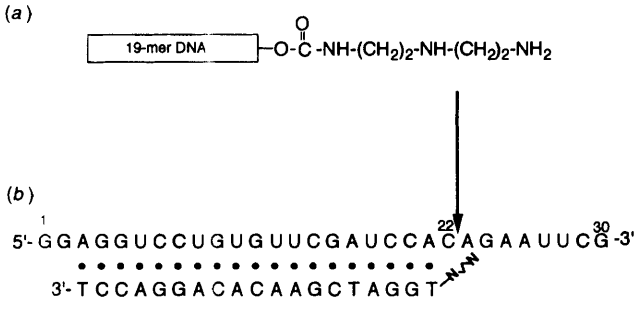

Fig. 1 N_2-DNA (*a*) and its duplex with the substrate 30-mer RNA (*b*). The scission position, obtained by densitometry on lane 4 in Fig. 2 (*a*), is represented by the arrow in (*b*).

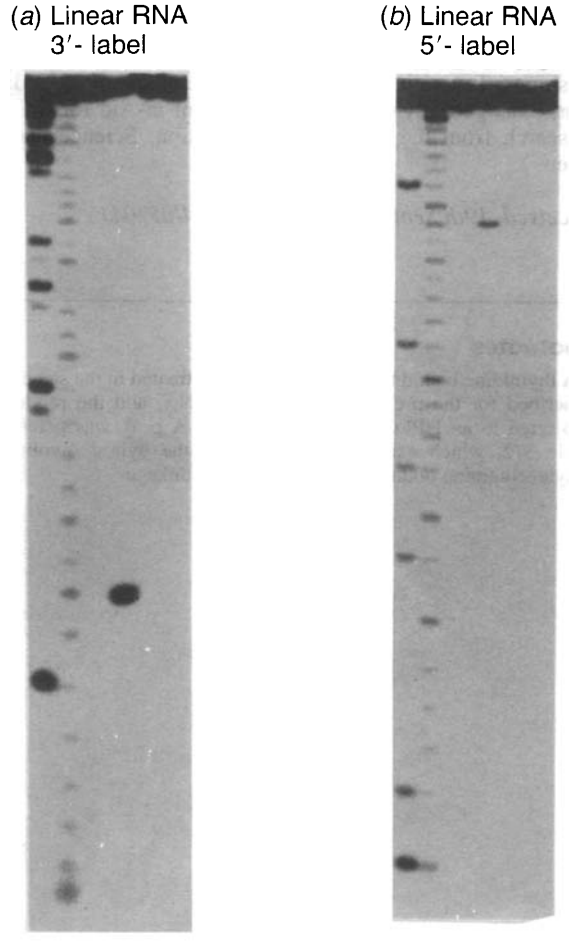

Fig. 2 Autoradiographs for the scission of the linear 30-mer RNA at pH 8 and 50 °C in the presence of EDTA (1 mmol dm^{-3}): (*a*) the RNA space ^{32}P-labelled at the 3′-end; (*b*) the 5′-end labelled RNA. For both (*a*) and (*b*), lane 1, digestion by ribonuclease T_1 lane 2, alkaline hydrolysis; lane 3, N_2-DNA (t = 0); lane 4, N_2-DNA (t = 4 h); lane 5, the DNA oligomer without N_2 (t = 4 h); lane 6, control (t = 4 h). $[RNA]_0 = 10^{-6}$ and $[N_2\text{-DNA or DNA}]_0 = 10^{-4}$ mol dm^{-3}.

consistently showed that only the hydrolytic products, adenosine monoposphates (2′- and 3′-) and adenosine (as well as adenosine 2′,3′-cyclic monophosphate as the hydrolysis intermediate), are formed when adenylyl (3′-5′)adenosine is incubated at pH 8 and 50 °C in the presence of ethylenediamine.

The participation of metal ions for the catalysis is quite unlikely, since the scissions were achieved in the presence of 1 mmol dm^{-3} EDTA (no deceleration effect was observed even at a concentration of 10 mmol dm^{-3}). Furthermore, dithiothreitol (1 mmol dm^{-3}), a well-known promoter for metal ion mediated redox reactions, showed no acceleration of the scission. Note that contamination by metal ions has been carefully avoided throughout the experiments.

Formation of the double helix between the 19-mer sequences in the N_2-DNA hybrid and in the RNA has been confirmed by use of ribonuclease H which specifically hydrolyses the RNA in RNA–DNA hetero-duplexes. When the RNA was treated with the enzyme in the presence of the N_2-DNA hybrid at pH 8 and 50 °C, all the linkages of the RNA in the U-9–C-19 region were hydrolysed. Scission at C-22, due to the catalysis by the N_2-DNA hybrid, occurred simultaneously. Thus the DNA, at least the portion near its 5′-end, forms a hetero-duplex with the RNA under the conditions employed for the RNA hydrolysis. In the absence of the N_2-DNA hybrid, the enzyme induced no RNA scission. A molecular modelling study has indicated that the ethylenediamine residue in the N_2-DNA hybrid is correctly placed near the selective scission site when the double helix is formed.§

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Footnotes

† A thymidine bound to a CPG column was treated in the same way as described for the preparation of the N_2-DNA, and the product was subjected to an HPLC-Mass spectroscopy. A peak was perceived at MH^+ 372, which exactly corresponds to the hybrid involving the ethylenediamine bound to T *via* a urethane linkage.

‡ Prior to the RNA hydrolysis, a mixture of the N_2-DNA and the substrate RNA was heated to 80 °C for 2 min, and then allowed to stand at 30 °C for 1 h to complete the double helix formation between the two 19-mer sequences. No selective hydrolysis proceeded during this process as confirmed by the electrophoresis at $t = 0$ (lanes 3 in Fig. 2).

§ A pinpoint prediction of the scission site by the molecular modelling was not successful. The single-stranded portion of the RNA in the N_2-DNA·RNA complex has a significant freedom in dangling motion and the linker moiety of the N_2-DNA also has a conformational freedom, preventing a definite conclusion.

References

1 A. M. Pyle and J. K. Barton, *Prog. Inorg. Chem.*, 1990, **38**, 413.
2 G. J. Murakawa, C. B. Chen, M. D. Kuwabara, D. P. Nierlich and D. S. Sigman, *Nucleic Acids Res.*, 1989, **17**, 5361.
3 J. A. Latham and T. R. Cech, *Science*, 1989, **245**, 276 and references therein.
4 J. K. Bashkin, J. K. Gard and A. S. Modak, *J. Org. Chem.*, 1990, **55**, 5125.
5 C. S. Chow, L. S. Behlen, O. C. Uhlenbeck and J. K. Barton, *Biochemistry*, 1992, **31**, 972 and references therein.
6 G. Wang and D. E. Bergstrom, *Tetrahedron Lett.*, 1993, **34**, 6721.
7 G. L. Eichhorn, in *Inorganic Biochemistry*, ed. G. L. Eichhorn, Elsevier, Amsterdam, 1973, vol. 2, ch. 34.
8 B. Barbier and A. Brack, *J. Am. Chem. Soc.*, 1988, **110**, 6880.
9 J. Ciesiolka, T. Marciniec and W. Krzyzosiak, *Eur. J. Biochem.*, 1989, **182**, 445.
10 M. K. Stern, J. K. Bashkin and E. D. Sall, *J. Am. Chem. Soc.*, 1990, **112**, 5357.
11 Y. Matsumoto and M. Komiyama, *J. Chem. Soc., Chem. Commun.*, 1990, 1050.
12 K. Yoshinari, K. Yamazaki and M. Komiyama, *J. Am. Chem. Soc.*, 1991, **113**, 5899.
13 R. Breslow and D.-L. Huang, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 4080.
14 M. Komiyama, K. Matsumura and Y. Matsumoto, *J. Chem. Soc., Chem. Commun.*, 1992, 640.
15 J. R. Morrow, L. A. Buttrey, V. M. Shelton and K. A. Berback, *J. Am. Chem. Soc.*, 1992, **114**, 1903.
16 R. N. Zuckermann, D. R. Corey and P. G. Schultz, *J. Am. Chem. Soc.*, 1988, **110**, 1614.
17 J. K. Bashkin, E. I. Frolova and U. Sampath, *J. Am. Chem. Soc.*, 1994, **116**, 5981.
18 D. Magda, R. A. Miller, J. L. Sessler and B. L. Iverson, *J. Am. Chem. Soc.*, 1994, **116**, 7439.
19 K. Matsumura, M. Endo and M. Komiyama, *J. Chem. Soc., Chem. Commun.*, 1994, 2019.
20 B. C. F. Chu and L. E. Orgel, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 963.
21 D. S. Sigman, *Acc. Chem. Res.*, 1986, **19**, 180.