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Sequence-dependent hydrolysis of RNA using modified oligonucleotide splints and RNase H

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We found that, in the presence of chimeric oligonucleotides containing complementary deoxyribo- and 2'-*O*-methylnucleosides, a nonaribonucleotide, [5'-^{32}P]pACUUACCUG, was cleaved specifically upon treatment with RNase H. When 3'm(UG)d(AATG)m(GAC)5' was used as a hybridization strand, *pACUUACCUG was cleaved between C6 and C7 to yield *pACUUAC. In the presence of 3'm(UGAA)d(TGGA)m(C)5', the nonaribonucleotide was hydrolyzed, mainly between U8 and C9, to give *pACUUACCU. This method will have a variety of applications in the field of RNA engineering.

2'-*O*-Methyloligonucleotide; Chimeric oligonucleotide; Heteroduplex; RNase H; Site-specific cleavage; RNA hydrolysis

1. INTRODUCTION

Hydrolysis of DNA by restriction endonucleases has provided an essential tool for gene manipulations. Restriction endonucleases recognize specific sequences in DNA [1]. No RNA endonucleases which recognize base sequences have been found, although there are several base-specific RNases, and partial digestions of RNA with RNases have been used to obtain larger RNA fragments [2]. A method for fragmenting RNA, analogous to the hydrolysis of DNA with restriction endonucleases, would be highly desirable.

RNase H has been found in calf thymus [3] and various organisms [4]. The enzyme is known to hydrolyze only RNA-DNA hybrids cleaving the RNA to yield a 3'-hydroxyl and a 5'-phosphate at the hydrolysis site. A region-specific cleavage of RNA with RNase H upon the hybridization of tetra- or hexadeoxyribonucleotides was reported, and the cleavage was found within, or immediately adjacent to, the hybrid [5,6]. To obtain a unique cleavage by RNase H, we investigated the use of a chimeric hybridization splint. Here, we describe sequence-specific hydrolyses of RNA using complementary 2'-*O*-methyloligonucleotides containing a tetradeoxyribonucleotide immediately upstream of the cleavage site.

We have previously found that 2'-*O*-methyloligonucleotides formed stable duplexes with complementary oligoribonucleotides [7]. X-ray analysis of 2'-*O*-methyl RNA fibers shows that the puckering of the sugar moiety in 2'-*O*-methyl RNA is similar to that of RNA [8]. Since RNase H does not cleave RNA duplexes, a region of 2'-*O*-Me-RNA/RNA hybrid ought not to be recognized by the enzyme.

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2. MATERIALS AND METHODS

The nonanucleotide duplexes were prepared by mixing appropriate strands obtained using the solid-phase phosphotriester method employing either 5'-linked ribonucleoside resin [9] or 3'-linked nucleoside resin [7]. The nonaribonucleotides were labelled at the 5'-end using [γ-^{32}P]ATP and polynucleotide kinase [10]. Each reaction mixture contained a 5'-labelled (2–3 ×

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10^4 cpm), non-labelled ribo-nonamer (0.002–0.005 A_{260} units) and a complementary nonamer (0.02–0.025 A_{260} units) in a total volume of 10–20 μl of 40 mM Tris-HCl (pH 7.7), 4% glycerol, 1 mM DTT and 0.003% BSA [11]. The mixture was incubated with *E. coli* RNase H (5 units, Takarashuzo) at 30°C for 16 h and analyzed by homochromatography [12] using Homo-mix VI [13] and identified by comparing them with the products obtained from the partial digestion of the nonanucleotide with venom phosphodiesterase.

3. RESULTS AND DISCUSSION

For testing with RNase H the four duplexes, I–IV in fig.1, were used as control for the reactions with duplexes containing the chimeric splints, V–X. In the RNA-DNA duplexes I and II, a non-specific cleavage occurred and the nonanucleotides were hydrolyzed at three points, as shown in fig.2. None of the ribo-oligonucleotide in duplex III nor any of the 2′-*O*-methyloligonucleotide in duplex IV was recognized by the enzyme, as expected. In the chimeric duplexes, V and VI containing three and five deoxynucleotides, respectively, the ribo-strand was cleaved at the 3′-end of the RNA-DNA region (fig.3). The rate of hydrolysis for V, containing three deoxynucleotides, was lower than that for VI, and another cleavage occurred as shown in lane 2 of fig.3. Duplex VIII, containing a tetradeoxynucleotide splint and also VI, were hydrolysed completely, as shown in fig.4 (lanes 4,5). From these results, a tetradeoxynucleotide cluster would seem to be sufficient for recognition, using the enzyme. Hydrolysis between U8 and G9 of the nonaribonucleotide was observed when a tetradeoxynucleotide splint was shifted towards the 3′-side (VII, fig.1; lanes 1,2, fig.4), and hydrolysis also seemed to occur between C6 and C7. Presumably the RNase H recognition site has

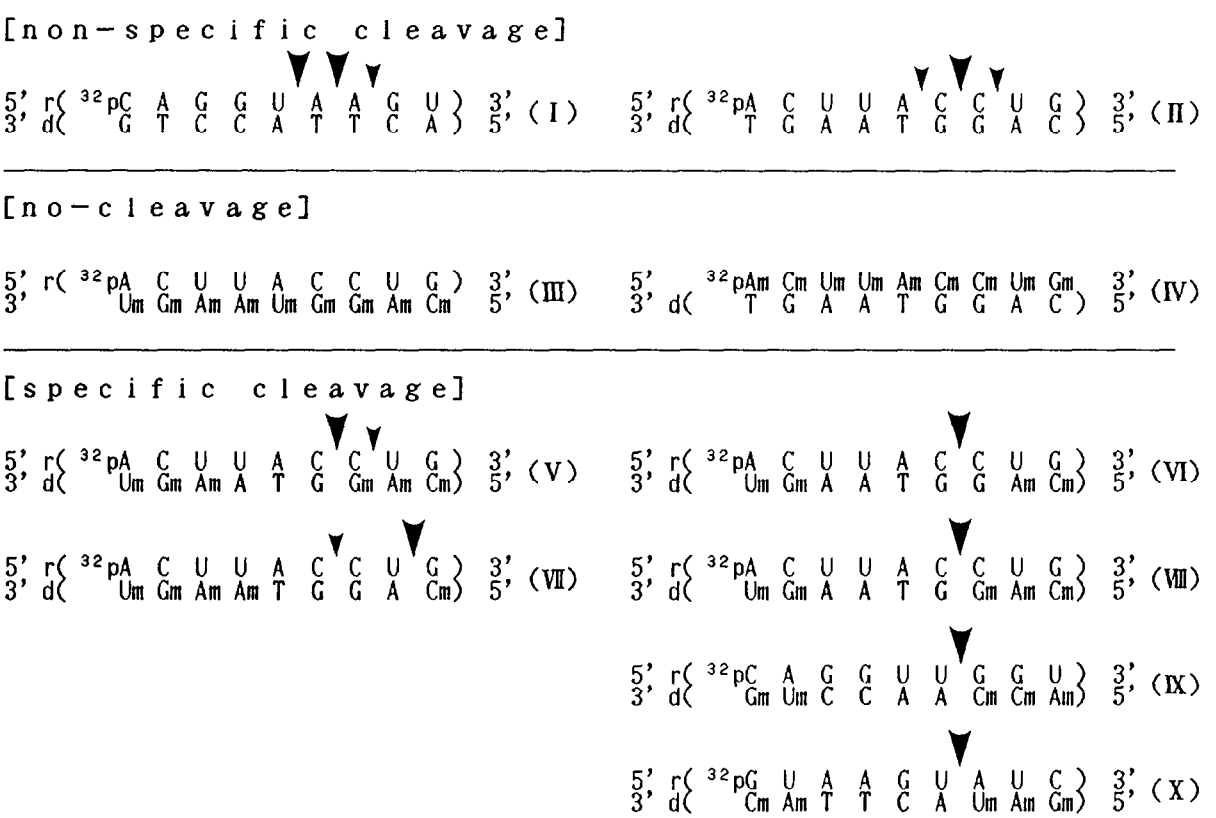

Fig.1. Reactions of duplexes with RNase H.

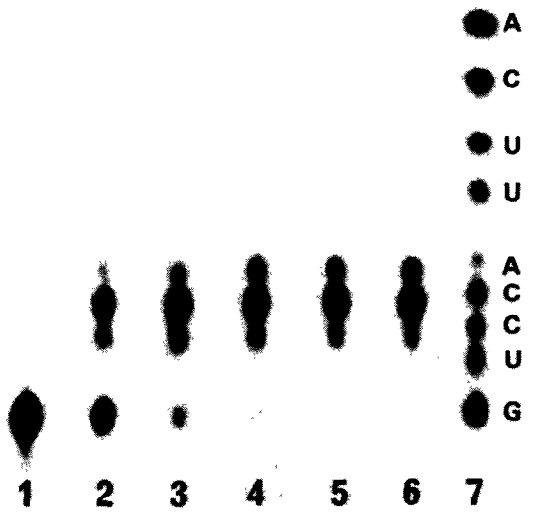

Fig.2. Autoradiogram of products obtained by the treatment of duplex II with RNase H at 20°C for various reaction times (h); lanes: 1, 0; 2, 0.5; 3, 1; 4, 2; 5, 3; 6, 6; 7, markers obtained by partial digestion of the 5′-labelled ribo-nonamer with snake venom phosphodiesterase [13].

been shifted too extremely to be cleaved at a normal rate of hydrolysis. In order to determine the exact nature of the hydrolysis, two nonaribonucleotides, having a different sequence, *pCAGGUUGGU and *pGUAAGUAUC, were tested in the presence of a complementary 2′-*O*-methyloligonucleotide which contained a tetradeoxynucleotide cluster in position 3–6. Cleavage of IX and X (fig.1) was observed at U6, as expected.

It is concluded that chimeric oligonucleotides containing 2′-*O*-methylnucleotides and tetradeoxyribonucleotides can be used to cleave a complementary RNA strand at specific positions in the presence of RNase H. The method is going to be extremely useful for obtaining larger RNA fragments. This will contribute to our understanding of the biological function of RNA.

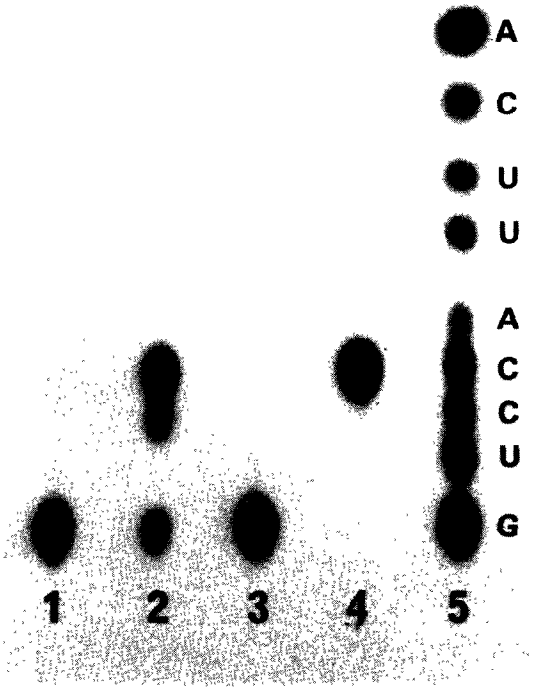

Fig.3. Autoradiogram of cleavage products of duplexes V and VI with RNase H under the conditions described in fig.1. Lanes: 1, V (RNase H−); 2, V (RNase H+); 3, VI (RNase H−); 4, VI (RNase H+); 5, markers (see fig.2).

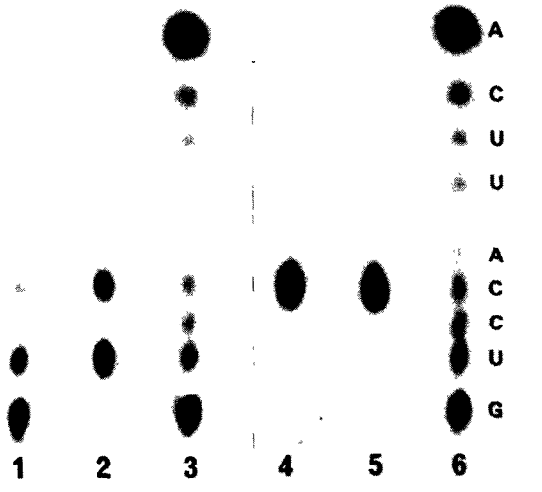

Fig.4. Autoradiogram of cleavage products with RNase H (10 units) of duplexes VII – lanes: 1, 20°C; 2, 30°C; and VIII – lanes: 4, 20°C; 5, 30°C. For lanes 3 and 6, see fig.2.

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