ELSEVIER

FEBS Letters 339 (1994) 67–72

FEBS 13635

FEBS LETTERS

Sequence-specific cleavage of RNA by a hybrid ribonuclease H

Chieko Nakai[a], Aritomo Konishi[b], Yasuo Komatsu[b], Hideo Inoue[b], Eiko Ohtsuka[b], Shigenori Kanaya[a,*]

[a]*Protein Engineering Research Institute, 6-2-3, Furuedai, Suita, Osaka 565, Japan*
[b]*Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan*

Received 31 July 1993

Abstract

Site-specific cleavage of the 22-, 132- and 534-base RNAs by the DNA/protein hybrid RNase H were examined. The 22-base RNA was chemically synthesized, and 132- and 534-base RNAs were prepared by run-off transcription. The hybrid enzyme cleaves these RNAs, which contain a single target sequence, primarily at the unique phosphodiester bond within the target sequence. The hybrid enzyme performs multiple turnovers, and at a substrate/enzyme ratio of 10:1 the RNAs are almost completely cleaved by the hybrid enzyme at 37°C within 1 h. We propose that hybrid RNase H molecules with various oligodeoxyribonucleotides function as RNA restriction enzymes and are useful for structural and functional studies of RNA.

Key words: RNase H; Hybrid enzyme; Sequence specificity; Protein engineering

1. Introduction

The ribonucleases H proteins are defined by their ability to degrade RNA hybridized to DNA (for a review, see [1]). They do not recognize either single- or double-stranded RNA as a substrate. This unique specificity of the enzyme has allowed us to develop various methods to cleave RNA site-specifically [2–7]. All of these methods consist of two successive procedures: the annealing of an oligodeoxyribonucleotide or chimeric oligonucleotide to the RNA to be cleaved, and the digestion with RNase H. We have recently shown, however, that a DNA/protein hybrid RNase H site-specifically cleaves a single-stranded RNA, without addition of the oligodeoxyribonucleotide [8]. This hybrid protein was originally constructed to test whether such a novel RNA cleaving enzyme with sequence-specific recognition could be rationally designed and constructed with protein engineering technology.

Among the various RNase H enzymes, the *E. coli* RNase HI has been most extensively studied for structure-function relationships (for a review, see [9]). The enzyme is composed of 155 amino acid residues and endonucleolytically cleaves the P-O3′ bond of RNA in the presence of Mg^{2+} ion to produce short oligonucleotides with 5′-phosphate and 3′-hydroxyl groups. We have chosen this enzyme as a parent molecule for the construction of hybrid RNase H molecules. The availability of the nonaribonucleotide (r9-mer) [10] allows us to select a complementary nonadeoxyribonucleotide (d9-mer) with the sequence 5′-GTCATCTCC-3′ as an adduct. Based on the model for the enzyme–substrate complex proposed by Nakamura et al. [11], we have designed the site to which the d9-mer should be linked to the protein and the size of the linker between the protein and the d9-mer. The cross-link was formed between a maleimide group attached to the 5′ terminus of the d9-mer through a 21 Å linker and the unique thiol group of Cys^{135} in the mutant *E. coli* RNase HI. The resultant hybrid enzyme, d9-C135/RNase H, cleaves the phosphodiester bond between the fifth and sixth residues of the r9-mer with Michaelis–Menten kinetics [8] (Fig. 1). However, it remains to be determined whether this hybrid RNase H is capable of efficiently cleaving large DNA molecules in a sequence-specific manner.

In addition to the methods mentioned above, several other approaches using ribozymes [12] and RNase A– or staphylococcal nuclease–DNA hybrid molecules [13–15] have been reported to cleave RNA site-specifically. Site-specific cleavage of RNA with a ribozyme is especially important in the application field, because it is expected to be a promising strategy to intercept a target mRNA in vivo. However, further improvement in the catalytic efficiency or the specificity seems to be required to use either of these methods practically as a tool for probing structures and functions of RNA.

Here we report that the hybrid RNase H, d9-C135/RNase H, acts as an artificial RNA restriction enzyme. Hybrid RNase H molecules with various oligodeoxyri-

*Corresponding author. Fax: (81) (6) 872 8210.

Abbreviation: RNase H, ribonuclease H.

SSDI 0014-5793(94)00019-R

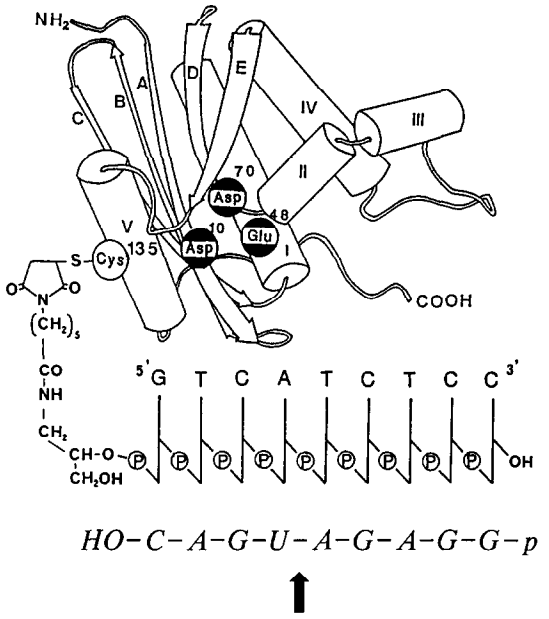

Fig. 1. Hybrid RNase H, d9-C135/RNase H. For the three-dimensional structure of *E. coli* RNase HI [22], β-strands are represented by arrows and α-helices are represented by cylinders. The three active site residues and the residue to which the d9-mer is covalently attached through a linker are shown by solid circles and an open circle, respectively, with residue numbers. The r9-mer hybridized to the d9-mer portion of the hybrid RNase H is cleaved at the site shown by a solid arrow [8].

bonucleotides may facilitate structural studies of RNA and prove useful as tools for RNA manipulations.

2. Materials and methods

2.1. Cells and plasmids

Plasmid pDR600 [10] and its derivative pAK600 [16] were constructed previously. Plasmid pSP64 was obtained from Promega. Plasmid pDR600 derivatives were grown in *E. coli* JM109 (*rec*A1, *end*A1, *gyr*A96, *thi*, *hsd*R17, *sup*E44, *rel*A1, λ^-, Δ[*lac-pro*]/F', *tra*D36, *pro*AB, *lac*I^q, ZΔM15) and plasmid pSP64 derivatives were grown in *E. coli* HB101 (F$^-$, *hsd*S20 r_B^-, m_B^-), *rec*A13, *ara*-14, *pro*A2, *lac*Y1, *gal*K2, *rps*L20 (Sm^r), *xyl*-5, *mtl*-1, *sup*E44, λ^-) in Luria Broth (LB) medium [17] containing 100 mg/liter ampicillin.

2.2. Plasmid construction

The plasmids pSPsub1 and pSPsub2, in which the transcription of the mutated *rnhA* gene with a single unique 5'-GGAGATGAC-3' sequence is controlled by the SP6 promoter (Fig. 2), were constructed by cassette mutagenesis. Introduction of the 5'-GGAGATGAC-3' sequence within the *rnhA* gene in plasmid pSPsub1 or pSPsub2 resulted in an alteration of the amino acid residues in the *E coli* RNase HI sequence from Leu^2 to Thr or from Leu^{107} to Gly and Ala^{109} to Asp, respectively.

Plasmid pSPsub1 was constructed as follows. Plasmid pSP700, in which the wild-type *rnhA* gene is controlled by the SP6 promoter, was first constructed by introducing the 700-base pair *Hin*dIII–*Sal*I fragment, containing the *rnhA* gene excised from plasmid pAK600, into the *Hin*dIII–*Sal*I site of plasmid pSP64. In this plasmid, the unique *Bgl*II site encompassing the sequences encoding amino acid residues 6–8 is located approximately 200 base pairs apart from the *Hin*dIII site. This 200-base pair *Hin*dIII–*Bgl*II fragment from plasmid pSP700 was then replaced with the 34-base pair DNA fragment shown in Fig. 2 to generate plasmid pSPsub1. In this plasmid, the *Acc*I site, which is not recognized by *Sal*I, is introduced between the *Hin*dIII site and the initiation codon of the *rnhA* gene.

Plasmid pSPsub2 was constructed as follows. First, plasmid pDR600BS, in which the unique *Bgl*II and *Sty*I sites were introduced into the *rnhA* gene in plasmid pDR600 by changing the codon for Val^{101} from GTA to GTC, and the codons for Ala^{110}-Leu^{111} from GCA-TTG to GCC-CTA, respectively, was constructed by PCR as described previously [18]. After replacing the 26-base pair *Bgl*II–*Sty*I fragment in plasmid pDR600BS with that containing the GGAGATGAC sequence, the 500-base pair *Acc*I fragment containing the mutated *rnhA* gene was excised from the resultant plasmid and substituted for that in plasmid pSPsub1 to generate plasmid pSPsub2. All oligodeoxyribonucleotides used for cassette mutagenesis and site-directed mutagenesis were synthesized with an Applied Biosystems Model 380A automatic synthesizer. The DNA sequences of the mutated *rnhA* genes were determined by the Sanger method [19].

2.3. Preparation of RNA

All buffers for RNA preparations were made with diethylpyrocarbonate-treated (0.1%) water and were filtered through 0.22-μm sterile filters prior to use. The 534-base RNA (r534-mer) and the 132-base RNA (r132-mer) were obtained by runoff transcriptions of *Sal*I-linearized plasmid pSPsub2 and *Eco*47III-linearized plasmid pSPsub1, respectively, with the MEGA$_{script}$ SP6 RNA Polymerase Large Scale RNA Transcription Kit (Ambion Inc.) according to the procedure recommended by the supplier. The transcripts were purified from 5% (for the r534-mer) or 10% (for the r132-mer) polyacrylamide gels containing 7 M urea as described previously [15], and then dephosphorylated by bacterial alkaline phosphatase prior to end-labeling. The 22-base RNA with the sequence 5'-AAGAUGUCUACGGAGAUGACCA-3' (r22-mer), in which the recognition sequence of the d9-C135/RNase H is underlined, was obtained from Toray Research Center, Inc. RNAs were ^{32}P-labeled and the 5' termini with T4 polynucleotide kinase and [γ-^{32}P]ATP (> 5000 Ci/mmol, Amersham) [20], and purified from 5–20% polyacrylamide gels containing 7 M urea. The labeled RNAs were mixed with an excess amount of unlabeled RNAs and used as a substrate. The concentration of the r22-mer was determined from UV absorption, using a molar absorption coefficient at 260 nm of 2.01×10^5. Concentrations of the r534-mer and the r132-mer were roughly estimated from UV absorption at 260 nm assuming that the molar absorption coefficient of an RNA increases in parallel with an increase in its size

2.4. Cleavage of RNA

Thr r534-mer (1.5 pmol), r130-mer (5 pmol), and r22-mer (10 pmol) were digested separately with 10 ng (0.5 pmol) of d9-C135/RNase H [8], or with 0.05 ng (2.5 fmol) of *E. coli* RNase HI in the presence of a 1.5-fold molar excess (2.5–15 pmol) of the d9-mer (5'-GTCATCTCC-3'), in 10 μl of 10 mM Tris-HCl (pH 8.0) containing 50 mM NaCl, 1 mM 2-mercaptoethanol, and 0.01% bovine serum albumin at 37°C for 1 h. The hydrolysates were separated on 5–20% polyacrylamide gels containing 7 M urea and were detected by autoradiography or ethidium bromide staining.

The cleavage sites of the r132-mer and the r22-mer were determined from the sizes of the 5'-terminal RNA fragments generated by the hydrolysis with the enzyme The sizes were determined by comparing their migrations on a gel with those of the oligonucleotides generated by the partial digestion of the ^{32}P-labeled r132-mer or ^{32}P-labeled r22-mer with phosphodiesterase from snake venom [21].

The cleavage site of the r534-mer was estimated by analyzing the size of the RNA fragments, which were separted on a 5% polyacrylamide gel containing 7 M urea and visualized with ethidium bromide staining. The sizes of the cleavage products were estimated by comparing their migrations on a gel with those of 0.16–1.77 kb RNA size markers (Bethesda Research Laboratories). For the purpose of determining the 5'-terminal nucleotide residue generated by the hydrolysis, the 3'-terminal RNA fragment was purified from the gel, dephosphorylated with bacterial alkaline phosphatase, and ^{32}P-labeled at the 5' end. After gel purification, the ^{32}P-labeled RNA fragment was completely digested with nuclease P1 (Pharmacia-LKB). The resistant ^{32}P-labeled 5'-mononucleotide was separated by paper electrophoresis with 51 A paper (Advantec Co.) and detected by autoradiography. Electrophoresis was performed at room temperature using 700 V and 0.2 M morpholinium acetate (pH 3.5).

3. Results and discussion

3.1. Cleavage of r534-mer

The r534-mer was first examined as a substrate for site-specific cleavage with the d9-C135/RNase H. The r534-mer contains the GGAGAUGAC sequence approximately 340-bases downstream from its 5′ terminus. If it were site-specifically cleaved at the phosphodiester bond between the A and U within this sequence, the sizes of the 5′- and 3′-terminal RNA fragments would be 340 and 194 bases, respectively (Fig. 2). Because the r534-mer was not cleaved by *E. coli* RNase HI (Fig. 3, lane 2) but was cleaved by the d9-C135/RNase H to produce two RNA fragments of approximately 200 and 350 bases (Fig. 3, lane 1), and because the sizes of these RNAs were identical with those generated by the hydrolysis with *E. coli* RNase HI in the presence of the d9-mer (*E. coli* RNase HI + d9-mer) (Fig. 3, lane 3), it is likely that the d9-C135/RNase H site-specifically cleaves the r534-mer within its recognition sequence. The 5′- and 3′-RNA fragments were identified by cleaving the 5′-^{32}P-labeled r534-mer with the d9-C135/RNase H. Only the 350-base fragment was visualized by autoradiography, as expected (data not shown). In addition, the identification of 5′-UMP as the major 5′-terminal mononucleotide of the 200-base RNA fragment (3′-terminal fragment) strongly suggests that the d9-C135/RNase H preferentially cleaves the A–U bond within the GGAGAUGAC sequence (Fig. 4).

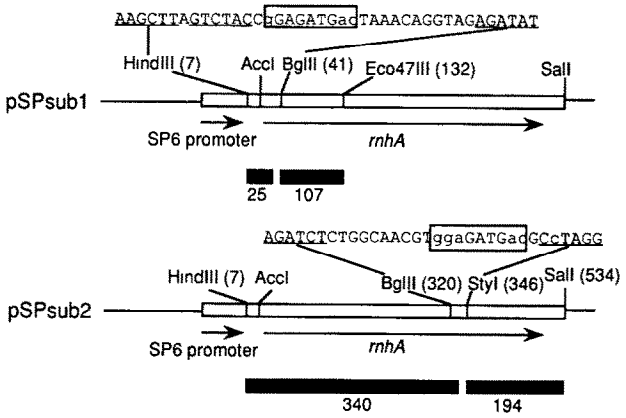

Fig. 2. Structures of plasmids pSPsub1 and pSPsub2. In these plasmids, the transcription of the structural gene of *E. coli* RNase HI (*rnhA*) is controlled by the SP6 promoter. The nucleotide sequences of the mutagenized cassette containing the GGAGAUGAC sequence, which is boxed, are shown. The mutated nucleotide residues resulting from the introduction of this sequence are shown by the lower case letters. The restriction sites that were used to construct these plasmids are also shown. Some of them are accompanied by numbers in parentheses, which represent the distance in bases from the initiation site of transcription. Solid thick lines represent the RNA fragments that are expected to be produced from an in vitro transcript with the cleavage of the A–U bond within the GGAGAUGAC sequence. The numbers below them indicate their sizes in bases.

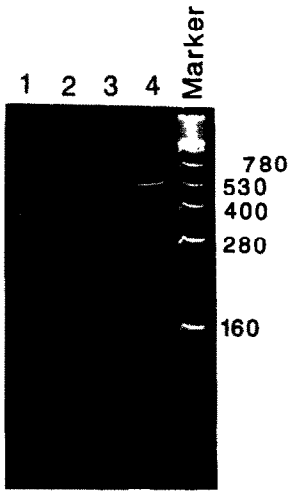

Fig. 3. Separation of r534-mer hydrolysates by polyacrylamide gel electrophoresis. Hydrolysates of the r534-mer (1.5 pmol) with 0.5 pmol of the d9-C135/RNase H (lane 1), 0.05 pmol of *E. coli* RNase HI (lane 2), and 2.5 fmol of *E. coli* RNase HI in the presence of 2.5 pmol of the d9-mer (lane 3) were separated on a 5% polyacrylamide gel containing 7 M urea and visualized with ethidium bromide staining. Lane 4, the untreated r534-mer; lane 5, 0.16–1.77 kb RNA size markers (Bethesda Research Laboratories). The numbers along the gel represent the size of each RNA marker in bases.

3.2. Cleavages of r132-mer and r22-mer

To determine the cleavage site by the d9-C135/RNase H, the 5′-^{32}P-labeled r132-mer and r22-mer were used as substrates. As shown in Fig. 5, the sizes of the major 5′-terminal RNA fragments produced from the r132-mer and the r22-mer were 25 and 16 bases, respectively, indicating that these RNAs were cleaved preferentially at the phosphodiester bond between A and U within the recognition sequence of the d9-C135/RNase H. For the hydrolysis of the r132-mer, production of the 3′-terminal fragment was confirmed by analyzing the hydrolysate on

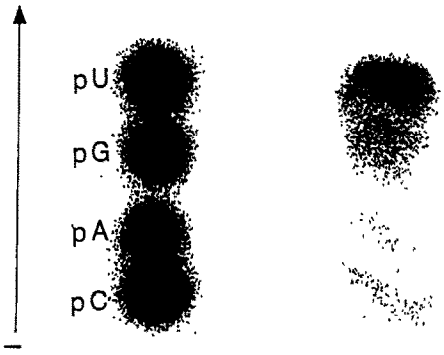

Fig. 4. Separation of 5′-mononucleotides on paper electrophoresis. The hydrolysate of the 5′-^{32}P-labeled RNA fragment (3′-terminal RNA fragment generated by the hydrolysis of the r534-mer with the d9-C135/RNase H) with nuclease P1 was fractionated by paper electrophoresis using a cellulose-acetate membrane (right lane). Electrophoresis was performed as described in section 2, and 5′-mononucleotides were detected by autoradiography. A mixture of four 5′-^{32}P-mononucleotides was also applied as a reference (left lane).

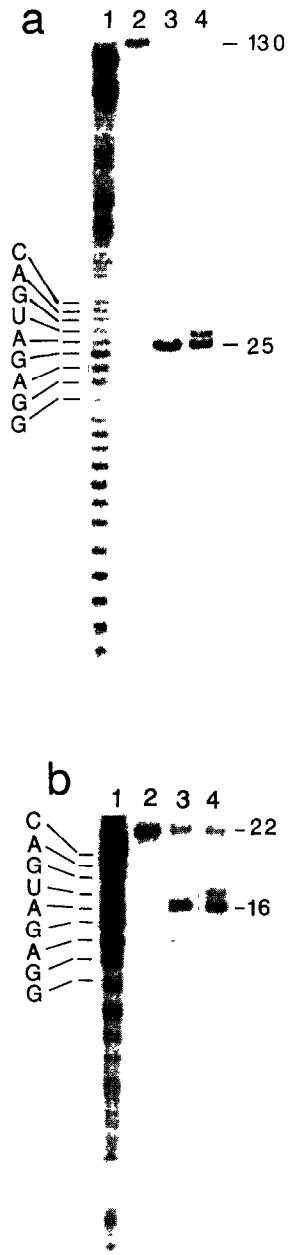

Fig. 5. Separation of r132-mer and r22-mer hydrolysates by polyacrylamide gel electrophoresis. Hydrolysates of 5 pmol of the r132-mer (a) or 10 pmol of the r22-mer (b) with 0.05 pmol of *E. coli* RNase HI (lane 2), 0.5 pmol of the d9-C135/RNase H (lane 3), and 2.5 fmol of *E. coli* RNase HI in the presence of 7.5 pmol (a) or 15 pmol (b) of the d9-mer (lane 4) were separated on a 10% (a) or 20% (b) polyacrylamide gel containing 7 M urea, and visualized by autoradiography. Lane 1, partial digest of the r132-mer (a), or r22-mer (b) with phosphodiesterase from snake venom. The numbers along the gel represent the size of RNA in bases. The RNA fragments resulting from the partial digest within the GGAGAUGAC sequence are also shown along the gel.

a 10% polyacrylamide gel containing 7 M urea with ethidium bromide staining (data not shown). The hybrid RNase H was shown to cleave these RNAs at the alternative phosphodiester bond as well, but with very poor efficiency, to produce the RNA fragment corresponding to the n−2 band. When these RNAs were cleaved with *E. coli* RNase HI + d9-mer, RNA fragments corresponding to the n + 1 and n + 3 bands, instead of the n − 2 band, were shown to be produced in addition to the major band, which results from the cleavage at the A–U bond within the recognition sequence.

3.3. Cleavage patterns

The r132-mer and r22-mer cleavage sites with the d9-C135/RNase H or *E. coli* RNase HI + d9-mer are summarized in Fig. 6. All of them are located within the GGAGAUGAC sequence. The major cleavage sites in these RNAs with the hybrid RNase H or *E. coli* RNase HI + d9-mer are basically the same as those in the r9-mer [8], indicating that the difference in the size and the sequence of the flanking region of the r9-mer sequence does not seriously affect the interaction between the enzyme and the RNA/DNA hybrid region.

The cleavage at the n − 2 position is of interest, although its efficiency is very poor, because the r9-mer is not cleaved at this position [8]. The major substrate-binding site of *E. coli* RNase HI is proposed to be located on the right of the catalytic site when a model for the three-dimensional structure of the enzyme is schematically drawn, as shown in Fig. 1 [11]. This suggests that the 5′-upstream region of RNA relative to the cleavage site, which forms the RNA/DNA hybrid, is recognized by the enzyme as a substrate. In addition, it has been proposed that the tetranucleotide duplex is the minimal size responsible for the interaction with the enzyme [2–5]. The n − 2 position of the r9-mer is not cleaved, probably because the size of the RNA/DNA duplex at the 5′-upstream region of the cleavage site is too short to effectively bind to the substrate-binding site of the enzyme. In contrast, the n − 2 position is cleaved by the hybrid RNase H when the r132-mer or the r22-mer is used as

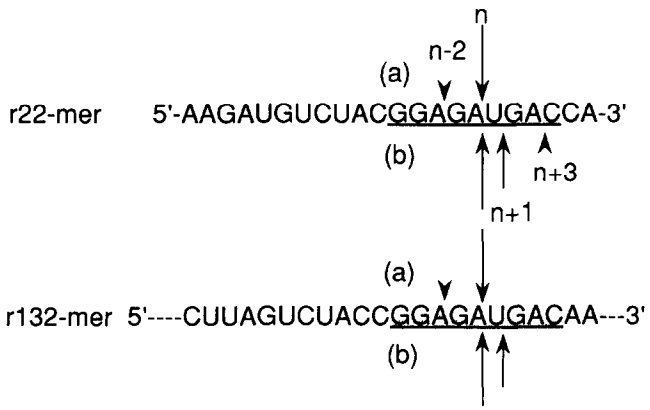

Fig. 6. Histograms of cleavage patterns. Cleavage sites of the r22-mer and the r132-mer, with the d9-C135/RNase H (a), or *E. coli* RNase HI + d9-mer (b), are shown by arrows. The difference in the height of arrows reflects the relative cleavage intensities at the indicated sites. The n − 2, n, n + 1, and n + 3 positions are also shown.

a substrate, probably because the interaction between the single-stranded RNA region extended to the 5′ terminus of the r9-mer sequence and the basic protrusion of the enzyme may help the trinucleotide duplex to associate with the substrate-binding site of the enzyme. Cleavage at the n − 2 position with *E. coli* RNase HI + d9-mer was not observed. In this case, the cleavage at the n + 1 position may dominate the cleavage at this position.

The observation of cleavage at the n − 2 position, instead of the n + 1 position, with the hybrid enzyme indicates that the cross-linking of the d9-mer to the enzyme restricts the interaction of the enzyme with the hybridized substrate. Relatively poor flexibility in the linker between the enzyme and the d9-mer due to its limited size probably forces the enzyme to cleave the RNA at this site. It is therefore expected that an increase in the size of the linker may alter the specificity of the hybrid RNase H.

3.4. A model for the action of a hybrid RNase H

The d9-C135/RNase H performs multiple turnovers for the site-specific cleavage of RNA, because a 10-fold molar excess of the substrate (r132-mer) over the hybrid RNase H is almost completely hydrolyzed at 37°C within 1 h (Fig. 5a). A model for the action of a hybrid RNase H is shown in Fig. 7. In this figure, the DNA attached to the enzyme is shown to exist as an independent domain, because the attachment of the d9-mer does not completely abolish the enzymatic activity against the M13 RNA/DNA hybrid [8]. The fact that the hybridization of the nonadeoxyribonucleotide to the d9-C135/RNase H does not affect the enzymatic activity against M13 RNA/DNA hybrid also supports this proposal (C. Nakai, personal communication). However, the possibility that the DNA attached to the enzyme interacts with the enzyme, probably with the basic protrusion, cannot be ruled out, because it seems likely that the interaction between the single-stranded or double-stranded DNA with the enzyme may be too weak to interfere with the interaction between the RNA/DNA hybrid and the enzyme.

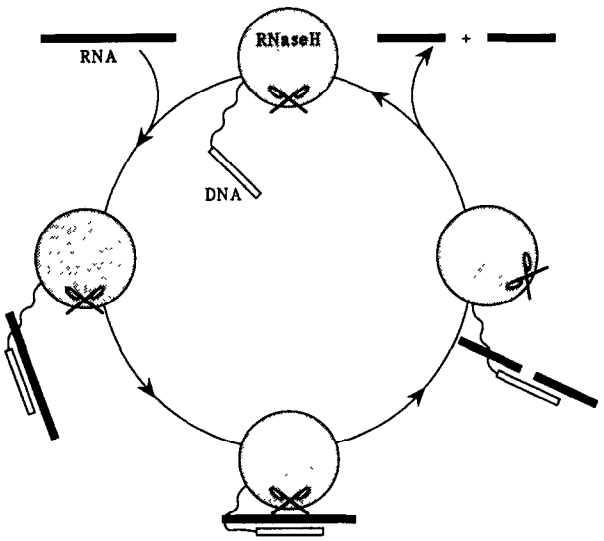

Fig. 7. A proposed mechanism for the sequence-specific cleavage of RNA by a hybrid RNase H.

According to the model shown in Fig. 7, hydrolysis of RNA is completed in four steps: (1) hybridization of the DNA portion with the RNA, (2) recognition of the resultant RNA/DNA hybrid with the RNase H portion, (3) dissociation of the RNase H portion from the cleaved RNA/DNA hybrid, and (4) dissociation of the DNA portion from the cleaved RNA. This last step results from a dramatic decrease in the stability of the RNA/DNA hybrid upon the cleavage of RNA within this hybrid. In fact, the Tm value of the r9-mer/d9-mer hybrid dropped from 49°C to less than 24°C upon cleavage between the fifth and sixth residues in the r9-mer [8]. The DNA/protein hybrid RNase H may be useful for structural and functional studies of RNA as an artificial RNA restriction enzyme, because an RNA restriction enzyme with any sequence-specificity can be constructed by attaching various oligodeoxyribonucleotides with different sizes and sequences. Therefore, it will be informative to examine whether alterations in the size and the sequence of an oligodeoxyribonucleotide attached to the enzyme affect the specificity or catalytic efficiency of the hybrid RNase H.

Acknowledgements: We thank to Dr. M. Ikehara for helpful discussions.

References

[1] Crouch, R.J. and Dirksen, M.-L. (1982) in: Nuclease (Linn, S.M. and Roberts, R.J., Eds.) pp. 211–241, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[2] Donis-Keller, H. (1979) Nucleic Acids Res. 7, 179–192.
[3] Inoue, H., Hayase, Y., Iwai, S. and Ohtsuka, E. (1987) FEBS Lett. 215, 327–330.
[4] Metelev, V.G., Zayakina, G.V., Ryabushenko, I.L., Krynetskaya, N.F., Romanova, E.A., Oretskaya, T.S. and Shabarova, Z.A. (1988) FEBS Lett. 226, 232–234.
[5] Atabekov, K.J., Tyulkina, L.G., Karpova, O.V., Metelev, V.G., Rodionova, N.P., Shabarova, Z.A. and Atabekov, J.G. (1988) FEBS Lett. 232, 96–98.
[6] Shibahara, S., Mukai, S., Nishihara, T., Inoue, H., Ohtsuka, E. and Morisawa, H. (1987) Nucleic Acids Res. 15, 4403–4415.
[7] Hayase, Y., Inoue, H. and Ohtsuka, E. (1990) Biochemistry 29, 8793–8797.
[8] Kanaya, S., Nakai, C., Konishi, A., Inoue, H., Ohtsuka, E. and Ikehara, M. (1992) J. Biol. Chem. 267, 8492–8498.
[9] Kanaya, S. and Ikehara, M. (1993) in: Nucleic Acids and Molecular Biology (Eckstein, F. and Lilley, D.M.J., Eds.) Vol. 7, pp. 285–302, Springer, Berlin, Heidelberg.
[10] Kanaya, S., Kohara, A., Miura, Y., Sekiguchi, A., Iwai, S., Inoue, H., Ohtsuka, E. and Ikehara, M. (1990) J. Biol. Chem. 265, 4615–4621.
[11] Nakamura, H., Oda, Y., Iwai, S., Inoue, H., Ohtsuka, E., Kanaya, S., Kimura, S., Katsuda, C., Katayanagi, K., Morikawa, K.,

Miyashiro, H. and Ikehara, M. (1991) Proc. Natl. Acad. Sci. USA 88, 11535–11539.
[12] Haseloff, J. and Gerlach, W.L. (1988) Nature 334, 585–591.
[13] Zuckermann, R.N. and Schultz, P.G. (1988) J. Am. Chem. Soc. 110, 6592–6594.
[14] Zuckermann, R.N., Corey, D.R. and Schultz, P.G. (1988) J. Am. Chem. Soc. 110, 1614–1615.
[15] Zuckermann, R.N. and Schultz, P.G. (1989) Proc. Natl. Acad. Sci. USA 86, 1766–1770.
[16] Kanaya, S., Katsuda-Nakai, C. and Ikehara, M. (1991) J. Biol. Chem. 266, 11621–11627.
[17] Miller, J.H. (1972) in: Experiments in Molecular Genetics, p. 433, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[18] Kanaya, S., Oobataka, M., Nakamura, H. and Ikehara, M. (1993) J. Biotechnol. 28, 117–136.
[19] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[20] Donis-Keller, H., Maxam, A. and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527–2538.
[21] Jay, E., Bambara, A., Padmanabham, P. and Wu, R. (1974) Nucleic Acids Res. 1, 331–353.
[22] Katayanagi, K., Miyagawa, M., Matsushima, M., Kanaya, S., Ikehara, M., Matsuzaki, T. and Morikawa, K. (1990) Nature 347, 306–309.