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Influence of probe structure on unique (regiospecific) cleavage of RNA by RNase H

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Chimeric oligo(ribo-deoxyribo)nucleotides with an internucleotide pyrophosphate bond are novel probes for regiospecific hydrolysis of RNA by RNase H. It has been shown that the use of d(TGTGTAT)ppGCCAU leads to unique hydrolysis of the TMV RNA fragment pAAUGGCAUACAC between C_{10} and A_{11}.

RNA hydrolysis; Regiospecific cleavage; Chimeric probe; RNase H

1. INTRODUCTION

RNase H, which hydrolyzes an RNA strand in the region involved in the formation of a hybrid duplex (DNA probe-RNA) [1,2], is widely used for studying the structure of ribosomal RNAs [3], and also the structure and functions of viral RNAs [4,5]. But the use of the method is limited in that it is impossible to predict the RNA fragmentation point.

It was shown earlier that the use of chimeric oligonucleotides for RNA hydrolysis [6,7] makes it possible to enhance the selectivity of RNA hydrolysis by RNase H. In the present work we suggest a method for regiospecific RNase H cleavage of RNA by using probes of specific design: the chimeric oligo(ribo-deoxyribo)nucleotides which contain a pyrophosphate bond between ribo- and deoxyribonucleotide blocks.

It has been shown in our laboratory that the insertion of pyrophosphate internucleotide groups into oligodeoxyribonucleotides does not interfere with the stability of DNA duplexes [8]. We have found that chimeric oligonucleotides containing the pyrophosphate internucleotide bond ensure a regiospecific cleavage of RNA by RNase H. For this purpose we investigated the cleavage of a synthetic dodecaribonucleotide (fragment of TMV RNA) in a complex with modified synthetic oligonucleotides, including the chimeric polymers (oligoribo-oligodeoxyribonucleotides), and also the chimeric oligonucleotides with internucleotide pyrophosphate bonds (fig.1).

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2. MATERIALS AND METHODS

A dodecaribonucleotide was obtained via the transcription reaction of d(GTGTATGCCATT) in the presence of RNA polymerase (core) from *E. coli* and AAU as primer according to [9]. Oligodeoxyribonucleotides were synthesized via a solid-phase phosphite method using a 'Victoria-4M' automated synthesizer. GCCAU was prepared by enzymatic synthesis [10]. The modified oligonucleotides (shown in duplexes **II–IV**) were obtained by the chemical ligation method from d(TGTGTATp) and GCCAU; d(TGTGTATp) and pGCCAU; d(TGTGTATp) and d(pGCCAT), respectively, as in [10].

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E. coli RNase H was purchased from Biolar (Olaine). The reaction mixture contained a 5′-end-labelled dodecaribonucleotide (3×10^4 cpm) and 0.01 A_{260} units of complementary oligodeoxyribonucleotide or modified oligonucleotide, 20 mM Tris-HCl (pH 7.9), 0.15 M NaCl, 0.1 mM EDTA, 1 mM $MgCl_2$, 0.05 mM DTT (total volume 10 μl). The reaction mixture was incubated in the presence of 10 units RNase H for 1 h at 0°C. The hydrolysis products were analysed by 20% PAAG-electrophoresis followed by autoradiography.

The points of RNA fragmentation were determined by comparison of the electrophoretic mobility of [5′-^{32}P]pAAUGGCAUACAC hydrolysis products after the treatment of duplexes **I–IV** with RNase H, and the products of the basic hydrolysis of the same dodecaribonucleotide.

3. RESULTS AND DISCUSSION

To study the site-specific cleavage of RNA by RNase H, the substrate characteristics of hybrid duplexes, shown in fig.1, were investigated.

Analysing 5′-^{32}P-labelled products of RNA dodecaribonucleotide hydrolysis by RNase H in duplexes **I–IV** (fig.2), we found that in all cases site-specific cleavage took place. Substitution of the DNA probe for the chimeric probe (duplex **II**) resulted in a decrease in the number of hydrolysis points and in the shifting of the process to the

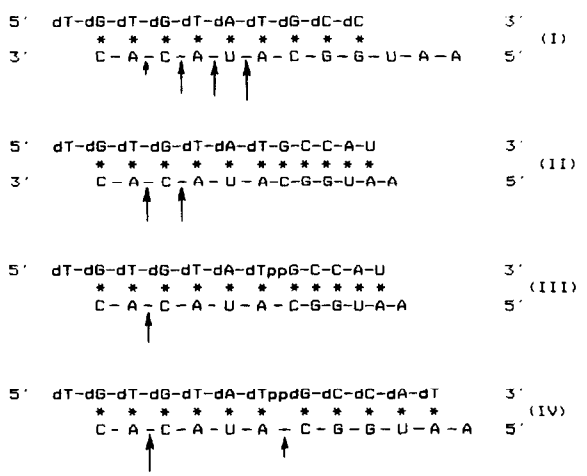

Fig.1. The scheme of pAAUGGCAUACAC hydrolysis by RNase H in duplexes **I–IV**.

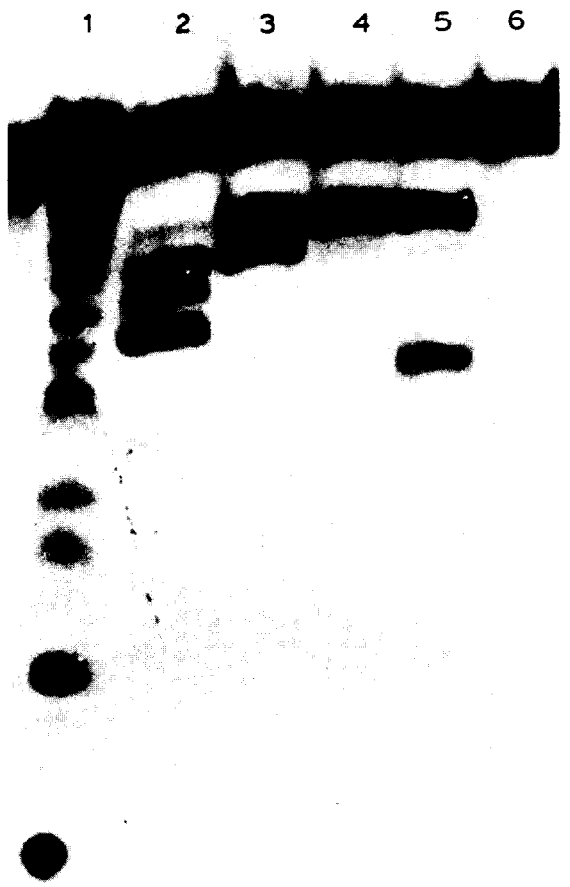

Fig.2. Cleavage products of duplexes **I–IV** with RNase H. Lanes: 1, partial basic hydrolysis of [^{32}P]-pAAUGGCAUACAC; 2, duplex **I**; 3, duplex **II**; 4, duplex **III**; 5, duplex **IV**; 6, [^{32}P]pAAUGGCAUACAC + RNase H.

3′-terminus of RNA. Thus, the products of hydrolysis in duplex **II** are pAAUGGCAUA and pAAUGGCAUAC in a ratio of 1:1 (fig.2), i.e., the hydrolysis is intensified, with a decaribonucleotide, heretofore present in minor amounts, being formed. If an additional phosphate group (a duplex **III**) is introduced into the oligonucleotide probe, hydrolysis proceeds only between C_{10} and A_{11} (fig.2). In this case regiospecific hydrolysis of RNA takes place at the 3′-terminus of RNA. The same shift in RNA hydrolysis is also observed in duplex **IV**. But the use of an oligodeoxyribonucleotide with an internucleotide pyrophosphate group does not result in unique cleavage (figs 1,2).

Thus, our method for regiospecific RNA

cleavage has allowed the above-described system to effect a unique cleavage of RNA, instead of a multiple one. Experiments with tobacco mosaic virus RNA and RNase H have shown that unique RNA cleavage also took place in the presence of the probe d(TGTGTAT)ppGCCAU (Atabekov, K.J., in preparation). The use of chimeric probes with internucleotide pyrophosphate bonds may certainly be of interest to researchers engaged in the RNA engineering.

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