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Construction of a series of several self-cleaving RNA duplexes using synthetic 21-mers

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Two fragments (21-mers) containing consensus sequences for the self-cleavage domain in transcripts of satellite DNA of the newt were chemically synthesized and found to be cleaved in the presence of Mg^{2+}. The cleaved product contained the 3'-terminal 2',3'-cyclic phosphate. Twenty-five combinations of partially double-stranded 21-mer RNA which contained different bases within the consensus sequences and at the cleavage sites were tested for self-cleavage. It seemed that guanosine 3'-phosphate was not susceptible to transesterification at the cleavage site.

Synthetic oligoribonucleotide; RNA self-cleavage; Satellite RNA; Hammerhead self-cleaving RNA; (Newt)

1. INTRODUCTION

A new class of self-cleavage RNA has been found in satellite RNA of the tobacco ring spot virus (STobRV RNA) [1,2], avocado sunblotch viroid (ASBV) [3] and lucerne transient streak virusoid (VLTSV) [4]. Transcripts of satellite 2 DNA of the newt were also found to be cleaved at a consensus sequence similar to the one found in infectious RNA in plants [5]. The newt RNA undergoes site-specific self-catalyzed cleavage in the presence of magnesium at neutral pH. Buzayan et al. [6] have found that one quarter of STobRV RNA is needed for cleavage. A 'hammerhead' model has been proposed by Symons and coworkers [3] for the secondary structure of the self-cleavage domain which contains consensus sequences. Model RNA containing these three conserved helices and 13 nucleotides have been prepared using T_7 RNA polymerase and found to undergo self-cleavage [7,8].

In this paper we describe the chemical synthesis of two oligoribonucleotides with a chain length of 21, which form the self-cleaving domain of the transcript of newt satellite 2 DNA, and their hydrolysis in the presence of magnesium ions. In order to determine the nature of the reaction, we prepared 15 21-mer strands to alter 6 positions in the domain, by forming 25 duplexes. We found that guanosine 3'-phosphate at the cleavage site was not susceptible. Complementarity in certain positions was also found to be important for efficient cleavage.

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2. MATERIALS AND METHODS

Oligoribonucleotides were synthesized by the phosphoramidite method using a DNA synthesizer (Applied Biosystems 380 A or 381 A) and dichloroacetic acid as the detritylating reagent [9]. Monomer units were prepared from 5'-*O*-dimethoxytrityl-2'-*O*-tetrahydropyranyl-*N*-protected nucleosides. Oligonucleotides were purified by reverse-phase and anion-exchange HPLC.

5'-labelling was performed using [γ-^{32}P]ATP plus polynucleotide kinase (Takara Shuzo Co.) and desalted by NENSORB 20 (Dupont).

Cleavage reactions were performed by the method which was essentially the same as that described previously [5]. The reaction mixtures contained 5'-labelled CL-1 (2.5 pmol), CL-2 (3.75 pmol), 25 mM $MgCl_2$, 40 mM Tris-HCl (pH 7.5), 20 mM NaCl in a volume of 2.3 μl, and were incubated at 37°C for 23 h unless otherwise specified. In control experiments without Mg^{2+}, 5 mM EDTA was added. The reaction was stopped by addition of 50 mM EDTA (3 μl) and the mixture analyzed by

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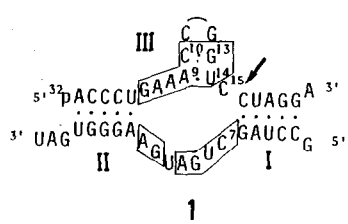
electrophoresis on 20% polyacrylamide in 8 M urea. The percentage of cleavage was estimated by counting radioactivity in gel fragments by a scintillation counter. The 2′,3′-cyclic phosphates in the oligonucleotides were characterized as described [5] and subjected to mobility shift analysis [10].

3. RESULTS AND DISCUSSION

Two partially paired 21-mers (1) (fig.1) containing the sequence found in the self-cleaving site of a transcript of a newt satellite DNA [5] were synthesized by the phosphoramidite method.

The self-cleaving domain was thus constructed using two separate oligonucleotides to facilitate a rapid automated synthesis. In the presence of Mg^{2+}, cleavage occurred at the site which is consistent with the in vitro reaction with the 600 base transcript [5]. The cleaved 3′-end had a 2′,3′-cyclic phosphate, which was characterized as described [5]. It was further confirmed by mobility shift analysis [10] (fig.2). The terminal cytidine 2′,3′-cyclic phosphate was identified by faster mobility in electrophoresis and slower mobility in homochromatography than that expected from a terminal cytidine. In order to find structural requirements of the common sequences (boxed in fig.1), the six numbered bases were altered. The extent of reaction of these sequence isomers as well as the wild type (1) is summarized in table 1. The maximum level of 80–85% cleavage obtained with the wild-type substrate may be due to incomplete duplex formation during the reaction. Four bases in stem III were considered to be important in forming the tertiary structure of the molecule for the reaction at cytidine 15. G:C pair at C-10 and G-13 can be exchanged with each other to give a reaction comparable to the wild type. However,



1

Fig.1. Two 21-mers forming the self-cleaving domain. The upper strand (CL1) was labelled with radioactive 5′-phosphate. Numbered nucleotides were altered to yield the 25 combinations listed in table 1. The 'consensus bases' and the cleavage site are boxed and indicated by an arrow, respectively. Three probable stems are numbered I–III.

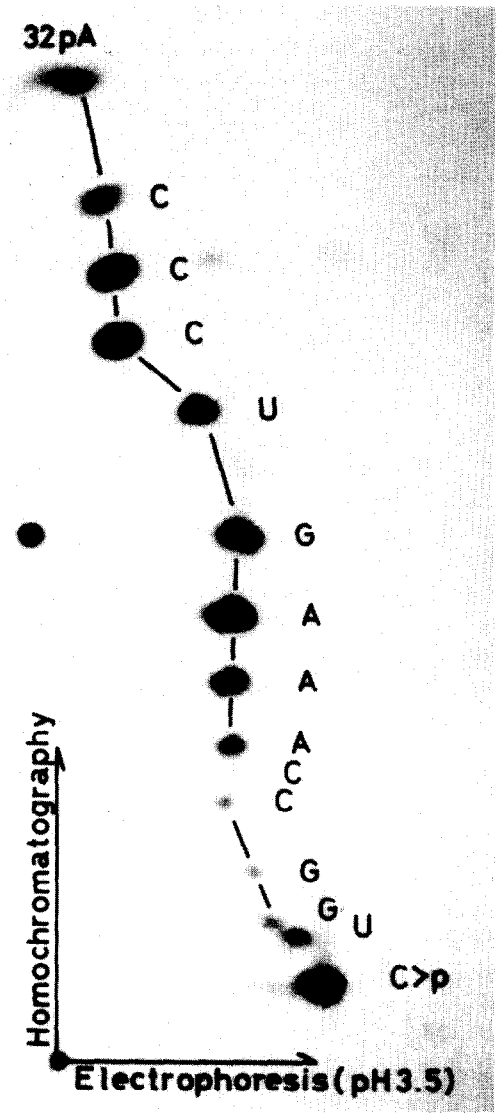


Fig.2. Mobility shift analysis of the cleaved product (p15-mer) using Homo-mixIII [11]. Partial digestion was performed using nuclease P_1 [12].

A-10 and U-13 yielded less cleavage (34%). A:U inversions at positions 9 and 14 as well as U-10 influenced the reaction. Some kind of purine stretch may be required to form the necessary tertiary structure. The cleavage site (C-15) next to stem I was replaced with three other bases. When C-7 was present in the lower strand (CL-2) cleavage at U-15 and A-15 occurred efficiently (86 and 88%). Although cleavage at A-15 has been reported [4],

Table 1

Self-cleavage of RNA duplexes in the presence of Mg^{2+}

Duplex	CL1					CL2	Percentage of cleavage
	9	14	10	13	15	7	
1	A	U	C	G	C	C	82, 52[a]
2	A	G					ND[b]
3	A	A					ND
4	A	C					ND
5	G	C					4
6	C	G					ND
7	U	A					11
8			G	C			83
9			A	U			34
10			U	A			8
11					G	C	ND
12					A	C	86, 52[a]
13					U	C	88, 55[a]
14					G	G	ND
15					A	G	ND
16					U	G	ND
17					C	G	ND
18					G	A	ND
19					A	A	ND
20					U	A	ND
21					C	A	ND
22					G	U	ND
23					A	U	ND
24					U	U	18
25					C	U	63

[a] For 4 h
[b] ND, not detected

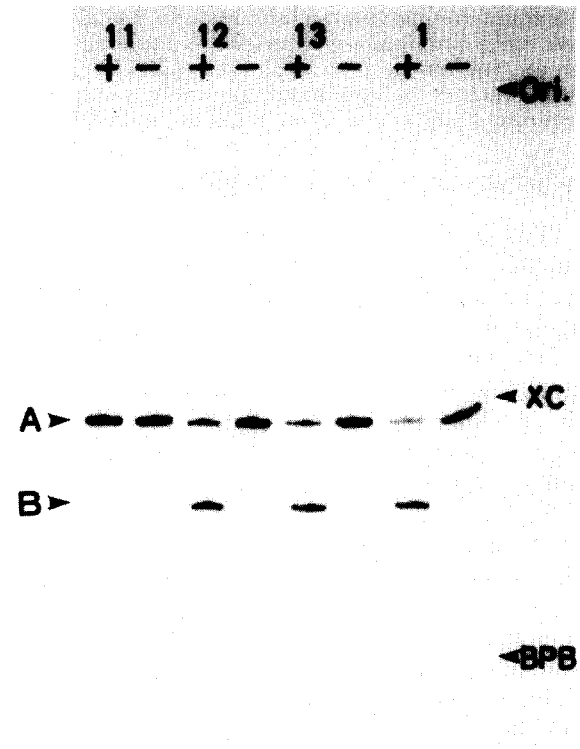


Fig.3. Autoradiogram of some of the cleaved mixtures shown in table 1. A and B show the 5′-labelled 21-mer (CL-1 in fig.1) and cleaved product (p15-mer), respectively. The reactions were performed for 4 h in the presence (+) and absence (−) of Mg^{2+}

transesterification at the 3′-position of uridine was observed for the first time. Autoradiograms of the products in these reactions are shown in fig.3. Oligomers containing G-15 were not recognized in the presence of oligonucleotides (CL-2) containing all possible 4 major nucleosides at position 7.

In conclusion, we found that self-cleavage of hammerhead-like RNA occurs in partially paired RNA 21-mers at the consensus Cp site and the base can be replaced with adenine or uracil but not with guanine. It may also be assumed that stem III must contain two basepairs, possibly with different stabilities, e.g. A:U and C:G.

From this evidence, it may be possible to design RNA molecules with catalytic activities for the structure-dependent cleavage of RNA.

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