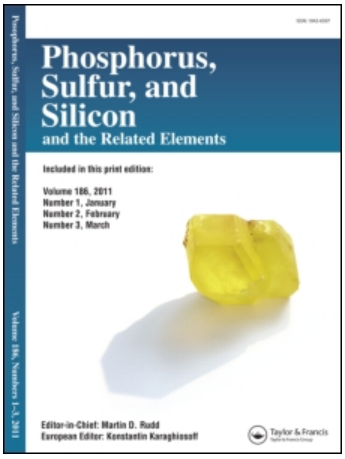

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

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To cite this Article Silnikov, Vladimir , Zuber, Guy , Behr, Jean-Paul , Giege, Richard and Vlassov, Valentin(1996) 'Design of Ribonuclease Mimics for Sequence Specific Cleavage of Rna', Phosphorus, Sulfur, and Silicon and the Related Elements, 109: 1, 277 — 280
To link to this Article: DOI: 10.1080/10426509608545144
URL: http://dx.doi.org/10.1080/10426509608545144

Phosphorus, Sulfur, and Silicon, 1996, Vols. 109–110, pp. 277–280
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DESIGN OF RIBONUCLEASE MIMICS FOR SEQUENCE SPECIFIC CLEAVAGE OF RNA

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Abstract. Hydrolysis of RNA by imidazole conjugates capable of binding to RNA have been investigated. Spermine-imidazole conjugate in the presence of imidazole buffer cleaves RNA at the single-stranded pyrimidine-purine sequences. Oligonucleotides with a diimidazole construction at the terminal phosphate cleave tRNA target in vicinity of the complementary sequences.

Key words antisense oligonucleotides; ribonuclease mimics; tRNA; RNA cleavage.

INTRODUCTION

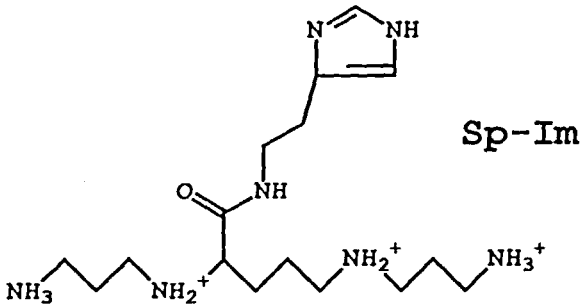
Small specific RNA cleaving agents are required for a number of important applications including development of probes for investigation of RNA structure [1] and design of efficient reactive groups for antisense oligonucleotide derivatives [2]. A few types of RNA cleaving groups including some complexes of metals, peptides and amines were conjugated to intercalating molecules and to oligonucleotides in order to prepare sequence specific artificial RNases [3-8]. Recently we have shown a possibility to mimic the active center of RNase A with small molecules containing two imidazole residues conjugated to an intercalating phenazine dye by linkers of variable length and flexibility (8). In this paper we describe hydrolysis of RNA by conjugates of imidazole with spermine and by oligonucleotides linked to a construction with two imidazole residues.

MATERIALS AND METHODS

Oligonucleotides **1** and **2** complementary to yeast phenylalanine tRNA were synthesized according to standard methods. Spermine- imidazole conjugate **Sp-Im** was synthesized as described in [9].

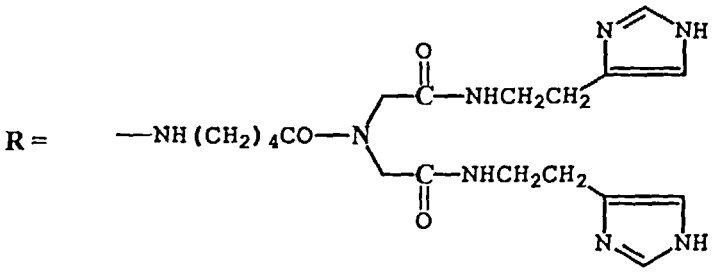
Synthesis of the diimidazole construction **R** will be described elsewhere. The construction was attached to the terminal phosphates of oligonucleotides by phosphorylation of the amino group with active 4-(N,N-dimethylamino)pyridinium derivatives of oligonucleotides. The latters were prepared by activation of the phosphate residue of oligonucleotides with mixture of triphenylphosphine and 2,2'-dipyridyldisulfide in the presence of 4-(N,N-dimethylamino)pyridine. The conjugates

were purified by reverse-phase HPLC and characterized by gel electrophoresis, UV spectroscopy and polyacrylamide gel electrophoresis.



R-1 R-pGATCGAACACAGGACCT

2-R TGGTGCGAATTCTp-R



Reaction mixtures contained 3'-end labeled $tRNA^{Phe}$ (50 to 100,000 Cerenkov counts) supplemented by 1 μg of carrier tRNA, dissolved in 20 μl of buffer. After the incubation, 20 μl of 0.3 M sodium acetate at pH 5.0 was added to each probe followed by 400 μl of a 2% solution of lithium perchlorate in acetone. The precipitated RNA samples were recovered by centrifugation, washed with acetone, dried and analyzed by electrophoresis.

RESULTS AND DISCUSSION

We expected that **Sp-Im** will bind to RNA electrostatically and deliver imidazole residue to the RNA backbone. The second imidazole needed for the reaction was expected to be provided as a free molecule by the buffer. Indeed it was found that **Sp-Im** cleaves tRNA in the presence of 10-50 mM imidazole buffer. **Sp-Im** alone as well as 50 mM imidazole buffer alone do not cleave tRNA, which proves specific nature of the reaction and eliminates a possibility of hydrolysis by some contaminants. The cleavage of phosphodiester bonds occurs predominantly within the single-stranded regions of the tRNA cloverleaf structure (Fig. 1 A). The major cleavages occur at positions 55 and 20. A reproducible cleavage was observed also at positions 8, 13, 36 and 43. The cleavage occurs after pyrimidines, in particular, in sequences CpA. This is in a general agreement

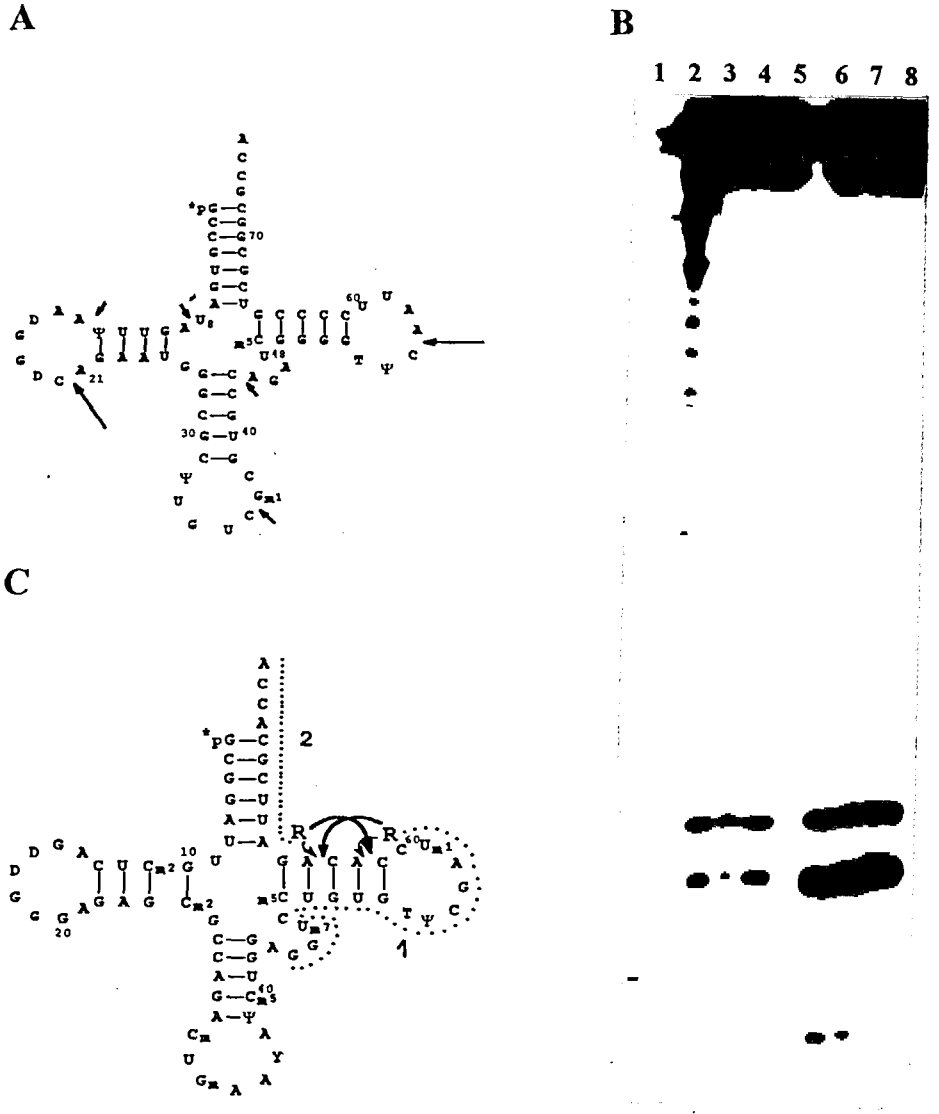


FIGURE 1. **A** $tRNA^{Asp}$. Phosphodiester linkages cleaved by the spermine-imidazole conjugate are indicated by arrows which lengths are proportional to the intensity of the cuts.

B. Cleavage of the 3'-end labeled $tRNA^{Phe}$ by oligonucleotides bearing diimidazole groups. Autoradiogram of a 15% denaturing polyacrylamide gel. The tRNA was incubated in 10 mM HEPES, 0.5 mM EDTA for 10 h at 37^0.

1, RNase T1 digest; 2, Ladder; 3-5 - **2-R**; 6-8 - **R-1**. Concentration of the conjugates was). 0.05 μM. In 4 and 7, the buffer contained 10 mM NaCl. In 8, oligonucleotide 1 was present (0.2 μM).

C. $tRNA^{Phe}$. Oligonucleotide conjugates are shown by zigzag lines along the complementary sequences. Arrows indicate the attacked phosphodiester bonds.

with fragile sites in RNA structure attacked most easily by RNase A and by bis-imidazole constructs tested earlier [8].

For oligonucleotide conjugates, yeast phenylalanine tRNA was used as a target. Results shown in Fig. 1 B,C evidence, that incubation of the tRNA with complementary oligonucleotide conjugates results in scission of phosphodiester bonds 61 and 63 of the target within the region where the imidazole groups were expected to be located. Cleavage occurs also at the phosphodiester bond 8, perhaps it juxtaposed of the sequence to the oligonuleotides binding site in the tertiary structure of the tRNA-oligonucleotide complex. Degradation of tRNA in in the absence of the reagents, in the presence of oligonucleotides without imidazole group or in the presence of the free group was negligible. Excess of the parent nonmodified oligonucleotide inhibited the cleavage which proves that the reaction was mediated by complementary complex formation.

The compounds investigated in this work represent a new family of RNA cleaving groups which can find applications as probes for investigation of RNA structure and function. The diimidazole groups can be used for design of the second generation antisense oligonucleotide derivatives for biological and therapeutic application.

ACKNOWLEDGEMENTS

This work was supported by International Science Foundation, CNRS, and Université Louis Pasteur as well as by the Ministry of Science, High Education, and Technical Policy of Russia in the frame of the State Program "Gene Targeted Biologically Active Compounds".

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