

Site-specific enzymatic cleavage of TMV RNA directed by deoxyribo- and chimeric (deoxyribo-ribo)oligonucleotides

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The TMV RNA molecule can be cleaved at a single site by RNase H directed by chimeric oligo(deoxyribo-ribo)nucleotide with an internucleotide pyrophosphate bond

Site specificity; RNA cleavage; RNase H; Oligo(deoxyribo-ribo)nucleotide

1. INTRODUCTION

The method of site-specific cleavage of large RNAs with *Escherichia coli* RNase H in the presence of oligodeoxyribonucleotides complementary to a given site of an RNA molecule has been proposed by our group [1-4]. This method was applied to the site-specific cleavage of RNA of satellite tobacco necrosis virus [5], RNA of barley stripe mosaic virus [6,7], RNAs of bacteriophages MS2 and R17 [3], tobacco mosaic virus (TMV) [4], brome mosaic virus [8] and potato virus X (Miroshnichenko et al., in preparation). RNase H was suggested to cleave the RNA at the 3'-end of the RNA-DNA heteroduplex [3,5]. However, cleavage of the selected RNA site by RNase H can occur ambiguously in more than one position within the RNA-DNA hybrid and in the case of homopolymeric sequences can even lead to the complete excision of homopolymeric RNA regions of RNA-DNA hybrids (e.g. see [9]). Recently, synthetic chimeric oligo(deoxyribo-ribo)nucleotides were used to direct site-specific attack of RNase H [10-12]. RNase H cleaved RNA (90-mer) at a

single site or two positions in the presence of chimeric oligonucleotides containing 2'-*O*-methyl-ribonucleotides and contiguous 3-5-deoxyribonucleotides [11]. Here, we describe experiments on TMV RNA cleavage at a position 3'-proximal to the first AUG initiation codon. RNase H was directed by oligodeoxyribonucleotides (7-10-mer) as well as by chimeric oligonucleotides containing pentaribonucleotide linked to seven contiguous deoxynucleotides at the 5'-end by either a phosphodiester or pyrophosphate bond (fig.1). In the latter case, TMV RNA within the heteroduplex was cleaved at a single site, two residues from the 3'-end of the RNA-DNA region.

2. MATERIALS AND METHODS

TMV strain U1 was isolated from *Nicotiana tabacum* var. Samsun EN. RNA was isolated using the phenol procedure from the purified virus [13]. Oligodeoxyribonucleotides and the modified chimeric oligo(deoxyribo-ribo)nucleotides were synthesized as in [12]. RNase H was isolated from cells of *E. coli* MRE-600 as described by Darlix [14]. RNase H cleavage reactions were carried out in buffer containing 10 mM Tris-HCl, pH 7.9, 150 mM NaCl, 1 mM MgCl₂, 0.1 mM DTT; 0.2 A₂₆₀ units of oligonucleotide were added to 10 µg TMV RNA, incubated for 3 min at 65°C, and cooled slowly at room temperature. The buffer and 0.01 U RNase H were then added and the reaction mixture was incubated for 1 h at 4°C. The reaction was stopped by addition of 2.5 vols ethanol. The

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III) or even one (oligonucleotide IV) cleavage site in contrast to oligodeoxynucleotides I and II (four and three cleavage sites, respectively).

Each of the cleavage products of TMV RNA was isolated and sequenced individually. All the products (bands 1–4 in fig.2) were found to correspond to the 5'-terminal fragments of TMV RNA in nucleotide sequence (not shown), i.e. in all cases site-specific oligonucleotide-directed cleavage of TMV RNA took place. However, exact determination of the 3'-terminal residues upon sequencing was complicated by the compression of bands due to the effect of the salts in the sample. Therefore, the 3'-terminally labeled nucleotides were determined in individual TMV RNA fragments derived from TMV RNA by RNase H in the presence of oligonucleotides I–IV (fig.3).

These data show that four TMV RNA fragments, designated 1–4 in fig.2, have 3'-terminal A, C, A and U, respectively. Since these fragments differ in length by one nucleotide, the tetranucleotide target sequence can be deduced.

Taken together, the results of direct sequencing and 3'-terminal nucleotide determination in individual RNA fragments allowed us to reveal the exact site(s) of TMV RNA cleavage by RNase H summarized in fig.1.

In the presence of deca- and hepta-oligodeoxynucleotides RNase H cleaved TMV RNA at four and three sites, respectively (fig.1). Formation of multiple cleavage products either can be due to the ability of RNase H to nick RNA at different sites (1–4 in fig.1) within the heteroduplex or can result from successive excision of nucleotides in the 3'–5'-direction of RNA after its cleavage at a single site (1 in fig.1). It should be noted that the number of cleavage sites can be decreased to only a single position (fig.1) if RNase H is directed by chimeric oligonucleotides.

Our experiments with TMV RNA cleavage are in general conformity with recent model experiments on site-specific cleavage of synthetic polyribonucleotides [10,11] and short oligoribonucleotides [12] by RNase H directed by chimeric polyribonucleotides. It should be mentioned that RNase H directed by chimeric oligonucleotides with an internucleotide pyrophosphate bond cleaved at a single site the native TMV RNA molecule (fig.1) as well as the dodecaribonucleotide pAAUGGCAUA-CAC [12]. However, the exact site of cleavage by

RNase H may be rather different in the cases of native TMV RNA and short oligo- [12] and polyribonucleotides [10,11].

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REFERENCES

- [1] Metelev, V.G., Stepanova, O.B., Chichkova, N.V., Rodionova, N.P., Smirnov, V.D., Bodganova, S.L., Sergeeva, N.F., Ratmanova, K.I., Bogdanov, A.A., Shabarova, Z.A. and Atabekov, J.G. (1978) *Biol. Nauki USSR* 8, 27–30.
- [2] Stepanova, O.B., Metelev, V.G., Chichkova, N.V., Smirnov, V.D., Rodionova, N.P., Atabekov, J.G., Bogdanov, A.A. and Shabarova, Z.A. (1979) *FEBS Lett.* 103, 197–199.
- [3] Metelev, V.G., Stepanova, O.B., Chichkova, N.V., Smirnov, V.D., Rodionova, N.P., Berzin, V.M., Jasone, I.V., Gren, E.J., Bogdanov, A.A., Shabarova, Z.A. and Atabekov, J.G. (1980) *Mol. Biol. USSR* 14, 200–211.
- [4] Rodionova, N.P., Karpova, O.V., Metelev, V.G., Bogdanova, S.L., Shabarova, Z.A. and Atabekov, J.G. (1983) *Mol. Biol. USSR* 17, 809–817.
- [5] Donis-Keller, H. (1979) *Nucleic Acids Res.* 7, 179–192.
- [6] Dolja, V.V., Lunina, N.A., Smirnov, V.D., Karpov, V.A., Hudjakov, Yu.E., Kozlov, Yu.V., Baev, A.A. and Atabekov, J.G. (1982) *Dokl. Akad. Nauk SSSR* 265, 474–477.
- [7] Agranovsky, A.A., Dolja, V.V. and Atabekov, J.G. (1982) *Virology* 119, 51–58.
- [8] Rodionova, N.P., Tyulkina, L.G., Karpova, O.V. and Atabekov, J.G. (1986) *Dokl. Akad. Nauk SSSR* 287, 1005–1008.
- [9] Tyulkina, L.G., Karpova, O.V., Rodionova, N.P. and Atabekov, J.G. (1986) *Virology* 159, 312–320.
- [10] Inoue, H., Hayase, Y., Iwai, S. and Ohtsuka, E. (1987) *FEBS Lett.* 215, 327–330.
- [11] Shibahara, S., Mukai, S., Nishihara, T., Inoue, H., Ohtsuka, E. and Morisawa, H. (1987) *Nucleic Acids Res.* 15, 4403–4415.
- [12] Metelev, V.G., Zayakina, G.V., Ruyabushenko, I.L., Krynetskaya, N.F., Romanova, E.A., Oretskaya, T.S. and Shabarova, Z.A. (1988) *FEBS Lett.* 226, 232–234.
- [13] Fraenkel-Conrat, H., Singer, B. and Tsugita, A. (1961) *Virology* 14, 54–58.
- [14] Darlix, J.L. (1975) *Eur. J. Biochem.* 51, 369–375.
- [15] Peattie, D.A. (1979) *Proc. Natl. Acad. Sci. USA* 76, 1760–1764.
- [16] Goelet, P., Lomonossoff, G.P., Butler, P.J.G., Akam, M.E., Gait, M.J. and Karn, J. (1982) *Proc. Natl. Acad. Sci. USA* 79, 5818–5822.