ADDRESSED FRAGMENTATION OF RNA MOLECULES

O. B. STEPANOVA+, V. G. METELEV, N. V. CHICHKOVA, V. D. SMIRNOV, N. P. RODIONOVA, J. G. ATABEKOV*, A. A. BOGDANOV, Z. A. SHABAROVA

Laboratory of Bioorganic Chemistry and Molecular Biology and Department of Virology of Moscow State University, Moscow 117234 and *+Institute of Applied Molecular Biology and Genetics, Moscow, USSR*

Received 23 April 1979

1. Introduction

Many problems of molecular biology require specific fragmentation of nucleic acids. For DNAs this can be done with the help of specific endonucleases (restriction enzymes). However, no reliable and efficient methods are known for strictly specific cleavage of RNA molecules.

This paper describes an experimental approach to addressed fragmentation of polyribonucleotides. It is known that RNase H splits RNA in RNA–DNA heteroduplexes [1] or in the heteroduplexes made of monotonous synthetic polyribonucleotides and complementary oligodeoxyribonucleotides [2–4]. In optimal conditions for double helix formation, oligodeoxyribonucleotides are capable of forming rather stable complexes with exposed complementary sites of native RNAs. It seems reasonable to assume then that treatment by RNase H of a heteroduplex composed of an RNA molecule and an oligodeoxyribonucleotide that is complementary to a certain part of this RNA, will result in the splitting of the RNA molecule in the binding site of the oligodeoxyribonucleotide. This approach has been realized using RNA of bacteriophage MS2, the primary structure of which is known [5], and RNA of a related phage, R17, which has the same genome structure (fig.1a).

* To whom correspondence should be addressed

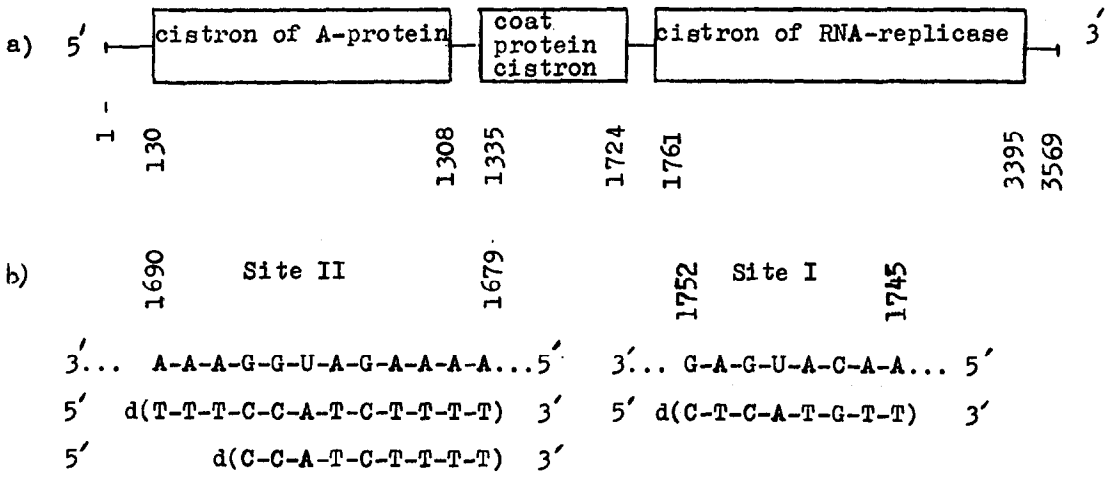

Fig.1. (a) The structure of the genome of bacteriophage MS2; (b) The regions of the MS2 RNA molecule complementary to the synthetic oligodeoxyribonucleotides. The figures correspond to the localization of the nucleotide residues in the 5′ → 3′ direction of the polynucleotide chain of the phage RNA.

The following oligodeoxyribonucleotides were synthesized and used:

(1) Octanucleotide d(C–T–C–A–T–G–T–T) that is complementary to the region of the RNA between the cistrons of the coat protein and RNA-replicase (site 1 corresponding to 1745–1752 nucleotides from the 5′-end of the bacteriophage RNA, see fig.1b);
(2) Nonanucleotide d(C–C–A–T–C–T–T–T–T);
(3) Dodecanucleotide d(T–T–T–C–C–A–T–C–T–T–T–T), that are complementary to a region of the bacteriophage RNA within the coat protein gene (site II corresponding to 1679–1690 nucleotides from the 5′-end, see fig.1b).

2. Materials and methods

The isolation of RNase H (EC 3.1.4.34) from *Escherichia coli* MRE-600 cells was carried out as in [1]. The activity of the enzyme was tested with *E. coli* DNA–[^{14}C]RNA hybrid used as substrate. The quantity of the enzyme that hydrolyses 1 nmol RNA in the hybrid in 20 min at 20°C in buffer A (10 mM Tris–HCl; 0.15 M NaCl; 1 mM $MgCl_2$; 2×10^{-4} M dithiothreitol; pH 7.9) was taken as a unit of enzymatic activity.

Phage R17 was isolated by the method in [6]. Phage MS2 was a kind gift of Dr V. N. Markushevich. RNA of phages R17 and MS2 was isolated by phenol deproteinization from purified preparations of the viruses [7]. RNA from brome mosaic virus was kindly given to us by Dr V. K. Novikov. The oligodeoxyribonucleotides were synthesized in this laboratory by chemical methods [8].

The enzymatic hydrolysis of RNA was done as follows: the samples containing 30–40 μg RNA and 0.03–0.05 A_{260} units of the oligonucleotide in buffer A were incubated for 30 min at 3°C, then 0.15 unit RNase H was added and the mixture was incubated for 5 h at 4°C. The hydrolysis was interrupted by addition of SDS to 0.1%. The products of the enzymatic reaction were examined by electrophoresis in 3% polyacrylamide gel [9] for 3 h, at 5 mA/tube; tube size 6 × 90 mm. The gels were scanned at λ = 258 nm in a Gilford-2500 spectrophotometer. To reveal hidden nicks in RNA, before electrophoresis the reaction mixtures were heated for 4 min at 70°C.

3. Results and discussion

We have shown that in buffer A at 4°C bacteriophage RNA formed complexes with complementary oligodeoxyribonucleotides that can be demonstrated by gel filtration [10]. Here we demonstrate that the cleavage of phage RNA by means of RNase H takes place in the binding sites of the oligodeoxyribonucleotides.

As seen in fig.2a, RNase H in the conditions employed by us does not cause the degradation of bacteriophage R17 RNA in the absence of the oligodeoxyribonucleotides. But in the mixture containing the bacteriophage RNA, an oligodeoxyribonucleotide complementary to a certain region of the latter and RNase H, there occurs effective fragmentation of RNA (in some experiments ⩽ 60–70%, see fig.2c), as shown by the appearance of one (fig.2b) or two (fig.2c,2d) bands that are not present in the control (fig.2a). In SDS gel electrophoresis these bands prove to have the same mobility as 16 S RNA of *E. coli* (the molecular weight of 16 S RNA can be estimated as 0.51×10^6 if the average molecular weight of a nucleotide is assumed to be 330 and the total number of nucleotides 1541–1542 [11,12]). Similar data were obtained in experiments with bacteriophage MS2 RNA. It can be concluded from this evidence that addressed fragmentation of bacteriophage RNA molecule took place in the region of formation of the

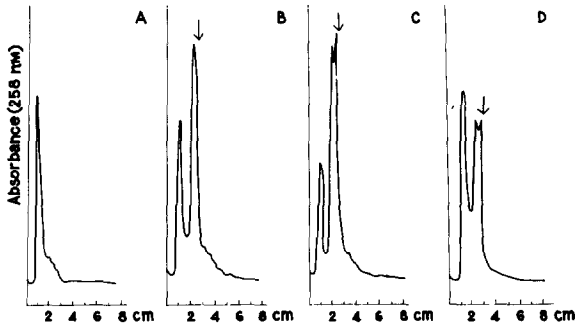

Fig.2. The electrophoretograms of the products of hydrolysis of RNA–oligodeoxyribonucleotide heteroduplexes by RNase: (a) Phage R17 RNA + RNase H; (b) Phage R17 RNA + RNase H + d(C–T–C–A–T–G–T–T); (c) Phage R17 RNA + RNase H + d(C–C–A–T–C–T–T–T–T); (d) Phage R17 RNA + RNase H + d(T–T–T–C–C–A–T–C–T–T–T–T). The arrow shows the localization of 16 S RNA of *E. coli*.

heteroduplex with the respective synthetic oligodeoxyribonucleotide. Indeed, octanucleotide d(C–T–C–A–T–G–T–T) is complementary to site I (see fig.1b) that is located approximately in the middle of the RNA molecule. If the RNA of bacteriophage R17 is cleaved in the region of the formation of the heteroduplex, RNA fragments of mol. wt 0.6×10^6 (1817×330) and 0.58×10^6 (1745×330) should be produced that cannot be resolved in the conditions of gel electrophoresis used by us (fig.2b).

Enzymatic cleavage of phage MS2 RNA with the use of nona- and dodecanucleotides that are complementary to site II, gives fragments of mol. wt 0.62×10^6 (1879×330) and 0.55×10^6 (1679×330) that can form discrete bands on electrophoresis in 3% polyacrylamide gel.

The fragments produced on cleavage of the R17 RNA–octanucleotide heteroduplex, could be resolved in 3% polyacrylamide gel with agarose in 7 M urea [13]. Determination of the molecular weights of the fragments produced upon R17 RNA cleavage was performed with the virion RNA of brome mosaic virus (four fragments of mol. wt 1.19×10^6, 1.07×10^6, 0.8×10^6 and 0.28×10^6 [14]), as well as ribosomal 16 S RNA and 23 S RNA of *E. coli* (mol. wt 0.56×10^6 and 1.1×10^6, respectively) used as markers.

The molecular weights of the fragments formed as a result of the cleavage of bacteriophage R17 RNA with RNase H (0.57×10^6 and 0.59×10^6 in the case of hydrolysis with the use of the octanucleotide and 0.54×10^6 and 0.60×10^6 after hydrolysis with the use of nona- and dodecanucleotide; data not shown) are close to the predicted values. These values allow us to contend that a specific fragmentation of the RNA polynucleotide chain by RNase H in the presence of a complementary oligodeoxyribonucleotide has taken place.

Analysis of the products of fragmentation of the complex formed by octanucleotide d(C–T–C–A–T–G–T–T) with a fragment of MS2 RNA (59 nucleotide residues) that contains site I [15], has furnished additional proof of the position-specific hydrolysis described above (unpublished data obtained in collaboration with Dr E. Ya. Gren and his coworkers).

The addressed fragmentation of RNA seems to be a promising method for limited and precise fragmentation of RNA molecules and also for the study of different aspects of the expression of genetic information of RNA viruses.

Acknowledgements

The authors are indebted to Drs N. F. Sergeeva and K. I. Ratmanova for the synthesis of the oligodeoxyribonucleotides used in this work.

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